Patrick F. Fox
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Fundamentals of Cheese Science

Second Edition

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Preface to Second Edition

Since the publication of the first edition of this book in 2000, the importance and popularity of cheese have increased further. Approximately 19×10^6 tonnes were produced in 2014, representing 35–40 % of milk production. The second edition covers mainly the same topics as the first edition. Two chapters, "Acceleration of Cheese Ripening" and "Analytical Methods for Cheese," have been omitted; the former has been incorporated in "Biochemistry of Cheese Ripening." One new chapter, "Legislation on Cheese" has been introduced, and a specialist has been recruited to write the chapter "Cheese Flavour."

Cheese remains an active subject of research, and considerable progress has been made on the cheese that is summarized here. Advances have been made in aspects of cheese sciences during the past 15 years, but some areas are quite "mature," and consequently new knowledge is limited. Significant advances have been made on the physico-chemical aspects of cheese, e.g. mechanism of the gelation of rennet-altered casein micelles, the rheology of rennet-induced milk gels, syneresis of rennet- or acid-induced milk gels and the functional properties of cheese. Advances are probably most noticeable, however, in the microbiology of cheese, made possible by advances in molecular biology techniques. Most cheese is consumed as "Table Cheese," but the importance of cheese as an ingredient in composite foods, e.g., pizza, sauces, etc., is increasing, and the functionality of cheese in such applications has attracted much attention.

Fundamentals of Cheese Science provides comprehensive coverage of the scientific aspects of cheese, appropriate for anybody working with cheese, from lecturers, researchers and technologists to undergraduate and postgraduate students in food science and technology. The book assumes familiarity with biochemistry, microbiology and dairy chemistry, and it emphasizes fundamental principles rather than technological aspects.

The book is extensively referenced. References are divided into ”Suggested Reading,” comprised mainly of textbooks and reviews, and “References”, i.e., primary references to support claims made.

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Chapter 1
Cheese: Historical Aspects

Summary Agriculture dates from about 6000 BC, when certain plants and animals were domesticated in Mesopotamia. Among the domesticated animals were goats, sheep and cattle, the milk of which was consumed by Man as a high-quality nutrient. Milk is also a very good growth substrate for bacteria, some of which produce lactic acid, which causes the milk to gel. The acidified milk was consumed as cultured milk or converted to acid-curd cheese. It was also discovered that milk could be coagulated by certain proteolytic enzymes, e.g., chymosin from the stomach of neonatal mammals; the coagulum was converted to rennet-curd cheese. Cheese has been produced since the earliest civilizations, e.g., Sumer and Egypt and was well established in Classical Rome. Cheese production spread throughout Europe and the Middle East and later to North and South America and Oceania and evolved as at least 1000 varieties. Cheese production was a farm-based operation until the mid-nineteenth century, and much cheese is still produced at an artisanal level. However, the principal varieties are now produced in very large highly mechanized factories by highly developed technology.

Current production of cheese is about 19×10^6 tonnes per annum, predominantly in Europe, North and South America and Oceania. Approximately 35 % of all milk is used for cheese production.

The objectives of this chapter are to provide a brief history of cheese production and identify the principal areas of production and consumption.

Keywords Discovery and spread of cheese • Production and consumption of cheese

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1.1 Introduction

Cheese is the generic name for a group of fermented milk-based food products, produced throughout the world in a great diversity of flavours, textures and forms. Sandine and Elliker (1970) suggest that there are more than 1000 varieties of cheese. Walter and Hargrove (1972) described about 400 varieties and listed the names of a further 400, Burkhalter (1981) classified 510 varieties and Harbutt (2009) includes photographs and descriptions of about 750 varieties.

It is believed that cheese evolved in the 'Fertile Crescent' between the Tigris and Euphratres rivers, in Iraq, some 8000 years ago during the "Agricultural Revolution", when certain plants and animals were domesticated. Among the earliest animals domesticated were goats and sheep; being small, gregarious and easily herded, these were used to supply meat, milk, hides and wool. Cattle were more difficult to domesticate; wild cattle were much larger and more ferocious than modern cattle and were also less well suited to the arid Middle East than goats and sheep. Apparently, cattle were used initially mainly as work animals and were not used as a major source of milk until relatively recently. The nutritive value of milk produced by domesticated animals was soon recognised and milk and dairy products became important components of the human diet.

Milk is also a rich source of nutrients for bacteria which contaminate the milk and grow well under ambient conditions. Some contaminating bacteria utilize milk sugar, lactose, as a source of energy, producing lactic acid as a by-product; these bacteria, now known as lactic acid bacteria (LAB), include the genera *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Leuconostoc*, *Pediococcus* and *Wiessla*. LAB are used in the production of a wide range of fermented milk, meat and vegetable products. They are generally considered to be beneficial to human health and have been studied extensively (see Chap. 6). Bacterial growth and acid production would have occurred in milk during storage or during attempts to dry milk in the prevailing warm, dry climate to produce a more stable product; air-drying of meat, and probably fruits and vegetables, appears to have been practiced as a primitive form of food preservation at this period in Man's evolution. When sufficient acid is produced, the principal proteins in milk, the caseins, coagulate at ambient temperature (21 °C) in the region of their isoelectric points (~pH 4.6) to form a gel in which the fat and aqueous phases of milk are entrapped. Thus, the first fermented dairy foods were probably produced accidentally. Numerous basically similar products are still produced in various regions of the world by artisanal methods, which are probably little different from those used several thousand years ago. Some "descendants" of these ancient fermented milks are now produced by scientifically based technology.

Fermented dairy foods were produced by a fortuitous combination of events, i.e., the ability of LAB to grow in milk and to produce just enough acid to reduce its pH to the isoelectric point of the caseins. Neither the lactic acid bacteria nor the caseins were designed for this function. The caseins were "designed" to be coagulated enzymatically in the stomach of neonatal mammals, the gastric pH of which is

around 6, i.e., much higher than the isoelectric point of the caseins. The ability of LAB to ferment lactose, a sugar specific to milk, is frequently encoded on plasmids, suggesting that this characteristic was acquired relatively recently in the evolution of these bacteria. Their natural habitats are vegetation, from which they presumably colonized the teats of mammals which had been contaminated with lactose-containing milk.

An acid-induced milk gel is quite stable if left undisturbed but, if broken, either accidentally (e.g., by movement of the storage vessels) or intentionally, it separates into curds and whey. Acid whey is a pleasant, refreshing drink for immediate consumption while the curds could be consumed fresh or stored for future use. The shelf-life of the curds can be greatly extended by dehydration and/or by adding salt; heavily salted cheese varieties (e.g., Feta and Domiati) are still widespread throughout the Middle East and the Balkans where the ambient temperature is high. Air/sun-dried varieties of fermented milk and cheese are not common today but numerous varieties survive throughout the hot dry areas of North Africa and the Middle East. Today, acid-coagulated cheeses, which include Cottage cheese, Cream cheese, Quarg, *Fromage frais* and some varieties of Quesco Blanco, represent ~22 % of total cheese production and in some countries are the principal varieties. They are consumed fresh (not ripened) and are widely used in other products, e.g., cheesecake, cheese-based dips and sauces.

Some proteolytic enzymes can modify the milk protein system, causing it to coagulate under certain circumstances, offering an alternative mechanism for coagulating milk; this mechanism was also recognized at an early date. Enzymes capable of causing this transformation are widespread and are found in bacteria, moulds, plant and animal tissues, but the most obvious source would have been animal stomachs. It would have been observed that the stomachs of slaughtered young animals frequently contained curds, especially if the animals had suckled shortly before slaughter; curds would also have been observed in the vomit of human infants. Before the development of pottery (~5000 BC), storage of milk in bags made from animal skins was probably common (it is still practiced in many regions of the world). Stomachs from slaughtered animals provided ready-made, easily-sealed containers; if stored in such containers, milk would extract coagulating enzymes (referred to as rennets) from the stomach tissue, leading to its coagulation during storage. The properties of rennet-coagulated milk curds are very different from those of acid-produced curds, e.g., they have better syneretic properties (ability to exude whey) which make it possible to produce low-moisture cheese curd without hardening. Rennet-coagulated curds can, therefore, be converted to more stable, low-moisture products and rennet coagulation has become the principal mechanism for milk coagulation in cheese manufacture; most modern cheese varieties and ~75 % of total world production of cheese are produced by this mechanism.

During the storage of rennet-coagulated curds, various bacteria may grow in the curd and the enzymes in rennet continue to act. Thus, the flavour and texture of the cheese curds change during storage. When controlled, this process is referred to as ripening (maturation), during which a great diversity of characteristics flavours and textures develop. Although animal rennets were probably the first enzyme coagulants

used, rennets produced from a range of plant species, e.g., figs and thistle, appear to have been common in Roman times. However, plant rennets are not suitable for the manufacture of long-ripened cheese varieties and gastric proteinases from young animals became the standard rennets until a shortage of supply made it necessary to introduce "rennet substitutes", which are discussed in Chap. 7.

While the coagulation of milk by in situ production of lactic acid was, presumably, accidental, the use of rennets to coagulate milk was intentional. It was, in fact, quite an ingenious development—if the conversion of milk to cheese by the use of rennets was discovered today, it would be hailed as a major biotechnological discovery! The use of rennets in cheese manufacture is probably the oldest, and is still one of the principal, industrial applications of enzymes.

The advantages accruing from the ability to convert the principal constituents of milk to cheese would have been apparent from the viewpoints of storage stability, ease of transport and, eventually, as a means of diversifying the human diet. Cheese manufacture accompanied the spread of civilization throughout the Middle East, Egypt, Greece and Rome. There are several references in the Old Testament to cheese, e.g. Job (1520 BC) and Samuel (1170–1017 BC), on the walls of tombs of Ancient Egypt and in classical Greek literature, e.g. Homer (1184 BC), Herodotus (484–408 BC) and Aristotle (384–322 BC). Cheese manufacture was well established in Classical Rome and cheese was included in the rations of Roman soldiers. The demand for cheese in Rome must have exceeded supply since the Emperor Diocletian (284–305 AD) fixed a maximum price for cheese. Many Roman writers, e.g., Cato the Elder (234–149 BC), Varro (116–27 BC), Columella (4–70 AD), Pliny the Elder (23–79 AD) and Palladius (c 400–470 AD) described the manufacture, quality attributes and culinary uses of cheese; Columella, in particular, gave a detailed account of cheese manufacture in his treatise on agriculture, *De Re Rustica*.

Movements of Roman armies and administrators contributed to the spread of cheese throughout the then known world. Cheesemaking practice appears to have changed little from the time of Columella and Palladius until the nineteenth century. The great migrations of peoples throughout Europe after the fall of the Roman Empire promoted the spread of cheese manufacture, as did the Crusaders and pilgrims of the Middle Ages. However, the most important contributors to the development of cheese 'technology' and to the evolution of cheese varieties during the Middle Ages were the monasteries and feudal estates. In addition to their roles in the spread of Christianity and in the preservation and expansion of knowledge during the Dark Ages, the monasteries were major contributors to the advancement of agriculture in Europe and to the development and improvement of food commodities, notably wine, beer and cheese. Many current cheese varieties were developed in monasteries, e.g., Wenslydale (Rievaulx Abbey, Yorkshire), Port du Salut or Saint Paulin (Monastery de Notre Dame du Port du Salut, Laval, France), Fromage de Tamie (Abbey of Tamie, Lac d'Annecy, France), Maroilles (Abbey Moroilles, Avesnes, France); Trappist (Maria Stern Monastery, Banja Luka, Bosnia). The inter-monastery movement of monks probably contributed to the spread of cheese varieties and to the development of new cheeses.

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The great feudal estates of the Middle Ages were self-contained communities which, in the absence of an effective transport system, relied on a supply of locally produced foods. Surplus food was produced in summer and preserved to meet the requirements of the community throughout the year. Especially in cool, wet Europe, fermentation and salting were the most effective principles for food preservation; well-known examples of such products include fermented and salted meat, salted fish, beer, wine, fermented vegetables and cheese, the manufacture of which exploits both fermentation and salting. Cheese probably represented an item of trade when amounts surplus to local requirements were available. Within large estates, individuals acquired special skills which were passed on to succeeding generations. The feudal estates evolved into villages and some into larger communities. Because monasteries and feudal estates were essentially self-contained communities with limited inter-community travel, it is readily apparent how several hundred distinct varieties of cheese could have evolved from essentially the same raw material. Traditionally, many cheese varieties were produced in quite limited geographical regions, especially in mountainous areas. The localized production of certain varieties is still apparent and indeed is preserved for those varieties with a Protected Designation of Origin (PDO). Regionalization of certain cheese varieties is still particularly marked in Spain, Italy, France and Greece, where the production of many varieties is restricted to very limited, sometimes legally-defined, regions. Almost certainly, most cheese varieties evolved by accident because of particular local circumstances, e.g., species or breed of dairy animal, a peculiarity of the local milk supply with respect to chemical composition or microflora, or an 'accident' during the manufacture or storage of the cheese, e.g., growth of moulds or other microorganisms. Presumably, those accidents that led to desirable changes in the quality of the cheese were incorporated into the manufacturing protocol; thus each variety has undergone a series of evolutionary changes and refinements.

The final chapter in the spread of cheese throughout the world resulted from the colonization of North and South America, Oceania and Africa by European settlers who carried their cheesemaking skills with them. Cheese has become an item of major economic importance in some of these "new" countries, notably the USA, Canada, Australia, New Zealand and Argentina, but the varieties produced are mainly of European origin, modified in some cases to meet local conditions and requirements. There is no evidence that cheese was produced in the Americas or Oceania prior to colonization; in fact, animals had not been domesticated for milk production in these countries.

Cheesemaking remained a craft until relatively recently. With the gradual acquisition of knowledge on the chemistry and microbiology of milk and cheese, it became possible to direct the changes involved in cheesemaking in a more controlled fashion. Although few new varieties have evolved as a result of this improved knowledge, existing varieties have become better defined and their quality more consistent. Although the names of many current varieties were introduced several hundred years ago (Table 1.1), it is very likely that those cheeses were not very comparable to their modern counterparts. Cheesemaking was not standardized until relatively recently; for example, the first attempt to standardize the well-known English varieties, Cheddar and Cheshire, was made by Joseph Harding in the mid-nineteenth

Table 1.1 First recorded date for some major cheese varieties[a]

Gorgonzola	897	Cheddar	1500
Schabzieger	1000	Parmesan	1579
Roquefort	1070	Gouda	1697
Maroilles	1174	Gloucester	1783
Schwangenkase	1178	Stilton	1785
Grana	1200	Camembert	1791
Taleggio	1282	St. Paulin	1816

[a]From Scott (1986)

century. Prior to that, 'Cheddar cheese' was that produced around the village of Cheddar, in Somerset, England, and probably varied considerably depending on the cheesemaker and other factors. Cheese manufacture was a farmstead enterprise until the mid-nineteenth century—the first cheese factory in the US was established near Rome, New York, in 1851 and the first in Britain at Longford, Derbyshire, in 1870. Thus, there were thousands of small-scale cheese manufacturers and there must have been great variation within any one type. When one considers the very considerable inter-factory, and indeed intra-factory, variation in quality and characteristics which still occur today in well-defined varieties, e.g., Cheddar, in spite of the very considerable scientific and technological advances, one can readily appreciate the variations that must have existed in earlier times.

Some major new varieties, e.g., Jarlsberg, Maasdamer, Regato and Dubliner have been developed recently as a consequence of scientific research. Many other varieties have evolved very considerably, even to the extent of becoming new varieties, as a consequence of scientific research and the development of new technology—notable examples are Quesco Blanco as produced in the USA, Feta-type cheese produced from ultrafiltered milk and various forms of Quarg. There has been a marked resurgence of farmhouse cheesemaking in recent years; many of the cheeses being produced on farms are not standard varieties and it will be interesting to see if some of these evolve to become new varieties.

A major cause of variation in the characteristics of cheese is the species from which the milk is produced. Although milk from several species is used commercially, the cow is by far the principal producer; worldwide, 85 % of commercial milk is bovine, with 11 %, 2 % and 1.5 % being produced by water buffalo, sheep and goats. However these species are major producers of milk in certain regions, e.g., the Mediterranean basin and India; goats and sheep are especially important in cheese production since the milk of these species is used mainly for the production of fermented milks and cheese. Many world-famous cheeses are produced from sheep's milk, e.g., Roquefort, Feta, Romano and Manchego; traditional Mozzarella is made from the milk of the water buffalo. As will be discussed in Chap. 4, there are very significant interspecies differences in the composition of milk which are reflected in the characteristics of the cheeses produced from them. There are also significant differences in milk composition between breeds of cattle and these influence cheese quality, as do variations due to seasonal, lactational and nutritional factors and of course the methods of milk production, storage and collection.

Brief histories of the history of cheese are provided by several authors, especially by Scott (1986). Kindstedt (2013) gives a very interesting account of the history of cheese and its place in Western culture.

1.2 Cheese Production and Consumption

World production of cheese is ~19×10^6 tonnes per annum (~35 % of total milk production) and has increased at an average annual rate of ~4 % over the past 30 years. Europe, with a production of ~11×10^6 tonnes per annum, is the largest producing block (Table 1.2). Thus, while cheese manufacture is practiced worldwide, it is apparent from Table 1.2 that cheese is primarily a product of European countries and those populated by European immigrants.

Cheese consumption varies widely between countries, even within Europe (Table 1.2). Cheese consumption in most countries for which data are available has

Table 1.2 Production and consumption of cheese, 2011 (IDF 2012)

Country	Cheese production (1000 tonnes)	Cheese consumption (kg per caput)
World	**18,833**	
Africa	*678*	
Egypt	620	10.1
South Africa	47	1.0
Nigeria	9	
Zimbabwe	2	
Oceania	*606*	
Australia	349	11.7
New Zealand	257	3.5
Asia	*981*	
Turkey	519	6.7
Iran	260	4.6
Israel	125	16.1
Japan	45	1.9
China	20	0.2
India	8	
South Korea	4	2.0
North & Central America	*5412*	
United States	4807	15.1
Canada	330	12.3
Mexico	275	3.1
South America	*1388*	
Brazil	675	3.6
Argentina	521	11.5

(continued)

Table 1.2 (continued)

Country	Cheese production (1000 tonnes)	Cheese consumption (kg per caput)
Chile	90	7.2
Uruguay	61	6.3
Colombia	41	0.9
European Union	*8634*	*17.1*
Germany	2196	22.9
France	1930	26.3
Italy	1094	21.8
Netherlands	750	19.4
Poland	650	11.4
United Kingdom	359	10.9
Denmark	276	16.4
Greece	190	23.4
Ireland	180	6.7
Austria	160	19.9
Spain	130	9.6
Czech Republic	110	16.3
Lithuania	104	14.2
Sweden	103	19.9
Finland	101	22.5
Belgium	76	15.3
Hungary	69	11.0
Romania	62	4.3
Estonia	41	19.6
Slovakia	32	10.3
Latvia	28	13.5
Cyprus	14	17.9
Luxembourg	–	24.1
Others	138	12.0
Other European	1132	
Russia	425	5.8
Ukraine	255	4.1
Switzerland	182	21.8
Belarus	149	–
Norway	84	17.4
Croatia	30	7.7
Iceland	7	24.1

increased consistently over many years; along with fermented milks, cheese is the principal growth product within the dairy sector. There are many reasons for the increased consumption of cheese, including a positive dietary image, convenience and flexibility in use and the great diversity of flavours and textures. Cheese can be regarded as the quintessential convenience food: it can be used as a major

component of a meal, as a dessert, as a component of other foods or as a food ingredient; it can be consumed without preparation or subjected to various cooking processes. The most rapid growth in cheese consumption has occurred in its use as a food component or ingredient; these applications will be discussed in Chap. 19.

1.3 Cheese Science and Technology

Cheese is the most diverse group of dairy products and is, arguably, the most academically interesting and challenging. While many dairy products, if properly manufactured and stored, are biologically, biochemically and chemically very stable, cheeses are, in contrast, biologically and biochemically active, and, consequently, undergo changes in flavour, texture and functionality, to a degree which is variety-dependent, during storage. Throughout manufacture and ripening, cheese production represents a finely orchestrated series of consecutive and concomitant biochemical events which, if synchronized and balanced, lead to products with highly desirable aromas and flavours but when unbalanced, result in off-flavours and odours. Considering that, in general terms, a basically similar raw material (milk from a very limited number of species) is subjected to a manufacturing protocol, the general principles of which are common to most cheese varieties, it is fascinating that such a diverse range of products can be produced. No two batches of the same variety are identical.

A further important facet of cheese is the range of scientific disciplines involved: study of cheese manufacture and ripening involves the chemistry and biochemistry of milk constituents, chemical characterization of cheese constituents, microbiology, enzymology, molecular genetics, flavour chemistry, rheology and chemical engineering. It is not surprising, therefore, that many scientists have become involved in the study of cheese manufacture and ripening. A voluminous scientific and technological literature has accumulated, including several textbooks (see suggested reading list) and chapters in many others. Many of these textbooks deal mainly with cheese technology or assume an overall knowledge of cheese. The present book is intended to provide a fairly comprehensive treatment of the scientific aspects of cheese.

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Chapter 2
Overview of Cheese Manufacture

Summary The objective of this chapter is to present a very brief description of the principal operations of cheese production so that the operations described in the following chapters can be seen in an overall context

Introduction

The production of all varieties of cheese involves a generally similar protocol (Fig. 2.1), various steps of which are modified to give a product with the desired characteristics. The principal general steps are

1. Selection, standardization and, in most cases, pasteurization of the milk.
2. Acidification, usually via the in situ production of lactic acid by selected bacteria.
3. Coagulation of the milk by acidification or limited proteolysis.
4. Dehydration of the coagulum to yield cheese curd, by a range of techniques, some of which are variety-specific.
5. Forming the curds into characteristic shapes.
6. For most varieties, ripening (maturation) of the curd during which the characteristic flavour and texture of the cheese develop.

The objective of this chapter is to present a very brief description of the principal operations so that the operations described in the following chapters can be seen in an overall context.

Keywords Selection and treatment of cheesemilk • Annato • Coagulation • Salting • Ripening • Processed cheese

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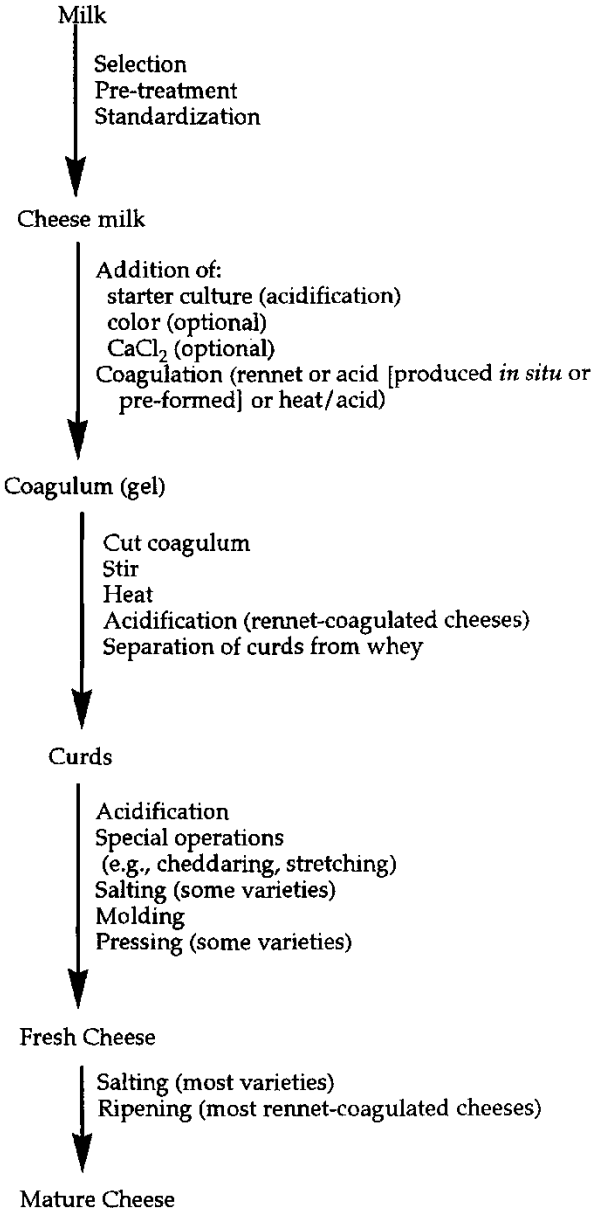


Fig. 2.1 General protocol for cheese manufacture

2.1 Selection of Milk

The composition of cheese is strongly influenced by the composition of the cheese milk, especially the content of fat, protein, calcium and pH. The constituents of milk, which are described in Chap. 4, are influenced by several factors, including species, breed, individuality, nutritional status, health and stage of lactation of the producing animal. Owing to major compositional abnormalities, milk from cows in the very early or late stages of lactation and those suffering from mastitis should be excluded. Somatic cell (leucocyte) count is a useful index of quality. Some genetic polymorphs of the milk proteins have a significant effect on cheese yield and quality and there is increasing interest in breeding for certain polymorphs. The milk should be free of chemical taints and free fatty acids, which cause off-flavours in the cheese, and antibiotics which inhibit bacterial cultures.

The milk should be of good microbiological quality, as contaminating bacteria will be concentrated in the cheese curd and may cause defects or public health problems. This subject will be discussed in Chap. 5.

2.2 Standardization of Milk Composition

Milk for cheese is subjected to a number of pre-treatments, with various objectives.

Different cheese varieties have a characteristic fat-in-dry matter content, in effect, a certain fat-to-protein ratio and this situation has legal status in the "Standards of Identity" for many cheese varieties. While the moisture content of cheese, and hence the level of fat plus protein, is determined mainly by the manufacturing protocol, the fat:protein ratio is determined mainly by the fat:casein ratio in the cheese milk. Depending on the ratio required, it can be modified by:

- removing some fat by natural creaming, as in the manufacture of Parmigiano Reggiano, or centrifugation
- adding skim milk
- adding cream
- adding micellar casein (prepared by ultrafiltration)
- adding milk powder, evaporated milk or ultrafiltration retentate. Such additions also increase the total solids content of the milk and hence cheese yield and will be discussed in Chap. 10.

Calcium plays a major role in the coagulation of milk by rennet and subsequent processing of the coagulum and hence it is common practice to add $CaCl_2$ (e.g., 0.01 %) to cheese milk.

The pH of milk is a critical factor in cheesemaking. The pH is inadvertently adjusted by the addition of 1.5–2 % starter culture which reduces the pH of the milk immediately by about 0.1 unit. Starter concentrates (sometimes called direct-to-vat starters), which are now used widely, have no immediate acidifying effect.

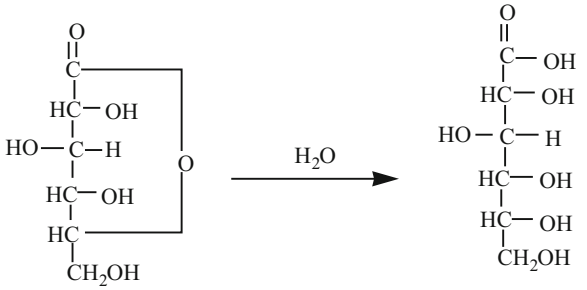
Previously, it was standard practice to add the starter to the cheese milk 30–60 min before rennet addition. During this period, the starter microorganisms began to grow and produce acid, a process referred to as "ripening". Ripening served a number of functions:

- it allowed the starter bacteria to enter their exponential growth phase and hence to be highly active during cheesemaking; this is not necessary with modern high-quality starters.
- the lower pH was more favourable for rennet action and gel formation.

However, the practice increases the risk of bacteriophage infection of the starter as phage become distributed throughout the liquid milk but is reduced after the milk has coagulated (see Chap. 6). Although ripening is still practiced for some varieties, it has been discontinued for most varieties.

The pH of milk on reception at the dairy is higher today than it was previously owing to improved hygiene during milking and the more widespread use of refrigeration at the farm and factory. In the absence of acid production by contaminating bacteria, the pH of milk increases slightly during storage due to the loss of CO_2 to the atmosphere. The natural pH of milk is ~6.6–6.7 but varies somewhat (e.g., it increases in late lactation and during mastitic infection).

To offset these variations and to reduce the pH as an alternative to ripening, the pre-acidification of milk by 0.1–0.2 pH units is recommended, either through the use of the acidogen, gluconic acid-δ-lactone, or by limited growth of a lactic acid starter, followed by pasteurization (referred to as pre-maturation).



Gluconic acid-δ-lactone Gluconic acid

2.3 Heat Treatment of Milk

Traditionally, all cheese was made from raw milk, a practice which remained widespread until the 1940s. Even today, significant amounts of cheese are made in Europe from raw milk. The use of raw milk may be undesirable due to:

- Public health safety
- The presence of undesirable microorganisms which may cause defects or variability in flavour and/or texture.

When cheese was produced from fresh milk on farms or in small, local factories, the growth of contaminating microorganisms was very low but as cheese factories became larger, storage of milk for longer periods became necessary and hence the microbiological quality of the milk varied. For public health reasons, it became increasingly popular from the beginning of the twentieth century to pasteurize milk for liquid consumption. The pasteurization of cheese milk became widespread about 1940, primarily for public health reasons, but also to provide a milk supply of more uniform bacteriological quality and to improve its keeping quality. Although a considerable amount of cheese is still produced from raw milk, on both an artisanal and factory scale, especially in southern Europe (including such famous varieties as Swiss Emmental, Gruyère de Comté, Parmigiano Reggiano and Grano Padano), pasteurized milk is now generally used, especially in large factories. The flavour of cheese made from raw milk is different from and more intense than that from pasteurized because beneficial indigenous LAB, which may contribute positively to cheese flavour, are killed by pasteurization, To counteract the loss of such LAB, it is becoming increasingly common to add a culture of selected LAB (lactobacilli) to cheese milk in addition to the main acid-producing culture. Some indigenous enzymes, e.g., lipase, which may contribute positively to cheese ripening, are also inactivated by pasteurization. A sub-pasteurization temperature, eg., 68–70 °C may be used for cheese milk and a temperature >72 °C × 15 s should not be used, owing to damage to the cheesemaking properties of milk (see Chaps. 7 and 8). Aspects of pasteurization are discussed in Chap. 5.

There are four alternatives to pasteurization for reducing the number of microorganisms in milk:

- treatment with H_2O_2
- Activation of the lactoperoxidase-H_2O_2-thiocyanate system.
- Bactofugation
- Microfiltration

These processes are also discussed briefly in Chap. 5.

2.4 Cheese Colour

Colour is a very important attribute of foods and serves as an index of quality, although in some cases, this is cosmetic. The principal indigenous pigments in milk are carotenoids which are obtained from the animal's diet, especially from fresh grass and clover. The carotenoids are secondary pigments involved in photosynthesis; the structure of β-carotene is shown in Fig. 2.2. Owing to the conjugated double bond system, carotenoids absorb ultraviolet and visible light, giving them colours ranging from yellow to red. They are responsible for the

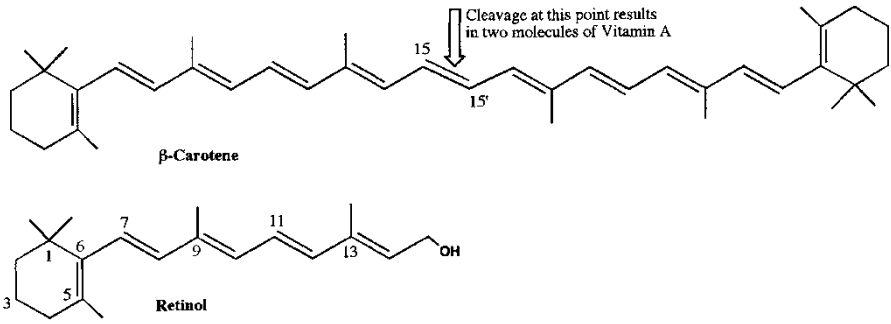


Fig. 2.2 Structures of β-carotene and retinol

colour of many foods, e.g., carrots, squashes, peppers, maize; they are also present in the leaves of plants in which their colour is masked by the green chlorophylls. Some carotenoids have pro-vitamin A activity and may be converted to retinol (vitamin A; Fig. 2.2) in the body.

Animals do not synthesize carotenoids but absorb them from plant materials in their diet. In addition to serving as pro-vitamin A, some animals store carotenoids in their tissues, which then acquire a colour, e.g., salmon, cooked lobster and egg yolk. Cattle transfer carotenoids to adipose tissue and milk but goats, sheep and buffalo do not. Therefore, bovine milk and products made therefrom are yellow to an extent dependent on the carotenoid content of the animal's diet. Products such as butter and cheese made from sheep, goat or buffalo milk are very white in comparison with their counterparts made from bovine milk. This yellowish colour may make products produced from cows' milk less acceptable than products produced from sheep's, goats' or buffalo milk in Mediterranean countries where the latter are traditional. The carotenoids in bovine milk can be bleached by treatment with H_2O_2 or benzoyl peroxide or masked by chlorophyll or titanium oxide (TiO_2), although such practices are not permitted in all countries.

At the other end of the spectrum are individuals who prefer highly coloured cheese, butter or egg yolk. Such intense colours may be obtained by adding carotenoids (synthetic or natural extracts) directly to the product or to the animal's diet. In the case of cheese and dairy products, annatto, extracted from the pericarp of the seeds of the annatto plant (*Bixa orellana*), a native of Brazil, is used most widely. Annatto contains two apocaroetnoid pigments, bixin and norbixin (Fig. 2.3). By suitable modification, the annatto pigments can be made fat-soluble, for use in butter or margarine, or water-soluble for use in cheese.

Initially, annatto may have been used in cheese manufacture to give the impression of a high fat content in partially skimmed cheese but some people believe that coloured ("red") cheese tastes better than its white counterpart of equivalent quality.

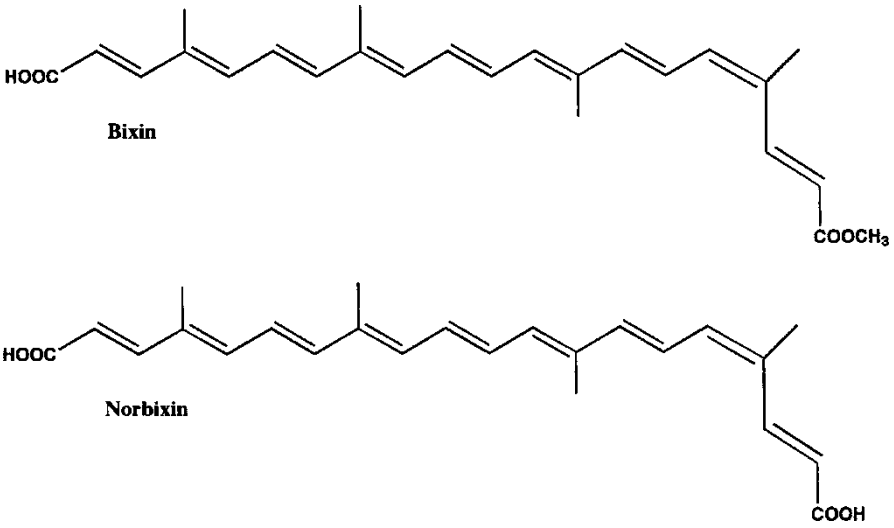


Fig. 2.3 Structures of *cis*-bixin and norbixin, the apocarotenoid pigments in annatto

2.5 Conversion of Milk to Cheese Curd

After the milk has been standardized, pasteurized or otherwise treated, it is transferred to vats (or kettles) which vary in shape (hemi-spherical, rectangular, vertical or horizontal cylindrical), may be open or closed and may range in size from a few hundred litres to 20,000–40,000 L [a selection of vats are shown in Fig 2.4]. Here, it is converted to cheese curd, a process that involves three basic operations: acidification, coagulation and dehydration.

2.5.1 Acidification

Acidification is usually achieved by the in situ production of lactic acid through the fermentation of the milk sugar, lactose, by lactic acid bacteria. Initially, the indigenous milk microflora was relied upon to produce acid but since this microflora is variable, the rate and extent of acidification are variable, resulting in cheese of variable quality. Cultures of lactic acid bacteria for cheesemaking were introduced commercially about 130 years ago and have been progressively improved and refined. The science and technology of starters are described in Chap. 6. The acidification of curd for some artisanal cheeses still relies on the indigenous microflora.

Direct acidification using acid (usually lactic or HCl) or acidogen (usually gluconic acid-δ-lactone) may be used as an alternative to biological acidification and is used commercially to a significant extent in the manufacture of Cottage, Quarg, Feta-type cheese from UF-concentrated milk and Mozzarella. Direct acidification is



Fig. 2.4 Examples of vats used for cheesemaking

more controllable than biological acidification and, unlike starters, is not susceptible to phage infection. However, in addition to acidification, the starter bacteria serve very important functions in cheese ripening (see Chaps. 11 and 12) and hence chemical acidification is used mainly for cheese varieties for which texture is more important than flavour.

The rate of acidification is fairly characteristic of the variety and its duration ranges from 5 to 6 h for Cheddar and Cottage to 10–12 h for Dutch and Swiss types. The rate of acidification, which depends on the amount and type of starter used and on the temperature profile of the curd, has a major effect on the texture of cheese, mainly through its solubilizing effect on colloidal calcium phosphate; this subject is discussed in Chap. 14.

Regardless of the rate of acidification, the ultimate pH of the curd for most hard cheese varieties is in the range 5.0–5.3 but it is 4.6 for the soft, acid-coagulated varieties, e.g., Cottage, Quarg and Cream, and some rennet-coagulated varieties, e.g., Camembert and Brie.

The production of acid at the appropriate rate and time is a key step in the manufacture of good quality cheese. Acid production affects several aspects of cheese manufacture, many of which will be discussed in more detail later:

- Coagulant activity during coagulation (Chap. 7).
- Denaturation and retention of the coagulant in the curd during manufacture and hence the level of residual coagulant in the curd; this influences the rate of proteolysis during ripening, and may affect cheese quality (Chaps. 8 and 12).
- Curd strength, which influences cheese yield (Chap. 10).
- Gel syneresis, which controls cheese moisture and hence regulates the growth of bacteria and the activity of enzymes in the cheese; consequently, it strongly influences the rate and pattern of ripening and the quality of the finished cheese (Chaps. 8, 12 and 15).
- The rate of acidification determines the extent of dissolution of colloidal calcium phosphate which modifies the susceptibility of the caseins to proteolysis during ripening and influences the rheological properties of the cheese, e.g., compare the texture of Emmental, Gouda, Cheddar and Cheshire cheese (see Chap. 14).
- Acidification controls the growth of many non-starter bacteria in cheese, including pathogenic, food-poisoning and gas-producing microorganisms; properly-made cheese is a very safe product from the public health viewpoint (see Chap. 19).

The level and time of salting have a major influence on pH changes in cheese. The concentration of NaCl in cheese (commonly 0.7–4 %, equivalent to 2–10 % salt in the moisture phase) is sufficient to halt the growth of starter bacteria. Some varieties, mostly of British origin, are salted by mixing dry salt with the curd toward the end of manufacture and hence the pH of curd for these varieties must be close to the ultimate value (~ pH 5.1) at salting. However, most varieties are salted by immersion in brine or by surface application of dry salt; salt diffusion in cheese moisture is a relatively slow process and thus there is ample time for the pH to decrease to ~5.0 before the salt concentration becomes inhibitory throughout the interior of the cheese. The pH of the curd for most cheese varieties, e.g., Swiss, Dutch, Tilsit, Blue, etc., is 6.2–6.5 at moulding and pressing but decreases to ~5–5.2 during or shortly after pressing and before salting. The significance of various aspects of the concentration and distribution of NaCl in cheese are discussed in Chap. 9.

In a few special cases, e.g., Domiati, a high level of NaCl (10–12 %) is added to the cheesemilk, traditionally to control the growth of the indigenous microflora. This concentration of NaCl has a major influence, not only on acid development, but also on rennet coagulation, gel strength and curd syneresis.

2.5.2 Coagulation

The essential characteristic step in the manufacture of all cheese varieties involves coagulation of the casein component of the milk protein system to form a gel which entraps the fat, if present. Coagulation may be achieved by:

- Limited proteolysis by selected proteinases (rennets);
- Acidification to ~pH 4.6;
- Acidification to a pH value >4.6 (perhaps ~5.2) in combination with heating to ~90 °C.

The majority of cheese varieties, and ~75 % of total production, are produced by rennet coagulation but some acid-coagulated varieties, e.g., Quarg, Cottage and Cream, are of major importance. The coagulation of milk by rennets or acid are discussed in Chaps. 7 and 16, respectively. Acid-heat-coagulated cheeses are of relatively minor importance and are usually produced from whey or a blend of whey and skim milk and probably evolved as a useful means for recovering the nutritionally-valuable whey proteins. Their properties are very different from those of rennet- or acid-coagulated cheeses and they are usually used as food ingredients. Important varieties are Ricotta and related varieties (indigenous to Italy), Anari (Cyprus) and Manouri (Greece) (see Chaps. 3 and 18).

A fourth, minor, group of cheeses is produced, not by coagulation, but by thermal evaporation of water from a mixture of whey and skim milk, whole milk or cream and crystallization of lactose. Varietal names include Mysost and Gjetost. These cheeses, which are almost exclusive to Norway, bear little resemblance to rennet- or acid-coagulated cheeses and probably should be classified as whey products rather than cheese, *sensu stricto*.

2.5.3 Post-Coagulation Operations

Rennet or acid-coagulated milk gels are quite stable if maintained under quiescent conditions but if cut or broken, they synerese, expelling whey. Syneresis essentially concentrates the fat and casein of milk by a factor of 6–12, depending on the variety. In the dairy industry, concentration is normally achieved through thermal evaporation of water and more recently by removing water through semi-permeable membranes. The syneresis of rennet- or acid-coagulated milk gels is thus a rather unique method for dehydration, dependent on special characteristics of the caseins.

The rate and extent of syneresis are influenced, *inter alia*, by milk composition, especially the concentrations of Ca^{2+} and casein, pH of the whey, cooking temperature, rate of stirring of the curd-whey mixture and, of course, time (see Chap. 8). The composition of the finished cheese is determined by the extent of syneresis and since this is under the control of the cheesemaker, it is here that the differentiation of the individual cheese varieties really begins, although the type and composition of the milk, the amount and type of starter and the amount and type of rennet are also significant in this regard.

A more or less unique protocol has been developed for the manufacture of each cheese variety. These protocols differ mainly with respect to the syneresis process. The protocols for the manufacture of the principal families of cheese are summarized in Chap. 3.

2.5.4 Removal of Whey, Moulding and Pressing of the Curd

When the desired degree of syneresis has been achieved and in some cases, the desired pH attained also, the curds are separated from the whey by a variety-specific method, e.g., transferring the curds-whey into perforated moulds (common for soft varieties, e.g., Camembert), allowing the curds to settle in the vat and sucking off the supernatant whey (eg., Gouda and Emmental), scooping the curds from the vat using heavy cloths and placing them in moulds (e.g., Parmigiano Reggiano), draining the whey form the curds using perforated screens (e.g., Cheddar and Pizza cheese).

Many cheeses are made into traditional shapes and sizes, eg., small flat cylinders (e.g., Brie and Camembert), taller cylinders, ranging in size from 5 to 40 kg (e.g., Cherddar and Parmesan), large low cylinders (e.g., Emmental), spheres (Edam). In some cases the traditional shapes have been abandoned, e.g., Cheddar and Emmental now frequently made as rectangular or square blocks.

In some cases, the size and shape of a cheese are cosmetic and traditional but the size of a cheese has important consequences for the ripening of many varieties. Surface-ripened varieties, e.g., Camembert, must be small since the surface microflora plays a critical role in ripening but are effective over only a short distance. The opposite is required for varieties in which eyes develop due to the propionic acid fermentation, e.g., Emmental, which must have a close texture and large enough to retain sufficient CO_2 for eye development, For an 80 kg Emmental cheese, 120 L of CO_2 are produced during maturation, 60 L remain dissolved in the cheese body, 40 L diffuse out of the cheese and 20 L are in the eyes.; too much CO_2 will be lost from a small or open cheese and eye formation will be poor or absent. A selection of cheese shapes is shown in Fig 2.5.

Curds for high-moisture cheeses form a congealed mass under their own weight but the curds for medium- and especially for low-moisture cheese must be pressed to form a well-matted body, e.g., Cheddar cheese is pressed at 2.7 kPa. As well as consolidating the curd mass, pressing removes some whey, eg., for Cheddar cheese, ~1.3 % of the volume of milk used is in the press whey.

2.5.5 Special Operations

The curds or pressed cheese curd for certain varieties are subjected to specific treatments to induce a characteristic texture or physico-chemical property or to induce the growth of certain microorganisms. Examples of such varieties are Cheddar, Pasta Filata, washed-curd varieties or Blue cheeses.



Cheddar



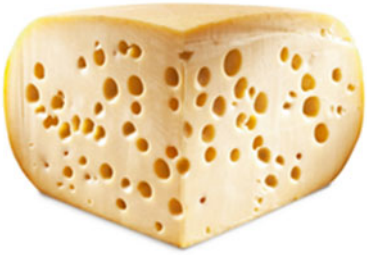
Camembert



Blue



Edam



Emmental



Gouda



Parmigiano-reggiano



Provolone

Fig. 2.5 Examples of the shape of cheese

2.5.6 Salting

Salting is the last manufacturing operation. Salting promotes syneresis but it is not a satisfactory method for controlling the moisture content of cheese curd which is best achieved by ensuring that the degree of acidification, heating and stirring in the cheese vat are appropriate to the particular variety. Salt has several functions in cheese, which are described in Chap. 9. Although salting should be a very simple operation, quite frequently it is not performed properly, with consequent adverse effects on cheese quality.

A low level of Na is essential in the diet (the RDA in the USA and UK is 2.4 g) but an excessive intake is undesirable. Although cheese contributes relatively little NaCl, even with a high consumption of cheese (consumption of 20 kg of cheese, containing 2 % NaCl, per annum, which is at the upper level of consumption, contributes 400 g NaCl per annum, i.e., about 1.1 g NaCl or 0.7 g of sodium daily), there is a commercial incentive to reduce the level of salt in cheese. Approaches are discussed in Chap. 9.

2.5.7 Application of Ultrafiltration in Cheesemaking

Since cheese manufacture is essentially a dehydration process, it was obvious that ultrafiltration would have applications in cheese manufacture, not only for standardizing cheese milk with respect to fat to casein, but also for the preparation of a concentrate with the composition of the finished cheese, commonly referred to as "pre-cheese". Standardization of cheese milk by adding UF concentrate (retentate) is now common but the manufacture of pre-cheese has to date been successful commercially for only certain cheese varieties, most notably UF Feta and Quarg. It seems very likely that ultrafiltration will become much more widespread in cheese manufacture, perhaps for the production of new varieties rather than modifying the process protocol for existing varieties.

2.6 Ripening

Fresh cheeses constitute a major proportion of the cheese consumed in some countries. Most of these cheeses are produced by acid coagulation and are described in Chap. 16. Although rennet-coagulated cheese varieties may be consumed at the end of manufacture and a little is (e.g., Burgos cheese), most rennet-coagulated cheeses are ripened (cured, matured) for a period ranging from ~3 weeks to >2 years; generally, the duration of ripening is inversely related to the moisture content of the cheese. Many varieties may be consumed at any of several stages of maturity, depending on the flavour preferences of consumers and economic factors.

Although curds for different cheese varieties are recognizably different at the end of manufacture (mainly as a result of compositional and textural differences arising from differences in milk composition and processing factors), the unique characteristics of the individual cheeses develop during ripening as a result of a complex set of biochemical reactions. The changes that occur during ripening, and hence the flavour, aroma and texture of the mature cheese, are largely predetermined by the manufacturing process, i.e., by composition, especially moisture, NaCl and pH, level of residual coagulant activity, the type of starter and in many cases by a secondary inoculum added to, or gaining access to, the milk or curd.

The biochemical changes that occur during ripening are caused by one or more of the following agents:

- coagulant
- indigenous milk enzymes, especially proteinase and lipase, which are particularly important in cheese made from raw milk
- starter bacteria and their enzymes
- secondary microorganisms and their enzymes
- non-starter lactic acid bacteria

The secondary microflora may arise from the indigenous microflora of milk that survive pasteurization or gain entry to the milk after pasteurization, e.g., some mesophilic *Lactobacillus spp.* especially *Lb casei* and *Lb paracasei,* and perhaps *Pediococcus* and *Micrococcus*. They may also be added as a secondary starter, e.g., citrate-positive *Lactococcus* or *Leuconostoc* spp. in Dutch-type cheese, *Propionibacterium* in Swiss cheese, *Penicillium roqueforti* in Blue varieties, *P. camemberti* in Camembert or Brie, or *Brevibacterium linens* in surface smear-ripened varieties, e.g., Tilsit and Limburger. In many cases, the characteristics of the finished cheese are dominated by the metabolic activity of these secondary microorganisms.

The primary biochemical changes involve catabolism of residual lactose and perhaps citrate, lipolysis and proteolysis but these are followed and overlapped by a host of secondary catabolic changes to the compounds produced in these primary pathways, including deamination, decarboxylation and desulphurylation of amino acids, β-oxidation of fatty acids, catabolism of lactic acid and even some synthetic reactions, e.g., esterification.

Although it is not yet possible to fully describe the biochemistry of cheese ripening, very considerable progress has been made on elucidating the primary reactions and these will be discussed in Chap. 12.

2.7 Processed Cheese Products

Depending on culinary traditions, a variable proportion of mature cheese is consumed as such, often referred to as “table cheese”. A considerable amount of natural cheese is used as an ingredient in other foods, e.g., Parmesan or Grana on pasta

products, Mozzarella on pizza, Quarg in cheesecake, Ricotta in ravioli. A third major outlet for cheese is in the production of a broad range of processed cheese products which in turn have a range of applications, especially as spreads, sandwich fillers or food ingredients. These products are discussed in Chaps. 17 and 18.

2.8 Whey and Whey Products

Only about 50 % of the solids in milk are incorporated into cheese; the remainder (90 % of the lactose, ~ 20 % of the protein and ~10 % of the fat) are present in the whey. Until recently, whey was regarded as an essentially useless by-product, to be disposed of as cheaply as possible. However, in the interest of reducing environmental pollution, but also because it is now possible to produce valuable food products from whey, whey processing has become a major facet of the total cheese industry. The principal aspects of whey processing are discussed in Chap. 22.

Chapter 3
Principal Families of Cheese

Summary Cheese is a very diverse group of foods (perhaps as many as 1500 varieties). To help consumers, retailers and cheese technologists, several schemes for the classification of cheese have been proposed and used. Criteria for classification include: coagulating agent (rennet or acid); texture/moisture content (very hard, hard, semi-hard, semi-soft, soft); matured or fresh; microflora (internal bacterial, surface/smear bacterial, internal or surface mould, propionic acid bacteria).

The principal families of cheese are described briefly in this chapter.

Keywords Cheese classification • Families and varieties of cheese • Cheese manufacture

3.1 Introduction

The diversity of cheese types is truly breathtaking! Despite starting from a limited range of raw materials (generally bovine, ovine, caprine or buffalo milk, starter cultures, coagulant and salt), a huge number (perhaps 1500 varieties) of cheeses are produced, including many local varieties. Indeed, it has been said that "there is a cheese for every taste preference and a taste preference for every cheese" (Olson 1990).

In order to facilitate their study, a number of attempts have been made to classify cheese varieties into meaningful groups or families. As discussed by McSweeney et al. (2004), traditional classification schemes have been based principally on their rheological properties (which, in practise are closely related to the moisture content): i.e., hard, semi-hard or soft (Table 3.1). Although this is a widely-used basis for classification, it suffers from a serious drawback since it groups together cheeses with widely different characteristics and manufacturing protocols. For example, **Cheddar**, **Parmesan** and **Emmental** are often grouped together as hard cheeses although they have quite different flavours and the methods for their manufacture are quite different. Attempts have been made to make this scheme more discriminating by including factors such as origin of the cheese milk, method of coagulation, cutting of the coagulum, scalding of the curds, drainage of whey, method of salting and moulding. Walter and Hargrove (1972), who classified cheeses on the basis of manufacturing technique, suggested that there are

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Table 3.1 Classification of cheese according to source of milk, moisture content, texture and ripening agents[a] (from Fox 1993a)

1.	Cows' Milk										
1.1	Hard (<42 % H_2O)	1.2	Semi-hard/semi-soft (43–55 % H_2O)	1.3	Soft (>55 % H_2O)	1.4	Fresh, rennet	1.5	Fresh, acid	1.6	Fresh
1.1.1	Grating cheese (extra hard)	1.2.1	Small round openings	1.3.1	Blue veined						
1.1.2	Large round openings	1.2.2	Irregular openings	1.3.2	White surface mould						
1.1.3	Medium round openings	1.2.3	No openings	1.3.3	Bacterial surface smear						
1.1.4	Small round openings	1.2.4	Blue veined	1.3.4	No rind						
1.1.6	No openings										
2.	Sheep's Milk Hard; semi-hard; soft; blue veined; fresh.										
3.	Goats' Milk										
4.	Buffalo's Milk										

[a]Unless otherwise stated, the cheeses are internal bacterially-ripened

only 18 distinct types of natural cheese which they grouped into eight families under the headings, very hard, hard, semi-soft and soft.

Probably the most comprehensive classification scheme for cheese developed to date is that of Ottogalli (1998, 2000a, b, 2001) which organizes cheeses into three main groups (indicated by the Latin words: "*Lacticinia*" [milk-like], "*Formatica*" [shaped], "*Miscellanea*" [miscellaneous]). The *Lacticinia* group includes products which are produced from milk, cream, whey or buttermilk by coagulation with acid (lactic or citric), with or without a heating step; however, a small amount of rennet is often used to increase the firmness of the resultant coagulum (e.g., Quarg and Cottage cheese). The *Lacticinia* group contains one class (A) comprised of seven families; Family A1 includes yogurt-like products from which some whey is removed. Family A2 contains somewhat similar products but from which a large volume of whey is removed and acid is added. Families A3 and A4 are whey cheeses produced by the combination of heat and acid (e.g., Ricotta) while cheeses in Families A5, A6 and A7 are similar to other products in the *Lacticinia* group except that they are made from cream, buttermilk or colostrum, respectively.

The second group, *Formatica*, contains most cheese varieties, all of which are coagulated by rennet. This is a large heterogeneous collection of varieties which are divided into six Classes (B-G), based essentially on the moisture content and the extent of ripening, and 31 families. Classes B and C include fresh cheeses and varieties with a short ripening period, respectively. The cheeses in Class D are soft surface-ripened varieties with a surface growth of moulds or smear bacteria. Blue cheeses are grouped in Class E while Classes F and G contain semi-hard and hard/extra-hard varieties, respectively.

The third group of cheeses, *Miscellanea*, is a heterogeneous collection of varieties and includes processed, smoked, grated and pickled cheeses, cheeses containing non-dairy ingredients (fruit, vegetables, spices), cheese analogues and cheeses made using ultrafiltration technology.

Unfortunately, none of these schemes is completely satisfactory and thus none is universally agreed. Fox (1993a) and McSweeney et al. (2004) proposed a number of "super-families" based on the method of milk coagulation, into which all cheeses may be grouped:

- Rennet-coagulated cheeses: most major international cheese varieties
- Acid-coagulated cheeses: e.g., **Cottage**, **Quarg**
- Heat/acid coagulated: e.g., **Ricotta**
- Concentration/crystallization: e.g., **Mysost**

In addition, there is a range of modified cheeses and cheese-like products, including enzyme-modified cheese, dried cheeses, cheese analogues and processed cheese. Leaving aside these products, Fox (1993a) and McSweeney et al. (2004) classified all cheeses into 13 super-families, although their classification scheme took no cognisance of the species of milk-producing animal (Fig. 3.1) and thus, too, is not completely satisfactory. However, for simplicity, the principal families of cheese will be discussed using this classification scheme.

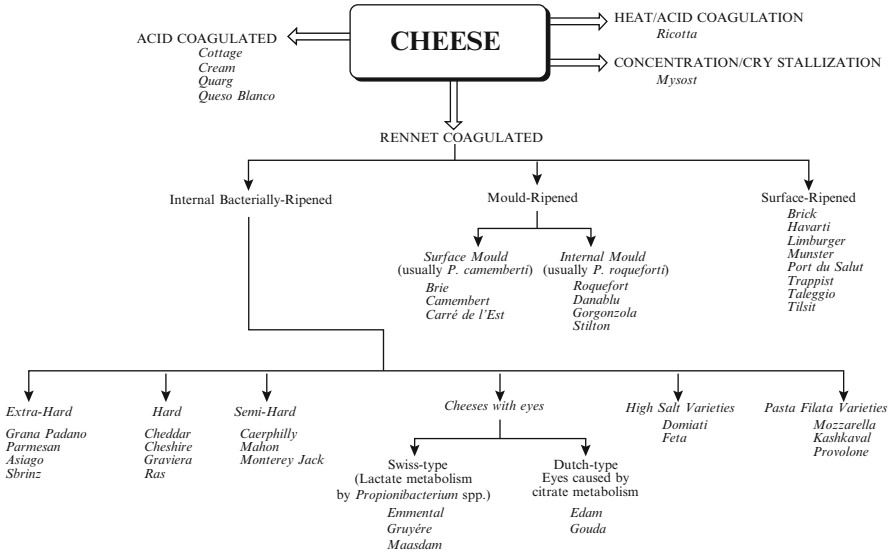


Fig. 3.1 The diversity of cheese. Cheese varieties are classified into super-families based on the method of coagulation and further subdivided based on the principal ripening agent and/or characteristic technology

3.1.1 Rennet-Coagulated Cheeses

All ripened cheeses are coagulated by rennet (~75 % of total world production). Acid-curd cheeses (Chap. 16) are the next most important group. Coagulation by a combination of heat and acid is used for a few varieties, including Ricotta. Concentration/crystallization is used in Norway to produce "whey cheeses" (e.g., Mysost).

There is a great diversity of rennet-coagulated cheeses and therefore they must be classified further (Fig. 3.1). Rennet-coagulated varieties are subdivided into relatively homogeneous groups based on the characteristic ripening agent(s) or manufacturing technology. The most diverse family of rennet-coagulated cheeses is that containing the internal bacterially-ripened varieties which include most hard and semi-hard cheeses. The term "internal bacterially-ripened" may be somewhat misleading since indigenous milk enzymes and residual coagulant also play important roles in the ripening of these cheese varieties. This group may be subdivided based on moisture content (extra-hard, hard or semi-hard) and on whether or not the cheese has eyes. Many varieties produced on a large industrial scale are included in this group. Parmesan (extra-hard) is used as a grating cheese and its manufacture is characterized by a high cook temperature. Cheddar and **British territorial varieties** (for which the curds are often textured and dry-salted) are classified as hard or semi-hard, internal bacterially-ripened cheeses. Internal bacterially-ripened cheeses with eyes are further subdivided on the basis of moisture content into hard varieties (e.g., Emmental) in which the numerous, large eyes are formed by CO_2 produced on

fermentation of lactate by *Propionibacterium freudenreichii* or semi-hard (e.g., **Edam** and **Gouda**) in which a few small eyes develop due to the formation of CO_2 by fermentation of citrate by a component of the starter (see Chaps. 11 and 12).

Most of the varieties which are classified in groups other than internal bacterially-ripened cheeses are soft or semi-hard. *Pasta filata* cheeses (e.g., **Mozzarella**) are characterized by stretching in hot water which texturizes the curd prior to salting. Mould-ripened cheeses are subdivided into surface mould-ripened varieties (e.g., **Camembert** or **Brie**), in which ripening is characterized by the growth of *Penicillium camemberti* on the surface, and internal mould-ripened cheeses ("**blue**" **cheeses**) in which *P. roqueforti* grows in fissures throughout the mass of the cheese. Surface smear-ripened cheeses are characterized by the development of a complex microflora consisting initially of yeasts and ultimately of bacteria (particularly coryneforms) on the cheese surface during ripening. White, brined cheeses, including **Feta** and **Domiati**, are ripened under brine and have a high salt content and, consequently, they are grouped together as a separate category.

The classification scheme in Fig. 3.1 is not without inconsistencies. A cursory glance will show that cheeses made from the milk of different species are grouped together (e.g., **Roquefort** and **Gorgonzola** are blue cheeses but the former is made from sheep's milk, the later from cows' milk) and that the sub-division between hard and semi-hard cheeses is rather arbitrary. There is also some cross-over between categories. **Gruyère** is classified as an internal bacterially-ripened variety with eyes but it is also characterized by the growth of a surface microflora, while some cheeses classified as surface-ripened (e.g., **Havarti** and **Port du Salut**) are often produced without a surface flora and therefore are, in effect, soft internal bacterially-ripened varieties. Likewise, *pasta filata* and high salt varieties are considered as separate families because of their unique technologies (stretching and ripening under brine, respectively) but they are actually ripened by the same agents as internal bacterially-ripened cheeses. However, we believe that the scheme in Fig. 3.1 is a useful basis for classification and therefore the diversity of cheeses will be discussed under these headings. Ultrafiltration technology is used for the manufacture of some cheese varieties which are discussed separately (Sect. 3.7). The following discussion is based largely on descriptions of cheeses given in Scott (1986), Fox (1993b), Robinson (1995), Kosikowski and Mistry (1997) and Robinson and Wilbey (1998), to whom the reader is referred for details of the manufacturing protocols. In some cases, the manufacturing protocol described in these sources for certain varieties is inconsistent, and therefore should be treated with due caution. This divergence is particularly true for minor varieties, which are probably ill-defined and variable in any case. The typical composition of a selection of cheeses is shown in Table 3.2.

A number of cheese varieties have Protected Designation of Origin (PDO) status which recognises a specific heritage and provides consumers with a guarantee of authenticity. Unlike commercial trademarks, PDO denomination constitutes a collective heritage and may be used by all producers of a particular cheese in a particular geographical area. PDO cheeses are protected by the European Union under various international agreements (see Bertozzi and Panri 1993). In addition to their

geographical origin, PDO denomination also certifies that the cheese has been made using specified (usually traditional) technology. A list of PDO cheeses is shown in Table 3.3.

Table 3.2 Compositions of selected cheese varieties (modified from Kosikowski and Mistry 1997)

Cheese	Fat	Total solids	Total protein	Salt	Ash	pH
	%	%	%	%	%	%
Asiago	30.8	72.5	30.9	3.6	6.6	5.3
Blue	29.0	58.0	21.0	4.5	6.0	6.5
Blue Stilton	33.0	61.7	24.8	3.5	3.2	5.2
Brick	30.0	60.0	22.5	1.9	4.4	6.4
Bulgarian White	32.3	68.0	22.0	3.5	5.3	5.0
Caciocavallo Siciliano	27.5	70.9	33.1	4.0	7.0	6.0
Caerphilly	34.0	67.7	27.2	1.5	3.4	5.4
Camembert	23.0	47.5	18.5	2.5	3.8	6.9
Cheddar (American)	32.0	63.0	25.0	1.5	4.1	5.5
Cheshire	33.0	66.7	26.7	1.8	3.9	5.3
Comte	30.0	66.5	30.0	1.1	4.1	5.7
Cottage	4.2	21.0	14.0	1.0	1.0	5.0
Cream	33.5	50.0	10.0	0.75	1.3	4.6
Edam	24.0	57.0	26.1	2.0	3.0	5.7
Emmental (Swiss)	30.5	64.5	27.5	1.2	3.5	5.6
Feta	20.3	40.3	13.4	2.2	2.3	4.2
Gouda	28.5	59.0	26.5	2.0	3.0	5.8
Grana (Parmesan)	25.0	69.0	36.0	2.6	5.4	5.4
Gruyère	30.0	66.5	30.0	1.1	4.1	5.7
Havarti	26.5	56.5	24.7	2.2	2.8	5.9
Leicester	33.0	64.7	25.5	1.6	3.5	6.5
Limburger	28.0	55.0	22.0	2.0	4.8	6.8
Manchego	25.9	62.1	28.1	1.5	3.6	5.8
Mitzithra	25.0	56.3	18.4	1.6	2.5	5.0
Mozzarella	18.0	46.0	22.1	0.7	2.3	5.2
Mozzarella-Low Moisture	23.7	53.0	21.0	1.0	3.0	5.2
Muenster	29.0	57.0	23.0	1.8	4.4	6.2
Pont L'Eveque	25.8	57.2	26.5	2.8	2.4	7.0
Provolone	27.0	57.5	25.0	3.0	4.0	5.4
Quarg	0.2	21.0	15.0	0.70	1.0	4.5
Queso Blanco	15.0	49.0	22.9	2.0–3.9	5.4	5.3
Ricotone (whey Ricotta)	0.5	27.5	11.0	<0.5	4.0	4.9
Ricotta	12.7	28.0	11.2	<0.5	–	5.9
Romano	24.0	77.0	35.0	5.5	10.5	5.4
Roquefort	31.0	60.0	21.5	3.5	6.0	6.4
Samsoe	27.0	59.9	26.5	1.8	3.7	5.5
Serra da Estrela	27.5	51.3	21.3	1.9	2.8	6.5
Svecia	28.3	56.0	21.8	2.5	4.1	5.5

Table 3.3 Cheeses with Protected Designations of Origin (modified from Bertozzi and Panari 1993)

Milk	Variety
France	
Bovine	Abondance
	Beaufort
	Bleu d'Auvergne
	Bleu des Causses
	Bleu de Gex-Haut Jura-Septmoncel
	Brie de Meaux
	Brie de Melun
	Camembert de Normandie
	Cantal
	Chaource
	Comté
	Epoisse de Bourgogne
	Fourme de'Ambert ou Montbrison
	Laguiole
	Langres
	Livarot
	Maroilles or Marolles
	Mont d'Or/Vacherin du Haut Doubs
	Munster or Munster Géromé
	Neufchâtel
	Pont l'Evêque
	Reblochon and Petit Reblochon
	Saint Nectaire
	Salers
Caprine	Chabichou du Poitou
	Crottin de Chavignol
	Picodon de l'Ardèche/Drome
	Pouligny Saint Pierre
	Sainte Maure de Touraine
	Selles sur Cher
Ovine	Ossau-Iraty-Brebis-Pyrénées etit
	Roquefort
Caprine-Ovine whey	Brocciu Corse or Brocciu
Spain	
Bovine	Cantabria
	Mahon
Ovine	Idiazabal
	Manchego
	Roncal
Mixed (cow, goat, ewe)	Cabrales
	Liebana
Portugal	
Bovine	San Jorge

(continued)

Table 3.3 (continued)

Milk	Variety
Ovine	Azeitao
	Serpa
	Serra da Estrela
	Castelo Blanco
	Picante da Beira Baixa Amarelo
Italy	
Bovine	Asiago
	Bra
	Castelmagno
	Fontina
	Formai de Mut
	Gorgonzola
	Grana Padano
	Montasio
	Murazzano
	Parmigiano Reggiano
	Raschera
	Robiola di Roccaverano
	Taleggio
Ovine	Canestrato Pugliese
	Casciotta di Urbino
	Fiore Sardo
	Pecorino Romano
	Pecorino Siciliano
	Pecorino Toscano
	Pecorino Sardo

3.2 Rennet-Coagulated Cheeses

3.2.1 Internal Bacterially-Ripened Varieties

Internal bacterially-ripened cheese varieties form a very diverse group of cheeses which are characterized by the absence of a surface microflora or internal mould growth. The agents which contribute to the ripening of these varieties originate from the milk (plasmin and other enzymes), the rennet (chymosin and/or other proteinases and, in certain cases, lipases) and the internal bacterial microflora (starter and non-starter bacteria in all cases and adjunct starter in some cheeses, particularly those in which eyes develop). Some internal bacterially-ripened varieties are easily classified into homogeneous groups based on some distinctive technology (e.g., *pasta filata* cheeses, varieties that develop eyes during ripening or cheeses ripened under brine). However, the classification used here for most varieties is based on texture (extra-hard, hard and semi-hard) and is therefore, somewhat arbitrary.

3.2.1.1 Extra-Hard Cheeses

The majority of extra-hard internal bacterially-ripened cheese varieties originated in Italy, are usually matured for a long period and often have a hard, grainy texture. They may be consumed as table cheeses when young or in grated form when mature. The hard texture of these cheeses results from the use of semi-skimmed milk in their manufacture, a high cooking temperature and evaporation of moisture during ripening.

Parmigiano Reggiano and **Grana Padano** are important members of this group; they are produced from raw milk in the Po valley in Northern Italy and are protected by designations of origin. A grainy texture in the mature cheese is desirable (hence they are referred to as "**Grana**" cheeses). "Parmesan-type" cheeses are made worldwide, particularly in the United States, from pasteurized milk; these cheeses are often smaller than Italian Grana-type cheeses, are cooked to a lower temperature (~50 °C) than the traditional product (54 °C), are salted more heavily, ripened for a shorter period and are usually used in grated form.

The manufacturing protocol for Parmigiano Reggiano is summarized in Fig. 3.2. The major features of its manufacture are the use of semi-skimmed raw milk produced by gravity creaming. In traditional manufacture, the evening milk is creamed in shallow vats, the lower skimmed milk layer drawn off and mixed with whole morning milk. Traditionally, the milk was coagulated and the curds cooked in copper vats shaped like an inverted bell, heated by a fire beneath the vat. Modern vats are often made from copper-plated steel with steam jackets for heating but the traditional shape is retained. Acidification is by a whey culture prepared by incubating whey from the previous day's manufacture. Calf rennet is used to coagulate the milk and the coagulum is broken by means of a wire basket-like implement ("spino"). The curds are cooked to 53–55 °C in 10–12 min and then transferred to a mould large enough to give a cheese of 25–40 kg. The cheeses may be subjected to light pressure and turned frequently to encourage whey expulsion. The cheeses are brine-salted for 20–23 days and ripened at 16–18 °C and 85 % equilibrium relative humidity (ERH) for 18–24 months. The rind of these cheeses is cleaned frequently.

The manufacturing protocol for Grana Padano cheese is generally similar to that for Parmigiano Reggiano except that it is made from raw milk from a single batch of milk which is partially skimmed after creaming for about 8 h. Grana Padano is brine-salted for about 25 days and ripened for 14–16 months.

Asiago is produced in the province of Vicenza, Italy, from partly skimmed raw cows' milk. Rennet paste is used to coagulate the milk and a natural whey starter is added for acidification. The cheeses, weighing 8–12 kg, are matured for various lengths of time, depending on the intensity of flavour desired. Asiago d'Allevo is a hard variant and Asiago Pressato, is a semi-hard, washed-curd cheese; Asiago d'Allevo Stravecchio (>15 months ripening) is hard but not grainy. Small eyes are characteristic of Asiago d'Allevo and Straveccio. **Montasio**, which originated in North Eastern Italy, is made from raw cows' milk and coagulated using calf rennet extract. Montasio may be consumed as a table cheese after 2–3 months or it may be

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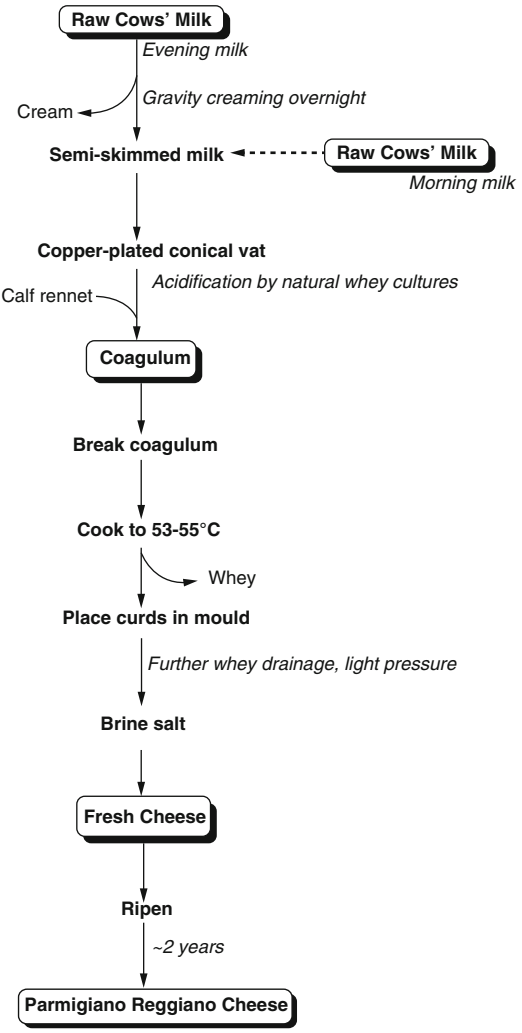


Fig. 3.2 Manufacturing protocol for Parmigiano Reggiano, an extra-hard Italian cheese variety

matured for a longer period (14–18 months) during which the texture becomes hard and the cheese is suitable for grating. **Sbrinz** is a hard grating cheese which originated in Switzerland. It is made from full-fat cows' milk, using rennet extract and a natural thermophilic whey starter. The curds are cooked to *ca*. 57 °C, placed in moulds, pressed for 2–3 days and either dry or brine-salted. The cheese may be consumed as a table cheese after a short ripening period or matured for up to 3 years and used for grating.

Prefixes "**Pecorino**", "**Vaccino**" or "**Caprino**" refer to Italian hard cheeses made from ewes', cows' or goats' milk, respectively. **Romano-type** cheeses are important members of this group and are made in central Italy and Sardinia. Similar cheeses manufactured in Sardinia are called "**Sardo**" and in Sicily, "**Siciliano**" but many regions of southern and central Italy make related local varieties. Italian Pecorino

Romano cheese is made from sheep's milk using a thermophilic starter (commercial or whey-based). Rennet paste is used as coagulant. The high lipolytic activity of the rennet paste results in the development of a strong, slightly rancid flavour in the mature cheese (the fat in ewes' milk has a high content of short, middle-chain and branched fatty acids which give it a characteristic flavour). The curds/whey mixture is cooked to 45–48 °C, followed by whey drainage. Blocks of curd are placed in moulds and pressed lightly before brine or dry salting. The cheeses are ripened for about 8 months. Pecorino Romano cheese is usually grated and used as a condiment.

3.2.1.2 Hard Varieties

Hard, pressed varieties include some of the most important commercial varieties produced worldwide (e.g., **Cheddar**). There is some heterogeneity in manufacturing technology for cheeses within this group and the definition of what constitutes a "hard cheese" is not always clear. However, these varieties usually have a moisture content in the range 30 to 45 % and are subjected to high pressure during manufacture to give a hard, uniform, close texture. According to Robinson (1995), the manufacture of these cheeses has a number of features in common, including renneting at ~30 °C, cutting the coagulum into small pieces and cooking to 39–40 °C, followed by whey drainage. In some cases, e.g., Cheddar and other **British varieties**, the curds are acidified and textured in the vat ("cheddared") and are then milled and dry-salted when sufficient acidity has developed. The salted curds are then moulded and pressed at a high pressure for 12–16 h or longer and matured at 6–8 °C for 3–12 months.

Hard cheese varieties include Cheddar, British Territorial varieties (**Cheshire**, **Derby**, **Gloucester** and **Leicester**), **Cantal** (French), **Friesian Clove cheese** and **Leyden** (the Netherlands), **Graviera** and **Kefalotiri** (Greece), **Manchego**, **Idiazabal**, **Roncal** and **Serena** (Spain) and **Ras** (Egypt). A wide variety of manufacturing techniques are used to produce hard cheeses; some are dry-salted while others are brine-salted.

Cheddar cheese originated around the village of Cheddar, England, and is now one of the most important cheese varieties worldwide. Cheddar is produced on a large scale in most English-speaking countries, particularly in the United States, United Kingdom, Australia, New Zealand, Canada and Ireland. Cheddar cheese is usually made from pasteurized whole cows' milk standardized to a casein:fat ratio of 0.67–0.72:1 and coagulated using calf rennet or a rennet substitute. The starter used is *Lc. lactis* subsp. *cremoris* or *Lc. lactis* subsp. *lactis*. Defined-strain starter systems are now used in large Cheddar factories in New Zealand, Australia, Ireland and USA but mixed, undefined cultures are also sometimes used (see Chap. 6). The milk is renneted at ~30 °C and the coagulum is cut and cooked to 39–40 °C over 30 min and held at this temperature for about 1 h. The whey is then drained and the curds are "cheddared" (Fig. 3.3). The traditional cheddaring process involves piling blocks of curd on top of each other, with regular turning and stacking of the curd blocks. The cheddaring process allows time for acidity to develop in the curd (pH decreases from *ca*. 6.1 to 5.4), thus solubilizing some colloidal calcium



Fig. 3.3 Cheddaring of cheese curd

phosphate and it also subjects the curds to gentle pressure which assists in whey drainage. During the cheddaring process, the curd granules fuse and the texture changes from soft and friable to quite tough and pliable. The curd should have a texture similar to cooked chicken-breast meat at the end of cheddaring. When the pH has reached *ca*. 5.4, the curd blocks are milled into small pieces and dry-salted. A "mellowing" period follows during which the salt dissolves in moisture on the surface of the curd chips. The curds are then moulded and pressed overnight at up to 200 kN m^{-2}. Cheddar is matured at 6–10 °C for a period ranging from 3 to 4 months to 2 years, depending on the maturity desired.

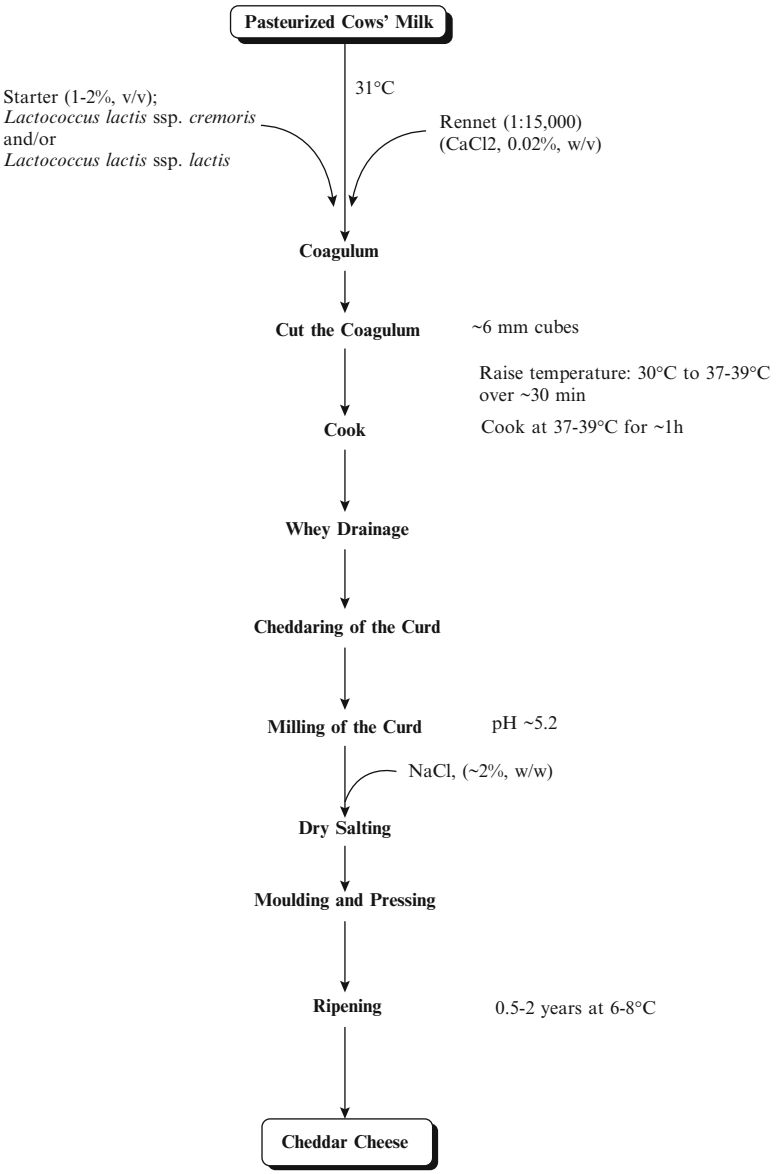


Fig. 3.4 Traditional protocol for the manufacture of Cheddar cheese

Although this traditional manufacturing process (Fig. 3.4) is still practised on a farmhouse scale, most Cheddar cheese is now manufactured in highly automated factories. The principal features of automated Cheddar production include the use of a number of cheese vats in which cheesemaking commences at 30 min intervals to provide a semi-continuous supply of curd. Whey drainage is mechanised and

automated, as is the cheddaring process which usually now is automated through a series of slowly moving belts (e.g., the Alfomatic system, Fig. 8.6, Chap. 8). The curds are then milled and salted mechanically on a belt system. Pressing and moulding are done automatically by pneumatically conveying the salted curds to the top of a "block former" which is a large tower (Wincanton tower, or block former) (Fig. 8.9, Chap. 8) in which the curds are compressed by their own weight; a close texture is ensured by applying a vacuum. As the curds exit the block former, 20 kg blocks are cut off by a guillotine and vacuum packaged in plastic bags, placed in card board boxes, stacked on a pallet and transferred to the cheese store. In many large factories, the boxed cheeses are cooled rapidly by being passed through a forced-air cooling tunnel before palleting; the objective is to retard the growth of non-starter lactic acid bacteria which may cause defects in flavour and texture. Most Cheddar is now produced in block form, although traditional Cheddar has a cylindrical shape. Annatto (see Chap. 4) or other colorant may be added to milk for Cheddar cheese; the resulting product is known as "**Red**" **Cheddar**.

Cheshire cheese is a **British Territorial variety** (a group of cheeses which originated in various parts of Britain) with a hard texture and perhaps some mechanical openings. Its manufacture is characterized by rapid acidification and a lower cook temperature (32–35 °C) than Cheddar which results in a lower pH, a higher moisture content and a shorter manufacturing time. The curd mat which develops while standing on the bottom of the vat after whey drainage is broken frequently to prevent the development of an extensive structure in the curd mass. Cheshire curds are dry-salted, placed in moulds, allowed to drain overnight and then pressed as for Cheddar, the cheeses are packaged and matured at 6–8 °C. **Leicester** is similar to Cheshire but is normally coloured with annatto. It is made from cows' milk with a mesophilic starter and cooked at ~37 °C. The curds are pressed after whey drainage and blocks of curd are placed on a draining rack and turned and cut to promote further whey drainage. The cheeses are dry-salted and matured for 4–8 months at 10–15 °C. Derby is similar to Leicester and is somewhat softer and more flaky than Cheddar. Gloucester, another British Territorial variety, has a cylindrical shape, about 40 cm in diameter. "**Single**" **Gloucester** is 6–8 cm high while a cylinder of "**Double**" **Gloucester** is 15–20 cm high. The manufacturing procedure for Gloucester is similar to that for Cheddar and annatto is added to the milk to colour the curds which are cooked to 35–38 °C. The curds are textured, milled twice, dry-salted, pressed and matured for 4–6 months.

Cantal is a hard French cheese from the Auvergne region, which is manufactured by a process somewhat similar to that used for British Territorial varieties. The milk is coagulated using standard calf rennet and acidified by a mesophilic lactic starter. The curds/whey mixture is not cooked but the whey is drained and the curds are cheddared. Weights may be placed on the bed of curd to assist in whey drainage. The blocks of curd are then milled, dry-salted, moulded, pressed and matured at 8–10 °C for 3–6 months.

Kefalotiri is a Greek cheese made from pasteurized sheep's or goats' milks standardized to *ca*. 6.0 % fat. The milk is inoculated with a thermophilic culture (usually *Sc. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*) and coagulated by calf rennet. The curds are cooked to 43–45 °C, transferred to moulds lined with cheese cloth and subjected to a low pressure, which is increased slowly. On removal from the moulds, the cheeses are dried overnight and brine-salted. After brining, dry salt is rubbed onto the surface of the cheese over the next few days to give a final salt content of *ca*. 4 %. During this time, the cheeses are washed with a brine-soaked cloth to control microbial growth on the surface and ripened for *ca*. 3 months. According to Robinson (1995), Kefalotiri has a hard texture and a strong, salty flavour.

Graviera is a relatively recently developed Greek variety, made principally from ewes' milk. It is acidified by a mixed mesophilic culture (1 %) containing *Lc. lactis* subsp. *lactis* or *Lc. lactis* subsp. *cremoris* and a smaller amount (0.1 %) of a thermophilic culture containing *Sc. thermophilus* and *Lb. helveticus*. The curds are cooked to ~50 °C and after whey drainage, the curds are moulded and pressed at an increasing pressure. The cheeses are then salted by frequent application of dry salt to the surface for 2–3 weeks and then ripened for 5–12 months. In some factories, the early stages of dry salting may be replaced by brining.

The Egyptian cheese, **Ras**, is produced in a manner similar in many respects to that for Kefalotiri. It is made from cows' milk standardized to 3 % fat. The curds are cooked to 45 °C, salted at a level of 1 % after whey drainage, moulded and pressed in a manner similar to Kefalotiri. The cheeses are brined for 24 h and rubbed with a small quantity of dry salt daily for several weeks. During this period, the cheeses are also washed with brine.

Manchego cheese, probably the most important Spanish variety, is made from ewes' milk, although generally similar cheeses (without PDO status) are manufactured from milk of other species. Two types of Manchego are produced; artisanal (made from raw milk without culture addition) or commercial (made from pasteurized milk) inoculated with a mesophilic starter (see Chap. 6). The milk is coagulated with standard calf rennet. The curds are cooked to ~38 °C, transferred to moulds and pressed for 12–16 h. Manchego has characteristic markings on its sides which are made by binding the cheeses in basket-work wrappings for ~30 min after pressing. The cheeses are then brine-salted and matured for at least 2 months at 10–15 °C and 85 % ERH.

Idiazábal cheese is produced in the Basque region of northern Spain from raw ewes' milk coagulated at 38 °C. The coagulum is allowed to cool to 25 °C and then broken and the curds ladled into moulds. The cheeses are salted by brining or by the application of dry salt and matured in caves for 2 months. The cheeses are then smoked in beechwood kilns and further matured for up to 1 year.

Roncal is made in the Navarra region of northern Spain from raw ewes' milk and acidified by the indigenous flora of the milk. The curds/whey mixture is cooked to ~37–40 °C and the curds then allowed to settle to the bottom of the vat. The whey is removed slowly and the curds pressed against the sides of the vat, moulded and

pressed before being dry-salted. Roncal is smoked and ripened at 6–8 °C and 100 % ERH for 45–50 days.

La Serena is a hard cheese made in western Spain from ewes' milk. Traditionally, raw milk is used although pasteurized milk is used in large-scale production. The milk is coagulated with rennet extracted from flowers of the cardoon thistle (*Cynara cardunculus*). A basically similar cheese, **Serra de Estrala**, is made in Portugal.

Mahon is produced in the Balearic island of Minorca from raw cows' milk containing 5 % ewes' milk and is acidified by a natural whey culture. Mahon is brine-salted and ripened at 18 °C for *ca*. 2 months. An extra-hard variant with 32 % moisture and a strong flavour is also produced, which is ripened for 10 months. The rind is oily as the cheese is coated with olive oil.

Other hard cheese varieties include **Leyden** and **Friesian Clove** cheeses from the Netherlands which are characterized by the addition of cumin seeds (Leyden) or cloves and cumin seeds (Friesian Clove).

3.2.1.3 Semi-Hard Varieties

The description of a cheese as semi-hard is very arbitary. The "semi-hard" group of cheeses is thus quite heterogeneous and the distinction between this and other groups of cheeses (e.g., hard cheeses, smear-ripened varieties or *pasta filata* cheeses) may not be clear. Semi-hard cheeses include **Colby** and **Monterey** (stirred-curd Cheddar-type cheeses), a number of British Territorial varieties (**Caerphilly**, **Lancashire** and **Wensleydale**) and cheeses such as **Bryndza** (Slovakia), and **Majorero** (Spain).

Caerphilly, which originated in Wales, is a crumbly, high-acid cheese. It is made from pasteurized cows' milk using calf rennet and a mesophilic starter. The curds are cooked to 32–34 °C and held at this temperature for about 1 h. The whey is drawn off and the curds collected on the bottom of the vat where rapid acid production occurs. Some (1 %) dry salt is added to the curds before moulding and pressing overnight. The pressed curds are then brine-salted for *ca*. 24 h and packaged. Caerphilly matures rapidly and is ready for sale after 10–14 days. **Lancashire**, another British Territorial variety, is made from cows' milk using rennet and a mesophilic starter. The curds and whey are not cooked but after draining the curds are allowed to develop acidity and held overnight during which time extensive acid production occurs. The next day, fresh curds are mixed with the acidified curds and the mixture is milled to ensure homogeneity. The curds are dry-salted, placed in moulds overnight at room temperature and pressed for 3 days. Lancashire is ripened at 13–18 °C for 3–12 weeks.

Wensleydale cheese, which originated in Yorkshire, England, is made from cows' milk, inoculated with a mesophilic starter; the curds are cooked at a low temperature. The whey is drained off and the curd mat is broken into pieces to assist whey drainage. The pieces of curd are dry-salted and moulded, held overnight at about 21 °C without pressing and then pressed lightly for 5 h. Wensleydale, which is matured for *ca*. 1 month, has mechanical openings and a mild, acidic taste.

Stirring Cheddar-type cheese curd prevents the development of curd structure and results in a cheese with a higher moisture content and thus in a softer texture. Two stirred-curd variants of Cheddar cheese are recognized, **Colby** and **Monterey**. The manufacture of Colby, which originated in the USA, follows a protocol similar to Cheddar until after cooking when some whey is removed and replaced by cold water. The curds/whey are stirred and most of the whey is removed; the curds and remaining whey are stirred vigorously. The remaining whey is then drained off and the curds are stirred further. Stirring prevents the development of an extensive structure while the curds are in the vat. Salt is added to the curds, which are moulded and pressed. Colby is ripened for 2–3 months at 3–4 °C. Monterey (Monterey Jack) cheese was first made in California and is similar to Colby. The whey is removed and the curds left on the bottom of the vat, with occasional stirring until the pH reaches 5.3. The curds are dry-salted and pressed lightly overnight. The cheeses are allowed to form a rind before waxing or packaging in films. Monterey, which is ripened for 5–7 weeks, has many mechanical openings.

Bryndza is made in south-eastern Europe, e.g., Slovakia, Romania and Moldova, from sheep's milk coagulated with rennet (sometimes with significant lipase activity) and acidified by the indigenous microflora of the milk or by a mesophilic starter. The curds are allowed to settle to the bottom of the vat, most of the whey is removed and the curds are consolidated into lumps by hand. The lumps of curd are placed into cloth bags and stored for 3 days while sufficient acidity develops. At this stage, the cheese is known as **Hrudka** and may be sold locally. However, most Hrudka is transported to a central factory, where it is broken into pieces, salted and passed between granite rollers to produce a smooth paste, which is placed in polythene-lined wooden tubs and matured.

Majorejo is made from goats' milk on Fuerteventura Island, one of the Canary Islands. In industrial practice, milk is acidified by a mesophilic starter and coagulated using rennet although artisanal cheesemakers rely on the indigenous microflora of the milk and use rennet paste as coagulant. The curds are moulded in braided palm leaves (which give its surface a characteristic pattern), pressed lightly and dry-salted. Majorejo develops a strong flavour during ripening.

3.2.1.4 Cheeses with Eyes

Mechanical openings, resulting from the incomplete fusion of curd pieces, are common in many cheese varieties and may be considered desirable (e.g., Monterey) or a defect (e.g., Cheddar). However, some internal bacterially-ripened varieties are characterized by the development of eyes caused by CO_2, produced by bacterial metabolism, being trapped within the curd. The development of eyes in cheese is governed by the rate of gas production by bacteria and the ability of the curd to retain the gas. There are two main families of cheese with eyes: **Dutch types** (**Edam**, **Gouda** and related varieties), which have small eyes, and **Swiss types**, which are characterized by large eyes. In the case of Edam and Gouda, CO_2 is produced from citrate by the

DL culture (see Chap. 6) while in Swiss varieties, CO_2 is produced by *Propionibacterium freudenreichii* from lactate during ripening (see Chaps.11 and 12).

Gouda (Fig. 3.5) originated in the Netherlands but it and similar cheeses are now produced worldwide from pasteurized cows' milk coagulated using calf rennet and acidified by a mesophilic DL starter. Nitrate may be added to the milk to suppress

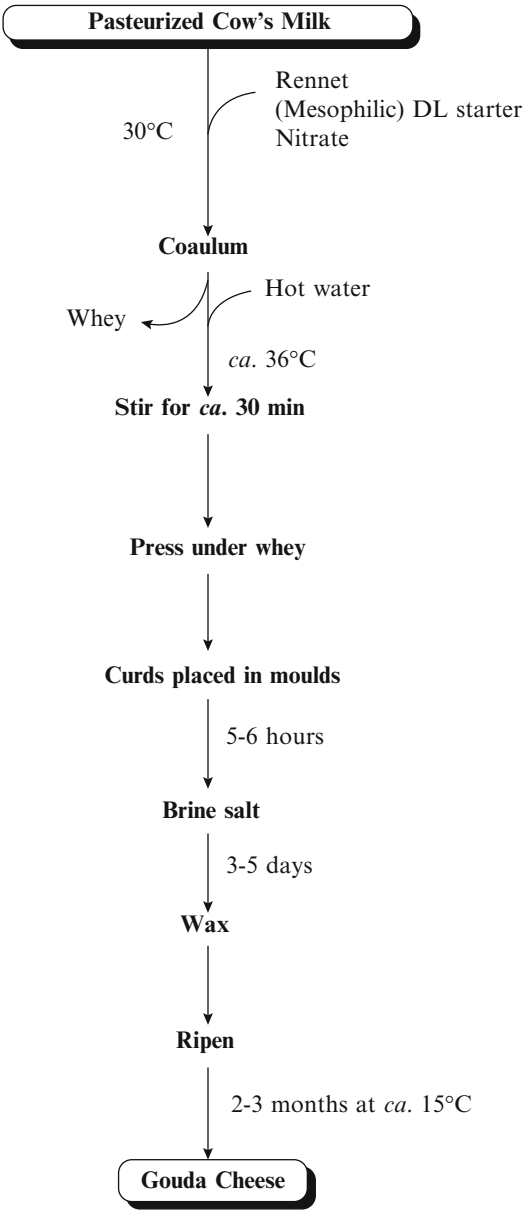


Fig. 3.5 Manufacturing protocol for Gouda cheese

the growth of *Clostridium* spp. which produce gas (H_2 and CO_2) from lactate during ripening, causing a defect known as late gas blowing; butanoic acid is also produced from lactate, causing off-flavours. The coagulum is cut and the curds-whey mixture stirred for 20–30 min before a portion (about 30 %) of the whey is drained off and replaced by hot water which raises the temperature to 36–38 °C. This washing step removes some of the lactose and consequently reduces the development of acidity after the curds are moulded. The curds are cooked at this temperature and then allowed to settle to the bottom of the vat where they are pressed under the whey. The bed of curd is cut into blocks which are transferred to moulds (wheel-shaped or rectangular, producing a cheese of 4–20 kg) and pressed at about 50 kPa for 5–6 h. Gouda is brine-salted, traditionally coated with yellow wax and ripened at 15 °C for 2–3 months or longer (up to 2 years) for extra-mature cheese.

Edam, a Dutch variety similar to Gouda but with a distinctive spherical shape, is coated with red wax. Variants of this cheese are made differing in fat content (typically 30, 40 or 45 % fat-in-dry matter) from milk that was standardised appropriately. A few small eyes develop in Edam which may be sold after ripening for 3–8 weeks. **Maribo** is a similar variety produced in Denmark from cows' milk and, in addition to eyes caused by a mesophilic DL starter, has numerous mechanical openings. Other similar varieties include **Danbo** (Denmark), **Colonia** and **Hollanda** (Argentina) and **Svecia**(**ost**) (Sweden).

Varieties with a propionic acid fermentation are usually referred to as Swiss-type cheeses (although not all originated in Switzerland) and are characterized by large eyes which are produced by *P. freudenreichii* which metabolizes lactate to propionate, acetate, H_2O and CO_2 (see Chaps. 11 and 12). Propionibacteria do not grow in the milk during cheesemaking but grow in the cheese during the early stages of maturation when it is transferred to a "hot room" (~20–22 °C). The curd of these cheeses is quite rubbery and is able to trap the CO_2 (which migrates through the curd until it reaches a fissure or weakness, at which an eye develops). The texture of these cheeses is influenced by a high cook temperature (~55 °C), which inactivates most of the coagulant, and a high pH at draining (which leads to a high concentration of calcium in the curd). Swiss-type cheeses are traditionally made as large wheels and are brine-salted. The relatively slow diffusion of salt in a large cheese allows the salt-sensitive propionibacteria time to grow.

Emmental is a typical Swiss cheese variety which is now made worldwide. Traditional Emmental is made (Fig. 3.6) from raw cows' milk which is acidified with a mixed thermophilic starter consisting of *Streptococcus thermophilus* and a *Lactobacillus* sp. *Lb. helveticus* was used traditionally but *Lb. delbrueckii* subsp. *lactis* is now more common. Propionibacteria may be added to the milk or contaminate milk from the environment. The milk, at 30 °C, is coagulated using calf rennet and the coagulum is cut into small pieces and cooked to ~55 °C until the curd grains are of the desired firmness. The curds and whey are transferred to moulds, where the whey is separated. The moulds are sufficiently large to give a wheel of cheese weighing up to 100 kg and ~1 m in diameter. The size of the Emmental wheel is significant since it determines the rate of cooling of the curd (and thus the activity

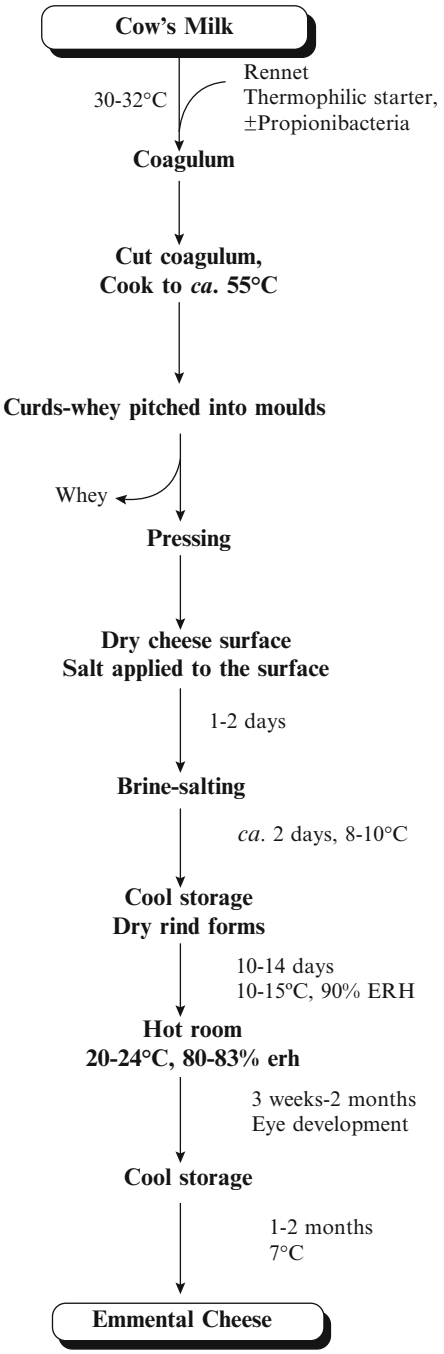


Fig. 3.6 Manufacturing protocol for Emmental, a Swiss-type cheese

of the starter; see Chap. 6), the diffusion of salt throughout the cheese mass and helps to trap gas within the cheese. Over the next 1–2 days, the wheels are pressed and turned frequently. During this time, the curds cool and acid production by the starter organisms (which were dormant during cooking) recommences. Complete fermentation of lactose and its constituent monosaccharides in Emmental takes *ca.* 24 h. After pressing, the cheese wheels are brine-salted, stored in a cool room (10–15 °C, 90 % ERH) for 10–14 days and brushed, dry-salted and turned daily until a smooth rind develops. The cheeses are then transferred to a hot room (20–24 °C, 80–83 % ERH) and held there for 3–6 weeks, until adequate eye formation has occurred (which is indicated by a hollow drum-like sound when the cheese is tapped). The cheeses are then matured at about 7 °C for a further 1 or 2 months. Emmental is very susceptible to late gas blowing, caused by CO_2 produced by *Clostridium tyrobutyricum*, a contaminant from soil or silage; in Switzerland, it is not permitted to feed silage to cows, the milk of which is to be used for Emmental. Elsewhere, the growth of *Clostridium* may be inhibited by addition of $NaNO_3$ or lysozyme to the milk.

Rindless (or Block) Emmental is also produced industrially by a protocol which is generally similar to that for Emmental but milk with a lower fat content and a lower cooking temperature are used. The cheese is wrapped in a plastic film and therefore no rind develops during maturation. Some large factories produce Emmental in square or rectangular blocks, cut from large (1000 kg) blocks.

Gruyère is another popular Swiss-type cheese which differs from Emmental in being smaller with a somewhat stronger flavour, fewer eyes and is characterized by the development of a surface flora (similar to that which develops on smear-ripened varieties, Sect. 3.2.3). The surface flora is encouraged by ripening for 2–3 weeks at 10 °C and then for 2–3 months at 15–18 °C and 90–95 % ERH, during which the cheeses are rubbed with a brine-soaked cloth. Further ripening at 12–15 °C is required before sale at 8–12 months of age.

Similar varieties include **Raclette** (which is manufactured from raw milk and is acidified by the indigenous milk microflora) and **Gruyère de Comté** which is produced in eastern France from raw bovine milk. Raclette has fewer eyes than Emmental and is often eaten melted over food. **Beaufort** is a French variety which is similar to, but larger than (*ca.* 45 kg), Gruyère and usually develops fewer eyes (which are due to the growth of mesophilic bacteria acting on citrate rather than *Propionibacterium*) than Gruyère. **Appenzeller**, which originated in Switzerland, is a small cheese (*ca.* 30 cm in diameter) with a soft texture which undergoes propionic acid fermentation resulting in the development of a few eyes. The curds are cooked at 43–45 °C. Appenzeller is immersed in cider or spiced wine or rubbed with a mixture of salt and spices during ripening which impart a distinctive flavour to the cheese. **Maasdammer**, a variety which was developed recently in the Netherlands, is characterized by the use of a mesophilic starter and extensive propionic acid fermentation which gives large eyes and a domed appearance to the cheese wheels. **Jarlsberg** is a relatively new cheese developed in Norway and made using a mesophilic starter and a propionic acid fermentation.

3.2.1.5 High Salt Varieties

White-brined cheeses originated around the Eastern Mediterranean and in the Balkans and Middle East and are characterised by ripening the cheese in brine (leading to a high salt content). The principal white-brined varieties today are **Feta** and **Telemes** (Greece), **Domiati** (Egypt) and **Turkish White-Brined** cheese. White-brined cheeses are now made worldwide and are major industrial products.

Feta cheese is made from sheep's milk or mixed sheep's/goats' milk. Strenuous efforts on the part of the Greek Government resulted in protected designation of origin status for Feta although similar cheeses are manufactured worldwide. Milk for Feta is usually pasteurized and standardized to a casein-to-fat ratio of about 0.7–0.8 (Fig. 3.7). A thermophilic or mesophilic starter culture is added to ensure rapid acidification. The rennet used is often a mixture of standard calf rennet and a local rennet extract which has some lipase activity. $CaCl_2$ may be added to the milk before renneting. The rennet-induced coagulum is cut (2–3 cm cubes) and the soft curds are ladled directly into moulds; the curds/whey mixture is not cooked. Whey drainage occurs in the moulds which are inverted after 2–3 h. The curd mass is then firm enough to be removed from the moulds and is cut into blocks which are dry-salted (or brined) before being transferred to a barrel or other container. The container is filled with brine (*ca*. 14 % NaCl) after *ca*. 7 days and held at 14–16 °C until the pH of the cheese has decreased to *ca*. pH 4.5, at which point the cheeses are stored at 3–4 °C for at least 2 months.

Feta-type cheese is now also manufactured industrially from cows' milk concentrated by ultrafiltration (UF; concentrated approximately fivefold using membranes with a cut off of 10–20 kDa). Less rennet is used in the manufacture of Feta-type cheese from UF retentate and the yield is higher than that made from unconcentrated milk due to the incorporation of whey proteins into the curd (see also Sect. 3.7).

Telemes cheese is made by a protocol similar to that for Feta except that some pressure is applied to the curds in the moulds to aid in the expulsion of whey and the curd blocks are brined after removal from the moulds.

Domiati is made in Egypt from cows' or buffalo milk or a mixture of both containing 2, 4 or 8 % fat (giving reduced- or full-fat cheese). Domiati may be made from pasteurized milk to which NaCl is added to 5–15 %. Alternatively, about one-third of the milk may be heated to ~80 °C and salt added to the remainder. At the level used, NaCl has a strong anti-bacterial effect and halotolerant lactobacilli are used as starter. Domiati may be consumed fresh or ripened in brine for a number of months.

Halloumi is a brined cheese made in Cyprus traditionally from sheep's milk or mixtures of sheep's and goats' milk (although large factories now also use cows' milk). The curds are cooked to 38–42 °C during manufacture and the cheeses are pressed. Blocks of curd are scalded by immersion in hot (90–92 °C) whey for 30 min but are not stretched. The cheeses are dry-salted and consumed fresh or after storage in brine.

Other white pickled cheeses include **White cheese**, **Kasar and Talum** (Turkey), **Lightvan** (Iran), **Beda** (Egypt) and **Sirene** (Bulgaria and elsewhere in the Balkans).

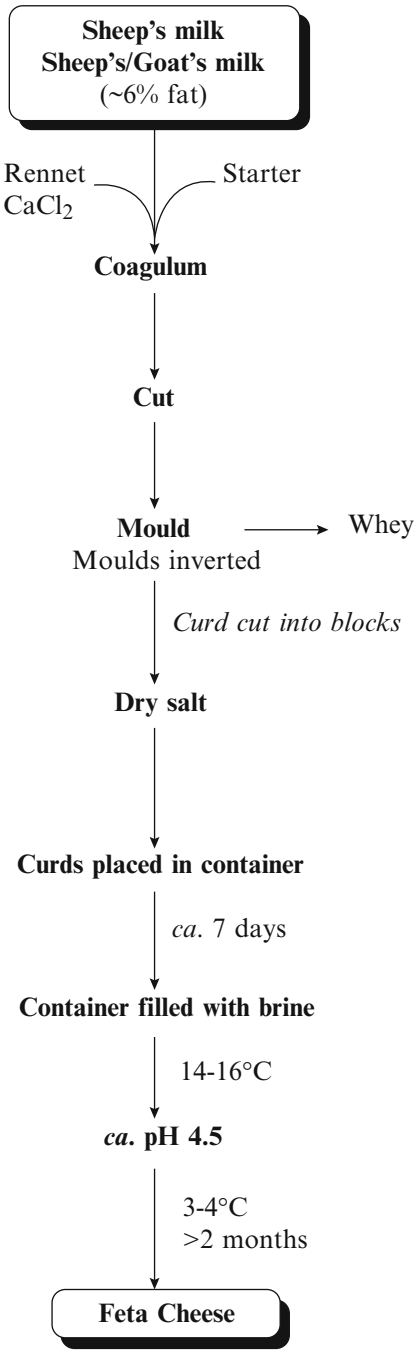


Fig. 3.7 Manufacturing protocol for Greek feta cheese

3.2.1.6 *Pasta Filata* Varieties

***Pasta filata* cheeses** are soft or semi-hard varieties, the curds for which are heated to ≥65 °C in hot (70–80 °C) water and mechanically stretched during manufacture. Stretching causes the curds to become fibrous and malleable. Most *pasta filata* cheeses originated in the Mediterranean region.

By far the most important member of this group is **Mozzarella** which originated in southern Italy and was manufactured originally from buffalo milk. **Mozzarella di Bufala** is hand-moulded into round pieces (100–300 g) during manufacture. This cheese is still manufactured in Italy but the type of Mozzarella now widely manufactured around the world is made from pasteurized, partly skimmed, cows' milk (Fig. 3.8) and is often referred to as **Pizza cheese** or (in the USA) as **low-moisture, part-skimmed Mozzarella**. This type of Mozzarella has a higher salt concentration (1.5–1.7 %) than Mozzarella di Bufala (<1.0 %). The production of low-moisture, part-skim Mozzarella cheese has increased greatly in recent years as a result of the increased popularity of pizza.

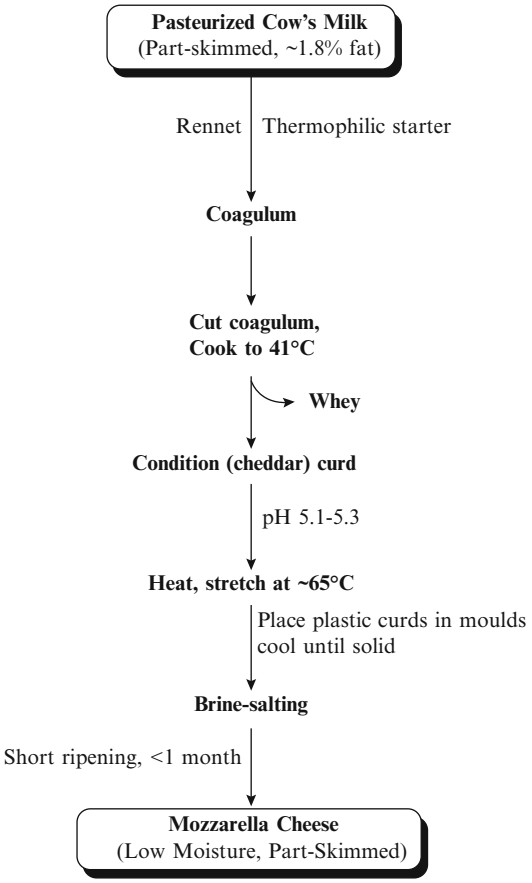


Fig. 3.8 Manufacturing protocol for low-moisture, part-skim Mozzarella (Pizza) cheese

The manufacturing process for Mozzarella for use as pizza topping (Fig. 3.8) involves standardizing pasteurized cows' milk to ~1.8 % fat. A higher fat content (~3.6 %) is used for Mozzarella intended to be consumed as a table cheese. A thermophilic starter (1–2 %) containing a combination of *Lactobacillus* spp. and *Streptococcus thermophilus* is used in the manufacture of Pizza cheese. The *Lactobacillus* is often omitted when Mozzarella is intended as a table cheese since the rate of acidification need not be as fast as for pizza cheese. Proteolytic enzymes of the *Lactobacillus* may make a minor contribution to the functionality of the final product by causing slight hydrolysis of the caseins. The milk is renneted after some acidity has developed and the coagulum is cut and cooked to ~41 °C. The whey is then usually drained off and texture developed in the curds (usually by a process similar to cheddaring) until the pH drops to around pH 5.1–5.3. Because the production and the treatment of the curds are quite similar to those used for Cheddar up to this stage, Cheddar plants may be modified easily to produce Mozzarella by altering the starter and temperature profile used and by the use of an appropriate stretcher. The next stages in Mozzarella manufacture are stretching and kneading which are characteristic of *pasta filata* varieties. The acidified curds are placed in hot water (*ca*. 70 °C) and kneaded, stretched and folded until the desired texture has been developed. The curds for pizza cheese are stretched more extensively than those for table Mozzarella. The former may also be salted during the stretching and forming stages. The hot, plastic curds are moulded (usually into rectangular blocks), cooled quickly in cold water or brine and, if salt was not added during the cooking/stretching process, the cheeses are then brine-salted. Mozzarella is usually consumed within a few weeks of manufacture; extensive ripening is undesirable since the functional properties of the cheese deteriorate.

So-called "**string**" **cheese** is produced from Mozzarella, Cheddar or similar cheese curd by cooking and extruding the plastic curd as long rods (1–2 cm in diameter), followed by brining (which is rapid due to the small cross-sectional area of the rods). The rods are then cut into convenient lengths and packaged. "Strings" of cheese may be torn from these rods; this novelty feature is the major selling point for string cheese, for which young children are the target market.

Kashkaval is a stretched-curd variety from the Balkans (known by several local names, some with PDO status) and was traditionally made from sheep's milk although cows' milk Kashkaval is now common. The cheese is usually brine-salted although some is dry-salted or stored in brine. Typically, the cheese is matured for 2–3 months at 12–16 °C before consumption. **Kasseri**, a Greek cheese similar to Kashkaval, is made from sheep's milk or a mixture of sheep's and goats' milks while Kasar cheese is made in Turkey.

Provolone, which is characteristically pear-shaped, originated in southern Italy where it is made from cows' milk. Rennet paste may be used in its manufacture, giving the resulting cheese (**Provolone piccante**) a stronger flavour than normal Provolone (**Provolone dulce**) which is manufactured using rennet extract. Provolone is ripened for 2–6 months at 12–14 °C. **Caciovallo** is a hard Italian cheese manufactured from cows' milk by a process somewhat similar to that used for Provolone. The curds are stretched in hot water and brine-salted; shaped like a large tear-drop.

Caciovallo is ripened for 3–4 months at about 10 °C, or longer (>12 months) if the cheese is to be grated. **Ostiepok**, a stretched-curd cheese from central Europe (Czech Republic and Slovakia), is made from sheep's milk (although cows' milk is used sometimes), brine-salted and smoked heavily.

3.2.2 Mould-Ripened Cheeses

Cheese varieties on which moulds grow during ripening fall into two broad categories: surface mould-ripened cheeses (e.g., **Brie** or **Camembert**), in which the mould grows as a mat on the surface, and **blue-veined** varieties which are characterized by the growth of *P. roqueforti* in fissures throughout the cheese. Although these two groups have mould growth in common, the methods used for their manufacture and the flavour and texture of the mature cheese are very different. Hybrid mould-ripened cheeses are also produced, e.g., **Cambozola (blue brie)**, which have a white mould growth on the surface and blue mould internally.

3.2.2.1 Surface Mould-Ripened Varieties

Surface mould-ripened cheeses are generally soft varieties characterized by the growth of the white mould, *Penicillium camemberti*, on the surface of the cheese. The surface flora is often more complex, particularly in cheeses made from raw milk by traditional technology. These curds are acidified to *ca*. pH 4.6 during manufacture using a mesophilic starter. Lactic acid produced by the starter is metabolized by the mould which also produces ammonia from amino acids, and therefore the pH of the surface layer of the cheese increases to *ca*. 7.0; if present, yeast also catabolise lactic acid. An important consequence of the increase in the pH of these cheeses is that there is considerable migration of calcium phosphate to the surface layer (which contains ~80 % of the calcium and ~55 % of the phosphate of the mature cheese). As in all cheeses containing active rennet, the coagulant plays a role in the development of texture. However, in surface mould-ripened cheeses the role of rennet is relatively minor compared to that of the mould; these cheeses soften from the surface towards the centre during ripening, due, not to proteolysis by mould enzymes, which diffuse only a few millimetres into the cheese, but to the establishment of pH and calcium phosphate gradients, which cause the cheese to soften from the surface towards the centre. Softening in these cheeses is often quite extensive, leading to a spreadable, almost fluid, consistently.

Many surface mould-ripened varieties originated in France. They are usually manufactured from cows' milk acidified by a mesophilic starter. The first microorganisms to become established on the surface are yeasts (including *Debaromyces* spp. and *Kluyveromyces* spp.). *Geotrichum candidum* also becomes established at this time, although its growth may be limited if the level of salt is high. *P. camemberti* is observed after 6–7 days of ripening and forms the characteristic mat on the cheese

surface. Once the surface of the cheese has been neutralized (after 15–20 days), aerobic bacteria (particularly micrococci and coryneforms), which are inhibited by the low initial pH, begin to grow.

Camembert, the most important surface mould-ripened variety, originated in Normandy, France, in the eighteenth Century. It is a small cheese (*ca*. 10 cm diameter; 200–250 g) manufactured from cows' milk (Fig. 3.9). Raw milk is used traditionally but industrial Camembert is now produced from pasteurized milk. A mesophilic starter (~0.1 %) is used and when the pH of the milk has fallen to ~6.1, rennet is added. Traditionally, the coagulum is not cut but is ladled into moulds where drainage occurs. To facilitate manufacture, the coagulum for industrial Camembert is first cut into large cubes and then transferred to moulds without cooking. Traditionally, the cheeses are dry-salted and *P. camemberti* spores are sprayed

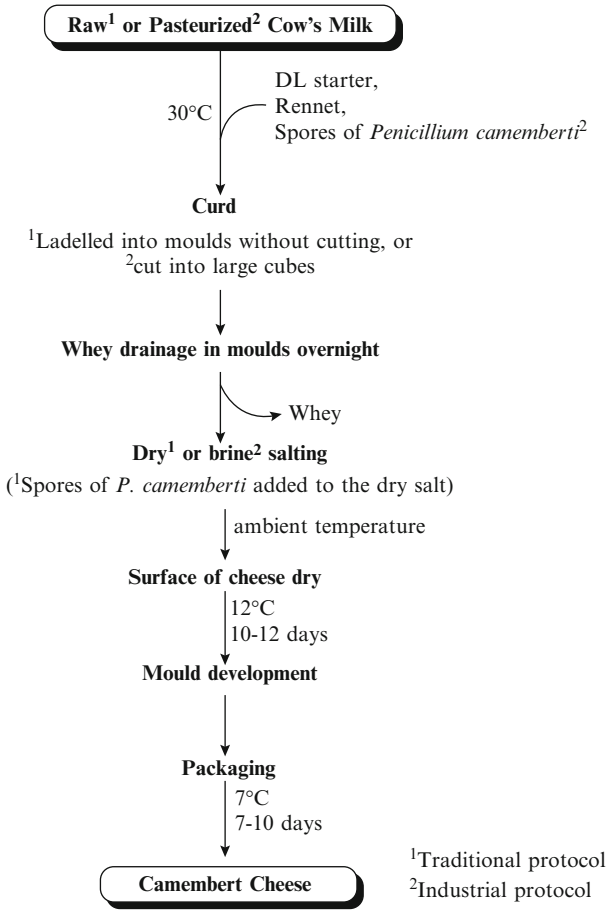


Fig. 3.9 Manufacturing protocol for Camembert, a surface mould-ripened cheese

on the surface although it is now industrial practice to inoculate the milk with mould spores and to brine-salt the cheeses. The surface of the cheese is allowed to dry at ambient temperature in a well ventilated room, after which the cheeses are transferred to a store at ~12 °C for 10–12 days for mould development. The cheeses are then packaged in waxed paper and placed in wooden or cardboard boxes prior to final ripening at 7 °C for 7–10 days.

Brie is a surface mould-ripened, flat cylindrical cheese with a larger diameter than Camembert, which it resembles closely in flavour, texture and manufacturing protocol. **Carré de l'Est** is a square, surface mould-ripened cheese which originated in eastern France. **Neufchâtel** originated near Rouen, France, where it is still produced, mainly on farms. Cows' milk is inoculated with a small quantity of an artisanal starter and renneted. The coagulum which forms overnight is transferred to muslin bags through which the whey is drained. After most of the whey has drained, the curds are removed from the bags, mixed, salted and placed in moulds. When the curds are firm enough to remove from the moulds, their surfaces are salted, dusted with *P. camemberti* spores and ripened for 2–3 weeks. In addition to the above-mentioned varieties, **St. Marcellin** (which was manufactured originally from goats' milk but is now made from cows' or sheep's milk) and a number of minor goats' milk cheeses also develop a surface mould growth during ripening. The microflora of these minor varieties is often uncontrolled.

3.2.2.2 Blue-Veined Cheeses

Blue-veined cheeses are characterized by the growth of *P. roqueforti* in fissures throughout the cheese. These cheeses usually have a soft texture and a flavour dominated by alkan-2-ones (methyl ketones) which are produced from free fatty acids by the mould via the first four steps of the β-oxidation pathway (Chap. 12). The pH of blue-mould cheeses increases during ripening, from 4.6–5.0 after moulding to 6.0–6.5, or higher, when mature.

Since *P. roqueforti* requires O_2 for growth, the manufacture of blue cheese is dominated by the need to provide a suitable environment for its growth. This is achieved by encouraging large mechanical openings in the cheese (by not pressing the cheese when moulded) and by piercing the cheese to allow air into its centre and to allow CO_2 produced by the mould to escape. Lipolysis is often encouraged in blue cheeses by separation of the raw cheese milk and homogenization of the cream, which encourages the action of the indigenous milk lipase. The level of starter used (usually a DL starter) is normally very low, and in some cases, the curds are acidified by the indigenous microflora, since the curds are usually held for an extended period in moulds or special drainers during which acidity develops. Spores of *P. roqueforti* are added, either to the milk or to the curds during manufacture. The curds for blue cheese are cooked to a low temperature and transferred to drainers or moulds where the whey is separated from the curds. Blue cheeses are dry-salted, either by the application of salt to the surface of the cheese or by milling the curds

after whey drainage and mixing with salt prior to moulding. The cheeses are ripened under conditions of temperature (usually 10–12 °C) and relative humidity which favour mould growth; they are pierced during ripening to facilitate uniform mould growth, turned and their surfaces cleaned regularly.

Roquefort is a blue cheese with PDO status manufactured from raw ewes' milk. Cheeses must be ripened in caves in a limited, defined area of south-eastern France. The absence of carotenoids from ewes' milk results in a very white cheese which highlights the contrast in colour between the curd and the mould. The manufacturing protocol for Roquefort is shown in Fig. 3.10. Usually, no starter is added to the milk which is acidified by its indigenous microflora. In addition to homofermentative lactococci, this microflora contains leuconostocs and other heterofermentative lactic acid bacteria which produce CO_2 as a by-product. The CO_2 causes small openings in the curd which favour the growth of the mould. Lamb rennet is added to the cheese milk and coagulation is complete in *ca*. 2 h, when the coagulum is cut. The curds are not cooked but are mixed with spores of *P. roqueforti* and placed in perforated metal moulds for whey drainage. The whey is allowed to drain for 4–5 days during which the cheeses are inverted periodically and acidity develops. The cheeses are then removed from the moulds and dry-salted over a period of 1 week, after which they

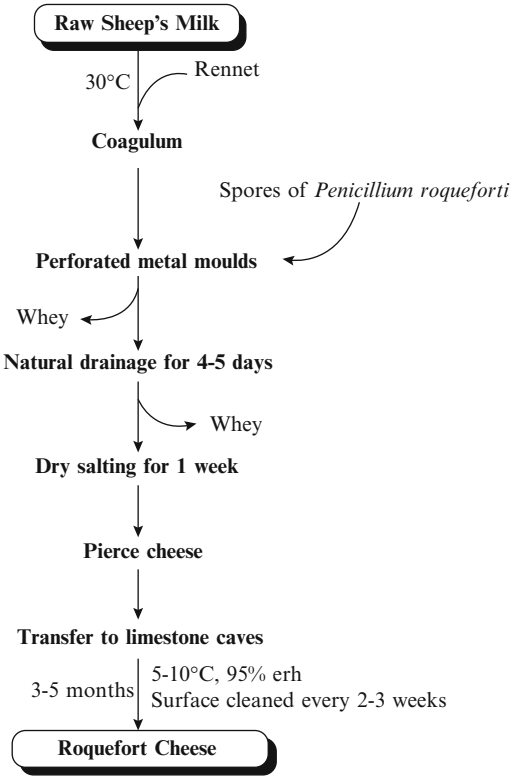


Fig. 3.10 Manufacturing protocol for Roquefort, an internal mould-ripened (blue) cheese

are pierced and placed in limestone caves, which have the correct temperature (10 °C) and relative humidity (95 %) to encourage mould growth. The cheeses are matured for 3–5 months during which their surface is cleaned to remove adventitious moulds or smear-forming bacteria.

Bleu d'Auvergne is a blue cheese manufactured in France from cows' milk. The Spanish blue cheese, **Cabrales**, is usually made from cows' milk and is ripened in local caves. Cabrales cheeses are often covered with sycamore (*Acer pseudoplatanus*) leaves to retain humidity around the cheese. The cheeses are not inoculated with *P. roqueforti* spores but become contaminated with mould spores from the environment during ripening. Thus, the degree of mould development in, and quality of, this variety can be variable.

Danablu (**Danish blue**) is perhaps the most commercially important blue cheese. It is manufactured from cows' milk, the cream from which is homogenized (to encourage lipolysis) prior to pasteurization. Pasteurized skim milk and cream are mixed and inoculated with starter and mould spores. The manufacturing protocol for Danablu is broadly similar to that for Roquefort, although chlorophyll is sometimes added to mask the yellow colour of the carotenoids in cows' milk, thus giving a whiter cheese. Danablu is ripened under controlled temperature (14–18 °C) and relative humidity (90–95 %) for up to 3 months. Most dairying countries produce Blue-mould cheese, which in many cases is modelled on Danablu.

Edelpilkäse is a blue cheese produced in Austria and Germany. **Mycella**, a blue cheese larger than Danablu produced in Denmark, is characterized by a yellow-white colour and intense mould growth. **Gorgonzola**, the traditional blue cheese of Italy, is also manufactured from cows' milk. The traditional protocol for the manufacture of Gorgonzola involves the separate production of curds from evening and morning milks. *P. roqueforti* spores are added to both batches of curd. The evening curds are stored overnight in cloth bags and used for cheesemaking the following morning. The morning curds are placed on the bottom and around the sides of the mould while still warm and the cool evening curds are placed in the centre. The top of the mould is then filled with warm morning curds. This layering encourages the development of mechanical openings in the cheese and yields a cheese with a smooth, firm surface. This two-curd system is not used as widely now and the cheese is softer and made more quickly.

Stilton is a blue cheese produced in the English counties of Leicestershire, Derbyshire and Nottinghamshire from pasteurized cows' milk. A mesophilic DL starter (<0.04 %) and *P. roqueforti* spores are added to the milk. After renneting, the curds are allowed to settle on the bottom of the vat and the whey is withdrawn slowly over the next 12–18 h; the curd mass is cut to facilitate drainage. The curd pieces are milled, dry-salted and placed in moulds. Whey drainage continues for about 7 days and is facilitated by frequent turning. During this time, the cheeses are kept warm (26–30 °C, 90 % relative humidity) so that the starter bacteria can produce sufficient acid in the cheese curd. The cheeses are then placed in a cooler room (13–15 °C, 85–90 % relative humidity) for 6–7 weeks during which the cheeses

cool and a rind develops on the surface. The cheeses are pierced and after sufficient mould growth has occurred (2–3 weeks), they are moved to a cold store at 5 °C.

3.2.3 Surface Smear-Ripened Cheeses

Cheeses ripened with a mixed surface microflora are perhaps the most hetereogeneous group of rennet-coagulated cheeses. Although most varieties in this group are soft or semi-hard, a surface flora may also develop on hard cheeses such as **Gruyère**. However, in the latter case, the contribution of the surface flora to cheese ripening is relatively minor.

The distinguishing feature of surface-ripened cheeses is the development of a mixed microflora on the cheese surface, forming a red-orange smear. These cheeses are manufactured using a mesophilic (most varieties) or a thermophilic (Gruyère and similar cheeses) starter and are usually brine-salted. Manufacturing protocols usually result in curds with a high-moisture content. After manufacture, a range of salt-tolerant yeasts (*Kluyveromeyces*, *Debaromyces*, *Saccharomyces*, *Candida*, *Pichia*, *Hansenula* and *Rhodotorula*), together with *Geotrichum candidum*, become established on the cheese surface where they metabolise lactate to CO_2 and H_2O. This change in environment favours the growth of other microorganisms. This microflora is complex and consists of Gram-positive bacteria, including *Micrococcus*, *Staphylococcus* and various coryneform bacteria (which are responsible for the colour of the smear). One component of the surface smear is the coryneform, *Brevibacterium linens*, which is widely used in smear inocula, although recent research has suggested that it may be only a relatively minor component of the complex surface flora.

The surface microflora may reach 10^{11} cfu cm^{-2} and thus the enzymatic activities of the smear microorganisms contribute significantly to the flavour of the cheese. Since enzymes do not diffuse through cheese curd, patterns of proteolysis in smear-ripened cheeses are similar to those in internal bacterially-ripened varieties except at the cheese surface. However, products of the metabolic activities of the smear diffuse into the cheese and influence its flavour. Soft surface-ripened cheeses usually have a strong flavour while semi-hard varieties have a milder flavour.

Soft surface-ripened cheeses mature quite rapidly. The rate at which they ripen is governed by the size of the cheese, its moisture content, ripening conditions and the composition of the surface microflora. The high-moisture content of these cheeses accrues from cutting the coagulum into large pieces and cooking the curds to a low temperature. Much whey is retained and therefore the curds have a relatively high-lactose content which favours the growth of the starter which acidifies the cheese to a low pH (~5). The ratio of surface area to volume (and thus the size and shape of the cheese) is very important in surface-ripened varieties. The smaller the cheese (and thus the higher this ratio), the greater will be the influence of the surface flora on the flavour of the cheese. The relative humidity (>95 %) and temperature (12–20 °C) of ripening rooms are controlled so as to favour the growth of the surface smear. In some

cases, cheeses are held in an environment with a lower relative humidity to encourage the development of a rind.

The smear develops initially as a series of colonies on the surface of the cheese. During ripening, the surface of these cheeses may be "massaged" (washed) with a brine solution which distributes the microorganisms evenly over the surface of the cheese and results in the development of a uniform smear. Although it is common practice to inoculate cheeses with *Br. linens*, the principal source of the surface microflora is the cheesemaking environment. To encourage this, young cheeses are often smeared with the same brine which had been used previously to smear older, high-quality cheeses.

The ripening period for smear-ripened cheeses depends on the desired flavour intensity but is usually relatively short. For some cheeses (e.g., **Brick**), the smear is washed from the surface and the cheeses coated with protective material before being transferred to a ripening room at a lower temperature (~10 °C) for further maturation. The degree to which smear is permitted to develop varies greatly between varieties. In some cheeses, smear development is desired principally to colour the cheese surface and is, therefore, very limited. These cheeses mature in a similar manner to internal bacterially-ripened varieties; in some cases, the surface is painted with dye to mimic smear colour.

The majority of bacterial surface-ripened cheeses originated in northern Europe but many are now produced worldwide. **Limburger**, one of the most important smear-ripened varieties, is named after the town of Limburg, Belgium, but the cheese is now manufactured widely in Germany and North America. The manufacturing protocol (Fig. 3.11) is similar to those for other smear-ripened varieties. Limburger cheese is produced from pasteurized cows' milk which is acidified using a mesophilic DL starter and coagulated using calf rennet. After cutting the coagulum, the curds and whey are cooked slowly to ~37 °C. Most of the whey is then drained off and, in some cases, replaced by dilute brine which firms the curds and removes lactose, thereby reducing the level of acid produced. The curds are then transferred to block-shaped moulds and whey drainage is assisted by frequent turning or the application of low pressure. During this time, the pH decreases and the curds mat sufficiently to retain the shape of the cheese when it is removed from the mould. The cheese is then either salted by the application dry salt to the surface or by immersion in brine (10–15 °C) for 1–2 days. The salted cheeses are transferred to ripening rooms at 10–15 °C and >95 % relative humidity where the characteristic surface microflora develops during the next 2–3 weeks. During this time, the cheeses are turned frequently and smeared with brine containing desirable microorganisms. After the development of the surface microflora, the cheeses are wrapped in foil and ripened for a further 3–8 weeks at ~4 °C to complete the development of flavour. Limburger is a strong-flavoured, soft, rindless cheese with mechanical openings. A number of cheeses similar to Limburger are produced, including **low-fat Limburger**, **Romadour** and **Weisslacker**.

Pont l'Evêque is a square (10–11 cm sides), smear-ripened cheese which originated in Normandy and is manufactured from pasteurized cows' milk. Curds are placed in cloth bags for initial draining and then in metal moulds standing on rush mats; the cheeses are dry-salted. The surface flora is dominated initially by *Geotrichum can-*

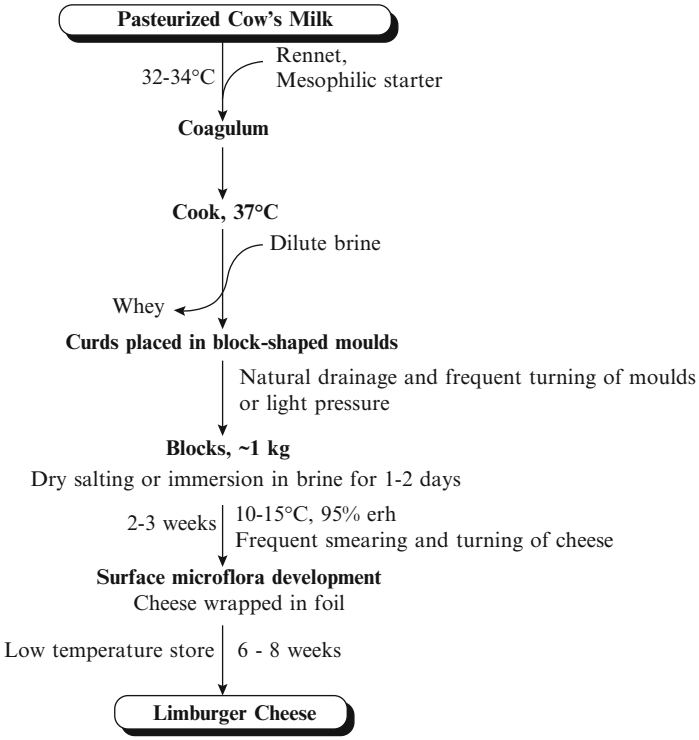


Fig. 3.11 Manufacturing protocol for Limburger, a bacterial surface-ripened cheese

didum, which gives the cheese a white appearance. Excessive mould growth is prevented by daily turning and washing with dilute brine; later during ripening, Pont l'Evêque develops a bacterial microflora characteristic of smear-ripened cheeses. **Port du Salut** and related varieties, e.g., **Saint Paulin**, are semi-hard cheeses with an elastic body. They are made from cows' milk and ripened at a relative humidity >90 %, and washed periodically with brine to restrict the development of the smear. **Brick** is a semi-hard smear-ripened cheese which originated in the USA. Smear development on this cheese is terminated after about 2 weeks by waxing or wrapping in film and ripening is completed at a low temperature for a further 2–3 months. **Butterkäse** is another surface-ripened variety with limited growth of smear. The cheese is brine-salted (which encourages the development of a rind) and smear growth is encouraged for 2–3 weeks. As suggested by its name, Butterkäse is a soft cheese with a butter-like consistency.

Trappist cheese is reputed to have originated in a Trappist monastery in Bosnia. Smear growth is limited by the short ripening time (2–3 weeks). **Tilsit** is an important smear-ripened variety which originated in Prussia. The cheese is somewhat similar to Limburger but its texture is firmer and there are more mechanical openings. The cheeses are brine-salted and a strong smear develops during maturation.

Taleggio is a soft smear-ripened variety which originated in Lombardy in the 1920s and has a characteristic square shape. **Serra da Estrela** is manufactured in Portugal from sheep's milk which is coagulated using an extract from flowers of the thistle, *Cynara cardunculus*. After manufacture, cheeses are first ripened at a high humidity which promotes the development of a yeasty smear ("reima"), which is removed about 14 days after manufacture; the cheeses are then ripening at a lower humidity to promote rind development. **Münster** cheese, which originated in Germany, is brine-salted and smear growth is encouraged to give a colour to the surface of the cheese. **Livarot** is produced in Normandy; some is sold as fresh cheese but most is matured, during which an intense smear develops; characteristic reed bands are placed around the cheese. **Havarti**, which originated in Denmark, has numerous irregular openings. The cheese is brine-salted and a dry surface develops when cheeses are held at room temperature for 1–2 days; a surface flora is then encouraged to contribute to flavour development.

Variants of some of the above smear-ripened cheeses (e.g., Havarti, Saint Paulin) are also produced in which a smear is not allowed to develop. These cheeses are semi-soft and internally-bacterially ripened. Such cheeses are sometimes covered with a red or orange coating to give the impression of smear growth.

3.3 Acid-Coagulated Cheeses

Acid-coagulated cheeses (e.g., **Cream**, **Cottage**, **Quarg**, some types of **Queso Blanco**) are those varieties which are produced from milk or cream by acidification to *ca*. pH 4.6 which causes the caseins to coagulate at their isoelectric point (*ca*. pH 4.6). Acid-curd cheeses were probably the first type of cheese produced since such products may result from the natural souring of milk. Acidification is usually achieved by the action of a mesophilic starter but direct acidification is also practised. A small amount of rennet may be used in certain varieties (e.g., Cottage or Quarg) but is not essential and serves to increase the firmness of the coagulum and to minimise casein losses in the whey. The coagulum may or may not be cut or cooked during manufacture but the curds are not pressed. Acid-coagulated cheeses are characterized by a high-moisture content and are usually consumed soon after manufacture. Acid-coagulated varieties are discussed in detail in Chap. 16.

3.4 Heat/Acid-Coagulated Cheeses

A small group of cheeses are coagulated by a combination of heat and acid. The most important member of this subgroup is **Ricotta**, an Italian cheese originally produced from whey. Ricotta (the name derives from the Italian *ricottura*, meaning "reheated") was produced originally from cheese (Mozzarella or Provolone) whey, perhaps with some milk added, by heat-induced coagulation (85–90 °C) and some

acidifying agent (e.g., lemon juice or vinegar). Ricotta curds are then transferred to moulds surrounded by ice, where drainage occurs.

However, much Ricotta cheese is now produced from full-fat or skimmed milk. The milk is acidified to *ca*. pH 6.0 by the addition of a large amount (typically >20 %) of bulk starter. Unlike other varieties, the starter does not produce acid in the cheese vat and is used simply as a source of pre-formed lactic acid. Alternatively, the milk may be acidified with acetic acid (white wine vinegar) or citric acid. The acidified milk is heated to *ca*. 80 °C by direct steam injection during which NaCl (0.2 %, w/v) and stabilizers may be added to the milk. Precipitation occurs in about 30 min at ~80 °C, after which the curds/whey are held for 15–20 min. During holding, the curd particles become firm, coalesce and float to the surface due to entrapped air; they are scooped into perforated moulds which are cooled with crushed ice. Ricotta has a high-moisture content (~73 %) and thus has a short shelf-life. It is normally consumed soon after manufacture as a table cheese or as an ingredient in lasagne, ravioli or desserts.

Ricottone cheese is produced by a similar procedure to Ricotta but from sweet whey to which some milk, skim milk or buttermilk is added. Since the pH of sweet cheese whey is *ca*. 6.2, little additional acidification is usually necessary. **Dry Ricotta**, which is a grating cheese, is produced by pressing Ricottone curd and by drying the cheese at 10–16 °C for several months (or 4 weeks at 21 °C). **Mascarpone** is similar to Ricotta except that cream is added to the milk and a slightly higher cooking temperature is used. The resulting cheese is more creamy than Ricotta and is usually salted at a low level, whipped and formed into a cylindrical shape. **Impastata** is made in a very similar way to Ricotta except that the curds are agitated gently as they form which causes them to sink to the bottom of the vat where they are cooked more efficiently than Ricotta curd, which remains on the surface. This results in a drier cheese, with a coarse texture, which is often ground to give a smooth, dough-like texture. Impastata is used mainly in the confectionery industry as an ingredient in pastry.

3.5 Concentration/Crystallization

A few Scandanavian cheeses are produced from whey by concentration and crystallization of lactose and concentration of other solids in the whey. One could argue that such varieties are not cheeses at all but rather by-products of cheese manufacture made from whey. These cheeses [“**Brunost**”, brown cheese, or **Mysost** (Norwegian), or **Mesost** (Swedish), **Mysuostur** (Icelandic), **Myseost** (Danish) or **Braunkäse** (German)] are characterized by having a smooth creamy body and a sweet, caramel-like flavour. Sweet whey is the usual starting material although acid whey may be used for some varieties. Sometimes, skim milk or cream is added to the whey to give a lighter-coloured product (which would otherwise be dark brown).

The manufacturing protocol for **Primost** is shown in Fig. 3.12. Primost (“premium quality cheese”) differs from the otherwise similar **Geitost** by the addition of

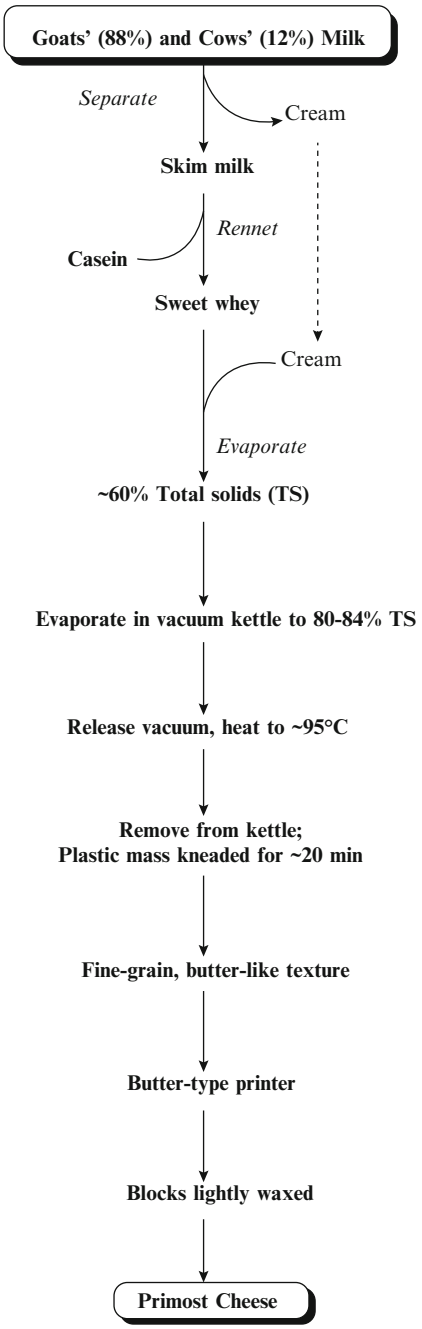


Fig. 3.12 Protocol for the manufacture of Primost from whey by concentration and crystallization (modified from Kosikowski and Mistry 1997)

cream to the whey from a mixture of goats' and cows' milks. The whey/cream mixture is first concentrated to ~60 % total solids (often in a multi-stage vacuum evaporator). A second concentration step (to >80 % total solids) follows which requires a higher vacuum. The resulting plastic mass is heated to ~95 °C. The Maillard reaction is encouraged during the manufacture of these cheeses and is important for the final colour and flavour of the product. The concentrate is then cooled, kneaded and packaged. Crystallization of lactose is controlled so as to avoid sandiness in the product. Several varieties (including **Mysost**, **Geitost**, **Niesost**, **Fløtemyost** and **Gudbrandsdalost**) are produced using this basic process; differences arise from the origin of the whey (cows' or goats' milk), the addition to skim milk, milk or cream to the mix or the use of sweet or acid whey. These cheeses have a high total solids content (<18 % moisture), are high in calories and are characterized by a long shelf-life.

3.6 Visual Appearance of Selected Cheeses

Historically, most cheese varieties evolved to have a characteristic shape and size, probably reflecting the facilities available. In most cases, the shape and size of a cheese are largely cosmetic but as discussed, in the case of some varieties, e.g., Emmental and Camembert, size does matter. In recent years, the shape of many varieties has changed, e.g., Cheddar was traditionally produced as cylinders but is now produced as rectangular blocks, because they are easier to stack, store and cut into consumer portions. The visual appearance of cheese also is characteristic, e.g. granular, with eyes or mechanical openings, with moulds, etc. The experienced consumer readily recognizes the type, and perhaps even the variety, of cheese from its visual appearance. The appearance of a selection of whole and cut cheeses, are shown in Fig. 3.13.

3.7 Ultrafiltration Technology in Cheesemaking

Ultrafiltration (UF), as a technology for cheese manufacture, was introduced in the early 1970s and has been investigated extensively and reviewed (Zall 1985; Ernstrom and Anis 1985; Ottosen 1988; Lelievre and Lawrence 1988; Lawrence 1989; Spangler et al. 1991; Mistry and Maubois 2004, 2016). It has attracted the attention of cheese and equipment manufacturers, primarily because of the potential to increase yield, through the recovery of whey proteins in the cheese curd. Other advantages include its potential to reduce production costs and to produce new cheese varieties with different textural and functional characteristics. In this section, some of the more important aspects of UF in cheesemaking are highlighted.

The most successful commercial applications of UF in cheese manufacture to date have been in the production of cast Feta in Denmark, fresh acid-curd varieties



Caciocavallo



Camembert



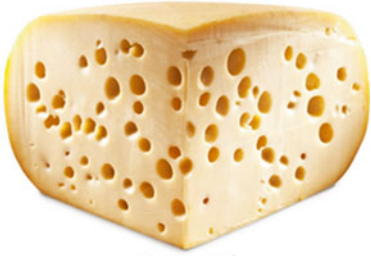
Cheddar



Cottage cheese



Edam



Emmental



Feta



Gouda



Gruyère



Limburger

Fig. 3.13 A selection of cheese varieties



Manchego



Mozzarella



Mysost



Parmigiano-Reggiano



Pecorino Rosso



Provolone



Ricotta



Roquefort

Fig. 3.13 (continued)

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(Quarg, Ricotta and Cream cheeses) and the standardization of milk protein, to 4–5 %, for the production of Camembert and other varieties.

Based on the degree of concentration and whether whey expulsion following concentration is necessary, UF in cheesemaking may be classified into three general approaches:

- **Low concentration factor (LCF) UF**, followed by cheesemaking and whey removal using conventional equipment. The main application of LCF-UF is the standardization of milk to a fixed protein level to obtain a more consistent end-product; variations in gel strength at cutting, buffering capacity and rennet-to-casein ratio are minimized. However, when using conventional cheesemaking vats, concentration appears limited to a maximum CF of ~1.5, or 4–5 % protein, because of difficulties in handling the curd and yield losses.

  An alternative approach is to concentrate a portion of the cheese milk, by UF, or preferably by MF in which only the casein is retained, to a medium or high CF and to use the retentate to standardize the remainder of the milk to the desired protein content or fat:casein ratio.
- **Medium concentration factor (2–6×) UF** to the final solids content of the cheese without whey expulsion. The main attraction of this type of UF technology is the increased cheese yield associated with retention of whey proteins and increased moisture when whey proteins are denatured prior to UF. The main commercial application is in the production of high-moisture, unripened cheeses (e.g., Quarg, Cream cheese) or are not very dependent on proteolysis during ripening for flavour development (e.g., Feta). Feta produced by this method (by addition of rennet to a concentrate, i.e., pre-cheese, without cutting the coagulum) has a smoother, more homogeneous texture than the more "curdy-textured" traditional product, hence the name "cast" Feta.

  There are numerous reports on the use of UF concentration to the final cheese dry matter level for the production of soft or semi-hard rennet-curd cheeses, including Camembert, Blue, Havarti and Mozzarella. Manufacture essentially involves pre-acidification, ultrafiltration/diafiltration, starter addition, rennet addition, coagulation and automated cutting of the coagulum using specialized equipment (e.g., Al-Curd or Ost Retentate coagulators), moulding, pressing and brining. To date, uptake of UF technology by the dairy industry for the production of the latter cheeses has been limited; apart from uncertainties concerning the regulatory status of such cheeses and the relatively small reported increases in yield, the main drawbacks include changes in cheese texture, flavour and functionality (i.e., meltability and stretchability).
- **High concentration factor UF**, followed by whey expulsion in novel equipment. Since the upper limit of concentration by UF is ~7 fold for whole milk, it is not possible to achieve the dry matter level required for hard cheeses such as Cheddar and Gouda; hence, further whey must be expelled following coagulation of the retentate and cutting the coagulum. Owing to the high curd-to-whey ratio, efficient curd handling (i.e., stirring and heat transfer) is not feasible in

conventional systems. The only continuous system capable of handling such concentrates is the Siro-Curd which was used for the production of Cheddar cheese in Australia during the 1980s but its use has been discontinued. The cheese produced by this process, which gave a yield increase of ~4–6 %, was claimed to be indistinguishable from Cheddar manufactured using standard equipment.

On renneting at a fixed dosage level, increasing the protein level in milk results in a reduced rennet coagulation time, an increase in the level of soluble (non-aggregated) casein at the point of gelation, increased rate of curd firming, reduced set-to-cut time when cutting at a given curd strength, a decrease in the degree of aggregation at cutting, and a coarser gel network. Micelles which are not modified, or aggregated, at the onset of gelation are presumably modified later and incorporated into the gel to greater or lesser degree.

Owing to the rapid rate of curd firming, it becomes increasingly difficult, as the milk protein level is increased, to cut the coagulum cleanly, without tearing, before the end of the cutting cycle. Reflecting the tearing of the coagulum and consequent shattering of curd particles, fat losses in the whey are greater than those predicted on the basis of volume reduction (due to UF) for milk with a protein concentration >5 %. Similar findings have been attributed partly to the poorer fat-retaining ability of higher protein curds which have coarser, more porous protein networks. Reducing the setting temperature, in the range 31–27 °C, and reducing the level of rennet added gives a set-to-cut times and curd firming rate for concentrated milks closer to those of the control milk.

Increasing the concentration of protein also results in slower proteolysis during ripening when an equal quantity of rennet on a milk volume basis is used. The slower rate of proteolysis in cheeses made from ultrafiltered milks may be attributed to a number of factors, including:

- the lower effective rennet concentration, i.e., rennet-to-casein ratio, and hence activity in the cheese,
- the inhibition of the indigenous milk proteinase, plasmin, by retained β-lactoglobulin in cheeses containing a significant quantity of whey proteins,
- the concentration during UF of indigenous proteinase/peptidase inhibitors, and/or
- the resistance of undenatured whey proteins to proteolysis in cheese where they represent a substantial portion (~18 %) of the proteins.

However, at an equal rennet-to-casein ratio, the level of α_{s1}-casein hydrolysis is higher in control Cheddar cheese than in cheese made from milk concentrated five-fold by UF. The reduced surface area-to-volume (SA/V) ratio of the protein network in cheeses made from concentrated milk, resulting from the coarser network, may also contribute to the observed reduction in proteolysis. It is conceivable that for a given level of enzyme activity in the cheese curd, casein degradation decreases as the SA/V ratio of the matrix decreases.

Cheese becomes progressively firmer (i.e., requires a higher compression force to induce fracture), more cohesive, mealier and drier, and the structure of the protein matrix becomes coarser and more compact (fused) with increasing concentration factor. The reduced rate of proteolysis results in slower softening and flavour development during maturation.

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Chapter 4
Chemistry of Milk Constituents

Summary Milk is a very complex fluid. It contains four principal constituents, water, lipids, proteins and lactose and perhaps 100 minor constituents, the most important of which from a cheesemaking viewpoint is calcium phosphate. The manufacture and quality of cheese depend, especially, on the properties of one of its protein groups, the caseins, and to a lesser extent on the lipids. Most (~90 %) of the water of milk is removed in the whey, which contains the soluble constituents, i.e., the whey proteins, lactose and some of the inorganic salts. Traditionally, whey was an almost worthless by-product but it is now the source of several very valuable products which are described in Chap. 22.

To better understand the cheesemaking process, the unique characteristics of the caseins, milk lipids, lactose and milk salts are described briefly in this chapter.

Keywords Lactose • Milk lipids • Milk proteins • Casein micelles • Milk salts

4.1 Introduction

Milk is a fluid secreted by the female of all mammals, of which there are >4000 species, for the primary function of meeting the complete nutritional requirements of the neonate of the species. It must supply energy [mainly from fats and sugar (lactose)], amino acids (from proteins), vitamins and inorganic elements (commonly, but inaccurately, referred to as minerals). In addition, some constituents of milk serve several physiological functions, including antimicrobial systems (immunoglobulins, lactoperoxidase, lactoferrin), enzymes and enzyme inhibitors, vitamin-binding carrier proteins, cell growth and control factors. Because the nutritional and physiological requirements of each species are more or less unique, the composition of milk shows very marked interspecies differences. The milk of only ~180 species have been analysed and of these, the data for only about 50 species are considered to be reliable (a sufficient number of samples, representative sampling, adequate coverage of the lactation period). Not surprisingly, the milk of the principal dairying

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species (i.e., cow, goat, sheep and buffalo) and the human are among those that are well characterized. The gross composition of milk from selected species is summarized in Table 4.1.

In addition to the principal constituents listed in Table 4.1, milk contains several hundred minor constituents, many of which, e.g., vitamins, inorganic and organic ions and flavour compounds, have a major impact on the nutritional, technological and sensoric properties of milk and dairy products.

Milk is a very variable biological fluid. In addition to interspecies differences (Table 4.1), the milk of any particular species varies with the individuality of the animal, the breed (in the case of commercial dairying species), health (mastitis and other diseases), nutritional status, stage of lactation, age, interval between milkings, etc. In a bulked factory milk supply, variability due to many of these factors is reduced but some variability persists and is quite large in situations where milk production is seasonal. In addition to variations in the concentrations of the principal and minor constituents due to the above factors, the chemistry of some of the constituents also varies, e.g., the fatty acid profile is strongly influenced by diet. Some of the variability in the composition and constituents of milk can be adjusted or counteracted by processing technology but some differences may persist. As will become apparent in later chapters, the variability of milk composition poses major problems in cheese production.

Physico-chemically, milk is a very complex fluid. The constituents of milk occur in three phases. Quantitatively, most of the mass of milk is an aqueous solution of

Table 4.1 Composition (%) of milk of some species (modified from Fox and McSweeney 1998)

Species	Total solids	Fat	Protein	Lactose	Ash
Human	12.2	3.8	1.0	7.0	0.2
Cattle	12.7	3.7	3.4	4.8	0.7
Buffalo	16.3	6.7	4.7	4.8	0.8
Goat	12.3	4.5	2.9	4.1	0.8
Sheep	19.3	7.4	4.5	4.8	1.0
Reindeer	27.1	11	10.4	3.0	1.5
Bison	14.6	3.5	4.5	5.1	0.8
Camel (dromedary)	12.2	3.8	4.7	4.5	–
Pig	18.8	6.8	4.8	5.5	–
Horse	11.2	1.9	2.5	6.2	0.5
Donkey	11.7	1.4	2.0	7.4	0.5
Dog	22.7	9.5	7.5	3.8	–
Domestic rabbit	32.8	18.3	11.9	2.1	1.8
Indian elephant	31.9	11.6	4.9	4.7	0.7
White rhino	8.8	0.74	1.4	6.6	–
Polar bear	47.6	33.1	10.9	0.3	1.4
Dolphin	41.4	30.0	10.3	~0	0.8
Grey seal	67.7	53.1	11.2	0.7	–
Fin whale	46.5	33.2	10.5	2.3	–

lactose, organic and inorganic salts, vitamins and other small molecules. In this aqueous solution are dispersed proteins, some at the molecular level (whey proteins), others as large colloidal aggregates, ranging in diameter from 50 to 600 nm (the caseins), and lipids which exist as an emulsion of globules ranging in diameter from 0.1 to 20 μm. Colloid chemistry is important in the study of milk, e.g., in the context of surface chemistry, light scattering and rheology.

Milk is a dynamic system owing to: the instability of many of its structures, e.g., the milk fat globule membrane; changes in the solubility of many constituents, especially the inorganic salts and proteins, with temperature and pH; the presence of various enzymes which can modify constituents through lipolysis, proteolysis or oxidation/ reduction; the growth of microorganisms, which can cause major changes either directly through their growth, e.g., changes in pH or redox potential (E_h) or through enzymes which they excrete; and the interchange of gases with the atmosphere, e.g., CO_2. Milk was intended to be consumed directly from the mammary gland and to be expressed from the gland at frequent intervals. However, in dairy operations, milk is stored for various periods, ranging from a few hours to several days, during which it is cooled (and perhaps heated) and agitated. These treatments cause some physical changes and permit some enzymatic and microbiological changes which may alter the processing properties of milk. It may be possible to counteract some of these changes.

Although many of the minor constituents of milk are very important from a nutritional viewpoint, the technological properties of milk are determined mainly by its macro-constituents, proteins, lipids and lactose, and some of its low molecular mass species, especially calcium, phosphate, citrate, and pH. The properties of these constituents, with emphasis on their significance in cheesemaking, will be discussed briefly in this chapter. For a more thorough discussion, the reader is referred to Jenness and Patton (1959), Webb and Johnson (1965),Webb et al. (1974), Fox (1982, 1983, 1985, 1989, 1992, 1995, 1997), Walstra and Jenness (1984), Wong et al. (1988), Jensen (1995), Fox and McSweeney (1998, 2003, 2006), Cayot and Lorient (1998), Walstra et al. (1999, 2005) and McSweeney and Fox (2009, 2013).

4.2 Lactose and Other Carbohydrates

Lactose is the principal carbohydrate in the milk of most mammals, which are the only sources. The milk of humans and some other species contains high levels of oligosaccharides which are believed to be very important nutritionally and physiologically (Urashima et al. 2009, 2011). However, the milk of commercial dairying species contains only trace levels of oligosaccharides which will not be considered further here. Milk also contains trace amounts of other sugars, including glucose, fructose, glucosamine, galactosamine and neuraminic acid, mainly as components of glycoproteins and polar lipids.

The concentration of lactose in milk varies widely between species (Table 4.2). The lactose content of cows' milk varies with udder infection and especially the

stage of lactation. The concentration of lactose decreases progressively and significantly during lactation (Fig. 4.1); this trend contrasts with the lactational trends for lipids and proteins, which, after decreasing during early lactation, increase strongly during the second half of lactation. In contrast, the concentration of lactose in equine and asinine milk decreases during lactation (Uniacke-Lowe and Fox 2011, 2012).

Lactose and soluble inorganic ions, e.g., Na^+, K^+, Cl^-, are the compounds mainly responsible for the osmotic pressure of milk. During mastitis, the concentration of NaCl in milk increases, resulting in an increase in osmotic pressure. This increase

Table 4.2 Concentration (%) of lactose in the milk of selected species (from Fox and McSweeney 1998)

Species	Lactose	Species	Lactose	Species	Lactose
California sea lion	0.0	Mouse (house)	3.0	Cat (domestic)	4.8
Hooded seal	0.0	Guinea pig	3.0	Pig	5.5
Black bear	0.4	Dog (domestic)	3.1	Horse	6.2
Dolphin	0.6	Sika deer	3.4	Chimpanzee	7.0
Echidna	0.9	Goat	4.1	Rhesus monkey	7.0
Blue whale	1.3	Elephant (Indian)	4.7	Human	7.0
Rabbit	2.1	Cow	4.8	Donkey	7.4
Red deer	2.6	Sheep	4.8	Zebra	7.4
Grey seal	2.6	Water buffalo	4.8	Green monkey	10.2
Rat (Norwegian)	2.6	Reindeer	3.0		

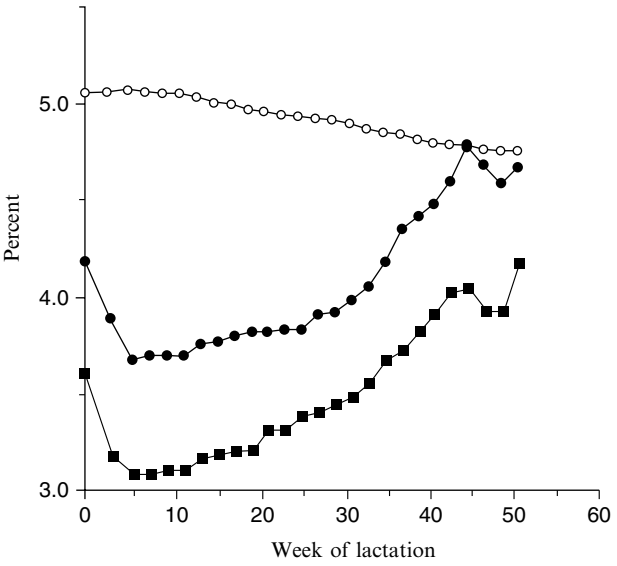


Fig. 4.1 Changes in the concentrations of fat (*filled circle*), protein (*filled square*) and lactose (*open circle*) in milk during lactation

is compensated for by a decrease in the lactose content, i.e., there is an inverse relationship between the concentrations of NaCl and lactose in milk which partly explains why certain milks with a high-lactose content have a low ash content and vice versa (see Table 4.1). The inverse relationship between the concentration of lactose and chloride is the basis of Koestler's chloride-lactose test for abnormal milk:

$$\text{Koestler Number} = \frac{\%\text{Chloride} \times 100}{\%\text{Lactose}}$$

A Koestler Number <2 indicates normal milk while a value >3 is considered abnormal.

In the mammocytes, lactose causes the influx of water into milk and hence leads to the dilution of other milk constituents; there is an inverse correlation between the concentrations of lactose and of lipids and protein in milk (Fig. 4.2).

Lactose plays an important role in milk and milk products:

- it is essential in the production of fermented dairy products, including cheese.
- it contributes to the nutritive value of milk and its products; however, only people of north European ancestry and some African tribes can consume lactose with

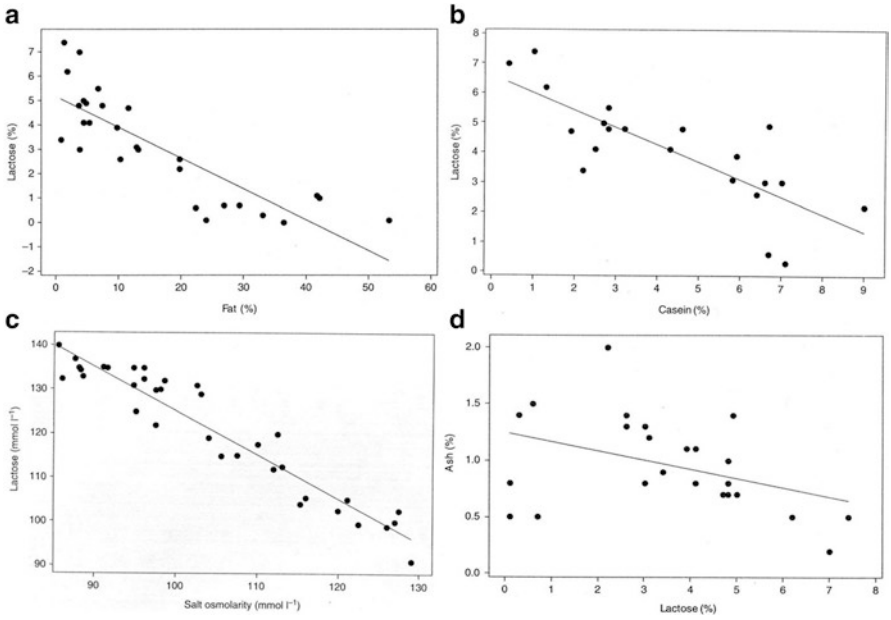


Fig. 4.2 Correlations between, (**a**) lactose and fat, (**b**) lactose and casein, (**c**) lactose and osmolarity and (**d**) lactose and ash in the milk of several species (modified from Fox, *Encyclopedia of Dairy Sciences*, Vol 3, pp 173–181, 2011)

impunity in adulthood; others have limited or zero ability to digest lactose, leading to a syndrome known as **lactose intolerance** (Ingram and Swallow 2009; Swallow 2011). Mature cheese is free of lactose and hence cheese is suitable for inclusion in the diet of lactose-intolerant individuals.
- it affects the texture of certain concentrated and frozen products.
- it is involved in heat-induced changes in the colour and flavour of highly heated milk products.

4.2.1 Structure of Lactose

Lactose is a disaccharide consisting of galactose and glucose, linked by a β1-4 glycosidic bond (Fig. 4.3). Its systematic name is *O*-β-D-galactopyranosyl-(1–4)-α-D-glucopyranose (α-lactose) or *O*-β-D-galactopyranosyl-(1–4)-β-D-glucopyranose (β-lactose). The hemiacetal group of the glucose moiety is potentially free (i.e., lactose is a **reducing** sugar) and may exist as an α or β anomer. In the structural formula of α-lactose, the hydroxyl group on the C_1 of glucose is *cis* to the hydroxyl group at C_2 (oriented downward) and vice versa for the ß-form (orientated upwards).

4.2.2 Biosynthesis of Lactose

Lactose is unique to mammary secretions. It is synthesized in the mammary gland from glucose absorbed from blood. One molecule of glucose is converted to UDP-galactose via the 4-enzyme Leloir pathway (Fig. 4.4). UDP-Gal is then linked to another molecule of glucose in a reaction catalysed by the enzyme, lactose synthetase, a two-component enzyme. Component A is a non-specific galactosyl transferase (EC 2.4.1.22) which transfers the galactose from UDP-gal to one of a number of acceptors. In the presence of the B component, which is the whey protein, α-lactalbumin, the transferase becomes highly specific for glucose (its K_M is reduced 1000-fold), leading to the synthesis of lactose. Thus, α-lactalbumin is an enzyme modifier and its concentration in the milk of several species is directly related to the concentration of lactose in those milks; the milk of some marine mammals contains neither α-lactalbumin nor lactose.

The presumed significance of this control mechanism is to enable mammals to terminate the synthesis of lactose when necessary, i.e., to regulate and control osmotic pressure when there is an influx of NaCl, e.g., during mastitis or in late lactation (milk is isotonic with blood, the osmotic pressure of which is essentially constant). The ability to control osmotic pressure is sufficiently important to justify an elaborate control mechanism and “wastage” of the enzyme modifier.

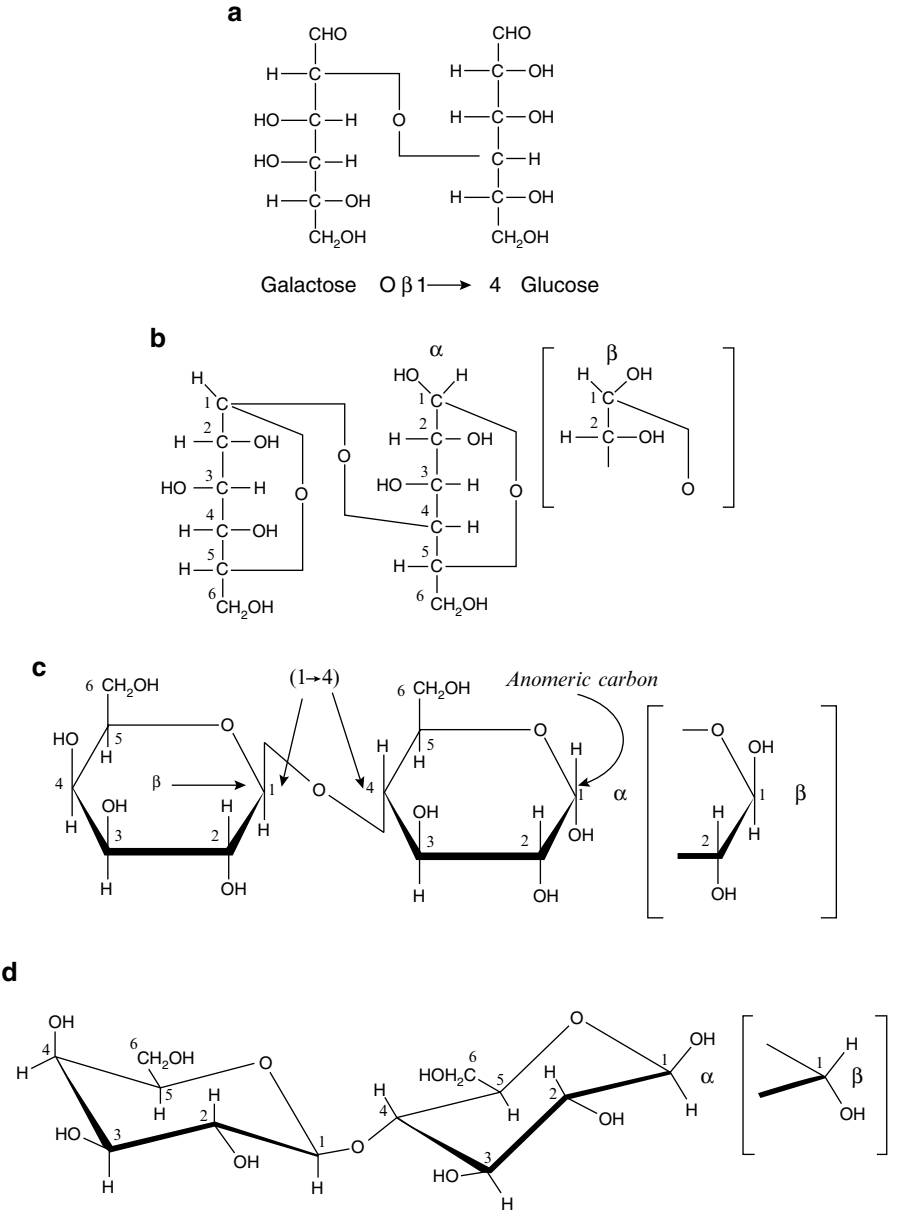


Fig. 4.3 Structural formulae of α- and β-lactose: (**a**) open chains, (**b**) Fischer projection, (**c**) Haworth projection and (**d**) conformational formula

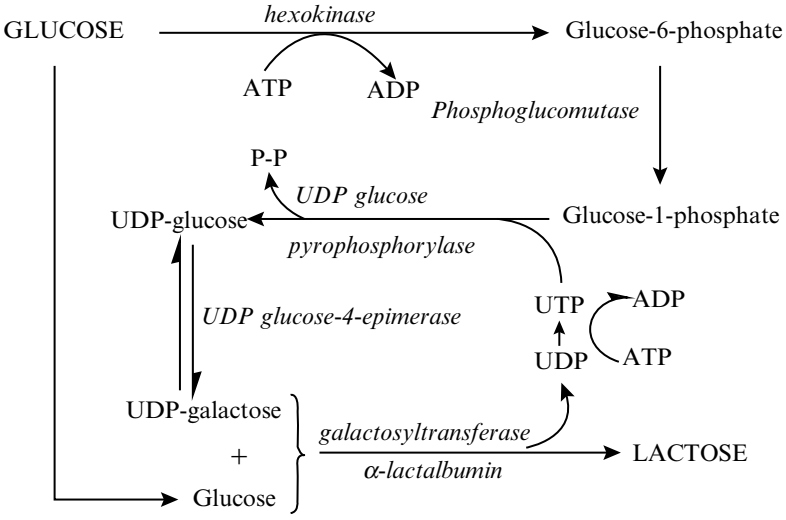


Fig. 4.4 Pathway for lactose synthesis

4.2.3 Lactose Equilibrium in Solution

The configuration around the anomeric C_1 of the glucose moiety of lactose is not stable and can readily change (**mutarotate**) from the α- to the ß-form and vice versa when the sugar is in solution as a consequence of the fact that the hemiacetal form is in equilibrium with the open-chain aldehyde form which can be converted to either of the two isomeric forms (Fig. 4.3). When either isomer is dissolved in water, there is a gradual change from one form to the other until equilibrium is established, i.e., mutarotation. These changes are reflected by changes in optical rotation from +89.4° for α-lactose or +35° for β-lactose to a value of +55.4° at equilibrium. These values for specific rotation indicate that at equilibrium, a solution of lactose contains 62.7 % of the β anomer and 37.3 % of the α anomer.

The α and β anomers of lactose differ markedly with respect to: solubility, crystal shape, hydration of the crystals, hygroscopicity, specific rotation and sweetness. α-Lactose is soluble to the extent of ~7 g/100 ml H_2O at 20 °C while the solubility of β-lactose is ~50 g/100 ml. However, the solubility of α-lactose is more temperature-dependent than that of β-lactose and their solubility curves intersect at ~94 °C (Fig. 4.5). Thus, α-lactose is the form normally produced by crystallization. α-Lactose crystallizes as a mono-hydrate while crystals of β-lactose are anhydrous. Although lactose has low solubility in comparison with other sugars, once in solution it crystallizes slowly and precautions must be taken in the manufacture of concentrated and dehydrated products to avoid hygroscopicity, caking and a grainy texture (due to the slow growth of lactose crystals to >15 μm). These physicochemical properties of lactose are of major concern to manufacturers of concentrated, dehydrated and frozen dairy products but problems can be avoided by proper

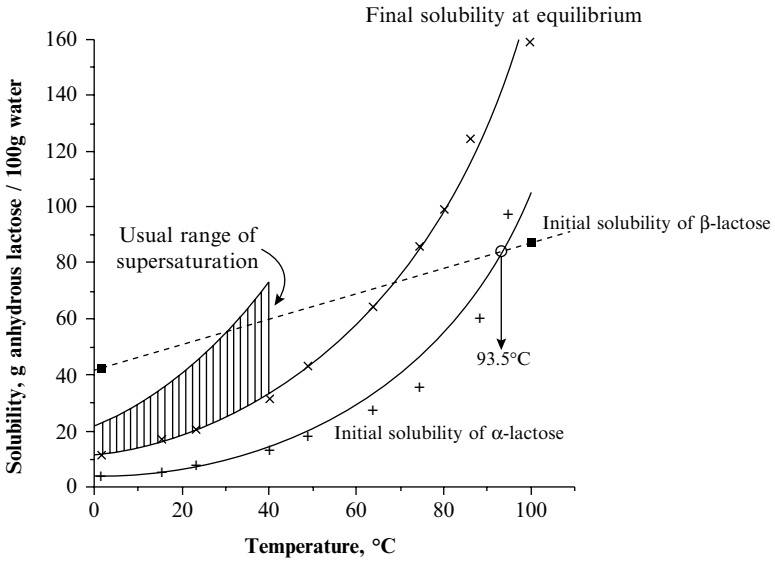


Fig. 4.5 Solubility of lactose in water

manufacturing procedures (see McSweeney and Fox 2009). Such properties are of no consequence in cheese in which all the lactose is utilized either during manufacture or early ripening (fresh curd contains ~1 % lactose) but are of major concern in the manufacture of whey powders since ~70 % of the total solids in whey are lactose and hence the properties of whey concentrates and powders (by-products of cheese production) are strongly influenced by the properties of lactose. The economic processing of whey is a very important component of the cheese industry; the production of lactose and its derivatives are discussed in Chap. 22.

In cheese, lactose is fermented to lactic acid by lactic acid bacteria, a process which has major, indeed vital, consequences for manufacture and quality of cheese, as will be discussed in Chaps. 6, 11 and 12.

For further discussion on the properties of, and the problems caused by, lactose, the reader is referred to Walstra and Jenness (1984), Fox (1985, 1997), Wong et al. (1988), Fox and McSweeney (1998), Walstra et al. (1999, 2005) and McSweeney and Fox (2009).

4.3 Milk Lipids

The lipid content of milk varies more widely than any other constituent; concentration ranges from ~<1 to >50 % (Table 4.3). The average fat content of bovine, goat, sheep and buffalo milk is 3.5, 3.5, 6.5 and 7 g/L, respectively. Within any particular species, there are considerable variations due to breed, individuality, stage of

Table 4.3 Fat content of milk from various species (g L^{-1})

Species	Fat content	Species	Fat content
Cow	33–47	Marmoset	77
Buffalo	47	Rabbit	183
Sheep	40–99	Guinea pig	39
Goat	41–45	Snowshoe hare	71
Musk ox	109	Muskrat	110
Dall sheep	32–206	Mink	134
Moose	39–105	Chinchilla	117
Antelope	93	White rhinoceros	74
Reindeer	100–200	Rat	103
Elephant	85–190	Red kangaroo	9–119
Human	38	Dolphin	62–330
Horse	19	Manatee	55–215
Monkeys	10–51	Pygmy sperm whale	153
Lemurs	8–33	Harp seal	502–532
Pig	68	Bear (four species)	108–331

Modified from Christie (1995)

lactation, age, animal health, nutritional status, interval between milking, etc. Among the common breeds of dairy cattle, Jersey cows produce milk with the highest fat content (6–7 %) and Holstein/Friesian, the lowest; within any breed, there are considerable individual-cow variations. The fat content of milk decreases for several weeks after parturition but then increases, especially towards the end of lactation (Fig. 4.1). If the interval between milkings is not equal, milk obtained after the shorter interval has the higher fat content. During a milking operation, the fat content increases considerably (e.g., from 1 to 10 %) due to the retardation of fat globules through the small ducts of the mammary gland; fat globules retained during one milking will be expressed at the next milking. The synthesis of all milk constituents, including fat, decreases during a mastitic infection and the fat content of milk decreases slightly as the animal ages.

The lipids in milk are predominantly triglycerides (triacylglycerols) which represent ~98 % of the total lipid fraction; the remaining 2 % is comprised of diglycerides, monoglycerides, fatty acids, phospholipids, sterols (principally cholesterol) and trace amounts of fat-soluble vitamins (A, D, E and K). Typical values for the concentration of the various lipids in milk are given in Table 4.4.

Ruminant milk fats contain a greater diversity of fatty acids than other fats; about 400 fatty acids, most at trace levels, have been identified in bovine milk fat. The predominant fatty acids have a straight carbon chain with an even number (4–22) of carbon atoms and may be saturated or unsaturated (1, 2 or 3 C=C double bonds). There are smaller amounts of fatty acids with an uneven number of carbon atoms, branched or cyclic hydrocarbon chains or hydroxyl or keto groups. The principal fatty acids in the milk fat of a selection of species are summarized in Table 4.5.

Table 4.4 Proportions (weight % of the total lipids) of individual lipids in the milk of some species

Lipid class	Cow	Buffalo	Human	Pig	Rat	Mink
Triacylglycerols	97.5	98.6	98.2	96.8	87.5	81.3
Diacylglycerols	0.36	–	0.7	0.7	2.9	1.7
Monoacylglycerols	0.027	–	T	0.1	0.4	T
Cholesteryl esters	T	0.1	T	0.06	–	T
Cholesterol	0.31	0.3	0.25	0.6	1.6	T
Free fatty acids	0.027	0.5	0.4	0.2	3.1	1.3
Phospholipids	0.6	0.5	0.26	1.6	0.7	15.3

From Christie (1995)
T trace

Table 4.5 Principal fatty acids in milk lipids of various species (%, w/w) (from Christie 1995)

Species	4:0	6:0	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	C_{20}-C_{22}
Cow	3.3	1.6	1.3	3.0	3.1	9.5	26.3	2.3	14.6	29.8	2.4	0.8	T[a]
Buffalo	3.6	1.6	1.1	1.9	2.0	8.7	30.4	3.4	10.1	28.7	2.5	2.5	T
Sheep	4.0	2.8	2.7	9.0	5.4	11.8	25.4	3.4	9.0	20.0	2.1	1.4	–
Goat	2.6	2.9	2.7	8.4	3.3	10.3	24.6	2.2	12.5	28.5	2.2	–	–
Musk ox	T	0.9	1.9	4.7	2.3	6.2	19.5	1.7	23.0	27.2	2.7	3.0	0.4
Dall sheep	0.6	0.3	0.2	4.9	1.8	10.6	23.0	2.4	15.5	23.1	4.0	4.1	2.6
Moose	0.4	T	8.4	5.5	0.6	2.0	28.4	4.3	4.5	21.2	20.2	3.7	–
Blackbuck antelope	6.7	6.0	2.7	6.5	3.5	11.5	39.3	5.7	5.5	19.2	3.3	–	–
Elephant	7.4	–	0.3	29.4	18.3	5.3	12.6	3.0	0.5	17.3	3.0	0.7	–
Human	–	T	T	1.3	3.1	5.1	20.2	5.7	5.9	46.4	13.0	1.4	T
Monkey (mean of six species)	0.4	0.6	5.9	11.0	4.4	2.8	21.4	6.7	4.9	26.0	14.5	1.3	–
Baboon	–	0.4	5.1	7.9	2.3	1.3	16.5	1.2	4.2	22.7	37.6	0.6	–
Lemur macaco	–	–	0.2	1.9	10.5	15.0	27.1	9.6	1.0	25.7	6.6	0.5	–
Horse	–	T	1.8	5.1	6.2	5.7	23.8	7.8	2.3	20.9	14.9	12.6	–
Pig	–	–	–	0.7	0.5	4.0	32.9	11.3	3.5	35.2	11.9	0.7	–
Rat	–	–	1.1	7.0	7.5	8.2	22.6	1.9	6.5	26.7	16.3	0.8	1.1
Guinea pig	–	T	–	–	–	2.6	31.3	2.4	2.9	33.6	18.4	5.7	T
Marmoset	–	–	–	8.0	8.5	7.7	18.1	5.5	3.4	29.6	10.9	0.9	7.0
Rabbit	–	T	22.4	20.1	2.9	1.7	14.2	2.0	3.8	13.6	14.0	4.4	T
Cottontail rabbit	–	–	9.6	14.3	3.8	2.0	18.7	1.0	3.0	12.7	24.7	9.8	0.4
European hare	–	T	10.9	17.7	5.5	5.3	24.8	5.0	2.9	14.4	10.6	1.7	T
Mink	–	–	–	–	0.5	3.3	26.1	5.2	10.9	36.1	14.9	1.5	–
Chinchilla	–	–	–	–	T	3.0	30.0	–	–	35.2	26.8	2.9	–

(continued)

Table 4.5 (continued)

Species	4:0	6:0	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	C_{20}-C_{22}
Red kangaroo	–	–	–	–	0.1	2.7	31.2	6.8	6.3	37.2	10.4	2.1	0.1
Platypus	–	–	–	–	–	1.6	19.8	13.9	3.9	22.7	5.4	7.6	12.2
Numbat	–	–	–	–	0.1	0.9	14.1	3.4	7.0	57.7	7.9	0.1	0.2
Bottle-nosed dolphin	–	–	–	–	0.3	3.2	21.1	13.3	3.3	23.1	1.2	0.2	17.3
Manatee	–	–	0.6	3.5	4.0	6.3	20.2	11.6	0.5	47.0	1.8	2.2	0.4
Pygmy sperm whale	–	–	–	–	–	3.6	27.6	9.1	7.4	46.6	0.6	0.6	4.5
Harp seal	–	–	–	–	–	5.3	13.6	17.4	4.9	21.5	1.2	0.9	31.2
Northern elephant seal	–	–	–	–	–	2.6	14.2	5.7	3.6	41.6	1.9	–	29.3
Polar bear	–	T	–	T	0.5	3.9	18.5	16.8	13.9	30.1	1.2	0.4	11.3
Grizzly bear	–	T	–	–	0.1	2.7	16.4	3.2	20.4	30.2	5.6	2.3	9.5

[a]*T* trace

The fatty acid profile of ruminant milk fats have a number of interesting features:

- They contain a considerable amount of butanoic acid ($C_{4:0}$); they are, in fact, the only fats that contain this acid. The high content of butanoic acid is due to the synthesis of 3-hydroxybutanoic acid (β-hydroxybutyrate) and its reduction to butanoic acid by bacteria in the rumen. The high concentration of butanoic acid in ruminant milk fats is the principle of the method commonly used to detect and quantify the adulteration of milk fat with other fats, i.e., the Reichert-Meissl (RM) number, the number of ml 0.1 M KOH required to neutralise the volatile water-soluble fatty acids released from 5 g fat on hydrolysis.
- Ruminant milk fats, in general, and ovine milk fat in particular, contain relatively high concentrations of middle-chain fatty acids [hexanoic ($C_{6:0}$) to decanoic ($C_{10:0}$)]. This is due to high thioacylhydrolase activity in the fatty acid synthetase complex which causes the early release of fatty acids during the chain elongation process.
- Hexanoic, octanoic and decanoic acids are rare in nature; high levels occur only in cocoa nut and palm kernal oils. They are relatively volatile but sparingly soluble in water. Their concentration in fats is expressed as the Polenske number (value), the number of ml of 0.1 M KOH required to neutralise the volatile, water-insoluble fatty acids released from 5 g of fat on hydrolysis. Ruminant milk fats have a high RM value but a low Polenske value; cocoa nut and palm kernel oils have a low RM but a high Polenske value. All other fats and oils have low RM and Polenske values.

- The short- and middle-chain acids ($C_{4:0}$–$C_{10:0}$) have relatively low flavour thresholds. They are esterified predominantly at the *Sn*3 position of glycerol, and hence are selectively released by lipases, especially by the indigenous lipoprotein lipase in milk. In milk and butter, the release of these highly flavoured short-chain fatty acids gives rise to off-flavours, referred to as **hydrolytic rancidity**. However, when present at an appropriate level, these short-chain acids contribute positively to the flavour of cheese, especially hard Italian and blue-mould varieties.
- The melting point, and consequently the "hardness" of fatty acids and triglycerides containing them, increase with the carbon chain length.
- Ruminant milk fats contain low levels of polyunsaturated fatty acids (PUFA; $C_{18:2}$, $C_{18:3}$) which are considered to be nutritionally desirable. However, the low level of PUFA makes milk fat relatively resistant to **oxidative rancidity**. The low concentration of PUFAs in ruminant milk fats is due to the hydrogenation of dietary fatty acids by bacteria in the rumen, although ruminant feed usually contains quite high levels of PUFAs. On the positive side, biohydrogenation of PUFAs results in lower levels of *trans* isomers than chemical hydrogenation, as practised in the processing of vegetable oils; trans fatty acids are considered to be nutritionally undesirable.

The concentration of PUFAs in ruminant milk fats can be increased by including protected lipids in the animal's diet. This involves encapsulating the dietary lipids in a layer of polymerized protein or using crushed vegetable seeds; encapsulation protects the PUFAs against hydrogenation in the rumen but the capsule is digested in the abomasum, liberating the encapsulated lipids which are then metabolized as in non-ruminants. Fat has a major effect on the rheological properties of cheese; polyunsaturated lipids, which have a low melting point, have an undesirable effect on cheese texture but a low level is acceptable.

In unsaturated fatty acids, there is usually a methylene ($-CH_2-$) group between each pair of double bonds, i.e., they are methylene interrupted. However, the double bond can be shifted by chemical or biological means to give a conjugated system. In ruminants, this is caused by an aborted saturation in the rumen by *Butyrivibrio fibrisolvens*. Linoleic acid (Δ 9, 12-octadecdienoic acid) is converted to four conjugated isomers (conjugated linoleic acid; CLA), one of which, Δ 9, 11-octadecdienoic acid, has anti-carcinogenic activity. Ruminant milk and adipose tissue fat are major sources of CLA (see Bauman and Lock 2006).

Although phospholipids are present at very low concentrations in milk, they play a very important role in the emulsification of fat in milk. Milk contains a relatively low concentration of cholesterol, a high level of which in the diet is considered to be nutritionally undesirable. The cow transfers dietary carotenoids to its milk and hence its milk fat has a yellow colour, the intensity of which depends on the concentration of carotenoids in the animal's feed—fresh grass and especially clover and lucerne are rich in carotenoids (see Fox and McSweeney 1998, 2006, for the structures of the principal phospholipids, cholesterol and fat-soluble vitamins). Buffalo, sheep and goats do not transfer dietary carotenoids to their milk and consequently their milk fat and fat-containing products (including cheese) made from these milks

are much whiter than their bovine counterparts. Products traditionally made from buffalo, ovine or caprine milk may be unacceptable, owing to their yellow colour, when made from bovine milk, especially if the cows are fed on fresh grass. However, it is possible to bleach or mask the colour of carotenoids, e.g., using H_2O_2, benzoyl peroxide, TiO_2 or chlorophyll. Some carotenoids are precursors of Vitamin A (retinol).

Milk contains a low level of Vitamin D and liquid milk products are commonly fortified with Vitamin D. Milk contains a substantial amount of Vitamin E (tocopherols), which is a potent antioxidant. The tocopherol content of milk may be increased by supplementing the animal's diet with tocopherols; this may be done for nutritional or stability reasons. However, lipid oxidation is not a problem in cheese, probably because of its low redox potential (E_h: −150 mV).

4.3.1 Milk Fat as a Emulsion

Lipids are insoluble in, and are less dense than, water (the specific gravity of fat and skimmed milk is ~0.9 and 1.036, respectively) and hence would be expected to form a layer on the surface of milk. However, lipids can be made compatible with water by forming an emulsion in which the fat is dispersed as small globules by homogenization, each of which is surrounded by a layer of emulsifier. An emulsion is defined as a two-phase system, one phase (the discontinuous, dispersed, phase) being dispersed in the other (continuous phase) and separated by a layer of emulsifier. In milk and cream, fat is the emulsified phase and water (or more correctly skim milk) is the continuous phase, i.e., milk is an oil-in-water emulsion. In butter (and margarine), the situation is reversed, i.e., water droplets are dispersed in a continuous oil/fat phase (i.e., water-in-oil emulsions).

Emulsifiers are ampiphatic molecules, with hydrophobic (lipophilic, fat-loving) and hydrophilic (water-loving) domains. The principal natural emulsifiers are polar lipids and proteins; in addition, numerous synthetic emulsifiers are available and are used widely in the manufacture of high-fat foods.

In milk, the fat exists as globules, 0.1–20 μm in diameter (mean diameter in bovine milk, 3–4 μm). Numerically, most of the globules have a diameter <1 μm but these small globules represent only a small fraction of the mass of milk fat. The globules are surrounded by a structured membrane, referred to as the milk fat globule membrane (MFGM), consisting mainly of phospholipids and proteins; the approximate composition of the MFGM is summarized in Table 4.6. The inner layers of the membrane are acquired within the secretory cell (mammocyte) as the fat globules move from the site of biosynthesis, i.e., the rough endoplasmic reticulum, located toward the base of the cell, toward the apical membrane, through which they are expressed into the lumen of the mammary alveoli by exocytosis (Fig. 4.6). During exocytosis, the fat globules become surrounded by the apical cell membrane which therefore forms the outer layer of the MFGM of freshly secreted milk fat globules. However, much of this membrane, which has a typical trilaminar fluid

Table 4.6 Gross composition of the milk fat globule membrane (from Fox and McSweeney 1998)

Component	mg 100 g^{-1} fat globule	mg m^{-2} fat globule surface	% (w/w) of total membrane
Protein	900	4.5	41
Phospholipid	600	3.0	27
Cerebrosides	80	0.4	3
Cholesterol	40	0.2	2
Neutral glycerides	300	1.5	14
Water	280	1.4	13
Total	2200	11.0	100

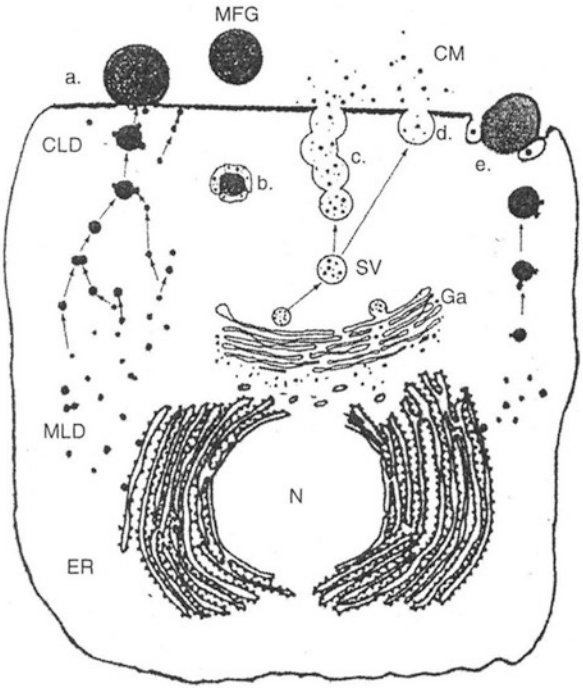


Fig. 4.6 Diagrammatic presentation of what is known about the intracellular origin, growth and secretion of milk fat globules. *Abbreviations*: *CLD* cytoplasmic lipid droplet, *CM* casein micelle, *ER* endoplasmic reticulum, *Ga* Golgi apparatus, *MLD* microlipid droplet, *MFG* milk fat globule, *N* nucleus, *SV* secretory vesicle (Keenan and Mather 2006)

mosaic structure, is lost as the milk ages and much of it accumulates as lipoprotein particles, sometimes referred to as "microsomes", in the skim milk phase.

The MFGM contains ~1 % of the total protein in milk. SDS-PAGE resolves the proteins of the MFGM into about 20 components which are unique to the MFGM, the principal of which are butyrophilin, xanthine oxidoreductase (XOR), adipophilin, mucin 1, mucin 15, periodic acid Schiff (PAS) staining protein 6/7 and

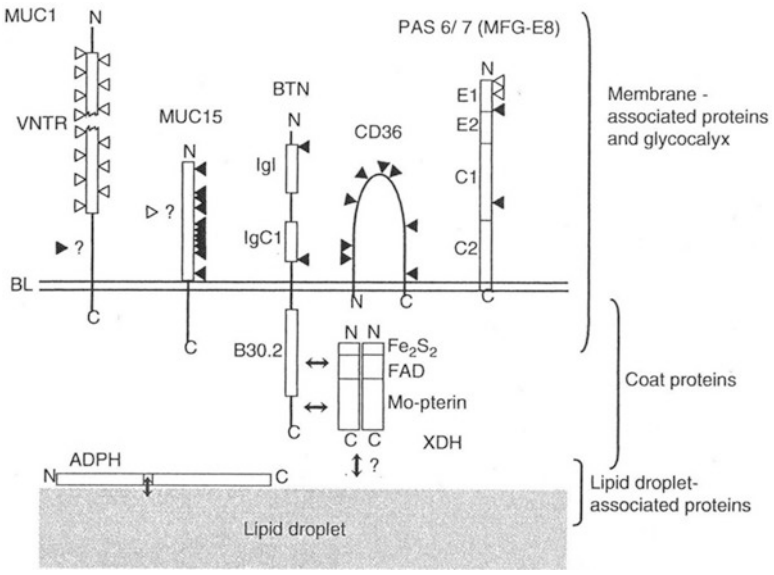


Fig. 4.7 Proposed structure of the milk fat globule membrane. *Abbreviations*: *BTN* butryophilin, *XDH* xanthynine oxidoreductase, *ADIP* adipophilin, *MUC* mucin, *CD 36* cluster of differentiation, *PAS 6/7* periodic acid Schiff, *VNTR* variable number tandem repeats, *BL* bilayer (Keenan and Mather 2006)

cluster of differentiation (CD) 36. A model of the putative arrangement of the principal proteins in the MFGM is shown in Fig. 4.7. Some proteins, e.g., adipophylin and XOR are on the inner face of the MFGM (somehow, XOR causes blebbing of the fat globule through the apical membrane), some, e.g., butyrophylin, are transmembrane, and some, e.g., the mucins which are very hydrophilic proteins, are on the outer face of the membrane and render the fat globules strongly hydrophilic.

Many of the indigenous enzymes in milk are constituents of the MFGM; consequently, isolated membrane [prepared by de-emulsification (churning) and washing] serves as the source material for the isolation of many indigenous milk enzymes. Xanthine oxidoreductase is one of the principal proteins of the MFGM. Two notable exceptions are the principal indigenous proteinase, plasmin, and lipoprotein lipase (LPL), which are associated mainly with the casein micelles. The MFGM isolates and protects the triglycerides from LPL but if the membrane is damaged, e.g., by agitation, the enzyme and its substrate come into contact and lipolysis and hydrolytic rancidity ensue, with undesirable consequences for the organoleptic quality of milk and many dairy products.

Although the milk fat emulsion is stable to phase separation, it does undergo rapid creaming due to the difference in density between the phases, i.e., the fat globules rise to the surface but remain discrete and can be redispersed by gentle agitation. The rate of creaming is governed by Stokes' law:

$$\nu = \frac{2r^2\left(\rho^1 - \rho^2\right)g}{9\eta}$$

ν = velocity of particle movement
r = radius of the globules
ρ^1 and ρ^2 = the density of the continuous and dispersed phases, respectively
g = acceleration due to gravity (9.8 m s^{-2})
η = viscosity coefficient of the emulsion

For milk, the parameters of Stokes' equation suggest that a cream layer would form after about 60 h but in fact it forms in about 30 min. This large discrepancy between the actual and the predicted rates of creaming is due to flocculation of the fat globules: the large globules rise faster than, and collide with, smaller globules and form clusters owing to the agglutinating action of immunoglobulin M. This protein is referred to as a "cryoglobulin" since it adsorbs onto the fat globules as the temperature is reduced. The cluster then rises as a unit, colliding with other globules as it does and, therefore, rises at an accelerating rate. The cryoglobulins solubilize as the temperature is increased and are fully soluble above 37 °C; consequently, creaming is promoted by low temperatures and is very slow above 37 °C. Cryoglobulins are denatured and inactivated by heating at time-temperature treatments >74 °C × 15 s; hence, severely pasteurized milk creams poorly or not at all. Sheep, goat and buffalo milks are devoid of cryoglobulins and hence cream very slowly.

If the MFGM is physically damaged by high temperatures and/or agitation, the globules coalesce and eventually phase inversion occurs, i.e., an oil-in-water emulsion is converted to a water-in-oil emulsion. Free (non-globular) fat will float on the surface. Such damage occurs to at least some extent during cheesemaking; the free fat is not incorporated into the coagulum and floats as quite large masses on the surface of the whey and is lost to the cheese; about 10 % of the fat in milk is normally lost in this way. It can be recovered from the whey by centrifugation and made into whey butter or other products.

Milk for many dairy products is "homogenized", usually by using a valve homogenizer. Homogenization reduces the size of the fat globules (average diameter, <1 μm) and denatures the cryoglobulins; thus, homogenized milk does not cream due to the combined effects of globule size reduction and denaturation of cryoglobulins. The membrane on the fat globules in homogenized milk is mainly casein and does not protect the triglycerides against lipolysis; therefore, homogenized milk must be pasteurized before or immediately after homogenization to prevent the occurrence of hydrolytic rancidity.

Milk for cheesemaking is not normally homogenized because on renneting homogenized milk forms a coagulum (gel) which has a lower tendency to synerese on cutting/stirring than that from non-homogenized milk and results in cheese with a higher moisture content. This situation arises because the casein-coated fat globules behave somewhat like casein micelles but they limit the contraction of the casein matrix. It may be advantageous to homogenise milk for low-fat cheese, so as to obtain a higher moisture content and thus soften the texture of the cheese. In some cases, milk for blue cheese is separated and the cream homogenized to promote

lipolysis (which is desirable in blue cheese); the lipolysed cream and skim milk are then recombined and pasteurized before cheese manufacture. Milk for yoghurt or cream cheese is also homogenized to:

- prevent creaming during the relatively long gelation period,
- to increase the effective protein concentration by converting the fat globules to pseudo-protein particles, thereby giving a firmer gel for a given level of protein, and
- to minimize syneresis.

Fat plays an essential role in cheese quality:

- it acts as a plasticiser in cheese texture; low-fat cheese has a hard, crumbly texture.
- it serves as a source of fatty acids which have a direct effect on cheese flavour and are changed to other flavour compounds, e.g., carbonyls, lactones, esters and thioesters.
- it acts as a solvent for flavour compounds produced from lipids, proteins or lactose.

With the objective of reducing the calorific content of cheese, there is considerable commercial interest in the production of low-fat cheeses but the quality of such cheese is reduced and consequently they have had only limited success (see Chaps. 12, 14, 17 and 18).

4.4 Milk Proteins

From a cheesemaking viewpoint, the proteins of milk are its most important constituents. The protein content of milk shows large interspecies differences, ranging from about 1 % for human milk to >20 % for milk from small mammals, e.g., mice and rats (Table 4.7). There is a good correlation between the protein content of milk and the growth rate of the neonate of that species (Fig. 4.8).

The proteins of milk belong to two main categories which can be separated based on their solubility at pH 4.6 at 20 °C. Under these conditions, one of the groups, the caseins, precipitates; the proteins that remain soluble at pH 4.6 are known as serum or whey proteins. Approximately 80 % of the total nitrogen in bovine, ovine, caprine and buffalo milks is casein but casein represents only ~40 % of the protein in human milk. Both caseins and whey proteins are quite heterogeneous and have very different molecular and physico-chemical properties.

4.4.1 The Caseins

Bovine casein consists of four protein with substantially different properties: α_{s1}-, α_{s2}-, β- and κ-casein; these represent approximately 38, 10, 34 and 15 %, respectively, of whole casein. The caseins are well characterized at the molecular level; some of the major properties are summarized in Table 4.8. The amino acid sequences are known (Figs. 4.9–4.12). Some of the more important properties of the caseins are as follows:

Table 4.7 Protein content (%) in the milk of some species (from Fox and McSweeney 1998)

Species	Casein	Whey protein	Total
Bison	3.7	0.8	4.5
Black bear	8.8	5.7	14.5
Buffalo	3.8	0.9	4.7
Camel (bactrian)	2.9	1.0	3.9
Cat	–	–	11.1
Cow	2.8	0.6	3.4
Domestic rabbit	9.3	4.6	13.9
Donkey	1.0	1.0	2.0
Echidna	7.3	5.2	12.5
Goat	2.5	0.4	2.9
Grey seal	–	–	11.2
Guinea pig	6.6	1.5	8.1
Hare	–	–	19.5
Horse	1.3	1.2	2.5
House mouse	7.0	2.0	9.0
Human	0.4	0.6	1.0
Indian elephant	1.9	3.0	4.9
Pig	2.8	2.0	4.8
Polar bear	7.1	3.8	10.9
Red kangaroo	2.3	2.3	4.6
Reindeer	8.6	1.5	10.4
Rhesus monkey	1.1	0.5	1.6
Sheep	4.6	0.9	5.5
White-tailed jack rabbit	19.7	4.0	23.7

- They are quite small molecules, with molecular masses of 20–25 kDa.
- All are phosphorylated: most molecules of α_{s1}-casein contain 8 mol PO_4/mol of protein but some contain 9 mol PO_4/mol; β-casein usually contains 5 mol PO_4/mol but some molecules contain 4 mol PO_4/mol; α_{s2}-casein contains 10, 11, 12 or 13 mol PO_4/mol; most molecules of κ-casein contain 1 mol PO_4/mol but some contain 2 or 3 mol PO_4/mol.
- The phosphate groups are esterified as monoesters of serine and most occur as clusters. The phosphate groups bind polyvalent cations strongly, causing charge neutralisation and precipitation of α_{s1}-, α_{s2}- and β-caseins at >6 mM Ca^{2+} at 30 °C. Because it contains little phosphate, κ-casein binds cations weakly and is not precipitated by them. It can stabilize up to 10 times its weight of calcium-sensitive caseins via the formation of micelles (see Sect. 4.4.2). In milk, the principal cation bound is calcium.
- Only α_{s2}- and κ-caseins contain cysteine which normally exists as intermolecular disulphide bonds. α_{s2}-Casein usually occurs as disulphide-linked dimers but up to at least 10 κ-casein molecules may be disulphide linked. The absence of cystine in α_{s1}- and β-caseins increases the flexibility of these molecules.
- All the caseins, especially β-casein, contain relatively high levels of proline; in β-casein, 35 of the 209 residues are proline which are uniformly distributed

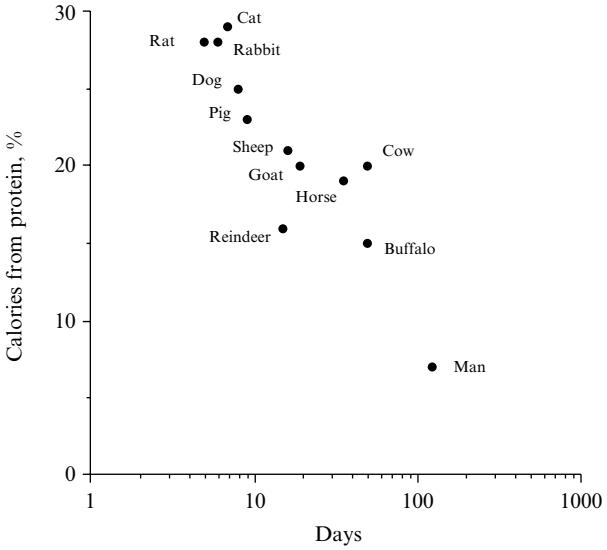


Fig. 4.8 Relationship between the growth rate (days to double birth weight) of the young of some species of mammal and the protein content (expressed as the percentage of total calories derived from protein) of the milk of that species (redrawn from Bernhart 1961)

throughout the molecule. The presence of a high level of proline limits the formation of secondary structures (α-helices, β-sheets and β-turns).

- Experimental techniques indicate that the caseins have low levels of secondary and tertiary structures although theoretical calculations indicate that they should have a substantial level of higher structure. It has been suggested that rather than lacking secondary structures, the caseins have very flexible structures which have been described as "rheomorphic". The lack of stable secondary and tertiary structures renders the caseins stable to denaturing agents, e.g., heat or urea, contributes to their surface activity properties and makes them readily susceptible to proteolysis, which is important in cheese ripening.
- The caseins have high surface hydrophobicity owing to their open structures. The hydrophobic, polar and charged residues are not uniformly distributed throughout the molecular sequences but occur as hydrophobic or hydrophilic patches (Fig. 4.13), giving the caseins strongly amphiphatic structures which make them highly surface active. The N-terminal 2/3 of κ-casein, which is particularly significant in cheese manufacture, is strongly hydrophobic while the C-terminal 1/3 is strongly hydrophilic. The hydrophobicity of the caseins explains why their hydrolyzates have a high propensity to bitterness, which is one of the principal defects in many cheese varieties.
- κ-Casein is glycosylated (α_{s1}-, α_{s2}- and β-caseins are not); it contains galactose, galactosamine and *N*-acetylneuraminic acid (sialic acid) which occur as either trisaccharides or tetrasaccharides attached to theronine residues in the C-terminal region. κ-Casein may contain 0–4, tri- or tetrasaccharides moieties, i.e., there

Table 4.8 Amino acid composition of the principal proteins in milk (from Fox and McSweeney 1998)

Amino acid	α_{s1}-Casein B	α_{s2}-Casein A	β-Casein A^2	κ-Casein B	γ^1-Casein A^2	γ^2-Casein A^2	γ^3-Casein A	β-Lactoglobulin A	α-Lactalbumin B
Asp	7	4	4	4	4	2	2	11	9
Asn	8	14	5	7	3	1	1	5	12
Thr	5	15	9	14	8	4	4	8	7
Ser	8	6	11	12	10	7	7	7	7
SerP	8	11	5	1	1	0	0	0	0
Glu	24	25	18	12	11	4	4	16	8
Gln	15	15	21	14	21	11	11	9	5
Pro	17	10	35	20	34	21	21	8	2
Gly	9	2	5	2	4	2	2	3	6
Ala	9	8	5	15	5	2	2	14	3
1/2 cys	0	2	0	2	0	0	0	5	8
Val	11	14	19	11	17	10	10	10	6
Met	5	5	6	2	6	4	4	4	1
Ile	11	11	10	13	7	3	3	10	8
Leu	17	13	22	8	19	14	14	22	13
Tyr	10	12	4	9	4	3	3	4	4
Phe	8	6	9	4	9	5	5	4	4
Trp	2	2	1	1	1	1	1	2	4
Lys	14	24	11	9	10	4	3	15	12
His	5	3	5	3	5	4	3	2	3
Arg	6	6	4	5	2	2	2	3	1
PyroGlu	0	0	0	1	0	0	0	0	0
Total residues	199	207	169	209	181	104	102	162	123
Molecular weight	23,612	25,228	19,005	23,980	20,520	11,822	11,557	18,362	14,174
$H\Phi_{ave}$ (kJ/residue)	4.89	4.64	5.12	5.58	5.85	6.23	6.29	5.03	4.68



Fig. 4.9 Amino acid sequence of α_{s1}-casein, showing the amino acid substitutions or deletions in the principal genetic variants (from Swaisgood 1992)



Fig. 4.10 Amino acid sequence of bovine α_{s2}-casein A, showing 11 of the 13 potential phosphorylation sites (from Swaisgood 1992)

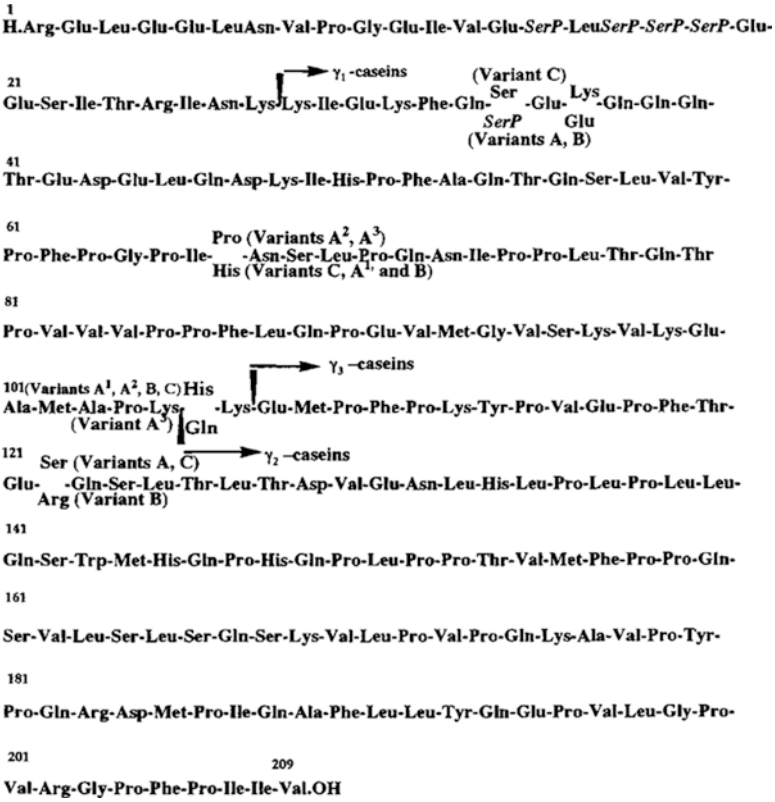


Fig. 4.11 Amino acid sequence of bovine β-casein, showing the amino acid substitutions in the genetic variants and the principal plasmin cleavage sites (*inverted filled triangle*) (from Swaisgood 1992)



Fig. 4.12 Amino acid sequence of bovine κ-casein, showing the amino acid substitutions in genetic polymorphs A and B and the chymosin cleavage site, (*inverted filled triangle*). The sites of post-transitional polymorphism or glycosylation are italicized (from Swaisgood 1992)

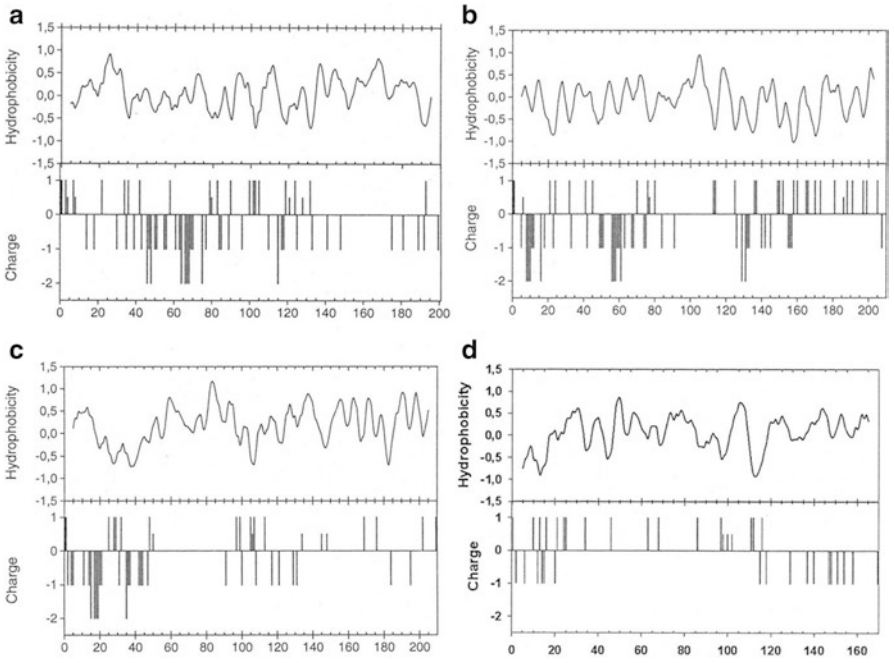


Fig. 4.13 Distribution of hydrophobicity (*top*) and charged residues (*bottom*) along the polypeptide of: (**a**) α_{s1}-CN B-8P; (**b**) α_{s2}-CN A-11P; (**c**) β-CN A^2-5P; (**d**) κ-CN A-1P (Huppertz 2013)

are nine variants of κ-casein. The presence of oligosaccharides attached to the C-terminal of κ-casein increases the hydrophilicity of that region.

- All the caseins exhibit genetic polymorphism which involves the substitution of 1 or 2 amino acids or rarely the deletion of a segment. The variant(s) present in milk is (are) determined by simple Mendelian genetics. To date, 9, 4, 12 and 15 genetic variants of α_{s1}-, α_{s2}-, β- and κ-casein have been reported in bovine milk (Martin et al. 2013). Since gel electrophoresis is usually used to detect and identify genetic variants, only substitutions involving a change in charge are detected. It is almost certain that substitution involving non-charged amino acids residues occurs but have not been detected; they could be detected by mass spectrometry. The presence of certain genetic variants in milk has a significant effect on the cheesemaking properties of milk.

The preceding discussion indicates that the casein system is very heterogeneous and a systematic nomenclature system is necessary. The following nomenclature has been adopted:

- The casein family is indicated by a Greek letter with a subscript, if necessary, i.e., α_{s1}-, α_{s2}-, β-, κ-.
- This is followed by CN.
- Then, the genetic variant is indicated by a Latin letter, A, B, C, etc, with a superscript, if necessary, e.g., α_{s1}-CN B, β-CN A^1.

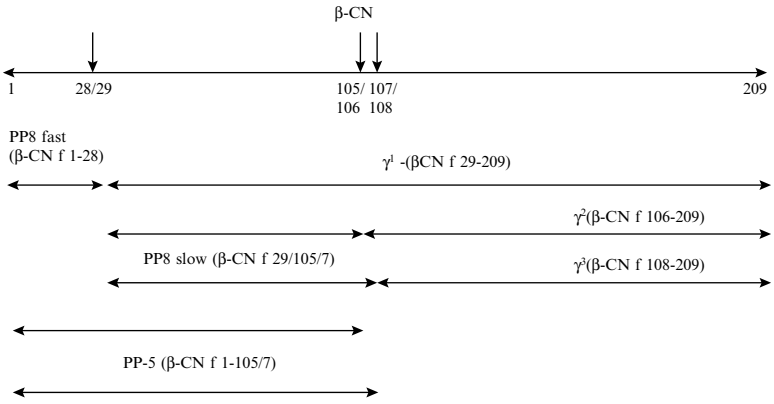


Fig. 4.14 Principal peptides produced from β-casein by plasmin

- The number of phosphate residues is indicated, e.g., α_{s1}-CN B-8P, β-CN A^1-5P.

Minor components of the casein system are the γ-caseins which are C-terminal fragments of β-casein produced through the action of the indigenous proteinase, plasmin. The N-terminal fragments are included in the so-called proteose peptone fraction of milk protein. These peptides are summarized in Fig. 4.14. Plasmin also hydrolyses α_{s1}-casein slightly and the resulting peptides include the λ-caseins and some proteose peptones. Isolated α_{s2}-casein is quite susceptible to plasmin but no α_{s2}-casein-derived peptides have been identified in milk.

Ovine, caprine and buffalo caseins exhibit similar heterogeneity to that of the bovine caseins but there are distinct interspecies characteristics (Fig. 4.15)

4.4.2 The Casein Micelle

The α_{s1}-, α_{s2}- and β-caseins, which together represent ~85 % of whole casein, are precipitated by Ca at concentrations >6 mM. Since bovine milk contains ~30 mM Ca, it might be expected that these caseins would precipitate in milk. However, κ-casein, which contains only 1 mol PO_4 per mol, is insensitive to Ca^{2+} and, moreover, can stabilize up to 10 times its mass of the Ca-sensitive caseins against precipitation by Ca^{2+}. It does this via the formation of a type of quaternary structure, referred to as the casein micelle.

The principal properties of the casein micelle are summarized in Table 4.9. Many attempts have been made to elucidate the structure of the micelle (see Fox and Brodkorb 2008; McMahon and Oommen 2013). It was widely accepted for many years that the micelles are composed of sub-micelles of mass $\sim 5 \times 10^6$ kDa. The core of the sub-micelles was considered to consist of the Ca-sensitive α_{s1}-, α_{s2}- and β-caseins with variable amounts of κ-casein located principally on the surface of the sub-micelles. It was proposed that the κ-CN-deficient sub-micelles are located in the centre of the micelles, with the κ-CN-rich sub-micelles concentrated at the surface. The hydrophobic N-terminal segment of κ-CN is considered to interact

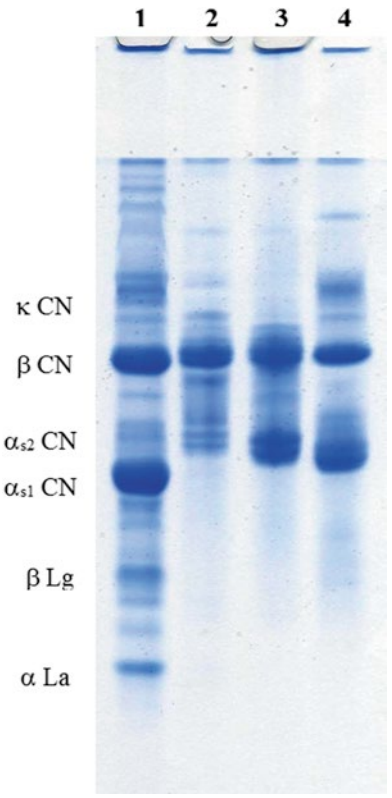


Fig. 4.15 Urea-PAGE of the proteins in bovine milk (1), caprine (2), ovine (3) and buffalo (4) casein (supplied by Dr T. Uniacke-Lowe, unpublished)

Table 4.9 Average characteristics of casein micelles (modified from McMahon and Brown, 1984)

Characteristic	Value
Diameter	120 nm (range: 50–500 nm)
Surface area	8×10^{-10} cm^2
Volume	2.1×10^{-15} cm^3
Density (hydrated)	1.0632 g cm^{-3}
Mass	2.2×10^{-15} g
Water content	63 %
Hydration	3.7 g H_2O g^{-1} protein
Voluminosity	4.4 cm^3 g^{-1}
Molecular weight (hydrated)	1.3×10^9 Da
Molecular weight (dehydrated)	5×10^8 Da
Number of peptide chains	10^4
Number of particles per ml milk	10^{14}–10^{16}
Surface area of micelles per ml milk	5×10^4 cm^2
Mean free distance	240 nm

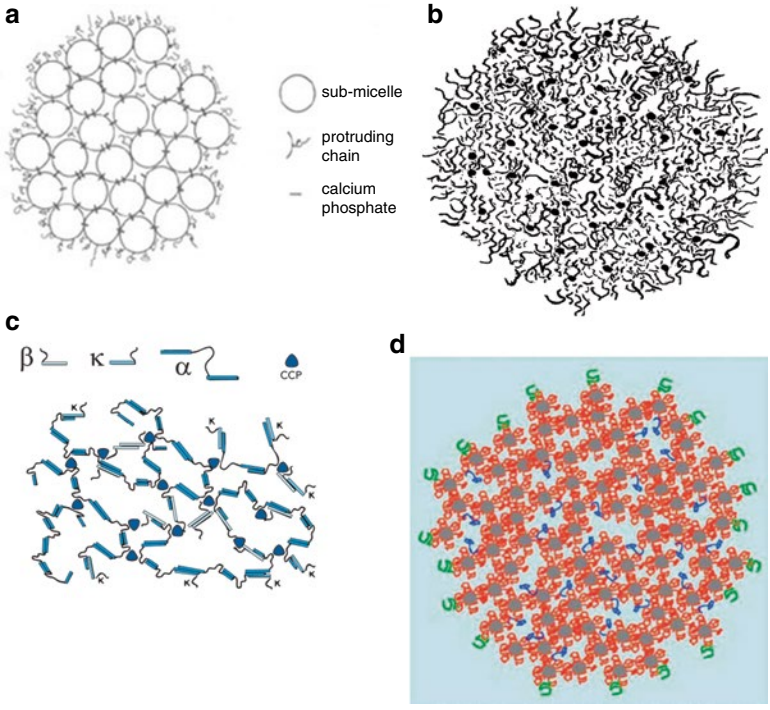


Fig. 4.16 A selection of sub-micelle models of the casein micelle: (**a**) Walstra and Jenness (1984), (**b**) Fox and McSweeney (1998), (**c**) Horne (1998), (**d**) Dalgleish (2011)

hydrophobically with the Ca-sensitive caseins, with the hydrophilic C-terminal segment protruding from the surface, giving the whole micelle a hairy appearance (Fig. 4.16). The colloidal stability of the micelles is attributed to a zeta potential of ~−20 mV at 20 °C and the steric stabilization provided by the protruding hairs. The sub-micelles are considered to be held together by microcrystals of calcium phosphate and perhaps hydrophobic and hydrogen bonds.

The sub-micellar hypothesis depended strongly on the results of electron microscopy but recent EM studies using improved techniques (e.g. McMahon and Oommen 2008 and Trejo et al. 2011) have failed to show discrete sub-micelles and alternative structures have been proposed (e.g., Horne 2002; McMahon and Oommen 2008, 2013). The newer models retain two important features of the older models: the integrating and stabilizing role of the colloidal calcium phosphate within the micelle and the predominant surface location of κ-casein. Rennet-induced coagulation follows the specific hydrolysis of the micelle-stabilizing κ-casein, as a result of which the stabilizing surface layer is lost.

As far as is known, the structure of the casein micelles in bovine, ovine, caprine and buffalo milks are essentially similar.

4.4.3 Whey Proteins

The whey protein fraction of bovine, ovine, caprine and buffalo milk contain four main proteins: β-lactoglobulin (β-lg, 50 %), α-lactalbumin (α-la, 20 %), blood serum albumin (BSA, 10 %) and immunoglobulins (Ig, 10 %; mainly IgG_1 with lesser amounts of IgG_2, IgA and IgM). Human milk contains no β-lg and the principal Ig is IgA. β-Lg and α-la are synthesised in the mammary gland and are unique to milk. BSA and IgG are imported from blood but IgA and IgM are milk-specific.

The principal properties of the whey proteins are summarized in Table 4.8. In contrast to the caseins, the whey proteins possess high levels of secondary, tertiary and quaternary structures. They are typical globular proteins and are denatured on heating, e.g., completely at 90 °C × 10 min. They are not phosphorylated and are insensitive to Ca^{2+}. All whey proteins contain intramolecular disulphide bonds which stabilize the structure of the proteins. β-Lg contains one sulphydryl group which under certain conditions can undergo sulphydryl-disulphide interaction with other proteins; the most important of these interactions is with κ-casein which occurs on heating to > ~75 °C × 15 s. This interaction markedly impairs the rennet coagulation properties of milk and alters the gel structure and rheological and syneretic properties of acid gel-based products such as yoghurt and fresh cheeses.

The whey proteins are not directly involved in cheese manufacture. However, they are indirectly involved in a number of cases, e.g.,

- Heat-induced interaction with κ-casein, with undesirable effects on rennet coagulation.
- They are incorporated into cheese made from milk concentrated by ultrafiltration.
- They are heat-denatured in the manufacture of some Quarg products.
- Valuable functional proteins are recovered from whey (see Chap. 22).

4.4.4 Minor Proteins

Milk contains numerous minor proteins which are found mainly in the whey but some also are found in the fat globule membrane, which contains ~1 % of the total protein in milk. These minor proteins include enzymes (about 60), enzyme inhibitors, metal-binding proteins (especially lactoferrin and osteopontin), vitamin-binding proteins and several growth factors. As far as is known, most of these are of no consequence in cheese. Some of the indigenous enzymes are active in cheese during ripening, especially plasmin and xanthine oxidoreductase and possibly acid phosphatase. Lipoprotein lipase is quite important in raw milk cheese and perhaps even in pasteurized milk cheese, since some probably partially survives HTST pasteurization. The significance of other indigenous enzymes in cheese has not been investigated and perhaps warrant study.

4.5 Milk Salts

After milk has been heated in a muffle furnace at ~600 °C for 5 h, a residue (ash), representing ~0.7 g/100 ml of the mass of the milk sample, remains. The ash contains the inorganic salts present in the original milk plus some elements, especially phosphorus, present originally in proteins and phospholipids and in lesser amounts in sugar and high-energy phosphates. The elements in the ash are changed from their original form—they are present, not as their original salts, but as oxides and sulphates. Organic salts, the most important of which is citrate, are lost on ashing. Fresh milk does not contain lactic acid but it may be present in stored milk, as a result of microbial growth. Although the salts of milk are quantitatively minor constituents, they are of major significance to its technological properties.

The typical concentration of the principal elements or compounds that constitute the salts of milk are summarized in Table 4.10. Some of the salts are present in milk at concentrations below their solubility limit and are therefore fully soluble. However, others, especially calcium phosphate, exceed their solubility and occur partly in solution and partly in the colloidal phase, associated mainly with the casein micelles. These salts are collectively referred to as micellar or colloidal calcium phosphate (CCP), although several other elements/ions are present also. Several elements are also present in the MFGM, mainly as constituents of enzymes. There are several techniques for partitioning the colloidal and soluble salts (see Fox and McSweeney 1998); typical distributions are indicated in Table 4.10.

It is possible to determine either experimentally or to calculate (after making certain assumptions), the concentration of the principal ions in milk; these are also indicated in Table 4.10.

From a cheesemaking viewpoint, the most important salts/ions are calcium, phosphate and, to a lesser extent, citrate. As shown in Table 4.10, bovine milk contains ~1200 mg Ca/L, i.e., 30 mM. About 30 % is soluble, most of which occurs as

Table 4.10 Concentration and partition of milk salts (from Fox and McSweeney 1998)

Species (%)	Concentration (mg L^{-1})	Soluble		Colloidal (%)
		%	Form	
Sodium	500	92	Completely ionized	8
Potassium	1450	92	Completely ionized	8
Chloride	1200	100	Completely ionized	–
Sulphate	100	100	Completely ionized	–
Phosphate	750	43	10 % bound to Ca and Mg 51 % $H_2PO_4^-$ 39 % HPO_4^{2-}	57
Citrate	1750	94	85 % bound to Ca and Mg 14 % $Citrate^{3-}$ 1 % $H.citrate^{2-}$	
Calcium	1200	34	35 % Ca^{2+} 55 % bound to citrate 10 % bound to phosphate	66
Magnesium	130	67	Probably similar to calcium	33

unionized salts of citrate but about 30 % exists as Ca^{2+}, i.e., 10 % of the total calcium exists as Ca^{2+} (2–3 mM). Although present at a low concentration, Ca^{2+} are of major significance in various aspects of the rennet coagulation of milk (see Chap. 7). The [Ca^{2+}] is inversely related to the citrate concentration.

The insoluble calcium occurs mainly associated with the casein micelles, either as colloidal calcium phosphate (CCP) or casein (micellar) Ca. CCP plays a major role in micellar integrity and has a very significant role in rennet coagulation. The precise composition and structure of CCP are not known. The simplest possible structure is tertiary phosphate, $Ca_3(PO_4)_2$ but the form for which the best experimental evidence exists is brushite, $CaHPO_4.2H_2O$, which forms microcrystals with organic casein phosphate (see Holt 1997).

Milk contains ~13 inorganic elements at trace levels [microminerals] (Hunt and Nielsen 2009). These are important nutritionally, some are components of enzymes, eg, Zn in alkaline phosphatase and superoxidase dismutase, Fe in lactoperoxidase, catalase, xanthine oxidoreductase and sulphydryl oxidase, Mo in xanthine oxidoreductase, Se in glutathione peroxidase, Mn in superoxidase dismutase.

4.6 pH of Milk

As will become apparent in subsequent chapters, pH is a critical factor in several aspects of the manufacture and ripening of cheese curd.

The pH of milk at 25 °C is usually in the range 6.5–7.0, with a mean value of 6.6. pH increases with advancing lactation and may exceed 7.0 in very late lactation; colostrum can have a pH as low as 6.0. The pH increases during mastitic infection due to increased permeability of the mammary gland membranes which permits greater influx of blood constituents into the milk; the pH of cow's blood is ~7.4. The difference in pH between blood and milk results from the active transport of various ions into the milk, precipitation of CCP, which results in the release of H^+ during the synthesis of casein micelles, higher concentrations of acidic groups in milk and the relatively low buffering capacity of milk between pH 6.0 and 8.0.

One of the key events during the manufacture of cheese is the production of lactic acid from lactose by lactic acid bacteria (see Chap. 6). Consequently, the pH decreases to about 5.0. While lactic acid is primarily responsible for the decrease in pH, the actual pH attained is strongly affected by the buffering capacity of the milk and curd.

Milk contains a range of groups which are effective buffers over a wide pH range. The principal buffering compounds in milk are its salts (particularly soluble phosphate, citrate and bicarbonate) and acidic and basic amino acid side chains of proteins (particularly the caseins). A typical buffering curve for milk is shown in Fig. 4.17. The contribution of these components to the buffering of milk was discussed in detail by Singh et al. (1997).

The buffering capacity of milk and curd is of significance during cheesemaking since it is the factor which determines the rate of decrease in pH caused by the production of lactic acid by the starter. The buffering capacity of milk is low near its

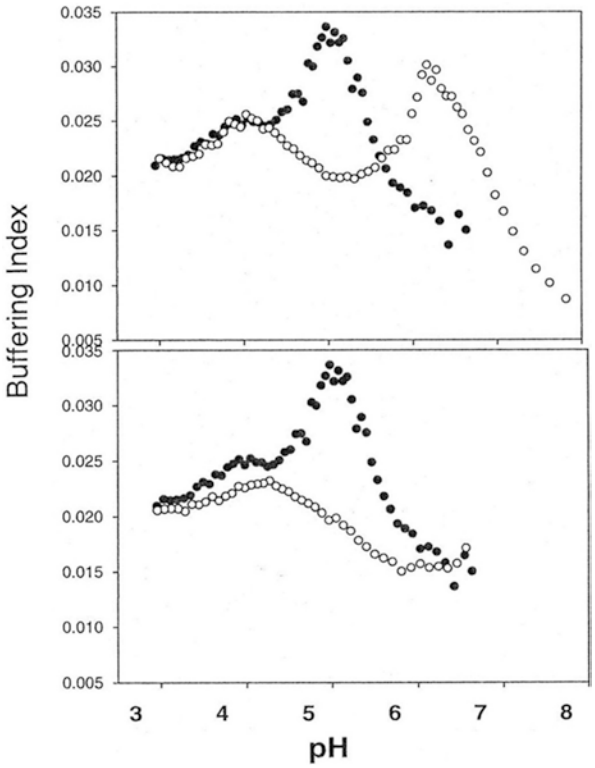


Fig. 4.17 Acid–base buffering curves of (**a**) milk titrated from its initial pH to pH 3.0 with 0.5 N HCl (*filled circle*) and back-titrated to pH 8.0 with 0.5 N NaOH (*open circle*); (**b**) milk (*filled circle*) and colloidal calcium phosphate-free milk (*open circle*) titrated from the initial pH to pH 3.0 with 0.5 N HCl (modified from Lucey and Horne 2009)

natural pH but increases rapidly to a maximum at ~pH 5.1. This means that given a steady rate of acid production by the starter, the pH of milk decreases rapidly initially and later slows down. One of the problems encountered in the production of cheese from UF retentate is its very high buffering capacity. Since all of the soluble and some of the colloidal calcium phosphate are lost in the whey, it is not surprising that the buffering properties of cheese differ from those of milk. Cheddar and Emmental cheeses have maximum buffering capacities at ~pH 4.8.

4.7 Physico-Chemical Properties of Milk

Information on the physico-chemical properties of milk is important when developing and processing dairy products, designing processing equipment and when using dairy products in food products. Some of the principal physico-chemical properties are summarized in Table 4.11.

Table 4.11 Some physical properties of milk (Fox and McSweeney 1998)

Osmotic pressure	~700 kPa
Water activity, a_w	~0.993
Boiling point	~100.15 °C
Freezing point	–0.522 °C (approximately)
Redox potential, E_h (in equilibrium with air at 25 °C and pH 6.6)	+0.25 to +0.35 V
Refractive index, n_D^{20}	1.3440–1.3485
Specific refractive index	~0.2075
Density (20 °C)	~1030 kg m^{-3}
Specific gravity (20 °C)	~1.0321
Specific conductance	~0.0050 ohm^{-1} cm^{-1}
Ionic strength	~0.07 M
Surface tension (20 °C)	~52 N m^{-1}
Coefficient of viscosity	2.127 mPa s
Thermal conductivity (2.9 % fat)	~0.559 W m^{-1} K^{-1}
Thermal diffusivity (15–20 °C)	~1.25×10^{-7} m^2 s^{-1}
Specific heat	~3.931 kJ kg^{-1} K^{-1}
pH (at 25 °C)	~6.6
Titratable acidity	1.3–2.0 meq OH^- per 100 ml (0.14–0.16 % as lactic acid)
Coefficient of cubic expansion (273–333 K)	0.0008 m^3 m^{-3} K^{-1}

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Chapter 5
Bacteriology of Cheese Milk

Summary Milk is a highly nutritious medium which can be easily contaminated by microorganisms. In this chapter, the sources of microorganisms, particularly bedding, faeces, the milking machine and milk storage tank are considered as is the role of disease, particularly mastitis. The most important source of contamination is the milking machine and bulk storage tank and the most important bacteria are psychrotrophs, i.e., bacteria that can grow relatively rapidly at <7 °C. *Pseudomonas* spp. are the dominant psychrotrophs. Pathogens in milk, the role of pasteurisation in controlling them and the alternatives to pasteurisation, e.g., bactofugation and microfiltration, are also considered. The production of cheese from raw milk and standards for production of milk hygienically are also examined.

Keywords Contamination of milk • Mastistis • Psychrotrophs • Pathogens in raw milk • Standards for raw milk

5.1 Introduction

Milk is highly nutritious, containing fat, protein and sugar, as well as significant amounts of the minerals and vitamins required for the growth of calves and humans. It is also an excellent medium for the growth of many bacteria. The temperature of milk in the udder is ~38.5 °C, which is ideal for bacterial growth. However, growth does not usually occur because milk in the udder is sterile, unless it becomes infected. During milking, the milk becomes contaminated with microorganisms and will, if maintained at a temperature >15 °C for several hours, coagulate due to growth and acid production by adventitious bacteria like lactic acid bacteria (LAB) and coliforms. Therefore, great care must be taken to ensure that milk is produced hygienically.

5.2 Sources of Microorganisms

The sources of microorganisms in milk are summarised in Fig. 5.1. Materials such as air, bedding, faeces, feed residues, soil, water used to wash the cows' teats and water residues left on the milking equipment, mastitis, the teat surfaces and the

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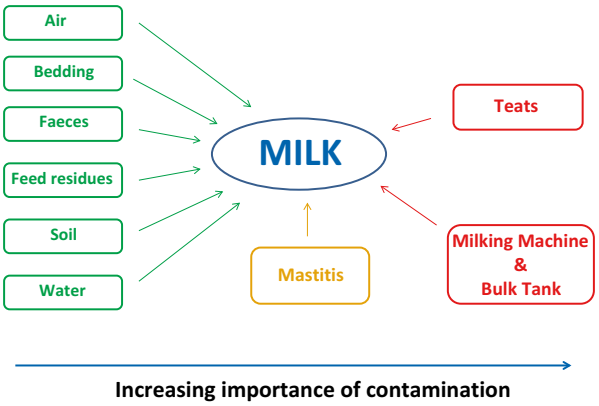


Fig. 5.1 Sources of contamination of raw milk. The most important sources are milking machines and bulk tanks

milking and bulk milk storage equipment are the main sources of contamination. The first six of these are probably the least important on well-run farms; the most important are the milking machine and bulk milk storage tank. Extremely dirty udders may contaminate milk with up to 10^5 cfu/mL. Dirty udders are more likely to occur in the winter, when cows are housed, than in summer, when cows are out on pasture. Where cows are milked by hand, the milker sometimes wets his hands with a few squirts of milk before starting to milk. Drops of contaminated milk dripping from the hands of the milker into the milk bucket can be a potent source of contamination in hand-milking operations.

Cows' faeces contain high numbers of bacteria and, in the past, were considered to be a major source of bacteria in milk. The cow lay on her own or another cow's faeces and so contaminated the udder and the teats. One of the reasons why teats are washed before milking is to reduce contamination from any faeces that may be present on the teats; another reason is to stimulate oxytocin production and milk letdown. It is also becoming more common for farmers to dip teats in disinfectants before putting on the teat cups of the milking machine as a mastitis control measure. A recent detailed study by Kagli et al. (2007) showed that contamination of milk by coliforms and enterococci present in faeces did not occur, at least when milking machines were used for milking. Numbers of coliforms in the faeces ranged from 20 to 4×10^6 cfu/g and were two orders of magnitude greater than those of enterococci which ranged from 10 to 32,000 cfu/g. The medium used to isolate the enterococci was Kanamycin Aesculin Agar and the enterococci in the faeces were identified mainly as *Aerococcus viridans* and small numbers of *Ec. faecium,* while those in the milk were identified as a single clone of *Ec. faecalis*. All the coliforms in the faeces were identified as *Escherichia coli*, while those in the milk were identified as *Enterobacter amnigenus, Hafnia alvei, Serratia liquefaciens* and *Yersinia enterocolitic*a. Thus, no enterococcus or coliform in the faeces was subsequently recovered from the milk. In a similar study on another farm, all the enterocooci in

the milk were identified as *Ec. casseliflavus* while those in the faeces were identified mainly as *Aerococcus viridians,* with small numbers of *Ec. hirae*.

Hay rather than silage is fed in the winter-time to cows, the milk of which is used to produce Comté, a French cheese, made from raw milk; silage is not fed because of the danger of contaminating the milk with clostridial spores, which could germinate and cause late gas formation during subsequent ripening of the cheese. Vacheyrou et al. (2011) evaluated the contamination of milk in winter from air, dust from hay and the teat surface as sources of contamination of milk in winter, on 16 farms producing the raw milk cheese, Comté, and found that useful cheesemaking bacteria, e.g., lactobacilli and propionic acid bacteria, were frequently identified on the teats and milk used to make the cheese, and that most of the fungi and other bacteria were also found in the stable and the milking parlour, indicating a large transfer of microorganisms from the stable to the milking parlour and then to milk. Not all the microorganisms were of environmental origin as 19 species present in the milk were not found in any environmental sample. They concluded that contamination of the milk from the stable environment was considerable even when a milking machine was used.

A recent study (Verdier-Metz et al. 2012) showed that the skin of cows' teats was a potent source of bacteria. Forty six different genera or species were identified among the 309 isolates, including *Leuconostoc mesenteroides*, and several species of staphylococci and actinobacteria found on the surface of smear-ripened cheeses, implying that teat surfaces could be potent sources of these bacteria in raw milk cheeses. In another study (Verdier-Metz et al. 2009), a link was found between milking practices and bacterial diversity in raw milk. Group A milks were characterised by a majority of corynebacteria and micrococci and a high level of milking hygiene. Groups B and C milks had less intensive hygienic practices with Group B milks being dominated by Gram-negative bacteria and the starter organism, *Lactococcus lactis* and Group C milks by *Leuc. mesenteroides,* which can be present in some mixed-starter cultures and *Brevibacterium linens,* which is a cheese surface ripening organism (Chap. 11).

Monthly removal of hair surrounding the teats of dairy cows did not result in an improvement in milk quality in a dairy herd over a 10 month period (Silk et al. 2003).

5.3 Mastitis and Other Diseases

Milk within the udder of healthy cows is sterile but small numbers of microorganisms can enter the teat canal of healthy animals from the outside of the teat through the teat orifice and are generally washed out in the first squirts of milk. These include streptococci, staphylococci, micrococci, corynebacteria and coliform bacteria. Less than 200 cfu/mL are probably added to the milk from the teat canal during milking.

Cows suffering from diseases like salmonellosis, tuberculosis (TB) or brucellosis may shed the causative bacteria, *Salmonella* enterica*, Mycobacterium bovis and Brucella abortus*, in their milk, potentially causing disease, when the milk is

consumed subsequently. Shedding into milk normally occurs only in extreme cases of the diseases or when the udder is directly infected, which generally occurs late in the course of the disease. Therefore, milk from cows suffering from these diseases is not a major source of these bacteria in raw milk. Inhalation is the normal route for transmission of TB among cows but infection by ingestion of contaminated drinking water also occurs in countries where TB in cows is endemic; however, a large infective dose is required. TB is endemic in badgers and this is a major source of TB in cows in Ireland and the UK. *M. bovis* also causes TB in humans.

Mastitis, an inflammatory infection of the mammary gland, is common in dairy cows; for a review, see Hillerton and Berry (2005). It can occur in sub-clinical (the majority of outbreaks) and clinical forms and is caused mainly by *Staphylococcus aureus* and *Streptococcus agalactiae* although *Streptococcus pyogenes*, *Escherichia coli* and *Corynebacterium bovis* may also be responsible. *S. aureus* is a Gram-positive coccus and many strains produce heat-stable toxins, called enterotoxins, which can cause food poisoning. Generally, growth to ~10^6 cfu/mL is necessary before sufficient toxin is produced to cause food poisoning. In sub-clinical mastitis, no physical change or abnormality is evident in the milk whereas in clinical mastitis, large clots consisting of a mixture of milk constituents, somatic cells and bacteria are produced in the milk. Sub-clinical mastitis is generally manifested by increased numbers of somatic cells in the milk and these are routinely checked for in herd samples at factories. High numbers of somatic cells (~10^6/mL) reduce cheese yield because they reduce the levels of fat and casein in the milk and increase the losses of these components in the whey.

The number of mastitic bacteria shed in the milk depends on the stage of the mastitis development and particularly on whether phagocytosis, i.e., engulfment of the bacteria by polymorphonuclear leucocytes and macrophages, has occurred or not. At the beginning of infection, before phagocytosis has occurred, several million bacteria/mL of milk may be present but as phagocytosis develops the numbers of bacteria added to the milk from the infected quarter will decrease rapidly to perhaps <1000/mL; in other words, the number of bacteria in the milk will be high at the beginning of infection and will decrease to low numbers as the infection progresses. Milk from cows with sub-clinical mastitis may contain 1000–10,000 of the causative bacterium/mL.

The treatment of mastitis in dairy cows generally involves the use of antibiotics, particularly penicillin or its various derivatives. This will result in contamination of the milk with antibiotics and a consequent decrease in the ability of the starter cultures to produce acid (Chap. 6). Therefore, milk from the treated quarter should be withheld from the rest of the milk for the prescribed period of time.

Antibiotic treatment of mastitis is being challenged on the basis that it results in the development of antibiotic-resistant microorganisms in man, which may limit the use of antibiotics in treating human disease. Despite this, antibiotic treatment is still the most effective method for treating mastitis in cows. Two important components of treatment are the use of a long-acting antibiotic on all quarters of cows at the end of their lactation to prevent new infection and the dipping of all teats of cows in an effective disinfectant after every milking (Hillerton and Berry 2005).

5.4 Milking Machines and Bulk Tanks

The major source of contamination of raw milk is improperly cleaned milking equipment. For this reason, considerable emphasis is placed on the satisfactory cleaning of the milking machine, the rubber hoses and associated pipework, and the bulk storage tank. The machine should be cleaned after each milking and the bulk storage tank after it is emptied. Hot and cold detergent washes are used and generally a hot acid rinse is given once a week to prevent the build-up of 'milk scale' which can harbour bacteria and make the equipment difficult to clean. Milk scale is composed mainly of calcium phosphate but sufficient nutrients may be present to allow significant microbial growth between milkings, if the ambient temperature is high (>15 °C). Heavily contaminated milking equipment is needed to cause a marked increase in the bacterial content of the raw milk. For example, to increase the bacterial count in 1000 L of milk by 1 bacterium/mL requires 1 million organisms; so, to increase the count by 10,000/mL would require the addition of 10^{10} bacteria. The milking machine and its associated pipe lines and rubber hoses have a large surface area and may contain such large numbers of bacteria, if they are not adequately cleaned. Immediately after milking, good quality milk produced using a properly cleaned milking machine and bulk storage tank should have a plate count of <2000 cfu/mL.

Gram-positive bacteria (e.g., *Micrococcus, Corynebacterium, Microbacterium, Lactobacillus, Lactococcus, Enterococcus,* etc) and Gram-negative bacteria (*Pseudomonas, Achromobacter, Enterobacter, Escherichia, Flavobacterium,* etc) are found in milk immediately after milking. Many of the Gram-positive genera include bacteria that are used as cheese starters. In the past, milk was either not cooled at all or cooled to ambient temperature with water. Under these conditions, growth of some of the Gram-positive bacteria, particularly LAB, e.g., *Lactobacillus, Lactococcus, Enterococcus* and *Streptococcus* spp. was quite rapid. Nowadays, milk is normally cooled to less <5 °C within 1–2 h of milking and the flora has changed from one dominated by Gram-positive bacteria to one dominated by Gram-negative, psychrotrophic bacteria, particularly *Pseudomonas* and *Achromobacter* species. Psychrotrophic bacteria are those bacteria capable of growth at <7 °C and are normally determined by incubating plates at 7 °C for 10 days. Psychrotrophic bacteria grow faster than Gram-positive bacteria at both 2 and 6 °C (Griffiths et al. 1987).

In modern milk production, the milk is held in the bulk tank at 4 °C for several days. Although, cooling significantly slows down the rate of multiplication of bacteria in raw milk (Fig. 5.2), slow growth, particularly of psychrotrophs, still occurs at 4 °C and significant numbers, e.g., 10^6 or 10^7 cfu/mL can be reached in 3 or 4 days. Raw milk may also be stored in silos for 1 or 2 days at the factory before use, during which further growth of psychrotrophs will occur. It is important to remember that rapid cooling of milk is no substitute for improperly cleaned milking machines and storage tanks, either on the farm or at the factory.

The growth of bacteria in four samples of raw milks during storage at 5 °C is shown in Fig. 5.3. Little or no growth occurred during the first 2 days of storage, after which two milks showed a significant increase in bacterial numbers while the

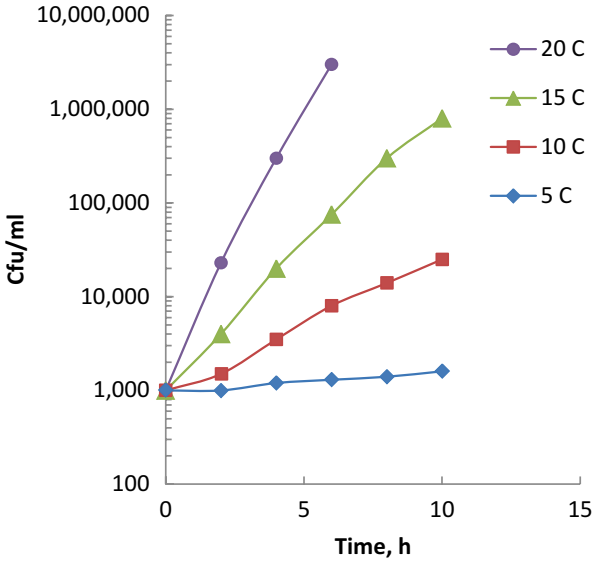


Fig. 5.2 Effect of temperature on the growth of bacteria in a sample of raw milk

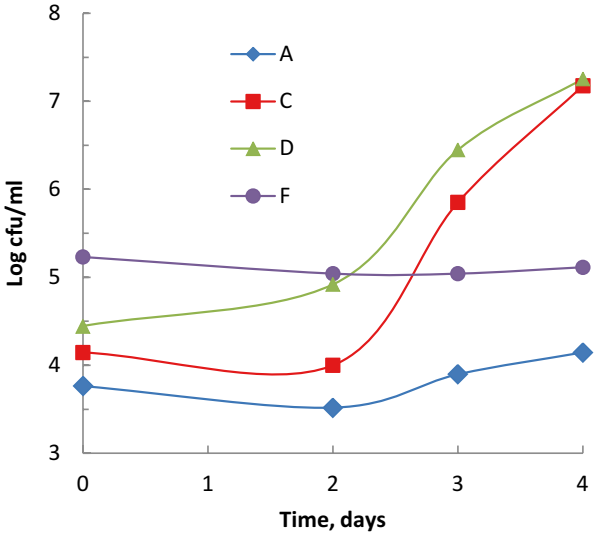


Fig. 5.3 Growth of bacteria in four samples of raw milk, coded A, C, D and F, incubated at 5 °C. (Modified from Bramley & McKinnon, 1993)

other two did not (Bramley and McKinnon 1990). This was probably due to differences in the species of organisms present and their ability to grow at 5 °C. The initial level of contamination had little effect on the subsequent rates of bacterial growth. After 4 days at 5 °C, counts in some cases were $>1 \times 10^7$ cfu/mL. Bacterial

counts of this magnitude are totally unacceptable in milk for cheesemaking or, indeed, for making any dairy product. A linear correlation has been found between the initial psychrotrophic count of raw milk and its shelf-life, defined as the number of days to reach 10^8 cfu/mL (Griffiths et al. 1987).

Raw milk is often stored in large silos for perhaps 24 h or longer and, therefore, further growth and contamination from improperly cleaned silos can occur so that milk before pasteurisation for cheesemaking, may have a count in excess of 10^5 cfu/mL. Such counts, while high, will not have a major effect on cheese quality. However, counts $>10^6$ cfu/mL in the milk before pasteurisation could affect cheese quality because many psychrotrophs, especially *Pseudomonas* spp., produce heat-stable lipases and proteinases, which withstand heating to 100 °C for 30 min, even though the bacteria that produce these enzymes are killed. These enzymes may be retained in the curd during cheesemaking and cause off-flavours to develop during ripening, especially in semi-hard and hard varieties which are ripened for a long time, e.g., Cheddar, Gouda, Comté, etc.

5.5 Natural Inhibitors

Raw milk contains several natural inhibitors of bacterial growth, e.g., lactoperoxidase, immunoglobulins, lysozyme and lactoferrin but they have limited roles in prolonging the shelf-life of raw milk. Lactoperoxidase requires H_2O_2 and SCN^- for maximum inhibitory activity (see below) while the immunoglobulins act by agglutinating bacteria. H_2O_2 can be produced by starter bacteria and SCN^- arises from plants that the cows eat. Lysozyme cleaves bonds in the peptidoglycan of bacterial cell walls and is more active on Gram-positive than on Gram-negative bacteria because of the high levels of peptidoglycans in the cell walls of Gram-positive bacteria. Lactoferrin acts by chelating the Fe necessary for the growth of some bacteria.

5.6 Pathogens in Raw Milk

Cows suffering from severe forms of TB, brucellosis and salmonellosis may shed the causative organisms, *M. bovis, Brucella abortus* and *Salmonella enteric* respectively, in their milk and thus these organisms may get into cheese. It is difficult to get information on the levels of *M. bovis* and *B. abortus* in milk. This may be due to the difficulty in isolating them, e.g., 6 weeks incubation is needed to enumerate *M. bovis* because it grows so slowly. Another possibility is that milk is no longer a source of these organisms due to the almost total eradication of these two diseases in dairy cows in developed countries. However, an outbreak of salmonellosis in cheese in Canada was traced to a single cow which was shedding 200 cfu salmonella/mL in her milk (D'Aoust et al. 1985). A cow has also been shown to shed 280 *L. monocytogenes*/ml of milk from one quarter without any signs of clinical disease in the cow or abnormality in the milk (Hunt et al. 2012). In addition, mastitis caused by

S. aureus can result in the presence of this organism in the raw milk. Cows' faeces can contain large numbers of salmonella and *E. coli* but, as already stated, the likelihood of these organisms gaining entry to milk is very low in modern milking practice.

Analysis of US records by Headrick et al. (1998) for the period 1973–1992 showed that there were 46 outbreaks of foodborne disease associated with the consumption of raw milk, which were caused mainly by *Campylobacter* and *Salmonella* spp. Outbreaks of foodborne disease, due to milk and milk products, over periods of 5–10 years, in the USA, Finland, The Netherlands, England and Wales, Germany, Poland and France were low, and ranged from 2.2 % in the USA to 6.1 % of all foodborne outbreaks in France (De Buyser et al. 2001).

E. coli 0157 and *Listeria monocytogenes* have become important causes of foodborne illness in recent years and both can occur in raw milk. *E. coli* 0157 causes bloody diarrhoea, haemolytic uremic syndrome and kidney failure while *L. monocytogenes* causes listeriosis in pregnant women and immune-compromised people; both organisms can cause death. Listeriosis may lead to meningitis and bacteremia. Oliver et al. (2005) have summarized surveys of the incidence of *Campylobacter jejuni*, enteropathogenic *E. coli, L. monocytogenes* and *Salmonella* spp. in bulk tank milk. The results (Table 5.1) show that significant variation occurs for each of the four pathogens examined. There have been three recent surveys of raw milk in the US, two of which involved milk samples from artisanal, raw milk, cheese producers and the third, a nation-wide survey. Each showed that the raw milk was of high microbiological quality. The first one (D'Amico et al. 2008) comprised milk from 11 artisanal cheese producers (5 from cow, 4 from goat and 2 from sheep milk) which were sampled weekly from June to September, 2006. *S. aureus* was found in 46 of the 133 samples (average level 250 cfu/mL), *L. monocytogenes* in three samples (two of which were from the same farm), *E. coli* 0157:H7 in one sample and salmonella in no sample; 61 % of samples contained <10 coliforms/mL. The second survey (D'Amico and Donnelly 2010) comprised milk samples from 21 artisanal cheese operations (12 from cow, 5 from goat and 4 from sheep milk) which were sampled weekly from July to September, 2008. No *L. monocytogenes*, *E. coli* O157:H7 or salmonella were recovered from any sample. Fourteen of the farm milks contained an average of 20 cfu of *S. aureus*/mL. Eighty-six % of samples had plate counts <10,000/mL and 42 % <1000/mL. The third survey (Jackson et al. 2012) was US wide and comprised 214 silo milk samples. Several different methods were used for each organism. By direct plating, *E. coli* O157:H7 was found in 1.1 % of samples, *Salmonella* in 13.6 % of samples, and *L. monocytogenes* in 12.5 % of

Table 5.1 Isolation rates of various pathogens in bulk tank milks[1]

Pathogen	No. of surveys	Range of isolation rates (%)
C. jejuni	7	0.4–12.3
Verotoxigenic *E. coli*	3	0.8–3.8
L. monocytogenes	13	1.0–12.6
Salmonella	8	0.2–8.9

[1]From Oliver et al. (2005)

samples. In the case of salmonella, there were significant differences between the four media used to detect them. Total counts ranged from 7.3×10^2 to $>4.7 \times 10^3$ cfu/mL, coliform, *E. coli* counts from 10 to 4.9×10^4/mL and *S. aureus* counts from <10 to 1.5×10^4/mL. The average log-transformed counts of total viable bacteria were slightly lower in samples containing no pathogens.

An organism gaining increasing importance in raw milk is *M. avium* subsp. *paratuberculosis* (MAP). It causes Johne's disease in cattle, which is characterised by reduced milk yield and weight loss in cows and has been associated with Crohn's disease, which is a chronic inflammation of the bowel in humans. Levels reported in milk are low and whether it is inactivated by pasteurisation is not clear. Quantifying the numbers of MAP in milk samples is not possible because very few articles provide quantitative information.

5.7 Raw Milk Cheeses

Many cheeses, including such famous varieties as Emmental, Gruyère, Comté, Parmigiano Reggiano, Reblochon and Roquefort are produced from raw milk. One of the important safety factors in these cheeses is that most of them (Reblochon and Roquefort are exceptions) are cooked to a high temperature (>50 °C) for up to 1 h during manufacture which kills many of the bacteria that may be present in the raw milk. Many countries require that cheese be (1) made from pasteurized milk or (2) aged for 60 days, during which food-poisoning or pathogenic bacteria die, or (3) the cheese itself be pasteurized, i.e., converted to processed cheese. The production of cheese from raw milk has been questioned because pathogens which might be present in the milk will also grow during manufacture. This is still a contentious issue but most pathogens will die off during ripening or will be inactivated by high cooking temperatures. Donnelly (2001) critically examined food-poisoning outbreaks due to raw milk cheeses and concluded that confounding parameters other than the use of raw milk contributed to the presence of bacterial pathogens in the majority of the outbreaks. These aspects are discussed more fully in Chap. 19.

5.8 Pasteurisation

Pasteurisation of cheese milk became widespread about 1940, primarily for public health reasons but also to provide a milk supply of more uniform bacteriological quality and improve its keeping quality. Prior to 1950, TB was prevalent in both cows and humans and the organism causing tuberculosis in cows, *M. bovis*, can also cause the disease in man. *M. tuberculosis* is still the major cause of TB in man. The minimum time/temperature combination required to kill *M. tuberculosis*, the most heat-resistant bacterial pathogen likely to be present in milk at that time, was the primary driving force for the development of pasteurisation. Batch pasteurisation (low temperature-long time, LTLT; 63–65 °C × 30 min) was used initially but was

replaced by continuous, high temperature-short time (HTST) pasteurisation (72 °C × 15 s) when plate heat exchangers were introduced in the 1930s. For a review of the history of pasteurisation see Westhoff (1978).

Most (>99.9 %) of the bacteria found in raw milk are heat labile and are killed by pasteurisation at 72 °C for 15 s and most milk for cheesemaking is given this heat treatment. Pasteurisation kills all potential pathogens which are likely to be present in milk but spores of *Clostridium* and *Bacillus* are not killed by this treatment. Whether vegetative cells of sporeformers are inactivated by pasteurisation is not clear. Recently, Pearce et al (2012) undertook a thorough study of the heat resistance of several pathogens. Approximately 30 strains each of *E. coli, L. monocytogenes, S. aureus, Yersinia enterocolitica, Cronobacter sakazakii* (formerly *Enterobacter sakazakii*) and various *Salmonella* species were screened initially and then the most heat-resistant strain of each species was selected for detailed study in a pilot-scale pasteuriser. The mean $\log_{10}$ reductions and temperatures of inactivation of the 6 pathogens during a 15 sec treatment in UHT milk were: >6.7 at 66.5 °C for *S. aureus*, >6.8 at 62.5 °C for *Y. enterocolitica*, >6.8 at 65 °C for *E. coli*, >6.7 at 67.5 °C for *C. sakazakii*, >6.9 at 66.5 °C for *L. monocytogenes* and >6.9 at 61.5 °C for *Salmonella* serotype Typhimurium. This data implies that pasteurisation of milk for 15 sec at 72 °C has a significant safety margin in inactivating pathogens in raw milk.

Micrococcus, Microbacterium, *Enterococcus*, *Arthrobacter* and *Lactobacillus* spp., as well as *Bacillus* and *Clostridium* spores, which withstand HTST pasteurisation, are found in raw milk. These are called thermoduric bacteria and invariably come from improperly cleaned equipment (Hull et al. 1992). Generally, thermoduric bacteria grow only slowly, if at all, in raw milk so that counts of thermoduric bacteria, even within 24 h of milking are a useful indicator of how well the milking equipment and bulk storage tanks have been cleaned. These bacteria are enumerated by heat-treating the milk at 63 °C for 30 min before plating on Plate Count Agar PCA). Thermophilic starters (see Chap. 6) can also be considered to be thermoduric but their incidence in raw milk is low and they grow poorly on Plate Count Agar, the medium used to estimate the bacteriological quality of milk. In cheese factories, *Streptococcus thermophilus,* a starter bacterium for many cheeses, has been shown to grow as a biofilm in the regeneration section of the pasteuriser during long pasteurization runs.

Pasteurisation also inactivates several enzymes in milk, including lipase and alkaline phosphatase. Lack of alkaline phosphatase activity in pasteurised milk is used as an index that raw milk has been properly pasteurised while milk lipase activity causes rancidity development in raw milk due to the production of free fatty acids, particularly butyric, when the milk is excessively agitated. Whether lipase activity plays a role in rancidity development in raw milk cheeses is not clear.

In some countries, e.g. Canada, significant amounts of cheese are made from milk heat-treated to time/temperatures lower than those of HTST pasteurisation. This heat treatment is called thermisation and generally involves heating the milk to 63 °C for 10–15 s. This treatment results in less inactivation of enzymes and non-starter lactic acid bacteria, which may be important in developing cheese flavour. It also kills only some pathogenic and food-poisoning microorganisms and the milk must be subsequently fully pasteurized to meet public health regulations. The purpose of thermization is to kill psychrotrophs, which dominate the microflora of refrigerated milk, and

which excrete potent proteinases and lipases, which may cause flavour and textural defects in cheese. Such treatments are also used in Europe to partially inactivate the microflora as the raw milk is delivered to the factory, and increase the keeping quality of the raw milk; in this case, the milk is usually pasteurised before cheesemaking.

While pasteurisation reduces the risk of producing poor quality cheese due to the growth of undesirable bacteria and destroys food-poisoning microorganisms, pasteurisation of cheese milk may damage the cheesemaking properties of milk if the heat treatment is too severe (due to heat denaturation of the whey proteins and their interaction with κ-casein; see Chap. 7). The extent of this damage is negligible under HTST pasteurisation conditions; some manufacturers heat-treat the cheese milk at sub-HTST conditions. The flavour of cheese made from pasteurised milk develops more slowly and is less intense than that made from raw milk, apparently because certain components of the microflora of raw milk contribute positively to cheese flavour. To overcome this deficiency, adjunct cultures of selected NSLAB are being recommended for use in the manufacture of long-ripened, low-moisture cheese made from pasteurised milk (see Chap. 11).

5.9 Alternatives to Heat Treatment

There are at least four alternatives to heat treatment which reduce the numbers of bacteria in cheesemilk: (1) treatment with hydrogen peroxide (H_2O_2), (2) activation of the lactoperoxidase-H_2O_2-thiocyanate system, (3) bactofugation and (4) microfiltration.

5.9.1 Treatment with Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is an effective bactericidal agent, the use of which is permitted in cheesemaking in some countries, including the USA. The excess H_2O_2 is usually destroyed by adding catalase. The use of H_2O_2 to treat cheese milk has received little attention recently and its use in developed dairying countries is very limited.

5.9.2 Lactoperoxidase-H_2O_2-Thiocyanate

Lactoperoxidase (LPO), an indigenous enzyme in milk, reduces H_2O_2 in the presence of a suitable reducing agent:

$$H_2O_2 + 2HA \rightarrow 2H_2O + 2A$$

One such reducing agent is the thiocyanate anion, SCN^-, which is oxidized to various short-lived but highly active inhibitory ions, particularly hypothiocyanite, $OSCN^-$, which are strongly bactericidal. The level of indigenous SCN^- in milk is low and varies significantly (0.02–0.25 mM) and 8–25 mM is needed for optimum activity.

SCN^- arises from the catabolism of glucosinolates, which are present in plants of the *Cruciferae* or cabbage family, by bacteria in the rumen. Milk contains no H_2O_2 which must either be deliberately added or produced *in situ* via oxidation of glucose by glucose oxidase, from xanthine by xanthine oxidase or by starter cultures grown in the presence of O_2. For the full inhibitory effect about 0.3 μM H_2O_2 is required.

The LPO system is very effective for the "cold-pasteurization" of milk. The process has been patented but has attracted very limited, if any, interest in developed dairy countries, possibly for economic reasons. It is practiced to a small extent in developing countries.

5.9.3 Bactofugation

A high percentage (98–99 %) of somatic and bacterial cells and bacterial spores in milk can be removed by centrifugation at high gravitational forces in a bactofuge. The cells and spores are heavier than the milk constituents and are concentrated in the sludge during bactofugation. The sludge may be sterilised subsequently to kill the spores and bacteria and added back to the milk to increase cheese yield. Bactofugation is not widely used in general cheesemaking but is commonly used to remove *Clostridium tyrobutyricum* from milk intended for Dutch- and Swiss-type cheeses which undergo a propionic acid fermentation (PAF). Cheeses which undergo a PAF are generally ripened at <13 °C for a few weeks after which the temperature is increased to ~22 °C for 3–4 weeks to promote the PAF. Clostridia, especially *Cl. tyrobutyricum*, can also grow under these circumstances and produce late gas (see Chap. 11) but bactofugation is very effective in eliminating spores of *Cl. tyrobutyricum* from the milk.

Cl. tyrobutyricum is an obligate anaerobe, which can ferment lactate, the major acid in cheese, and the anaerobic cheese environment is ideal for its growth. The major source of the organism in raw milk is improperly fermented silage. For this reason, feeding of silage to cows is prohibited in the areas of Switzerland where Emmental cheese is made. Contamination with spores is much greater in the wintertime, when the cows are fed indoors with silage, than in the summertime when they are out on pasture. The vegetative cells are probably killed by pasteurisation (though scientific proof of this appears to be lacking), but the spores are heat resistant, requiring several minutes at 100 °C to kill them.

When silage contaminated with clostridia is eaten by cows, the spores pass through their gastrointestinal tract. Cows lying in their own or other cows dung, pick up faecal material containing clostridial spores on their teats and udders from which contamination of the milk with clostridia occurs. Proper cleaning of the teats and udders will reduce contamination from this source. Less than 10 spores/100 mL of milk is sufficient to cause late gas in Dutch-type cheeses. A lower number is required in cheeses which undergo a PAF because of the higher temperature used in ripening these cheeses.

The design of the bactofuge is essentially similar to that of separators used to separate the fat from milk but is modified such that only the bacteria and spores, which are more dense than skim milk, are forced outwards and move down along the lower side

of the upper of a pair of discs and eventually through orifices in the bowl of the centrifuge as a bacterial concentrate (bactofugate) representing ~3 % of feed volume. Some large, dense, casein micelles are also removed by this process, perhaps as much as 6 % of the total casein. This loss of casein will cause a decrease in cheese yield which may be avoided by heat-sterilizing the bactofugate and returning it to the milk or by otherwise supplementing the casein content, e.g., by adding UF retentate.

5.9.4 Microfiltration

Microfiltration is a membrane separation process, in principle like reverse osmosis, nanofiltration or ultrafiltration, except that large pore size (0.8–1.4 μm) membranes are used. The semi-permeable membranes used retain bacteria but allow milk constituents, including most of the casein micelles, to pass through into the permeate. The process can be applied only to skim milk as the fat globules in whole milk block the pores of the membrane and reduce its efficiency. Therefore, the cream must be separated from the milk, pasteurised and added back to the microfiltered skim milk before cheesemaking.

Microfiltration is very efficient at removing bacterial cells (>99 %) and is being used increasingly in the dairy industry, e.g., in the production of extra-long life pasteurized milk. It is not yet widely used for cheese milk, except for the removal of spores from milk for Swiss and similar cheeses. The technique has been very useful in studying the effect of the indigenous raw milk microflora and of enzymes inactivated by pasteurisation on cheese flavour. The quality of Cheddar and Comté cheeses made from microfiltered milk is similar to that made from pasteurized milk and different from that made from raw milk which indicates that the differences in flavour between raw and pasteurized milk cheeses are due to the indigenous microorganisms which are efficiently removed by microfiltration or killed by pasteurization rather than to the inactivation of indigenous enzymes or other heat-induced change.

A microfiltration system known as the "Bactocatch System" has been developed by the Alfa Laval Company for the decontamination of milk as an alternative to pasteurization.

5.9.5 Pre-Maturation

In some countries, particularly France, the starter may be added to the raw milk which is then incubated at 8–10 °C for 12–15 h (overnight). This process is called pre-maturation. The rationale is that sufficient growth of the starter (and a decrease in pH of 0.1 units) occurs during the overnight incubation to bring it out of the lag phase of growth so that exponential growth and acid production begin as soon as the temperature is brought up to the renneting temperature (~30 °C). Pre-maturation is believed to suppress the growth of psychrotrophs. Problem-causing bacteria may also grow during pre-maturation so, sometimes, the milk is repasteurised before more starter is added to inactivate them and avoid subsequent potential defects in the cheese.

5.10 Standards for Raw Milk

Microbiological standards for raw milk have evolved over the years as scientific research has shown what can be accomplished by relatively simple procedures. The European Union (EU) operates an integrated approach to food safety through assuring a high level of animal health, animal welfare and food safety through coherent farm-to-table measures and adequate monitoring. This ensures a high level of consumer protection with regard to food safety. Three recent documents (Anon 2004a, b, 2005) have consolidated various EU regulations on the general hygiene of food, specific rules on the hygiene of food and microbiological criteria for food. These lay down the responsibilities of the primary producer and the processor to ensure the safety of the food.

Regarding raw milk, the regulation (Anon 2004b) covers the health requirements for milk production, hygiene in milk production units, and the microbiological standards and temperature to which raw milk should be cooled. According to the regulation, raw milk should have a plate count of <100,000 cfu/mL and a somatic cell count of <400,000/mL when received at the manufacturing plant and should be cooled quickly to not more than 6 °C until it is processed (Anon 2004b). Raw milk can be kept at a higher temperature if processing begins immediately or within 4 h of acceptance at the processing establishment. Testing to ensure compliance is conducted once or twice a month. These criteria are very easy to meet in practice and to-day it is easy to produce milk with <2000 cfu/mL, whereas 40 years ago it was difficult to produce it with <500,000 cfu/mL. These improvements in the microbial quality of raw milk are due to better hygiene during milking, improved design of milking equipment making it easier to clean, and cooling to and storage of milk at 6 °C within a few hours of production in easily cleaned, stainless steel, bulk storage tanks until it is collected and transported to the factory.

Traditionally culture-dependent methods based on plating decimal dilutions of raw milk on suitable non-selective or selective media have been used to determine the levels of microbial contamination of raw milk with microorganisms. More recently, DNA based technologies including culture-dependent methods, e.g., Randomly Amplified Polymorphic DNA (RAPD), and Restriction Fragment Length polymorphism (RFLP), and culture-independent methods, e.g., Real Time PCR, Single Stranded Conformation Polymorphism (SSCP), Denaturing gradient Gel Electrophoresis (DGGE) and Fluorescence *in situ* Hybridisation (FISH) and high-throughput DNA sequencing, have been used (for reviews see Quigley et al. 2011 and 2013). Such methods have confirmed that psychrotrophs are the important contaminants in raw milk but they have also identified bacteria which are usually not associated with milk, e.g., *Bacteroides, Faecalibacterium, Prevotella* and *Catenibacterium* species. Many of these methods do not distinguish between live and dead cells but this disadvantage can be overcome by treating the sample with stains, e.g., ethidium bromide or propidium monoazide, which are able to penetrate dead cells and interact with their DNA so that the DNA is not amenable to amplification by the Polymerase Chain Reaction.

A French survey (Desmasures et al. 1997) of milks in Normandy, around the area where raw milk Camembert cheese is made, showed that the microbiological quality

Table 5.2 Microbiological quality (cfu/mL) of milk produced in Normandy, France[1]

		Winter		Spring/Summer
	n		n	
Total count	39	71,000 ± 27,000	30	86000 ± 21,000
Enterococci	37	74 ± 150	25	79 ± 400
Coliform	29	57 ± 2400	19	77 ± 5000
S. aureus	25	450 ± 1700	18	350 ± 280
L. monocytogenes	39	4 positive samples	30	No positive samples
Salmonella	39	1 positive sample	30	1 positive sample
Y. entercoitica[2]	39	19 positive samples	30	6 positive samples
Campylobacter	39	1 positive sample	30	No positive sample

[1]From Desmasures et al. (1997)
[2]Only 1 of 61 isolates was a potential pathogen

of the milk was generally very good; 69 milks were sampled and 83 % of them had a total bacterial count <20,000 cfu/mL and an average somatic cell count of 176,000/mL (Table 5.2). The average numbers of coliforms, enterococci and *S. aureus* were 77, 79 and 350/mL, respectively, in milk produced in summer and 57, 74 and 450/mL in milk produced in winter, respectively. However, the incidence of *Yersinia entecolitica* was relatively high and 14 of the 43 milks examined did not meet the EU criteria for *S. aureus* in milk (<500/mL) destined for cheesemaking from raw milk. Despite this, these data suggest that hygiene and milk cooling were effective.

Application of Hazard Analysis and Critical Control Points (HAACP) can be applied to cheese production units but is not generally feasible at milk production level (Anon 2004a). Nevertheless, guides to good practice should be developed to encourage the use of appropriate hygiene practices at farm level. Included in these should be details of how udders and cows' teats should be cleaned before milking and how milking machines and bulk storage tanks should be cleaned and disinfected.

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Chapter 6
Starter Cultures

Summary In this chapter, the various types of starters, viz., mesophilic, thermophilic, defined- and mixed-strain, natural cultures, etc. and the analysis of these cultures by molecular approaches are considered. Then, the taxonomy and phylogeny of the important species of *Lactococcus*, *Streptococcus*, *Leuconostoc*, *Lactobacillus* and *Enterococcus* found in different cultures are analysed. Proteolysis and transport of the amino acids and peptides produced from it are important for the growth of starters in milk. The important pathways used by different starters to transport and metabolise arginine, lactose and citrate are treated. Respiration can be undertaken by lactococci but not by streptococci or lactobacilli and could be important in retaining activity when growing cultures in cheese plants. Some details are given on exopolysaccharide production, the importance of plasmids and genome sequences. Major consideration is given to the importance of phage in inhibiting cultures, the sources of phage and how they may be controlled in cheese factories, and phage-resistance mechanisms. The production and role of bacteriocins in controlling spoilage and pathogens, which are common in lactic acid bacteria is considered. Finally, the production of starters in cheese plants is detailed and a summary of the use of frozen cultures which can be added directly to the milk in the cheese vat is given.

Keywords Taxonomy • Metabolic pathways • Phage sources and control • Bacteriocins • Starter production

6.1 Introduction

Lactic acid production from lactose is probably the most important step in cheesemaking and is carried out by carefully selected cultures of different species of lactic acid bacteria (LAB) which are added to the milk shortly before renneting. These cultures are called starters because they initiate (start) the production of acid. They are also called lactic cultures because they produce lactic acid which is their major function; however, their enzyme systems are also important in developing flavour formation during the ripening of cheese (see Chap. 12) and some starter

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LAB also produce other compounds, particularly acetaldehyde, acetic acid, and diacetyl, which are important in flavour perception in fresh fermented products like Cottage cheese, Quarg and yoghurt. Lactic acid production during cheesemaking causes the pH of the curd and whey to decrease and has three functions: it promotes rennet activity, it aids the expulsion of whey from the curd, thus reducing the moisture content of the cheese, and it helps to prevent the growth of undesirable bacteria in the cheese.

Starter LAB are the primary cultures used in cheese manufacture. In some cheeses, other microorganisms, e.g., brevibacteria and corynebacteria in surface-ripened cheeses, propionibacteria in the case of Emmental, and moulds in the case of Brie and Camembert, are used. These secondary cultures function only during ripening and are considered in Chap. 11.

Until the end of the nineteenth century, cheese was made without the deliberate addition of starters. Today some cheeses, especially those made in Spain and Greece, are still made without the deliberate addition of starters to the milk. In these cheeses, the cheesemaker relies on adventitious LAB, present in the raw milk, to grow and produce lactic acid in the cheese during manufacture and the early days of ripening. 'Backslopping' with some of yesterday's whey was probably practiced and variations of this procedure are still used in the production of cheese today, especially in Italy (see Sect. 6.2.4 on natural cultures). Starters were first introduced about 1890 in Denmark for butter production. When they were first used in cheesemaking is not clear.

More detailed information on the general aspects of starter cultures can be found in Parente and Cogan (2004), on starter genetics in Callanan and Ross (2004) and on bacteriophage or phage in McGrath et al. (2004), McGrath and van Sinderen (2007) and Mahony et al. (2012).

6.2 Types of Starters

Starter cultures are commonly divided into mesophilic, with an optimum temperature of ~30 °C and thermophilic, with an optimum temperature of ~42 °C. Mesophilic cultures comprise mainly strains of *Lactococcus lactis* subsp. *cremoris* but sometimes also small numbers of *Lc. lactis* subsp. *lactis* and/or *Leuconostoc* sp. Thermophilic cultures comprise *Streptococcus thermophilus* and either *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lb. delbrueckii* subsp. *lactis* or *Lb. helveticus*. Recently, a new species, *Streptococcus alolyticus* subsp. *macedonicus*, has been isolated from the Greek cheese, Kasseri, which is thermophilic and is more closely related to *Str. equinus* than to *Sc. thermophilus* (de Vuyst and Tsakalidou 2008). It is not clear whether this species should be considered a starter culture or not as the cheese, from which it was isolated, is made without the deliberate addition of the organism. Each type of starter can be further subdivided into mixed- and defined-strain cultures.

6.2.1 Mesophilic Mixed Cultures

If raw milk is incubated at a temperature in the range 20–40 °C, it will coagulate within 10–24 h. This physical transformation is due to growth and consequent acid production by adventitious LAB present in the raw milk. The source of these bacteria is plant material fed to cows and the milking environment, particularly the milking equipment. Many mixed cultures in use today are probably sub-cultures of such coagulated milks which produced good quality cheese at the end of the nineteenth Century when cheese was beginning to be produced on a large scale. The main species of *Lactococcus* in mesophilic mixed cultures is *Lc. lactis* subsp. *cremoris*. In addition, many of them also often contain small numbers of citrate-utilising strains (Cit^+) of *Lactococcus lactis* subsp. *lactis* and Cit^+ *Leuconostoc* sp.

Lc. lactis subsp. *lactis* can be differentiated from *Lc. lactis* subsp. *cremoris* by its ability to grow at 40 °C and produce NH_3, ornithine and citrulline from arginine (Table 6.1). In addition, *Lc. lactis* subsp. *lactis*, contains glutamate decarboxylase, which produces γ-aminobutyric acid from glutamate, while *Lc. lactis* subsp. *cremoris* does not (Nomura et al. 1999). The exact species of *Leuconostoc* found in starter cultures is not clear but in French cheeses *Ln. mesenteroides* and *Ln. citreum* have been found (Cibik et al. 2000). The function of the *Leuconostoc* spp. and the Cit^+ strains of *Lc. lactis* is to metabolise citrate to CO_2, diacetyl and acetate. CO_2 is responsible for eye formation in Edam and Gouda cheeses, while diacetyl and acetate are important flavour components of Quarg, fromage frais and Cottage cheese. For this reason, the citrate utilisers in mesophilic mixed cultures are often called aroma producers.

Cit^- *Lc. lactis* dominates these cultures and generally comprise ~90 % of the organisms present while the Cit^+ lactococci and leuconostocs comprise the remaining ~10 %. Depending on the aroma producers present in the culture, mesophilic, mixed-strain starters containing only Cit^+ *Leuconostoc* are called L cultures (L, the first letter of *Leuconostoc*), those containing only Cit^+ *Lactococcus* are called D cultures (D, from *Streptococcus diacetilactis*, an old name for Cit^+ *Lactococcus*). Cultures containing both Cit^+ *Lactococcus* and *Leuconostoc* are called DL cultures, and those containing no aroma producer (i.e., only Cit^- *Lactococcus* are present) are called O cultures. DL cultures were the common cultures used in the past for production of cheese and, even though they contain phage (see Sect. 6.13.1), are still used in the manufacture of Gouda and Edam cheeses.

6.2.2 Thermophilic Mixed Cultures

Thermophilic mixed cultures almost always consist of two organisms, *Streptococcus thermophilus* and either *Lactobacillus helveticus*, *Lb. delbrueckii* subsp. *lactis* or *Lb. delbrueckii* subsp. *bulgaricus*. These are often referred to as the coccus and rod, respectively. *Sc. thermophilus*, *Lb. helveticus* and *Lb. delbrueckii* subsp. *lactis* are

Table 6.1 Some distinguishing characteristics of the lactic acid bacteria found in commercial and natural starter cultures

Name	Type[a]	Shape	% Lactic acid produced in milk[b]	Metabolism of citrate	NH_3 from arginine	Growth at:				Growth in presence of 4 % NaCl[d]	Fermentation of sugar	Isomer of lactate	Allosteric lactate dehydrogenase[d]
						10 °C	15 °C	40 °C	45 °C				
Commercial cultures													
Streptococcus thermophilus	T	Coccus	0.6	–	–	–		+	+	–	Homo	L	
Lactobacillus helveticus	T	Rod	2.5	–	–	–	–	+	+	–	Homo	DL	–
Lactobacillus delbrueckii subsp. *bulgaricus*	T	Rod	1.8	–	±	–	–	+	+	–	Homo	D	–
Lactobacillus delbrueckii subsp. *lactis*	T	Rod	1.8	–	±	–	–	+	+	–	Homo	D	–
Lactococcus lactis subsp. *cremoris*	M	Coccus	0.8	-	–	+	+	–	–	–	Homo	L	+
Lactococcus lactis subsp. *lactis*	M	Coccus	0.8	±	+	+	+	+	–	+	Homo	L	+
Leuconostoc lactis	M	Coccus	<0.5	+	–	+	+	–	–		Hetero	D	–
Leuconostoc mesenteroides subsp. *cremoris*	M	Coccus	0.2	+	–	+	+	–	–		Hetero	D	–
Natural cultures, (above plus:)													
Lactobacillus casei subsp. *casei*		Rod	1.4	±	–		+		–		Homo	L	+

Lactobacillus paracasei subsp. *paracasei*		Rod		±	–	+	+	+	±		Homo	L	+
Lactobacillus paracasei subsp. *tolerans*[e]		Rod	0.3–1.2	±	–		+		–		Homo	L	+
Lactobacillus rhamnosus		Rod			–	d	+	+	+		Homo	L	+
Lactobacillus plantarum		Rod		±	–		+		–		Homo	DL	–
Lactobacillus curvatus		Rod			–		+		–		Homo	DL	+
Lactobacillus fermentum		Rod			+		+	+	+		Hetero	DL	–
Enterococcus faecalis		Coccus		+	+	+	+	+	+	+	Homo	L	
Enterococcus faecium		Coccus			+	+	+	+	+	+	Homo	L	

[a]*T* thermophilic, *M* mesophilic

[b]Approximate values; individual strains vary

[c]Activated by fructose-1,6-phosphate and in lactobacilli also by Mn^{2+}

[d]All grow in media containg 2 % NaCl; *Ec. faecalis* and *Ec. faecium* grow in media containing 6.5 % NaCl

[e]d, 11–89 % of strains positive

used in Swiss cheese production while *Sc. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* are used in yoghurt production. Bulk starters of the rod and coccus are generally grown individually for cheese manufacture and they are usually grown together for yoghurt production. For some products, e.g., Mozzarella cheese, the rod:coccus ratio is important and it is much easier to control this ratio by growing the cultures separately.

The yoghurt starter organisms, viz., *Sc. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* grow faster together than when they are grown individually (Fig. 6.1). However, the growth of the rod and coccus are more difficult to control when they are grown together. In this figure, acid production is used as the indicator of growth. It is a simple and accurate way of measuring growth since, in LAB, acid production is directly proportional to the increase in cell numbers. The improved growth of both organisms when grown together is called symbiosis and, in this case, is due to the production of amino acids, particularly leucine, isoleucine and valine, from the casein in the milk by the proteolytic system of *Lb. delbrueckii*, which stimulate the growth of *Sc. thermophilus*. The latter organism, in turn, produces small amounts of formic acid from lactose, which stimulate the growth of the *Lactobacillus*. A recent transcriptome analysis (Sieuwerts et al. 2010) of a mixed culture of *Sc. thermophilus* and *Lb. delbrueckii* showed that *Sc. thermophilus* also produces CO_2, folic acid and long-chain fatty acids which stimulate the growth of *Lb. delbrueckii* and that proteolysis by *Lb. delbrueckii* was insufficient to meet the biosynthetic demands for the sulfur and branched chain amino acids, because genes associated with these amino acids were upregulated in the mixed culture. Genes coding for exopolysaccharide production and some of the genes involved in iron uptake were also upregulated in both organisms in mixed culture compared to the individual cultures.

Whether symbiosis occurs between the *Leuconostoc* and the *Lactococcus* in mesophilic cultures is not clear; there is some evidence in the old literature that a

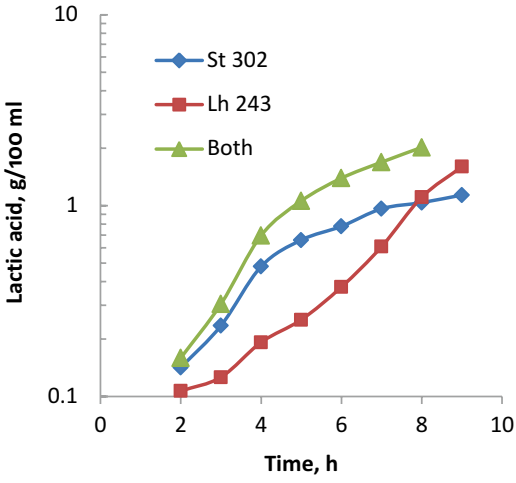


Fig. 6.1 Acid production by *Lactobacillus helveticus* 243, *Streptococcus thermophilus* 302 and their combination in milk at 42 °C. (Redrawn from Accolas et al. 1971)

certain amount of symbiosis occurs but the exact nature of the interaction has not been determined. A certain amount of symbiosis must occur since many strains of lactococci in mixed cultures do not have a proteinase system (Prt^-) and depend on the Prt^+ strains to produce the amino acids they require for growth.

6.2.3 Defined-Strain Cultures

Defined-strain cultures are pure cultures of known and stable physiological characteristics. They were developed initially in New Zealand in the 1930s to combat open texture development in Cheddar cheese due to CO_2 production from citrate by the Cit^+ strains present in the mesophilic, mixed-strain cultures then in use. Cit^- strains were isolated from the mixed cultures and used as single cultures; however, they were susceptible to attack by phage, which resulted in poor acid production. This was overcome to some extent by using pairs of phage-unrelated strains in 4 day rotations, i.e. strains A and B on day 1; strains C and D on day 2, etc. The limiting factor was the lack of phage-unrelated strains. As factory size grew, this system was put under severe pressure from inhibition of acid production by phage and at least two pairs had to be used each day for cheese manufacture.

In 1976, Heap and Lawrence developed a very simple test to identify phage-resistant strains for Cheddar cheese manufacture. The strains were grown in milk for 5 h, in the presence of both purified phage and cheese factory wheys, over the cheese temperature profile viz., incubation at 32 °C for the first 70 min, followed by increasing the temperature to 39 °C over the next 30 min; the temperature was then reduced to 38 °C for the next 160 min before cooling to 32 °C over the next 40 min. After incubation, the pH of the culture containing whey and phage and a control (not containing whey or phage) were compared. If the difference was <0.2 pH units, the test was repeated, including whey from the previous cycle in addition to the factory and purified phage. This test was repeated 7 times and a strain that was still resistant to phage at the end of the 7th cycle was considered to be phage resistant. Isolated colonies, were then tested for acid production, salt tolerance, temperature sensitivity and lack of off-flavour development after incubation in pasteurised milk for several hours. A strain that passed all of these tests was deemed to be suitable for cheesemaking. These strains were used in pairs in 3 day rotations. Subsequently, three pairs were combined into a 6-strain culture which was then used daily. These strains were quite phage resistant and when a phage developed for a strain that strain was removed from the mixture and replaced by a different phage-resistant strain or by a phage-resistant mutant (see later). Cultures comprising 2–3 strains are more commonly used today.

Defined cultures are used in most Cheddar cheese-producing countries like, Australia, New Zealand, USA, UK and Ireland, whereas mixed-strain cultures are used for Gouda cheese manufacture in The Netherlands.

6.2.4 Natural Cultures

In some countries, e.g., Italy, France and Switzerland, other types of mixed cultures, called natural whey cultures (NWCs), are used. These are derived mainly from the practice of 'back-slopping', where some of the previous batch of whey is incubated under prescribed conditions for use as the inoculum for the new batch of cheese. No special precautions are taken to prevent contamination from the cheesemaking environment and, as a result, natural cultures are continuously evolving. These cultures are used in Parmigiano Reggiano, Grana Padano, Emmental and Comté cheeses, which are normally made from raw milk. Commonly, whey from today's cheesemaking is incubated at a high temperature (45–52 °C) for an extended period of time (4.5–18 h), depending on the cheese, for use in tomorrow's cheesemaking. Occasionally, milk rather than whey is used. Where milk itself is used it is usually heat-treated at 62–65 °C for 10–15 min before being incubated. Such cultures depend on the presence of LAB in the raw milk and they are called whey starter cultures or NWCs. In Italy, these cultures are called *siero-innesto* or *siero fermento*. In the case of Pecorino cheese, the whey may be deproteinized before incubation. These cultures are called *scotta innesto*. In Switzerland and France, calf vells are sometimes added to the whey before incubation so that rennet is extracted from them during the incubation. Such cultures are called *Présure a la recruite* or *Fettsirtenmagenlab*.

The temperature of incubation and pH exert selective pressure on the types of bacteria which grow under these conditions. The composition of these cultures is extremely complex and very variable. *Lb. helveticus*, is the dominant organism in NWCs but one or more of the following organisms, *Lb. delbrueckii* subsp. *lactis*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. plantarum*, *Lb. casei*, *Lb. paracasei*, *Lc. lactis*, *Sc. thermophilus*, *Ec. faecalis*, *Ec. faecium* and/or *Leuconostoc* sp. can also be found in them (Table 6.1). In some cheeses, a plant-specific microflora may be present. NWCs have many of the attributes of mixtures of both mesophilic and thermophilic cultures.

All LAB found in starters are Gram-positive and catalase-negative. Some important phenotypic properties which can be used to distinguish between the species of bacteria found in starters are shown in Table 6.1. These include shape, growth at different temperatures, the way lactose is fermented (homofermentatively or heterofermentatively), and the isomer of lactate produced. Growth in the presence of 4 % NaCl and the ability to produce NH_3 from arginine are useful to distinguish between *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris*. Examples of the types of cultures used to produce some important cheeses are shown in Table 6.2.

Table 6.2 Starter cultures used in the manufacture of different cheeses and fermented milks

Cheese	Starter cultures	Other cultures	Important products other than Lactic Acid
Emmental cheese	*Sc. thermophilus* and *Lb. helveticus*. Galactose-positive *Lb. delbrueckii* subsp. *lactis* may be used also	*Propionibacterium freudenreichii*	CO_2, propionate and acetate
Mozzarella and other Italian cheeses	*Sc. thermophilus* and *Lb. helveticus* or natural whey cultures		
Cheddar cheese	Defined strains of *Lc. lactis* subsp. *cremoris* and *Lc. lactis* subsp. *lactis* or O, L or DL mesophilic mixed cultures. Sometimes thermophilic cultures are included		
Edam and Gouda cheeses	Mainly DL mesophilic mixed cultures		CO_2 and acetate
Camembert and Brie cheeses	O, L or DL mesophilic mixed cultures	*Penicillium camemberti Geotrichum candidum*	
Tilsit, Limburger and Munster cheeses	O, L or DL mesophilic mixed cultures	*B. linens Geotrichum candidum*	Sulphur compounds, e.g., methional
Yoghurt	Mainly thermophilic mixed cultures or Defined strains of *Sc. thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus* and *Lb. delbrueckii* subsp. *lactis*		Acetaldehyde
Fromage Frais and Quarg	Mainly DL mesophilic mixed cultures		Diacetyl and acetate

6.2.5 Molecular Analysis of Cultures

Until the advent of molecular techniques, the question as to whether mixed cultures contained several strains or not was not easy to answer. Isolates from mesophilic mixed cultures produced acid at widely different rates, e.g., pH values within the range 5–6.5 in milk after 6 h incubation were common, had different phage-host patterns and showed different plasmid profiles. However, these properties provide only circumstantial evidence that strains are different since low-acid production may reflect a lack of the plasmid encoding proteinase activity and different phage sensitivities and plasmid profiles may simply imply acquisition or loss of plasmids.

Several molecular methods have been developed to distinguish genetically different strains of the same species and these allow determination of the number of strains in a mixed culture. The two most common ones are Randomly Amplified Polymorphic DNA (RAPD) and Pulsed Field Gel Electrophoresis (PFGE). In RAPD, arbitrary pieces of DNA are used as templates for amplification in a PCR reaction. The amplified DNA fragments are separated by gel electrophoresis and the bands detected after illumination with UV light. This method, when applied to 283 strains of *Lc. lactis* allowed them to be separated into 12 groups (Taillez et al. 1998); one cluster contained 175 of the 283 strains, indicating how closely related strains actually are. The other clusters contained between 2 and 22 strains. Further analysis of 113 of the 283 strains by RAPD analysis using 3 different primers, differentiated them into 3 major groups, G1, G2 and G3. Based on phenotypic analysis, groups G1 and G3 were strains of *Lc. lactis* subsp. *lactis* and group G2 strains of *Lc. lactis* subsp. *cremoris*. The taxonomic structure within *Lc. lactis* is therefore unusual. Two genetically distinct groups, G1 and G3, showed indistinguishable phenotypes, while, conversely, two phenotypically distinct groups, G2 and G3, were genetically homologous. Plant material is the original source of *Lc. lactis* subsp. *lactis* and Taillez et al. (1998) suggested that *Lc. lactis* subsp. *cremoris* arose as a result of adaptation to the dairy environment, through loss of several genes, the products of which are not required for growth in milk.

PFGE analysis of DNA after cutting it with rare cutting restriction enzymes is the gold standard to determine whether isolates of the same species are similar or different strains. In this technique, isolates which differ by not more than one or two bands are considered to be identical. To our knowledge, this technique has not been used to analyse strains from mixed cultures used for cheesemaking. However, Kahala et al. (2008) studied three mesophilic mixed cultures used in the production of the Finnish fermented milk, Viili. This product is like cultured buttermilk except that it also has a ropy texture, due to exopolysaccharide (EPS) production by some of the lactococci present. *Geotrichum candidum* is also used in its production to consume lactate and result in a product with a less astringent taste. Several different PFGE patterns were found, indicating that different strains were present, which could be resolved into three major clusters. Clusters 1 and 3 contained isolates from mixed cultures A and C, indicating that the same strains were present in cultures A and C, while Cluster 2, which consisted of 5 different PFGE patterns strains, contained isolates from only culture B, indicating that this was a mixed culture and different from the other two cultures.

Recently, the detailed composition of a commercial mixed-strain starter culture, which has a long history of use in Gouda cheese production, was undertaken, using a newly developed, high-resolution AFLP (amplified fragment length polymorphism)–based fingerprinting method, which allows the resolution of isolates below the subspecies level (Erkus et al. 2013; Smid et al. 2014). The 140 isolates were resolved into eight distinct genetic lineages, viz., one *Leuc. mesenteroides* lineage, two citrate-utilising (Cit^+) *Lc. lactis* subsp. *lactis* lineages, two proteinase-negative (Prt^-) *Lc. lactis* subsp. *cremoris* lineages, which were unable to hydrolyse casein,

and three proteinase-positive (Prt$^+$) *Lc. lactis* subsp. *cremoris* lineages, which were able to hydrolyse casein. One of the Prt$^-$ *Lc. lactis* subsp. *cremoris* lineages comprised 90.2 % of the isolates and so dominated the culture, while the other 6 *Lactococcus* lineages ranged from 0.2–2.7 % of the isolates; the *Leuconostoc* lineage comprised 1.8 % of the isolates. This technique was also used to follow the development of the different lineages during cheese ripening (see Chap. 11).

Lc. lactis subsp. *lactis* can be distinguished from *Lc. lactis* subsp. *cremoris* by its ability to grow at 40 °C, in the presence of 4 % NaCl and at pH 9.6, and produce NH_3 from arginine. The PFGE patterns of 289 strains of *Lc. lactis* subsp. *cremoris* from various sources were compared by Kelly et al. (2010). The strains could be divided into 12 groups indicating that many strains were related. This was not surprising since phage-host relationships had previously indicated that only a relatively small number of different strains exist. An example of the PFGE patterns of several strains of *Lc. lactis* related to strain AM1 is shown in Fig. 6.2. In PFGE analysis, strains showing a one or two band differences are considered to be identical. Strain AM1 is considered to produce very good flavoured Cheddar cheese and strain SK11, the genome of which has been completely sequenced, is a phage-resistant mutant of it. Both of these strains as well as strains 134, US3 and R6 have been used in cheesemaking and had almost identical PFGE patterns, indicating that they are, in fact, the same strain. In addition, several strains (MSS 1–4) isolated from various mixed-strain starters had the same PFGE patterns as strain AM1, indicating that this par-

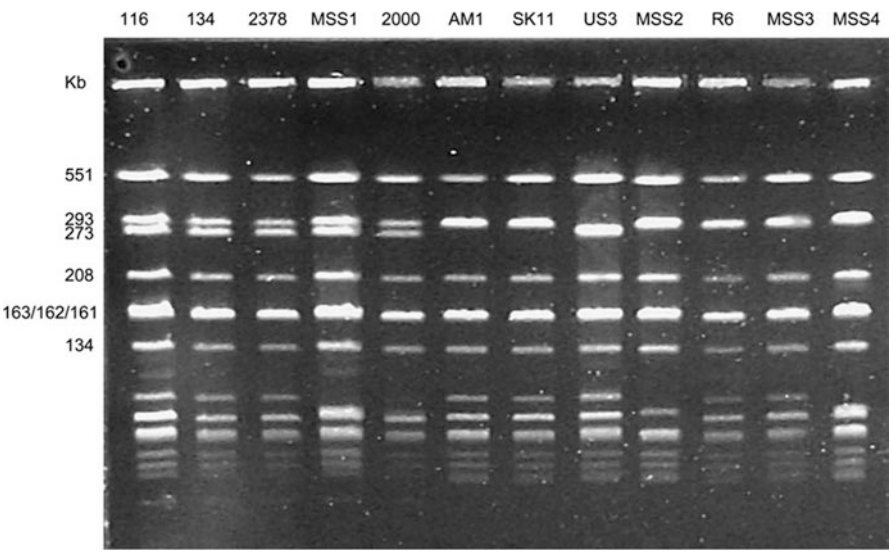


Fig. 6.2 Pulsed Field Gel Eletrophoretograms of *Sma* digested genomic DNA from *Lactcoccus lactis* AM1 and related strains. Strain SK11 is a phage-resistant mutant of strain AM1. The mixed-strain isolates (MSS1, MSS2, MSS3 and MSS4) were from different mixed-strain starters (From Kelly et al. 2010)

ticular strain is common in mixed-strain starter cultures. The PFGE patterns of 72 strains of Cit$^+$ *Lc. lactis* subsp. *lactis* also showed that many strains were related and also contained the same prophage. The chromosomal lengths of 80 strains showed that the smallest chromosomes were associated with strains of dairy origin and larger lengths with strains of plant origin (Kelly et al. (2010). This implies that the origin of all strains of *Lc. lactis* is plant material and that the shorter length of the dairy strain chromosomes is due to the loss of genes, the protein products of which are not necessary for growth in milk. To complicate things further, a few strains of *Lc. lactis* subsp. *cremoris* exist which have the *cremoris* genotype but the *lactis* phenotype (the genetically well-known strain, MG 1363 is one of these strains) and a few strains of *Lc. lactis* subsp. *lactis* exist which have the *lactis* genotype but the *cremoris* phenotype.

Molecular techniques have also been applied to thermophilic cultures and NWCs. PFGE and Multiplex PCR were applied by Jenkins et al. (2002) to 9 commercial cultures of *Sc. thermophilus* and 12 of *Lb. helveticus* from three US suppliers. Significant genetic diversity was found within both species, with eight distinct genotypes of *Sc. thermophilus* and five of *Lb. helveticus*. Based on their PFGE patterns, all the *Sc. thermophilus* and eight of the *Lb. helveticus* cultures were pure cultures (i.e., single, defined strains) with different abilities to produce lactic acid in milk. The other four *Lb. helveticus* cultures were mixed cultures, containing either two or four strains; in addition, two of the *Lb. helveticus* cultures were identified as strains of *Lb. delbrueckii*.

The use of molecular techniques to study NWCs has been reviewed by Neviani et al. (2013). These cultures are dominated by *Lb. helveticus* followed by *Lb. delbrueckii* and *Sc. thermophilus*; in addition, *Lc. lactis*, *Ec. faecalis* and *Ec. faecium* were found in low numbers in some of them. Two new species of enterococci have been isolated from Italian cheeses, *Ec. lactis* (Morandi et al. 2012) and *Ec. italicus* (Fortina et al. 2004), but whether they occur in the starter cultures used in manufacture of these cheeses is unclear. Because many enterococci are of faecal origin their use in starter cultures has been questioned. They can grow during cheese manufacture and many scientists believe that they have a positive role in cheese flavour development (see Franz et al. 2003 for a review).

In addition to the above techniques, other techniques for identifying LAB have been developed, e.g., amino acid composition of the cell wall peptidoglycan, the type of menaquinones present in the cell walls, PAGE of the whole cell proteins and 16S rRNA gene sequencing, etc. during the past 30 years. All of these techniques are very sophisticated and are either too slow or require standardised conditions or elaborate equipment for routine use. Species-specific probes based on short oligonucleotide sequences, called signature sequences, within the 16S rRNA molecule, and which are unique to each bacterial species and RAPD techniques, have been, or are being, developed for all LAB. These methods are relatively simple to use and are becoming routine tools for identifying these commercially important bacteria. These complement the biochemical and physiological tests which are normally used to identify bacteria.

6.2.6 *New Sources of Starters*

There is a continuous need for the isolation of new strains because different starter strains, particularly mesophilic ones, can be attacked by the same phage. Cheeses made without the deliberate addition of a starter culture can be a useful source. Such cheeses are traditionally made in many parts of Southern Europe, particularly Spain and Greece. These strains are natural contaminants of the milk which grow to high numbers during cheesemaking. Other sources of these 'wild' starters include fermented milks, particularly the less common ones like Koumiss and Kefir. The normal habitat of *Lc. lactis* subsp. *lactis* is plant material and strains of this organism, but not *Lc. lactis* subsp. *cremoris*, have been isolated from red nettles, common sow thistle, Himalayan blackberries, potato, maize, cucumber, sweet pea, beans, cantaloupe and broccoli; many of them were good acid producers, coagulating milk in 18 h at 21 °C (Salama et al. 1993).

The most important property of these isolates is the ability to produce acid rapidly Any good acid producers must then be checked for salt tolerance, phage sensitivity, ability to use citrate and produce good flavoured cheese. Some of them can produce unusual flavours. For example, the combination of a 'wild' starter which had low proteolytic activity and high amino acid decarboxylase activity with a commercial strain which had the opposite properties resulted in the production of chocolate flavour in milk, due mainly to production of the branched chain aldehyde, 3-methylbutanal (Wouters et al. 2002).

6.3 Adjunct Cultures

Much commercial hard cheese made today is thought to lack flavour. The probable reasons for this are low bacterial numbers in the raw milk and, more importantly, vastly improved hygiene in cheese factories. Because of this, various methods for improving flavour have been developed. Traditionally, only mesophilic cultures were used in the production of Cheddar and other low-cooked cheeses. However, in recent years, thermophilic starters, particularly *Sc. thermophilus* and *Lb. helveticus*, are also being used with the mesophilic starter because they improve the flavour of the cheese. These organisms are very resistant to the cook temperature ($\leq$38 °C) used for these cheeses but their growth in these cheeses is limited to temperatures >25 °C. At temperatures below this, little growth and, consequently little acid production, occurs. In addition they have a protective effect on acid production as phage outbreaks for the mesophilic part of the culture will not affect acid production by the thermophilic component and *vice versa*.

Carefully selected strains of mesophilic lactobacilli, particularly *Lb. paracasei and Lb. casei*, are also used to improve the flavour of some cheeses, especially Cheddar. The basis for this development is the fact that large numbers (10^8 cfu/g) of

these bacteria are found in ripened cheese and thus they must have some role in flavour development. However, despite extensive research over several decades, the role of these bacteria in cheese flavour development is still unclear (see Chap. 11).

6.4 Measurement of Growth

The extent of bacterial growth is usually determined either by measurement of the colony count or the optical density (OD). The use of OD is of no value for milk because of its opacity. Two useful methods for estimating the extent of starter growth are measurement of lactic acid production by titration, e.g., with 0.1 N NaOH to the phenolphthalein end-point, and determination of the pH, which is both faster and easier. The basis for these methods is as follows. Lactic acid production by LAB is directly proportional to the increase in cell number, so the amount of lactic acid produced is an accurate estimate of the extent of growth. As lactic acid is produced, the pH of a culture decreases. This relationship is shown in Fig. 6.3 for a mesophilic culture grown in milk at 21 °C. However, the relationship between the increase in lactic acid production and pH is not linear but polynomial and depends on the buffering capacity of the milk (the higher the concentration of solids-not-fat in the milk, the greater is the buffering capacity). *Sc. thermophilus* and the starter lactobacilli would give similar growth curves to that of the mesophilic culture but they would be grown at higher temperatures, e.g. 42 °C, and the time axis would be much shorter, of the order of 10 h or so.

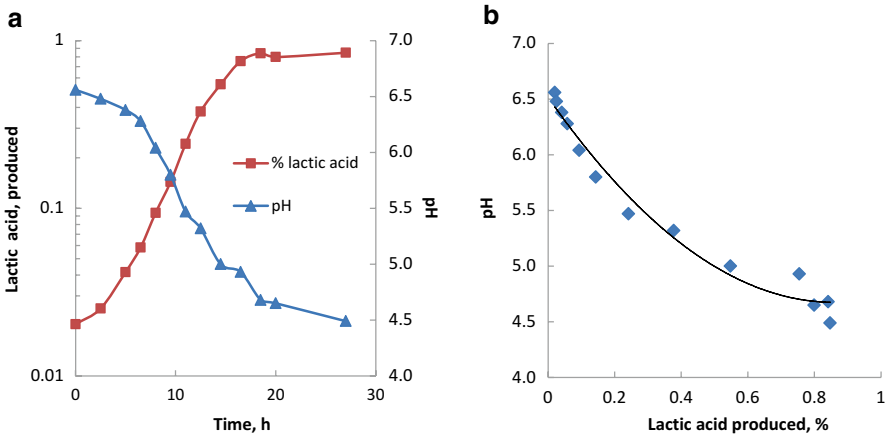


Fig. 6.3 (a) Relationship between lactic acid production and pH in a mesophilic culture grown in 10 % (w/v) non-fat milk solids at 21 °C. (b) The relationship between lactic acid production and the decrease in pH. The trend line shown is a 2nd power polynomial

6.5 Effect of Temperature

Temperature has a major effect on the growth of starter bacteria (Fig. 6.4). In this figure, two different measurements, the generation time and the decrease in pH after 5.5 h, are used as indicators of growth. The former is the time for the number of cells to double. The latter is a quite acceptable estimator of growth where the initial number of cells is the same in the cultures being compared. Under this circumstance the effect on pH is a function of the temperature of incubation. The optimum temperatures for the growth of *Lc. lactis*, *Leuc. mesenteroides*, *Sc. thermophilus* and *Lb. helveticus* were 30, 25, 42 and 42 °C, respectively. The behaviour of these starters at the cooking temperature of cheese is also important. *Lc. lactis* subsp. *lactis* grew slowly at 38 °C, which is the cooking temperature for Cheddar cheese, whereas growth of most strains of *Lc. lactis* subsp. *cremoris* is markedly inhibited at this temperature. A common cooking temperature for Swiss-type cheese is 54 °C and strains of *Sc. thermophilus* and *Lb. helveticus* produce little acid at this temperature (Fig. 6.4). However, they withstand this temperature and begin to grow again once the temperature decreases to ~47 °C.

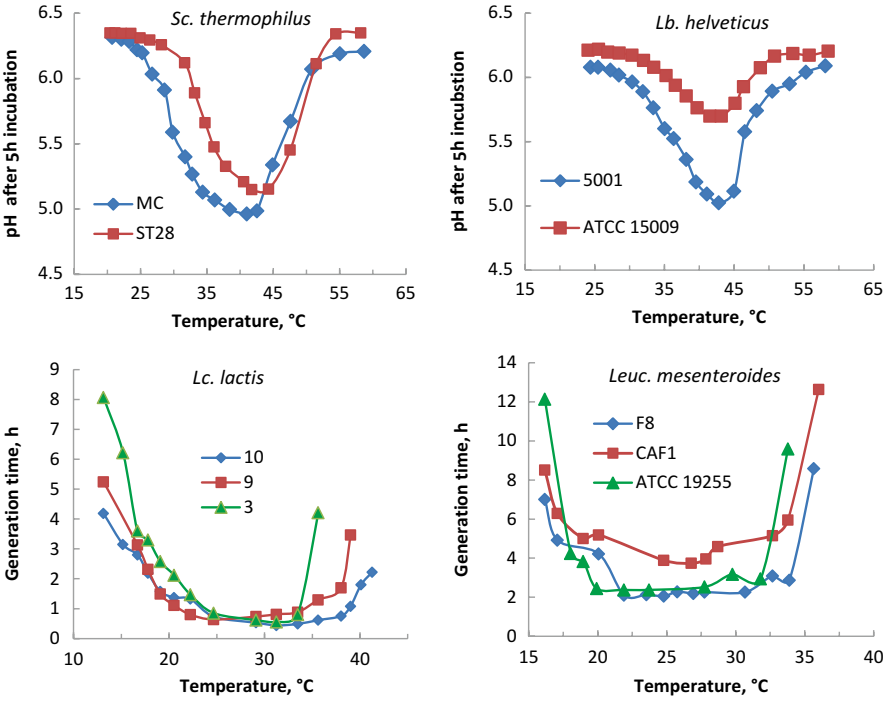


Fig. 6.4 Effect of temperature on the growth of two strains of *St. thermophilus* in milk, two strains of *Lb. helveticus* in milk, three strains of *Lc. lactis* (*Lc. lactis* subsp. *lactis* 10 and *Lc. lactis* subsp. *cremoris* 3 and 9) in a complex medium, and 3 strains of *Lc. mesenteroides* subsp. *cremoris* in a complex medium. Note differences in *y* axes. (Redrawn from Lee and Collins 1976; Cooper and Collins 1978 and Martley 1983)

6.6 Taxonomy

All the LAB used in starter cultures are Gram-positive, catalase-negative, non-motile, non-sporforming bacteria and their taxonomy has gone through several major revisions during the past 40 years as a result of more detailed and sophisticated analyses. The current names are used in Table 6.1. In the past, various phenotypic tests, e.g., their ability to grow at different temperatures, whether they metabolised sugars homo- or heterofermentatively, the isomer of lactic acid they produced, etc., were used but nowadays these have been replaced by molecular analyses, including DNA:DNA and DNA:RNA hybridisations, comparative oligonucleotide cataloguing, serological studies with superoxide dismutase and sequencing of the16S rRNA gene. The latter is now the most common way for identifying bacteria. The lactococci have probably gone through the greatest number of changes and the historic details were summarized by Schleifer and Kilpper-Balz (1987).

6.6.1 Lactococcus

Lc. lactis was isolated from soured milk in 1873 by Lister and named *Bacterium lactis* (Latin for bacterium of milk). It was the first bacterium isolated in pure culture. In 1909, it was renamed *Streptococcus lactis* by Lohnis and placed in the genus *Streptococcus*. In the 1890s, Storch, a Danish microbiologist, isolated a very similar organism from cream, which Orla-Jensen, another Danish microbiologist, named *Sc. cremoris* (of cream). In 1937, Sherman divided the streptococci into four groups, pyogenic, lactic, viridians and faecal, based on growth in media containing 6.5 % NaCl, at 10 and 45 °C, at pH 9.6 and in milk containing 0.1 % methylene blue and placed *Sc. lactis* and *Sc. cremoris* in the lactic group.

DNA homology studies showed that *Sc. lactis* and *Sc. cremoris* were closely related to each other and, in 1982, they were reclassified as subspecies of *Sc. lactis* and named *Sc. lactis* subsp. *lactis* and *Sc. lactis* subsp. *cremoris* respectively (Garvie and Farrow 1982). In 1985, it was realized that these organisms were only distantly related to the genus *Streptococcus* and they were therefore transferred to a new genus, *Lactococcus*, as *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris* respectively (Schleifer et al. 1985). Comparison of the genome sequences shows that *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris* share over 85 % similarity. Since 1985, six new species and one subspecies, *Lc. raffinolactis*, *Lc. garviae*, *Lc. plantarum*, *Lc. piscium*, *Lc. chunganensis Lc. fugiensis* and *Lc. lactis* subsp. *hordniae*, which were isolated from spontaneously-soured raw milk, from a cow suffering from mastitis, frozen peas, fish, activated sludge foam, leaves of Chinese cabbage and the leaf hopper insect, respectively, have been added to the genus. None of these new species grows well in milk and hence they are of little value as starter cultures.

In 1936, an organism similar to *Sc. lactis* was isolated from fermenting potatoes and named *Sc. diacetilactis* and later renamed *Sc. lactis* subsp. *diacetylactis*. It differs from *Sc. lactis* only in its ability to metabolise citrate. The transport of citrate is

plasmid-encoded and, so, it was named *Sc. lactis* subsp. *lactis* biovar. *diacetylactis*. "Biovar" is not an accepted taxonomic epithet, and this organism is now called citrate-utilising (Cit^+) *Lc. lactis* subsp. *lactis* or Cit^+ *Lactococcus* to distinguish it from the vast majority of lactococci which are unable to metabolise citrate (Cit^-).

Lc. lactis subsp. *cremoris* and *Lc. lactis* subsp. *lactis* are the main species isolated from mesophilic starters and from raw milk incubated at 18–30 °C. It is generally believed that *Lc. lactis* subsp. *cremoris* gives a better flavoured cheese than *Lc. lactis* subsp. *lactis. Lc. lactis* subsp. *lactis* is able to grow at 40 °C and in the presence of 4 % NaCl, produce NH_3 from arginine and ferments maltose whereas *Lc. lactis* subsp. *cremoris* cannot (Table 6.1); however, the latter organism can grow in the presence of 2 % NaCl. The concentration of salt in many cheese is greater than 4 % in the moisture of the cheese and is therefore inhibitory to strains of *Lc. lactis* subsp. *cremoris*.

Lactococci are spherical or ovoid cells which occur singly, in pairs or in chains elongated in the direction of the chain. The latter property can sometimes cause them to be misidentified as *Lactobacillus* spp. They grow at 10 °C, but not at 45 °C or in the presence of 6.5 % NaCl and ferment sugars by the glycolytic pathway to L-lactate. They are also non-motile; motile strains of *Lc. lactis* have been transferred to the genus *Vagococcus*.

6.6.2 *Enterococcus*

There is considerable debate as to whether enterococci should be considered as starter cultures since the major sources of some of them are animal and human faecal material. Because of this, they are used in foods as indicators of faecal pollution. Occasionally, they cause endocarditis and urinary tract infections. Some of them, especially *Ec. faecalis*, are promiscuous and easily pick up antibiotic resistance genes, especially for vancomycin, from plasmids or transposons. They are considered here because they are common in cheese made with NCWs (Table 6.1) and in cheese made without the deliberate addition of a starter, where they play a positive role in flavour development. However, their use in these cheeses has been questioned because they can indicate faecal contamination of food (Franz et al. 2003). Unlike lactococci, enterococci are not killed by pasteurisation.

Ec. faecalis was first identified in 1906 by Andrews and *Ec. faecium* in 1919 by Horder and Orla-Jensen, as *Sc. faecalis* and *Sc. faecium*, respectively. In 1984, DNA hybridisation studies showed that these two organisms were only distantly related to the streptococci, and they were transferred to a new genus, *Enterococcus*, as *Ec. faecalis* and *Ec. faecium*, respectively (for a review see Schleifer and Kilpper-Balz 1987).

When the genus *Enterococcus* was established, it contained only these two species but since then, 50 new species have been added, including some from dairy environments, e.g., *Ec. lactis and Ec. italicus*, from Italian cheese and *Ec. malodoratus*, from a Gouda cheese showing off-flavour development. Not all enterococci are of faecal origin, e.g., *Ec. mundtii*, *Ec. sulfureus* and *Ec. casseliflavus* have been

isolated from plants, *Ec. durans* from milk and meat, *Ec. pseudoavium* from the tissue of a cow showing mastitis, *Ec. raffinosus* from a clinical source and *Ec. lactis and Ec. italicus* from cheese. Therefore, the usefulness of enterococci as indicators of faecal pollution is questionable.

Unfortunately, there are no simple, biochemical or physiological tests which will categorically separate *Lactococcus* from *Enterococcus*. This can be done only by sophisticated molecular techniques, e.g., protein profiling of extracts from whole cells by SDS-PAGE or by genus-specific DNA probes. *Ec. faecalis* and *Ec faecium* are easily separated from *Lactococcus* by their ability to grow at pH 9.6, at 10 and at 45 °C and in the presence of 6.5 % NaCl. The more recently recognised *Enterococcus* species do not give positive results with some of these tests, e.g., *Ec. dispar* and *Ec. sulfureus* do not grow at 45 °C; growth of *Ec. italicus* at this temperature is variable while *Ec. cecorum*, *Ec. columbae* and *Ec. italicus* do not grow at 10 °C or in the presence of 6.5 % salt and therefore could be confused with *Lactococcus* spp. Growth of *Ec. italicus* is variable at 45 °C is slow at 10 °C and does not occur in 6.5 % salt while *Ec. lactis* grows at 10 and 45 °C, and in the presence of 6.5 % salt. In addition, a few (mainly human) isolates of *Lactococcus* grow at 45 °C and in the presence of 6.5 % NaCl. However, the likelihood of isolating the latter species from starters and cheese is low.

In the 1930's, Lancefield introduced a method, based on the antigenic structure of the cell wall, to help in identifying what were then *Streptococcus* spp. Prior to the separation of *Streptococcus* into *Lactococcus*, *Enterococcus* and *Streptococcus*, the terms, faecal streptococci, Group D streptococci and enterococci were used interchangeably. Group D species are found in both *Enterococcus* (e.g., *Ec. faecalis*, *Ec. faecium*, *Ec. durans*, etc.) and *Streptococcus* (e.g., *Sc. bovis*, *Sc. equinus*, *Sc. gallolyticus*) and therefore the Group D descriptor is illogical and the term, faecal streptococci, is now defunct. *Lactococcus* spp. react with Group N antiserum. The newer species of *Enterococcus*, e.g., *Ec. dispar*, *Ec. caecoroum* and *Ec. columbae*, do not have a Lancefield antigen. Based on these findings, Lancefield groupings are of little relevance today for distinguishing these particular groups.

Enterococci occur in pairs or chains and are salt and heat tolerant and generally grow in the presence of 6.5 % NaCl and at 45 °C. These properties make them ideal starters for cheesemaking. Enterococci ferment sugars by the glycolytic pathway to L-lactate.

6.6.3 Streptococcus

These are also spherical to ovoid cells, occurring in pairs or chains. Currently, 79 species of *Streptococcus* are recognised but only one of them, *Sc. thermophilus*, is used as a starter culture. It grows at 45 °C but not at 10 °C and in the presence of 2.5 % but not 4 % NaCl, and was originally included in the viridans group of streptococci by Sherman. It is closely related to *Sc. salivarius*, an inhabitant of the mouth and a few years ago, it was renamed *Sc. salivarius* subsp. *thermophilus*

but it is now restored to full species status. It ferments sugars by the glycolytic pathway to L-lactate and does not have a Lancefield antigen.

In 1998, another organism, *Sc. gallolyticus* subsp. *macedonicus*, was isolated from the Greek cheese, Kasseri, produced without the deliberate use of a starter culture (de Vuyst and Tsakalidou 2008). Kasseri is a *pasta filata* (i.e., stretched-curd) type cheese produced from ewes' milk or a mixture of ewes' and goats' milk. This organism ferments lactose and grows at 45 °C and in 4 % but not in the presence of 6.5 % NaCl and coagulates milk within 24 h at 37 °C. Genetically, it is more closely related to *Sc. equinus* than to *Sc. thermophilus*.

6.6.4 *Leuconostoc*

Leuconostocs are spherical or lenticular-shaped cells which occur in pairs and chains and are found in some mesophilic mixed cultures. Within a chain, some cells can appear elongated or coccobacillary in shape. Thus, they can be confused with lactococci and with heterofermentative lactobacilli. *Leuconostoc* spp. do not hydrolyse arginine (Table 6.1). They have been divided into two genera, *Leuconostoc sensu stricto* and a new genus, *Weissella*, into which the *Lb. paramesenteroides* group and some heterofermentative lactobacilli like *Lb. viridescens*, *Lb. confusus* and *Lb. halotolerans* have been transferred.

Leuconostocs differ from lactococci in three fundamental respects: they ferment sugars heterofermentatively rather than homofermentatively, producing equimolar amounts of lactate, ethanol and CO_2; they produce the D rather than the L isomer of lactate, and, except for *Ln. lactis*, show no visual evidence of growth in litmus milk, unless yeast extract (0.3 g/100 mL) is added; *Ln. lactis* will produce acid in litmus milk but does not reduce the litmus, unlike lactococci, which reduce the litmus before coagulating the milk.

Currently, 13 species of *Leuconostoc* are recognised and despite the fact that leuconostocs were first identified in starter cultures in 1919, the exact species found in many starters is still not clear. Different species have been implicated but they have never been identified in the taxonomic sense, because of the difficulty in identifying them phenotypically and also researchers were more interested in how they behaved in milk fermentations than in their taxonomy. Cibik et al. (2000) looked at the diversity within 221 strains of *Leuconostoc*, isolated mainly from raw milk French cheeses (73 % of isolates) or milk (7 % of isolates), using three molecular techniques, viz., RAPD, 16 s rDNA sequencing and a species-specific PCR method which distinguishes between *Ln. mesenteroides*, *Ln. citreum*, *Ln. lactis* and *Weissella paramesenteroides*, which was originally called *Ln. paramesenteroides*. Many of the cheeses from which these strains were isolated were made from raw milk and so the isolates may have been contaminants of the milk just as easily as components of the starter. RAPD divided the strains into two major families, corresponding mainly to *Ln. mesenteroides* and *Ln. citreum*. The latter organism was isolated originally from a clinical source, tomatoes and rye and this was the first time it has been

reported in dairy products. The results of the species-specific probe showed that phenotypic testing had misidentified many strains. This probe could not distinguish between the three subspecies of *Ln. mesenteroides*, viz., *Ln. mesenteroides* subsp. *mesenteroides*, *Ln. mesenteroides* subsp. *dextranicum* or *Ln. mesenteroides* subsp. *cremoris*. Such a probe was subsequently developed by Perez et al. (2002). It would be interesting to apply these techniques to isolates of mixed mesophilic cultures especially since starter cultures are the only known source of *Ln. mesenteroides* subsp. *cremoris*, which is an unusual organism in that it ferments only lactose and its component monosaccharides, glucose and galactose.

6.6.5 *Lactobacillus*

These are rod-shaped cells, which may be long and slender, or short and sometimes bent, and often occur in chains. Currently, 125 species of *Lactobacillus* are recognised. The genus is divided into three groups: obligately homofermentative, facultatively heterofermentative and obligately heterofermentative, depending on whether they contain aldolase and/or phosphoketolase. The latter group can be coccobacillary in shape and are often confused with *Leuconostoc* spp.

Group 1, the obligate homofermenters, contain aldolase but not phosphoketolase and hence cannot ferment pentoses or gluconate; they ferment hexoses exclusively by the glycolytic (homofermentative) pathway to DL-, L- and/or D-lactate. This group includes all the thermophilic lactobacilli found in starter cultures, *Lb. helveticus*, *Lb. delbrueckii* subsp. *bulgaricus* and *Lb. delbrueckii* subsp. *lactis*. All strains of *Lb. delbrueckii* subsp. *bulgaricus* and most strains of *Lb. delbrueckii* subsp. *lactis* excrete galactose in proportion to the amount of lactose taken up by the cell. *Lb. helveticus* produces DL-lactate while *Lb. delbrueckii* produces only the D isomer.

Group 2, the facultative heterofermenters, contain aldolase and phosphoketolase. They ferment hexoses almost exclusively to lactate by the EMP pathway and pentoses to equimolar concentrations of lactate and acetate *via* an inducible phosphoketolase. Growth on glucose represses the formation of phosphoketolase. This group includes *Lb. casei*, *Lb. paracasei*, *Lb. plantarum* and *Lb. curvatus* which are found in NCWs and in ripening cheese, where they are called the non-starter lactic acid bacteria (NSLAB) (see Chap. 11).

Group 3, the obligate heterofermenters, possess phosphoketolase but not aldolase and hence, like *Leuconostoc*, ferment sugars heterofermentatively to equimolar concentrations of lactate, ethanol and CO_2. Small amounts of acetate may be produced also. Almost invariably, members of this group produce NH_3 from arginine. The only Group 3 lactobacilli reported in cheese are *Lb. brevis* and *Lb. fermentum*; they occur only in low numbers and are considered to be NSLAB.

Generally, group 1 lactobacilli do not grow at 15 °C but they will grow at 45 °C, while those in groups 2 and 3 grow at 15 °C but not at 45 °C. This is not an absolute rule but applies well to most of the lactobacilli found in starters and cheese. *Lb. fermentum* is an exception as it is the only Group III *Lactobacillus* which grows at

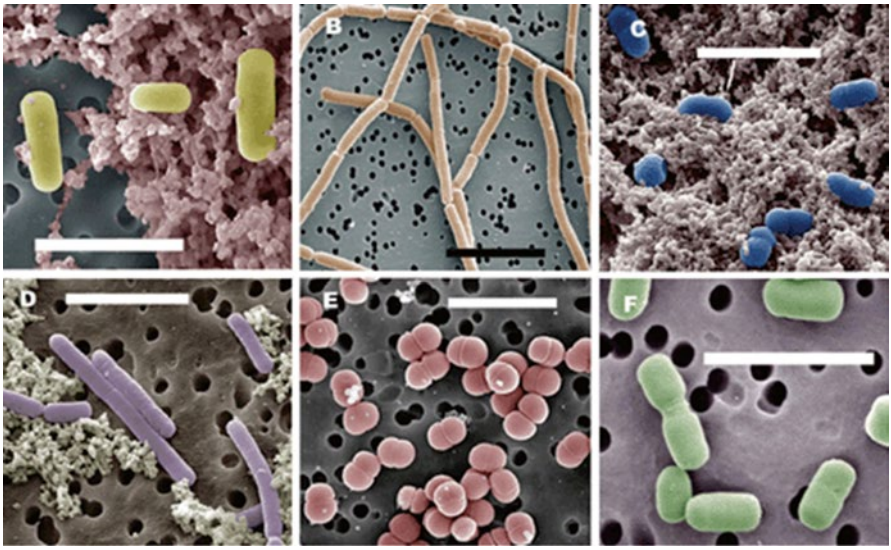


Fig. 6.5 Colour enhanced scanning electron micrographs of some starter and non-starter bacteria. a, *Lactobacllus helveticus*; b, *Lb. delbrueckii* subsp. *bulgaricus*; c, *Lactococcus lactis*; d, *Lb. casei*; e, *Pediococcus pentosaceus* and f, *Lb. brevis*. (From Broadbent and Steele 2005)

45 °C. The lactobacilli found in starter cultures are often called thermophilic because their optimum growth temperature is ~42 °C. They are not true thermophiles since they do not grow at 55 °C; however, they are able to withstand the cooking temperature (52–54 °C) used in Swiss cheese manufacture.

Colour enhanced scanning electron micrographs of some species of LAB found in starter cultures are shown in Fig. 6.5. The considerable variation that occurs in the shape of these bacteria is evident.

6.7 Phylogeny

There is sound scientific evidence that all living organisms, including animals, plants and microorganisms, evolved from a common ancestor. The phylogeny or evolutionary history of bacteria, including LAB, has received a lot of attention during the past 20 years in attempts to understand the relationships between different microorganisms. To study phylogeny, one selects and compares the sequences of a macromolecule which is present and has the same function in all cells. Such molecules have been called evolutionary chronometers. 16S rRNA is probably most widely used for this purpose because it is a relatively large molecule (~15,000 nucleotides) and some sequences are highly conserved while others are variable. A comparison of the sequences has allowed the construction of the universal tree of life.

Bacteria form one of three major domains (Archea and Eucarya are the other domains) and, within the bacterial domain, there are at least 12 distinct phylogenetic lineages, of which the Gram-positive bacteria are one. Gram-negative bacteria do not form a single group. The Gram-positive bacteria are divided into two branches, the clostridial branch with a % mol guanine + cytosine (GC) of <50 and the actinomycete branch with a % mol GC of >55. All starter bacteria and the bacteria found in ripening cheese are Gram-positive *Sc. thermophilus* and the species of starter LAB found in the genera *Lactococcus*, *Lactobacillus*, *Enterococcus* and *Leuconosoc* belong to the clostridial sub-division whereas the bacteria commonly found on the surfaces of washed-rind cheeses (*Micrococcus*, *Corynebacterium*, *Brevibacterium* and *Arthrobacter*) belong to the actinomycete branch of the Gram-positive bacteria (Chap. 11). The 16S rRNA sequences have also shown that *Enterococcus* are more closely related to *Carnobacterium* and *Vagoccoccus* than to *Lactococcus* and *Streptococcus*.

Normally, 16S rRNA gene sequences are used to study the phylogeny of bacteria. The availability of complete genome sequences for all food related LAB enables one to use concatenated ribosomal protein sequences to construct phylogenetic trees. This improves the resolution and increases the robustness of the analysis (Makarova et al. 2006). The phylogenetic relationships between starter and other LAB, based on this approach is shown in Fig. 6.6. As expected, *Lc. lactis* subsp. *cremoris* and *Lb. lactis* subsp. *lactis*, are closely related to each other and only distantly to *Sc. thermophilus*. The *Lactobacillaceae* are quite a disparate group, with

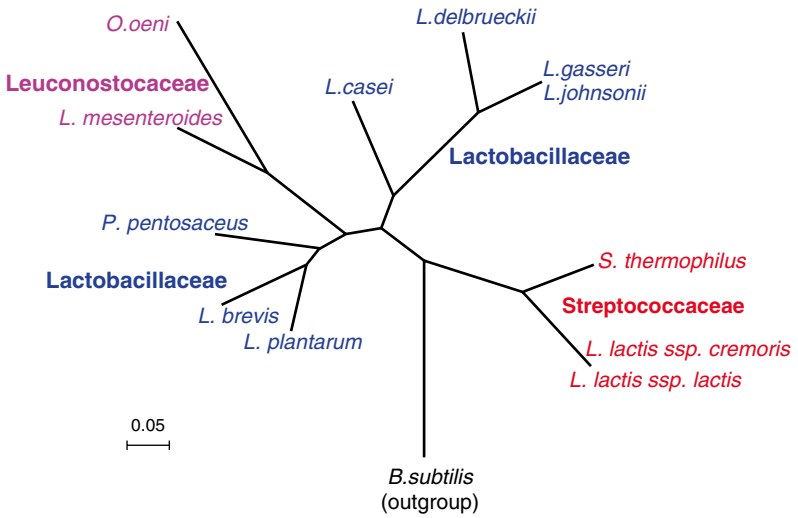


Fig. 6.6 Phylogenetic tree of lactic acid bacteria constructed on the basis of concatenated alignments of ribosomal protein sequences. Species are coloured according to their current taxonomy amongst the families, *Lactobacillaceae*, blue; *Leuconostocaceae*, magenta; *Streptococcaceae*, red. (From Makarova et al. 2006)

Lb. delbrueckii being closely related to *Lb. johnsonii*, which is found in human blood and in chicken, mice, calf and pig faeces, and *Lb. gasseri*, which is found in the human mouth and vagina, but only distantly related to *Lb. plantarum*. *Lactobacillus*, *Pediococcus* and *Leuconostoc* are morphologically quite dissimilar (rods, tetrads and cocci, respectively), yet phylogenetically they are quite intermixed. *Pediococcus pentosaceus*, a homofermentative coccus is closely related to *Lb. brevis*, a heterofermentative rod. One would think that the type of fermentation would be a fundamental distinguishing characteristic of LAB. Thus, the common tests used to distinguish between starter LAB (shape, the type of fermentation, growth at 10, 15 or 45 °C) give no absolute information on the relationships of these bacteria to each other.

6.8 Metabolism of Starters

6.8.1 Proteolysis

All LAB are auxotrophic and require several amino acids and vitamins for growth and specific strains of LAB are used to assay foods for vitamins, e.g., *Lb. delbruckii* ATCC 7830 for vitamin B_{12} and *Ec. faecalis* ATCC 8043 for folic acid. In lactococci, the requirements for amino acids are strain-specific and vary from as few as 4 to perhaps 12 or more. Most lactococci require glutamate, methionine, valine, leucine, isoleucine and histidine for growth and many strains have additional requirements for phenylalanine, tyrosine, lysine and alanine. The amino acid requirements of *Sc. thermophilus* and *Leuconostoc* spp. are less demanding than lactococci, with *Sc. thermophilus* requiring six amino acids, including leucine, valine and cycteine. Only one strain of *Lb. helveticus* has been studied; it required all the amino acids except glycine, alanine, serine and cysteine. Whether the requirement for amino acids is due to the lack of the necessary genes required for biosynthesis or to mutation within the genes depends on the organism. *Lc. lactis* IL 1403 possesses all of the genes for the synthesis of the 20 standard amino acids and its amino acid requirements are likely to be the result of frameshift mutations, caused, in part, by the treatments used in curing the strain of plasmids (Bolotin et al. 2001). *Lc. lactis* MG1363, the main strain used in genetic studies of lactococci, also contains the genes necessary to synthesize five of the six essential amino acids for its growth (Wegmann et al. 2007). In contrast, the requirement of *Lb. helveticus* CNRZ 32 for amino acids is due to the lack of the genes necessary for amino acid biosynthesis (Christensen et al. 2008).

Fully grown milk cultures of starter bacteria contain ~10^9 cfu/mL. The concentrations of amino acids and peptides in milk are low (the free amino acid content of milk is ~100 mg/L) and sufficient to sustain only ~25 % of the maximum number of starter cells present in a fully grown starter culture. Therefore, starter bacteria must

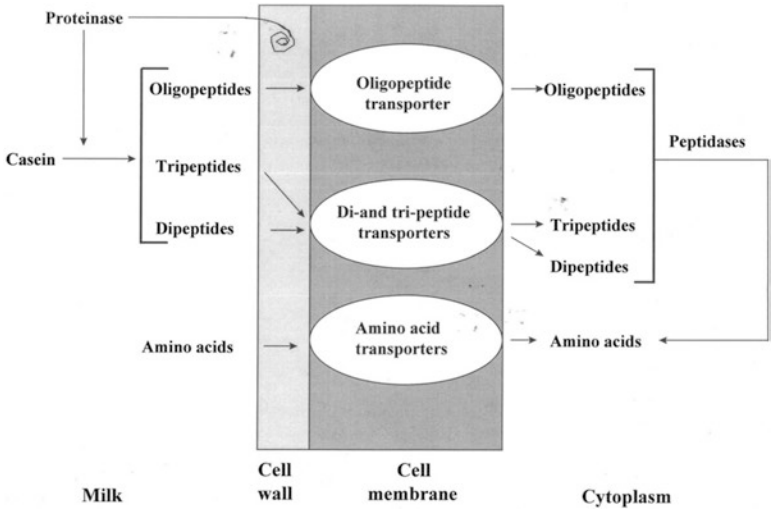


Fig. 6.7 An overview of the proteolytic system of *Lactococcus*

have a proteolytic system to hydrolyse the milk proteins to the amino acids required for good growth in milk. The proteolytic system of *Lactococcus* involves a cell wall-associated proteinase, amino acid and peptide transport systems and peptidases. A simplified consensus of the system is shown in Fig. 6.7. It includes a proteinase, various transport systems and numerous intracellular peptidases.

The lactococcal proteinase (PrtP) is one of the most intensively studied enzymes of starter bacteria, and it is likely that other starter bacteria have similar systems. It is a serine proteinase which is synthesised in the cell as a pre- pro-proteinase which is transformed into the mature, active proteinase by a process not yet completely understood. It is not a truly extracellular enzyme but is anchored to the cell membrane by its extremely hydrophobic C-terminal region. The mature proteinase, called Prt P, contains ~1800 amino acid residues, has a molecular mass of ~185 kDa and an optimum pH of ~6. The gene encoding the proteinase (*prtP*) has been sequenced in several strains of lactococci. The sequences are very similar (98 % identical), implying that there is only one proteinase but there are two specificities, P_I and P_{III}, The primary substrates for the P_I–type are β-casein and to a lesser extent κ-casein while the P_{III}-type degrade α_{s1}-, β- and κ-caseins (Kunji et al. 1996). The different specificities are most likely due to subtle differences in the amino acid sequences of the different proteinases.

Only limited information is available on the proteinases of the other starter bacteria, but the available data indicate that they are similar to the lactococcal proteinases., e.g., the PrtP of *Lc. lactis* and *Lb. pararcasei* share 95 % similarity. Unusually, no proteolytic activity has been detected in *Sc. thermophilus*, except in the so-called Asian strains, which were isolated in Mongolia, India and Japan. This lack of

proteolytic activity may explain the symbiotic relationship between *Lb. delbrueckii* subsp. *bulgaricus* and *Sc. thermophilus* in yogurt cultures (Fig. 6.3). When growing in milk, *Lb. delbrueckii* subsp. *bulgaricus* produces peptides and amino acids, especially histidine and glycine, which stimulate the growth of *Sc. thermophilus*, while the latter organism produces formic acid which stimulates the former organism.

There are four different caseins in milk, α_{s1}-, α_{s2}-, β- and κ-, occurring in the ratio of ~4:1:3:1 and they comprise ~80 % of the total protein in cows' milk (see Chap. 4). Hydrolysis of these proteins by lactococcal proteinases results in production of numerous oligopeptides of different sizes. For example, hydrolysis of β-casein, which contains 209 amino acid residues, by the PI proteinase results in the production of 100 peptides, the majority of which contain between 4 and 30 amino acid residues. Peptides containing up to eight amino acid residues can be transported, across the cell membrane, into the cell. Various transport systems, including an oligopeptide transport system, a di/tripetide transport system and at least ten amino acid transport systems, which have a high specificity for structurally-related amino acids, e.g. Glu/Gln, Leu/Ile/Val, have been described in lactococci. The driving forces for transport include the proton motive force, antiport and symport systems and ATP hydrolysis.

Inside the cell, the peptides are hydrolysed by peptidases to the individual amino acids for the synthesis of the proteins required for cell growth. Numerous peptidases have been identified in starter LAB, including at least two aminopeptidases (Pep N and Pep C), 2 tripeptidases (Pep T and Pep 53) and two dipeptidases (Pep V and Pep D) which release single amino acids from the N-terminal of the relevant substrates and an oligopeptidase (Opp). Two different endopeptidases (Pep O and Pep F) have also been indentified in *Lc. lactis* which hydrolyse internal peptide bonds in peptides but not in the caseins. The proline content of casein is quite high and many proline-containing peptides taste bitter. Specific peptidases, called prolidases (Pep Q), aminopeptidase P (Pep P), X-prolyl-dipeptidyl amino peptidase (Pep X), prolinase (Pep R) and proline iminopeptidase (Pep I) hydrolyse proline from peptides and help to reduce bitterness. Some of the important properties of the peptidases are summarised in Table 6.3. Generally, the peptidases were isolated from several strains of the same species and the data shown is a summary. Pep R has been found only in *Lb. helveticus*. Carboxypeptidase activity is not found in LAB.

The peptidases are either serine-, metallo- or thiol-enzymes and have pH optima within the range 6.0–8.0. All of them are located inside the cell and, acting together, hydrolyse the peptides transported into the cell by the peptide transport systems to their constituent amino acids for use in protein synthesis. They are also important in the ripening of cheese. During ripening, the starter bacteria gradually lyse releasing their intracellular peptidases, which then act on any peptides present near them. The amino acids produced are the precursors of the flavour compounds necessary for the development of good flavoured cheese (see Chap. 13). For reviews of different aspects of proteolysis of LAB see Kunji et al. (1996); Christensen et al. (1999) and Kok et al. (2011).

Table 6.3 Properties of the various peptideses found in starter lactic acid bacteria[a]

Peptidase	Name	Substrate $n=1,2,3\ldots$	Organism	Mw kDa	Type[b]	pH Optimum	Location[c]
General:							
Aminopeptidase N	Pep N	X ↓$(X)_n$	*Lc. lactis*	95	M	7	I
			Lb. delbrueckii	95	M	7	I
			Lb. helveticus	97	M	6.5	CW and I
Aminopeptidase C	Pep C	X ↓$(X)_n$	*Lc. lactis*	50	T	7	I
			Lb. delbrueckii	52	T	7	I
			Lb. helveticus	50	T		I
Tripeptidase	Pep T	X ↓X-X	*Lc. lactis*	46–52	M	7.5	I
	Pep 53		*Lc. lactis*	>23	M	5.8	CW
Dipeptidase	Pep V	X ↓X	*Lc. lactis*	50	M	8	I
			Lb. delbrueckii	51	M	7.5	
			Lb. helveticus	50	M	8	I
	Pep D	X ↓X	*Lb. helveticus*	54	T	6	
Proline Specific:							
Prolidase	Pep Q	X ↓Pro	*Lc. lactis*	42	M	7–8	I
			Lb. delbrueckii	41	M		I
Aminopeptidase P	Pep P	X ↓Pro-$(X)_n$	*Lc. lactis*	43	M	8	I
X-prolyl-dipeptidyl	Pep X	X ↓Pro-$(X)_n$	*Lc. lactis*	59–90	S	7–8.5	CW and I
aminopeptidase			*Lb. delbrueckii*	82–95	S	6.5–7	I
			Lb. helveticus	88–95	S	6.5	I
Prolinase	Pep R	Pro ↓X	*Lb. helveticus*	35		7.5	I
Proline iminopeptidase	Pep I	Pro ↓X-$(X)_n$	*Lc. lactis*	30–>50	S		I
			Lb. delbrueckii	33	S	6.5	CW
			Lb. helveticus	34	S		I

Adapted from Kunji et al. (1996)

[a]Many of the enzymes were isolated from several strains of the species and the data given is a summary

[b]*M* metallo-enzyme, *S* serine peptidase, *T* thiol peptidase

[c]*I* intracellular, *CW* cell wall

6.8.2 *Arginine Metabolism*

An important distinguishing characteristic of some starter and non-starter LAB is the production of NH_3 from arginine (Table 6.1). The pathway used (Fig. 6.8) is called the arginine deiminase pathway and 1 mol of ATP per mol of arginine metabolised is produced. Arginine is hydrolysed to NH_3 and citrulline by arginine deiminase. Ornithine carbamyltransferase then catalyses the phosphorolysis of citrulline

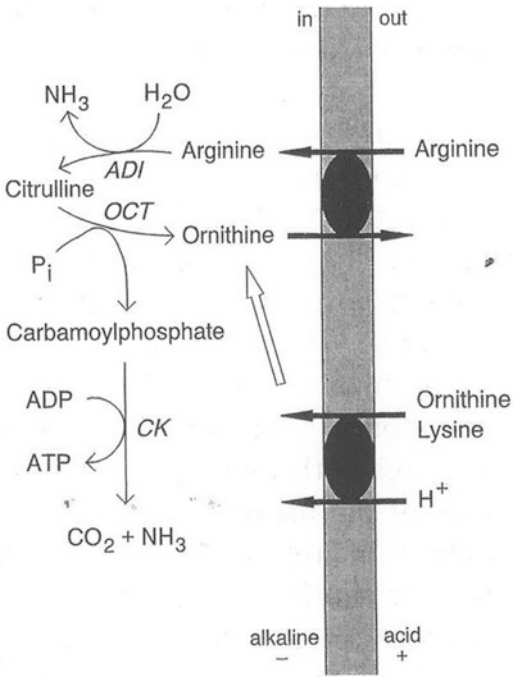


Fig. 6.8 The arginine deiminase pathway. Accumulation of ornithine (lysine) via the Δp-driven lysine transport system is also shown. *ADI* arginine deiminase, *OCT* ornithine carbamoyl-transferase, *CK* carbamate kinase. (From Poolman 1993)

to ornithine and carbamyl phosphate; the latter is then hydrolysed to NH_3 and CO_2, with the concomitant production of ATP by carbamate kinase. The uptake of arginine is driven by an antiport transport system in which ornithine is exchanged for arginine.

Lc. lactis subsp. *lactis* produces NH_3 from arginine *via* this pathway whereas *Lc. lactis* subsp. *cremoris* does not; this is due to the lack of at least one of the three enzymes of the pathway in the latter organism.

6.8.3 Lactose Metabolism

Starter LAB cannot grow without an energy source. The main energy source in milk is lactose which they ferment to lactic acid, simultaneously producing sufficient energy to sustain cell multiplication and growth.

Lactose, is a disaccharide composed of one molecule of galactose and one of glucose connected in a β1,4 linkage (see Chap. 4). Before it can be fermented, it must be first transported into the cell. Lactococci use the phosphoenolpyruvate (PEP)-phosphotransferase (PTS) system to transport lactose, in which the energy in PEP is transferred to lactose in a complex series of reactions involving enzyme one (EI), three phosphoryl transfer proteins (IIA, IIB and IIC), and a heat-stable protein, HPr,

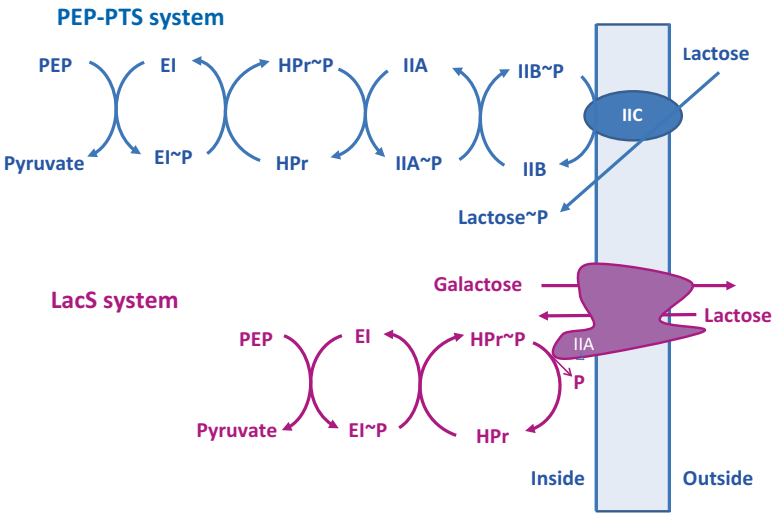


Fig. 6.9 Phosphoenol pyruvate-phosphotransferase (PEP-PTS) and Lac S lactose transport systems for transport of lactose. The PEP-PTS system operates in *Lactococcus lactis* and the LacS system in *Sc. thermophilus*

ultimately forming lactose-phosphate (Fig. 6.9). Protein IIC is located in the membrane and both it and proteins IIA and IIB are specific for lactose. In contrast, *Sc. thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus* and Gal⁻ strains of *Lb. delbrueckii* subsp. *lactis*, use the LacS system for transport of lactose. In this system, lactose is transported into the cell without modification, and, at the same time, galactose is transported out of the cell, by a lactose-galactose exchanger (Fig. 6.9). The energy required for transport is derived from the concentration gradients of the two sugars across the cell membrane. *Sc. thermophilus* can also transport lactose by a lactose-H^+ symport system, in which the energy for transport is provided by the proton motive force, but the LacS system is used *in vivo* as it is much faster than the lactose/H+ symport system. In the PTS system, the high-energy phosphate is transferred to the next protein in the sequence on histidine residues while the LacS system uses serine residues. HPr and E1 are also involved in the LacS system of lactose transport but its main function is not to transfer phophoryl groups rapidly but rather to control transport activity. The lactose transport systems used by other starter bacteria are not clear. Further information on transporters in LAB can be found in Poolman (2002).

The initial enzyme involved in the metabolism of lactose depends on the transport system used. In the PTS system, the lactose-P formed during transport is hydrolysed by phospho-β-galactosidase (*p*βgal) to glucose and galactose-6-P, while in the LacS transport system, lactose is hydrolysed by β-galactosidase (βgal) to glucose and galactose .

Two different pathways are used to ferment sugars, the glycolytic pathway (Fig. 6.10), which is used by all starter LAB, except leuconostocs, and the phosphoketolase (PK) or pentose phosphate pathway (Fig. 6.11), which is used by

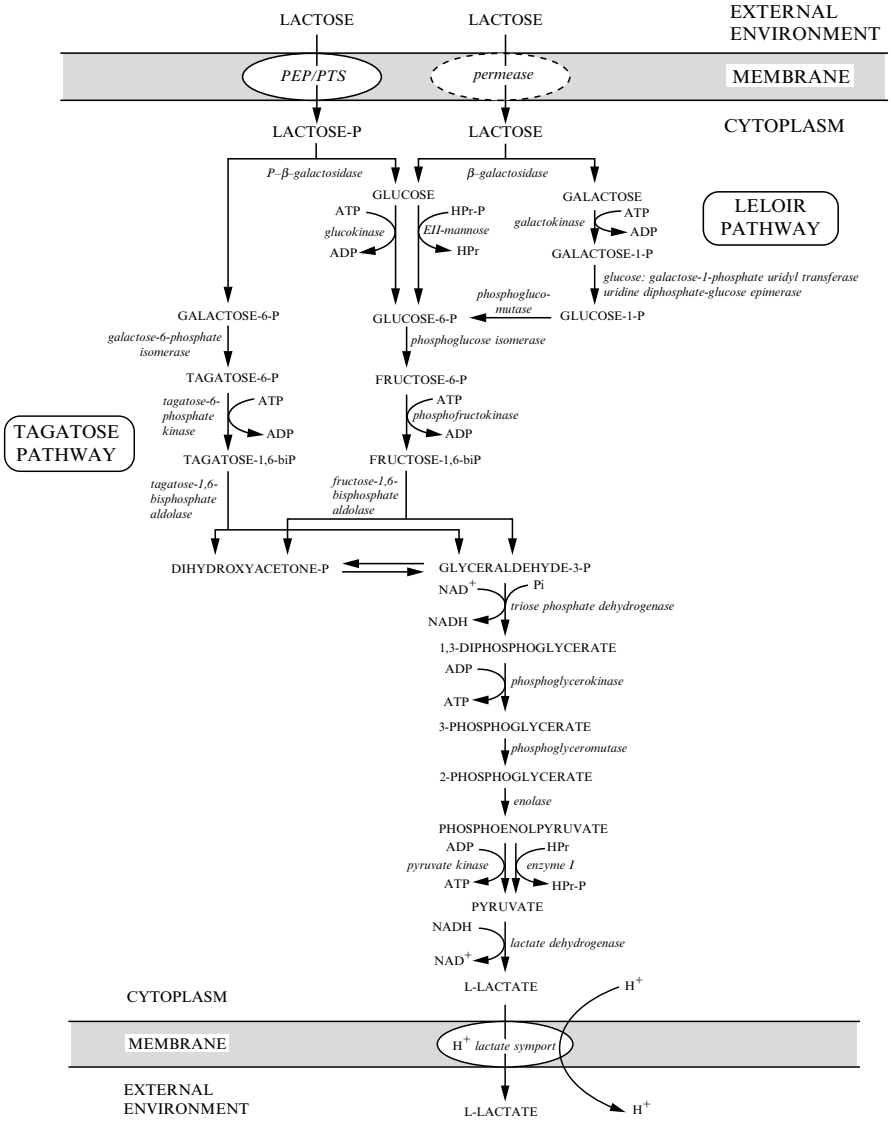


Fig. 6.10 Glycolytic pathway for lactose metabolism in lactic acid bacteria

leuconostocs. Homofermentative NSLAB ferment lactose *via* glycolysis while heterofermentative NSLAB use the PK pathway. In lactococci, the galactose-6-P, produced by *p*βgal activity, is metabolised through several tagatose derivatives to dihydroxy acetone phosphate. Tagatose is a stereoisomer of fructose. Fermentation of glucose and galactose by thermophilic starter LAB also occurs *via* glycolysis but some of them, including *Sc. thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus*

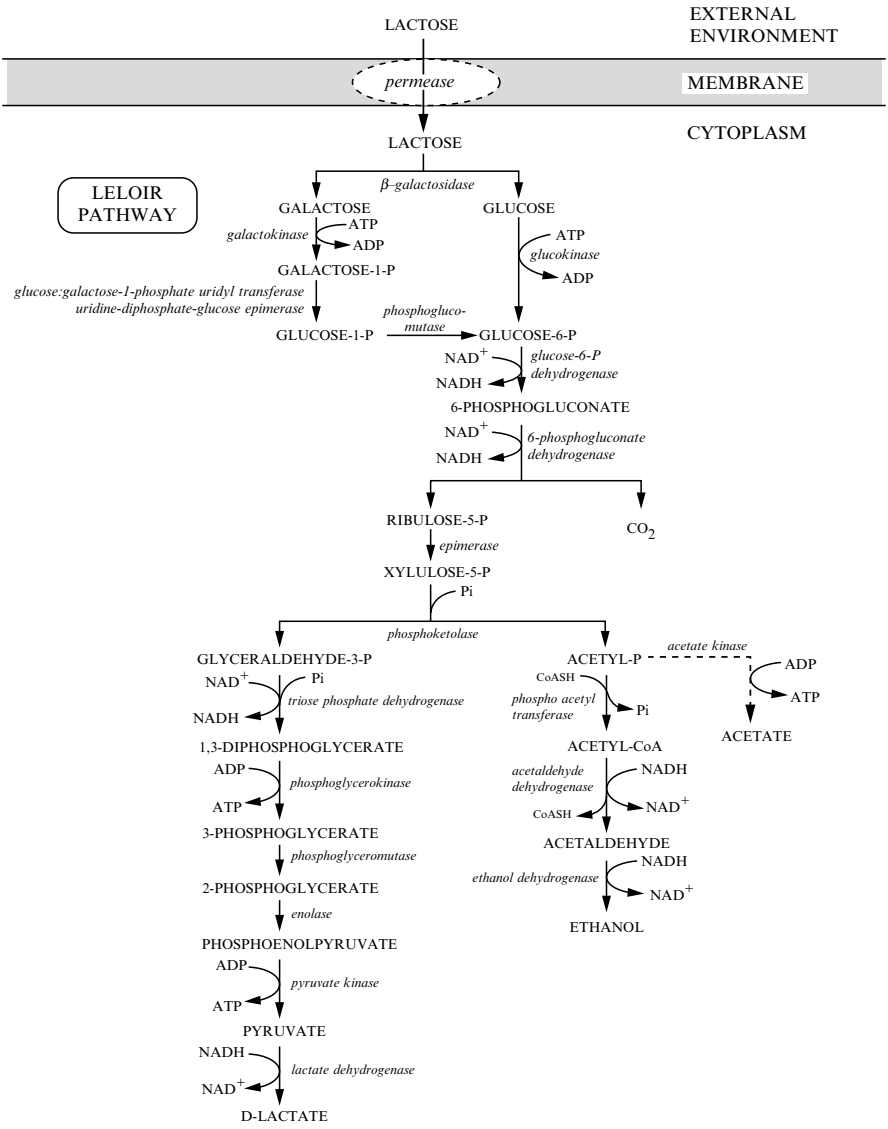


Fig. 6.11 Phosphoketolase pathway for lactose metabolism in lactic acid bacteria

and some strains of *Lb. delbrueckii* subsp. *lactis*, ferment only the glucose moiety of lactose and excrete galactose in proportion to the amount of lactose transported. *Leuconostoc* transform galactose to glucose-1-P via the Leloir pathway, before fermenting it by the PK pathway.

A more detailed study of 49 strains of *Sc. thermophilus* (de Vin et al. 2005) showed that the strains could be divided into four groups depending on how they metabolised galactose after lactose depletion. For this purpose they were grown in

a medium containing 0.5 % lactose so that the pH after lactose had been depleted decreased to ~5.6, which did not prevent continued growth of the strain. One strain was able to metabolise galactose and did not excrete it into the medium and 9 strains consumed none of the excreted galactose; 32 strains (the majority) were able to consume part of the galactose as fermentation continued while the remaining 7 strains consumed all the excreted galactose within 8.5 h. This has implications for cheesemaking since it would be desirable to use strains which either do not excrete galactose or deplete it rapidly, ultimately resulting in a sugar free cheese.

The products of both pathways are quite different. In glycolysis, 1 mol of lactose is transformed to 4 mol of lactic acid (or 2 mol in the case of *Sc. thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus* and some strains of *Lb. delbrueckii* subsp. *lactis*, because of the excretion of galactose) while in the PK pathway it is transformed to 2 mol each of lactate, ethanol and CO_2.

Lactic acid contains an asymmetric carbon and hence can exist as two isomers, D and L. The isomer of lactate produced is useful in the identification of the various genera and species of starter LAB, e.g., *Leuconostoc* and *Lb. delbrueckii* produce only the D isomer, *Lactococcus* and *Sc. thermophilus* produce only the L form and *Lb. helveticus* produces a mixture of the D and L isomers, due to the presence of two lactate dehydrogenases in the cell, one of which is specific for the L and the other for the D isomer. The salient features of lactose metabolism in the different starter bacteria are summarised in Table 6.4

The reason for the production of lactate in both pathways and ethanol in the PK pathway is the need to re-oxidise the NADH and NADPH produced in the early steps of the pathway to allow fermentation to continue. The purpose of the fermentations is to produce sufficient ATP to sustain growth. Production of ATP by fermentation is much less efficient than the production of ATP by respiration, e.g., in glycolysis, 4 mol of ATP are produced per mole of lactose fermented compared with a possible 76 mol by respiration. Therefore, to produce the same amount of ATP by fermentation as by respiration, a large amount of sugar must be fermented and, consequently, large amounts of lactic acid are produced.

The growth of some strains of *Lactococcus* on galactose or low levels of glucose leads to the production of other compounds from pyruvate besides lactate, e.g.,

Table 6.4 Salient features of lactose metabolism in starter organisms

Organism	Transport[a]	Pathway[b]	Cleavage enzyme	Products (mol/mol lactose)	Isomer of lactate
Lactococcus	PEP-PTS	GLY	pβgal	4 lactate	LD
Leuconostoc	Permease	PK	βgal	2 lactate + 2 ethanol + 2 CO_2	D
Sc. thermophilus	Permease	GLY	βgal	2 lactate[c]	L
Lb. delbrueckii	Permease	GLY	βgal	2 lactate[c]	D
Lb. helveticus	Permease	GLY	βgal	4 lactate	DL

[a]PEP-PTS, phosphotransferase system

[b]*GLY* glycolysis, *PK* phosphoketolase

[c]These species metabolize only the glucose moiety of lactose

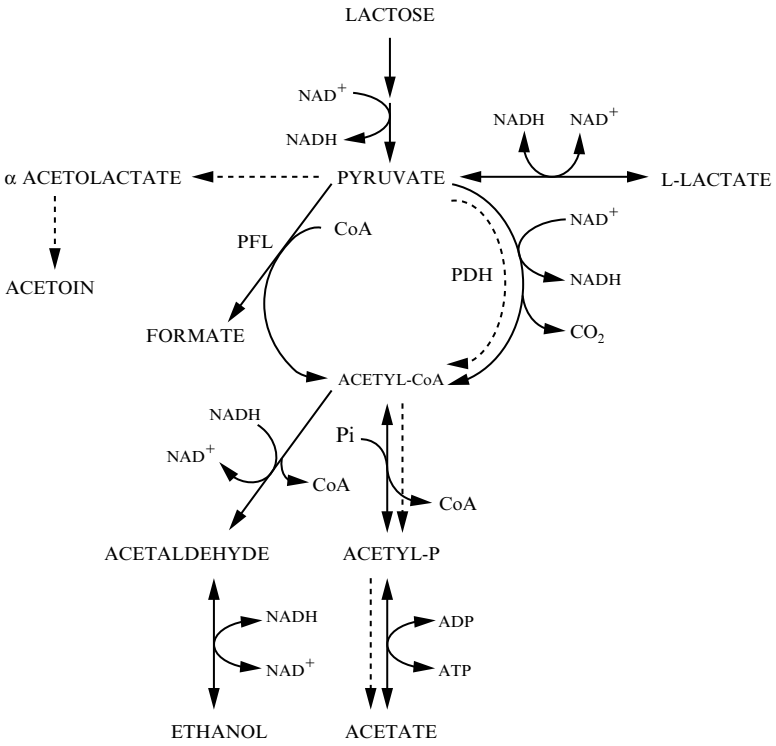


Fig. 6.12 Pathways for pyruvate metabolism in lactic acid bacteria

ethanol, acetate and acetaldehyde *via* pyruvate dehydrogenase (PDH) and/or pyruvic/formic lyase (PFL) (Fig. 6.12). In these bacteria, lactic dehydrogenase (LDH) and PFL are allosteric enzymes. LDH requires the presence of fructose-1,6-bisphosphate for activity while PFL activity is inhibited by triose phosphates. Normally, both effectors are present at high concentrations in the cell which favours LDH activity and lactate production. Growth at low sugar concentrations results in lower intracellular concentrations of the effectors and therefore LDH activity is reduced and PFL activity increased which allows pyruvate to be channelled to ethanol and acetate rather than lactate. Normally, during anaerobic growth, PFL activity is favoured over PDH activity in the initial formation of acetyl CoA.

Cells of *Lc. lactis* starved of carbohydrate enter a non-culturable state. Cells lose their ability to form colonies on solid media fairly quickly, within 1 week, but maintain an intact cell membrane and retain their metabolic activity for at least 3.5 years (Ganesan et al. 2007). The addition of branched chain amino acids (BCAA) to the culture medium increases intracellular ATP levels and new metabolic products, indicating that BCAA catabolism results in energy and metabolic product formation to support survival during starvation. Cheese is devoid of carbohydrate relatively

early in ripening and the role of this system in cheese ripening does not appear to have been investigated.

For a recent review of LAB physiology and energy metabolism see Poolman et al. (2011).

6.8.4 Excretion of End-Products

The end-products of lactose fermentation are mainly acidic and will, unless excreted, acidify the cell cytoplasm. LAB have two mechanisms for excreting lactate and protons. One involves the transmembrane reversible F_oF_1-ATPase and is responsible for the secretion of protons. The second involves the simultaneous secretion of lactate anions and protons in symport with each other. This mechanism occurs especially when the external concentration of lactate is low and the internal concentration is high. Energy can also be derived from this reaction through the creation of a proton motive force.

6.9 Respiration in Lactic Acid Bacteria

Starter bacteria do not contain a functional cytochrome system and thus cannot oxidise sugars completely to CO_2 which is much more energy efficient. Instead, they produce lactic acid, which is much more energy inefficient. Their metabolism of sugars is strictly fermentative rather than respirative and ATP is produced by substrate-level phosphorylation rather than by the more energy-efficient oxidative phosphorylation. Despite their lack of a cytochrome system, most LAB are aerotolerant and grow quite well in the presence of air, even though they are unable to use the O_2 in the air as a terminal electron acceptor. However, *Lc. lactis* will respire if grown in the presence of 10 μM heme (Gaudu et al. 2002). Heme is a complex of Fe^{2+} and protophorphin IX, the coenzyme component of cytochrome oxidase, the terminal enzyme of respiration. *Lc. lactis* contains the gene encoding cytochrome oxidase but lacks the functional pathway for the formation of heme which is an integral part of a functional cytochrome oxidase. *Lc. lactis* grown in the presence of haem produces more biomass and retains its acid-producing ability much longer during subsequent storage at 4 °C, than cultures grown in the absence of haem. In addition, it produces less acid during growth in the presence of haem because pyruvate, the precursor of lactate, is oxidised to CO_2 and H_2O during respiration. Therefore, the pH does not decrease as much during growth in the presence of haem as it does during the absence of haem.

These findings have practical implications and are being used by one of the major culture suppliers, Chr. Hansen, to improve the production of *Lc. lactis* cultures (Pedersen et al. 2005). Cit^+ *Lc. lactis*, *Ln. mesenteroides* and *Ec. faecalis* respond to the presence of heme in a similar manner to *Lc. lactis* but thermophilic

cultures, including *Sc. thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus* and *Lb. helveticus* do not. These results suggest that bulk cultures of *Lc. lactis* should be grown in the presence of haem, but to our knowledge this is not done in cheese factories.

6.10 Citrate

The Cit$^+$ *Lactococcus* and *Leuconostoc* spp. present in mesophilic starters metabolise citrate to acetate, CO_2, diacetyl, acetoin and 2,3-butanediol by the pathway shown in Fig. 6.13. Citrate is not metabolised by thermophilic starters. It is not used as an energy source but is co-metabolised with lactose or some other sugar. The CO_2 produced is responsible for the small eyes in Edam and Gouda cheese, while diacetyl and acetate are important contributors to the flavour of many fermented products, including Quarg, Fromage Frais and Cottage cheese. Diacetyl is produced in only small amounts (<10 mg/mL or 0.11 mM in milk) and acetoin production is generally 10–50 times greater than that of diacetyl. One mole of acetate is produced from each mole of citrate used but recent studies suggest that ~1.2 mol of acetate are produced per mole of citrate used. This is probably due to the production of small amounts of acetate from sugar metabolism also. There are very little quantitative data on the levels of 2,3-butanediol produced by starters.

There is still controversy on how diacetyl is actually produced. One of the reasons for this is that the putative enzyme, diacetyl synthase, has never been found in LAB. Another, and probably more important, reason is that acetolactate (AL) is very unstable and easily autodecarboxylates, non-oxidatively to acetoin or oxidatively to diacetyl. AL is so unstable that it is available commercially only as a double ester to protect it from autodecarboxylation; it is normally hydrolysed with two equivalents of NaOH just before use. Acetoin is produced by AL decarboxylase (ALD) activity but it is generally believed that diacetyl is produced chemically, rather than enzymatically, from AL. Despite this, it is difficult to see how diacetyl can be produced oxidatively from AL by starter cultures which essentially grow anaerobically and produce very low E_h values (~−250 mV).

Growth and metabolite production by a DL mesophilic mixed-strain culture in milk at 21 °C is shown in Fig. 6.14. Cell numbers and the production of end-products are plotted semi-logarithmically because bacteria grow and produce end-products exponentially. Arithmetic plots are quite different. This is an example and it is important to remember that individual cultures will show some variation. Production of the various metabolites is in the order: lactate>acetate>acetoin >>diacetyl. Production of diacetyl and acetoin ceases as soon as all the citrate has been used, after which the levels of acetoin and diacetyl may decrease due to the activity of acetoin dehydrogenase. The same enzyme is probably responsible for the reduction of diacetyl to acetoin and acetoin to 2,3-butanediol. Therefore, to retain the maximum amount of diacetyl in unripened cheese, the product should be cooled as soon as possible after citrate utilisation is complete. Citrate utilisation is generally slower

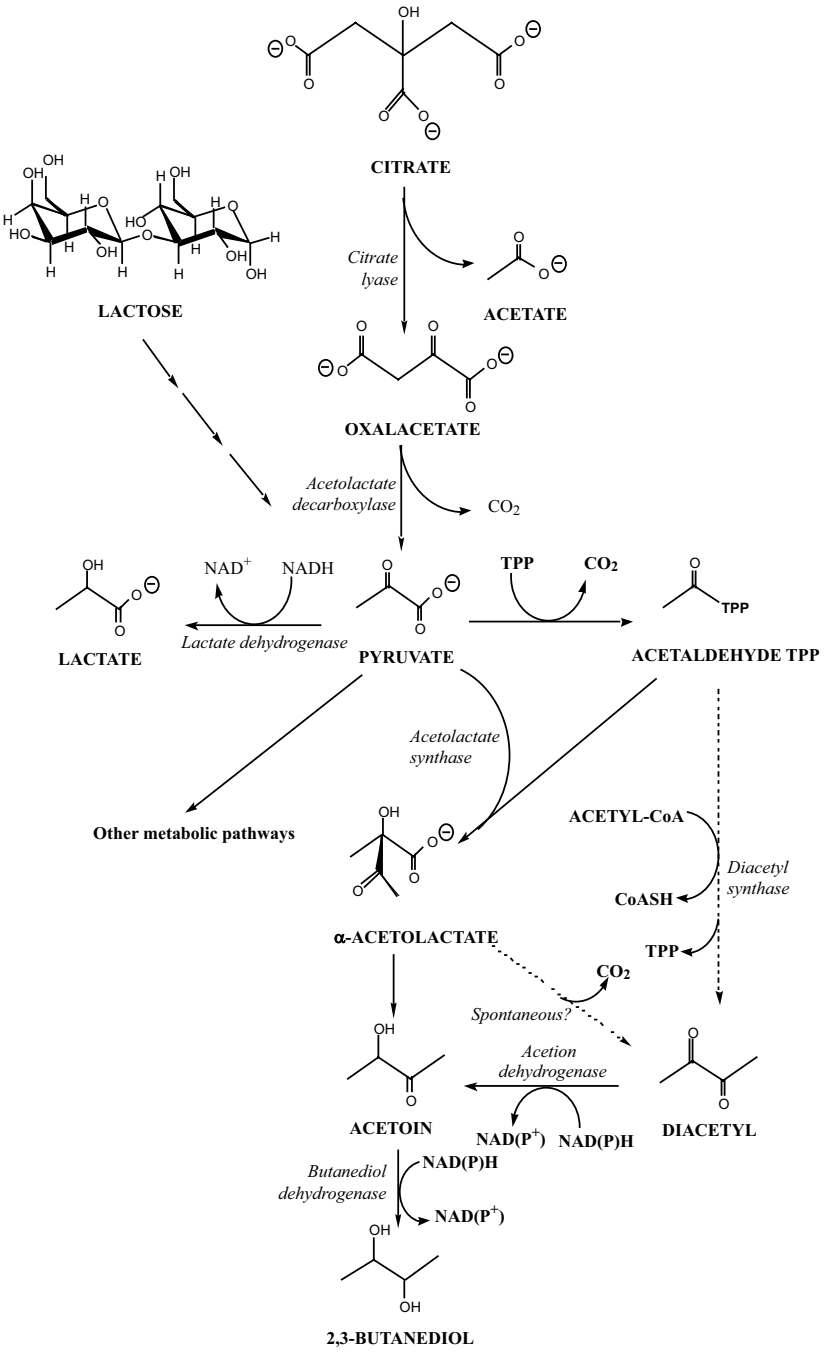


Fig. 6.13 Pathway for citrate metabolism in lactic acid bacteria. Pyruvate can also be formed from lactose

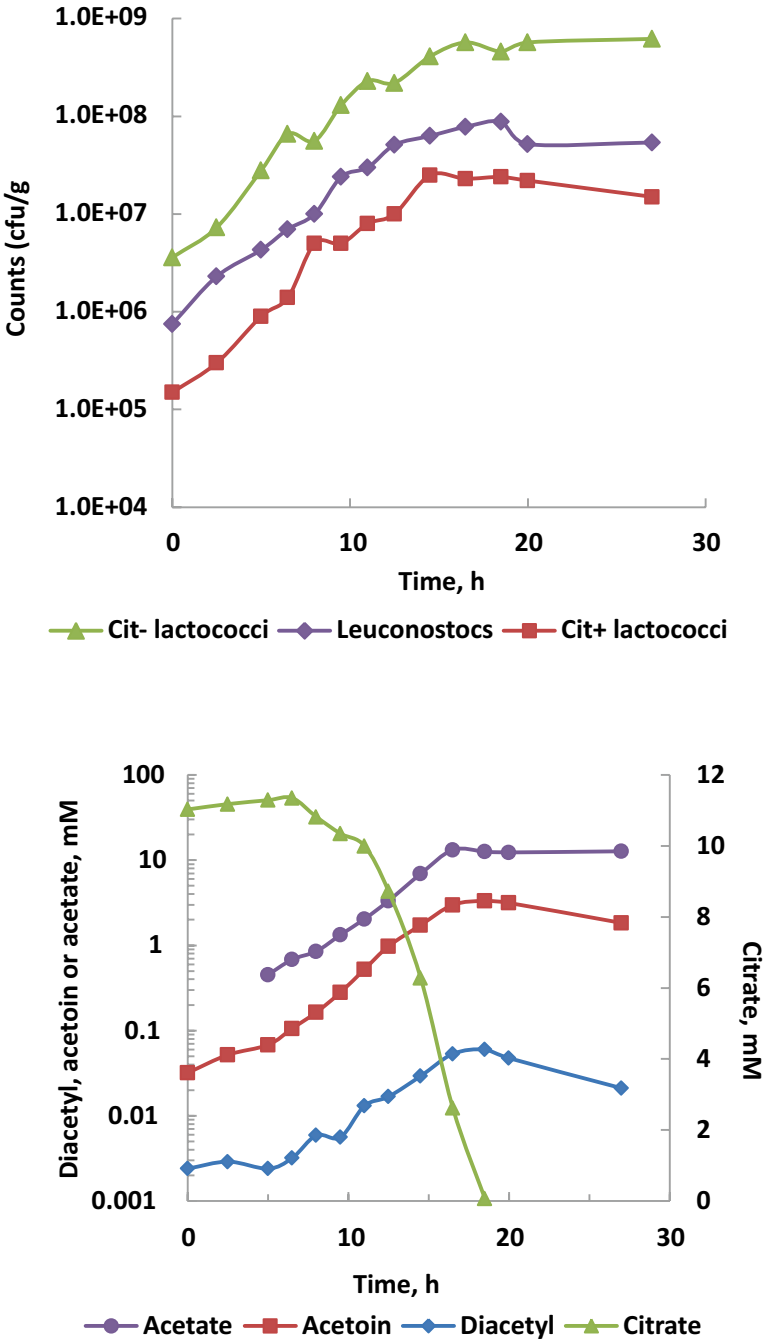


Fig. 6.14 Growth of Cit⁺ and Cit⁻ lactococci and leuconostocs, decrease in pH, production of acetate, diacetyl and acetoin and utilisation of citrate by DL culture 505 in reconstituted skim milk (100 g/L) at 21 °C

in L than in D or DL cultures because of the slower growth of the Cit⁺ *Leuconostoc* compared with Cit⁺ *Lactococcus*.

Little, if any, acetolactate (AL) accumulates in cultures because most Cit⁺ lactococci contain an active ALD; in addition, AL is unstable and readily decarboxylates to acetoin and diacetyl. However, an important D culture, called 4/25, which is used in the NIZO process for the production of lactic butter in most European countries, contains an inactive ALD and hence accumulates AL. Nucleotide sequences of the *ald* gene in this strain and in a strain containing ALD activity showed only one nucleotide substitution in the gene (cytosine for thymine at position 659), which results in a change of histidine to tyrosine in a motif conserved in all ALDs. It is thought that this change is sufficient to inactivate the enzyme (Goupil et al. 1996). Autodecarboxylation of AL to acetoin (mainly) and diacetyl probably occurs throughout growth but is noticed only as soon as AL production ceases when all the citrate is used. The level of diacetyl produced from autodecarboxylation can be increased by aeration at acid pH and this property is used to produce diacetyl in the manufacture of lactic butter by this particular culture in the NIZO process.

Pure cultures of Cit⁺ *Lactococcus* and *Leuconostoc* differ in the products produced during co-metabolism of citrate and lactose. Cit⁺ *Lactococcus* produce lactate, acetoin, acetoin and CO_2, in a similar manner to mixed-strain mesophilic cultures. In contrast, the Cit⁺ *Leuconostoc* spp. produce no acetoin or diacetyl. Instead, they produce increased amounts of lactate and acetate. Pyruvate is an intermediate in the metabolism of both lactose and citrate. In *Leuconostoc*, the pyruvate produced from the metabolism of both substrates is converted to lactic acid. This relieves the cells from forming ethanol to regenerate the pyridine nucleotides. Instead, the acetyl-P produced from lactose is used to produce acetate and ATP:

$$\text{Acetyl} - \text{P} + \text{ADP} \rightarrow \text{Acetate} + \text{ATP}$$

This extra production of ATP also results in much faster growth of the organism. However, *Leuconostoc* will produce diacetyl and acetoin from citrate in the absence of an energy source and proportionately more of the citrate is converted to these compounds as the pH decreases. The question then may be asked, how do *Leuconostoc* produce diacetyl and acetoin in mixed cultures? The answer is not clear but it may be due to the fact *Leuconostoc* do not take up much lactose at pH values below 5.5.

6.10.1 Acetaldehyde

Acetaldehyde is produced by both mesophilic and thermophilic cultures and is an important component of the flavour of yoghurt. The amount produced varies and can reach 30 μg/mL. It is generally considered to be a product of carbohydrate (pyruvate) metabolism (Fig. 6.12) but it can also be produced from threonine *via* threonine aldolase activity:

$$\text{Threonine} \rightarrow \text{glycine} + \text{acetaldehyde}$$

This is the mechanism by which acetaldehyde is produced by lactococci and the physiological function of this reaction is thought to be to provide glycine for growth. The only strain of *Lactococcus* which has a requirement for glycine also lacks threonine aldolase. The pathway by which acetaldehyde is produced by thermophilic cultures is not clear.

Both organisms present in yoghurt cultures produce acetaldehyde but *Sc. thermophilus* produces greater amounts than *Lb. delbrueckii* subsp. *bulgaricus* and both organisms produce more when grown together than when grown individually, due to the symbiosis between the two organisms.

In some fermented dairy products, particularly those prepared with mesophilic cultures, the ratio of diacetyl:acetaldehyde is important. The optimum ratio is 4:1 but when the ratio falls to 3:1, a "green" off-flavour defect, reminiscent of yoghurt, develops. One of the functions of *Leuconostoc* spp. in mixed cultures is to reduce the acetaldehyde produced by *Lactococcus* to ethanol, which, at the concentrations found in cultures, has no effect on their flavour.

6.11 Exopolysaccharide Production

Many strains of all species of starter LAB produce exopolysaccharides (EPS) which are responsible for the thickening of yoghurt and several Scandinavian fermented milk products, e.g., Taette, Skyr and Villi. These give a ropy property to the product and a simple way to test for EPS producing cultures is to determine if long strands of coagulated milk can be pulled from milk-grown cultures using an inoculation loop; individual colonies can be tested in a similar manner. The ability to produce EPS is plasmid-encoded and EPS may be produced as capsules that are tightly associated with the producing cell or they may be liberated into the medium as a loose slime.

EPSs are divided into homopolymers, which are produced mainly by *Lc. mesenteroides* and heteropolymers, produced by the other species. Homopolymers are comprised of only one sugar, e.g., dextran is an α-1,6 linked glucose molecule, while heteropolymers comprise several sugars, most commonly, glucose, galactose and rhamnose in different ratios to each other and different linkages (α and β) depending on the strain.

As well as their use to improve the mouth-feel and creaminess of fermented milks, they have also been used to improve the texture of reduced-fat cheeses which are often rubbery. They do this by binding water and thus increasing the moisture in the non-fat substance of the cheese. One of the downsides of their use is that the EPS is also found in the whey and clogs the membranes used in further processing of the whey. For reviews on EPS of LAB see de Vuyst et al. (2001, 2011) and Hassan (2008).

6.12 Plasmids

Plasmids are extrachromosomal pieces of DNA which are much smaller than chromosomes; their molecular mass ranges from ~2 to 100 kDa while the chromosomes range from 1.8 to 2.5 Mb. Several of the commercially important properties of starters, including the genes encoding proteinase, transport of citrate, several of the proteins involved in transport and metabolism of lactose, exopolysaccharide production, bacteriocin production and resistance to phage are commonly encoded on plasmids. In some strains, the lactose plasmid encodes the enzymes of the tagatose pathway (galactose-6-phosphate isomerase, tagatose-6-phosphate kinase and tagatose-1,6-bisphosphate aldolase), *p*βgal and Enzyme I and Enzyme III of the PTS system,

Starter LAB can contain several plasmids which are easily lost on sub-culture and, once this occurs, the properties encoded by the genes on that plasmid are also lost. For this reason, sub-culturing of starters should be limited. Instead, several aliquots of the starter can be frozen. When required, an aliquot is thawed and, after sub-culturing it two or three times, it is discarded and replaced it with another frozen aliquot. Cells which lose the proteinase plasmid become proteinase negative (Prt^-) and consequently grow poorly in milk; those that lose the lactose plasmid are unable to metabolise lactose and therefore cannot grow in milk while those that lose the citrate plasmid are unable to transport citrate, even though they contain the enzymes necessary to metabolise it. In some strains of starters, the proteinase and lactose genes are encoded on the same plasmid but in other strains, two separate plasmids are involved. Prt^- strains are often isolated from mesophilic mixed-strain starters. In these cultures, Prt^- strains rely on Prt^+ strains to produce the amino acids and peptides required for growth. Conjugative plasmids (i.e., plasmids that can mediate their own transfer to other strains of the same species) have played a major role in our understanding of the genetics and metabolism of LAB; they have also proven to be useful in generating phage-resistant strains particularly of lactococci (see section on bacteriophage).

6.13 Genome Sequences

The first complete genome sequence of a lactic acid bacterium was that of *Lc. lactis* subsp. *lactis* IL 1403, a plasmid-free derivative of *Lc. lactis* IL594, which was isolated originally from a cheese starter (Bolotin et al. 2001). As of November, 2015, complete genome sequences are available for 15 strains of *Lc. lactis*, 13 strains of *Sc. thermophilus*, 4 strains of *Lb. delbrueckii* and 8 strains of *Lb. helveticus*, 1 strain

of *Leuconostoc citreum* and 4 strains of *Leuconostoc mesenteroides* (see www.genomesonline.org/ for details). In addition, the genomes of a representative of each of the eight lineages of the mixed culture was studied by Erkus et al. (2013), have been sequenced. NSLAB strains have also been completely sequenced but none of these strains were isolated from cheese. This is a huge amount of data and mining it should give a much greater understanding of the metabolism and role of these bacteria as starters and in cheese ripening.

The genomes are all single, circular chromosomes and are relatively small compared with other bacteria, ranging from about 1.8 Mb for *Lb. helveticus* to about 2.5 Mb for *Lc. lactis*. It has been suggested that this is due to the loss of many biosynthetic genes not necessary for growth in nutritionally-rich media like milk and cheese (Makarova et al. 2006). *Lc. lactis* KF147 was isolated from a plant source and is the largest of the genomes sequenced (Siezen et al. 2010). It has 98 % sequence similarity with that of *Lc. lactis* IL403, which is a cheese starter. Strain KF147 has many genes, the products of which allow it to grow on substrates derived from plant cell walls, including gene clusters that code for the degradation of complex plant polymers, such as xylan and arabinan, and the uptake and conversion of typical plant cell wall degradation products, such as α-galactosides, arabinose and xylose. Many of these activities are associated with a transposon (a DNA sequence that can change its position within the genome, sometimes creating or reversing mutations and altering the cell's genome size), which is lost spontaneously when the strain is grown in milk.

Comparison of the 4 *Sc. thermophilus* genomes revealed that they are very similar, except for 73 genes which encode transposase, glutamate decarboxylase, acetyltransferase, glycosyltransferase, polysaccharide biosynthesis protein, and EPS biosynthesis that are unique to strain ND03, which was isolated from fermented yak milk (Sun et al. 2011a). The *Lb. delbrueckii* genomes were also very similar, except for 416 genes, many of which are involved in EPS production that were unique to strain ND02, which was also isolated from fermented yak milk (Sun et al. 2011b). Some of the sequenced strains also contained prophage sequences.

6.14 Inhibition of Acid Production

Slow acid development during cheesemaking, where acid production does not occur as rapidly as it should, is an important cause of poor quality cheese. There are four causes of slow acid production: bacteriophage, the presence of antibiotics in the milk, bacteriocins and indigenous inhibitors. Of these, bacteriophage is the most important.

6.14.1 Bacteriophage

Bacteriophage, or phage are found in all environments where bacteria multiply and are particularly prevalent in industries, like the dairy industry, which rely on bacterial growth to manufacture products. Phage for *Lactococcus lactis* were first reported in New Zealand in 1935 and since then they have been described for all starter LAB. Infection of the starter culture by phage is the most common problem in the manufacture of cheese and fermented milks. It can significantly upset manufacturing schedules and, in extreme cases, can result in complete failure of the culture to produce acid resulting in 'dead' vats. Phage are viruses which can multiply only within a bacterial cell; they cannot multiply outside it. They are ubiquitous in nature, are found throughout the cheesemaking environment and are so small that they can be 'seen' only using an electron microscope. All have a head, which contains the nucleic acid, and a tail, which is composed of protein and which can be contractile or non-contractile. Many of them also can have a collar between the head and the tail, a base plate at the end of the tail, and fibres on the base plate but such structures are not found on all phage. All LAB phage contain DNA but phage for other bacteria may contain RNA.

Phage are divided into different families on the basis of their morphology, e.g., *Myoviridae*, which have a contractile tail, *Siphoviridae*, which have a long, non-contractile tail and *Podoviridae*, which have a short, non-contractile tail. The length of the tail varies from 20 to 500 nm. The majority of phage infecting LAB belong to the *Siphoviridae* family, three of which, 936, with a small isometric (spherical) head, c2, with a moderately elongated (prolate) head and P335, with a large isometric head, are particularly prevalent. Micrographs of examples of *Siphoviridae and Podoviridae* phage which attack *Lc. lactis* are shown in Fig. 6.15. Phage for *Lc. lactis* are divided into ten 'species' The most frequently isolated phage are those of the 936 type, and these have been found in cheese whey in countries as geographically apart as Australia, Argentina, the US, Ireland and Norway (Mahony et al. 2012a). Forty-five 936, ten P335 and two C2 phage have been sequenced. While there are localised regions of variability, the genomes are generally highly conserved (Mahony et al. 2014). All phage for *Sc. thermophilus* belong to the *Siphoviridae* family, with isometric heads and long, non-contractile tails.

Host range can be broad, where one phage attacks several strains of the same species, or narrow, where the phage attacks only one or two strains; phage do not cross the species barrier but they may cross the subspecies one. Host range is of considerable practical significance since starter cultures which are attacked by the same phage should not be used in culture rotations. Phage for *Sc. thermophilus* usually have a narrow host range.

Because different hosts have different phage sensitivities does not mean that the hosts are different strains. Ward et al. (2004) compared the sensitivity of 9 strains of *L. lactis*, 6 of which had been used as starter cultures, to 19 phage. The strains had identical PFGE patterns, implying that they were the same strain, even though all of them, except one, had been isolated from different sources. The nine strains were divided into six groups based on their phage sensitivities, with one strain being resistant to all 19 phage and three strains sensitive to all 19. The other strains showed varying degrees of sensitivity.

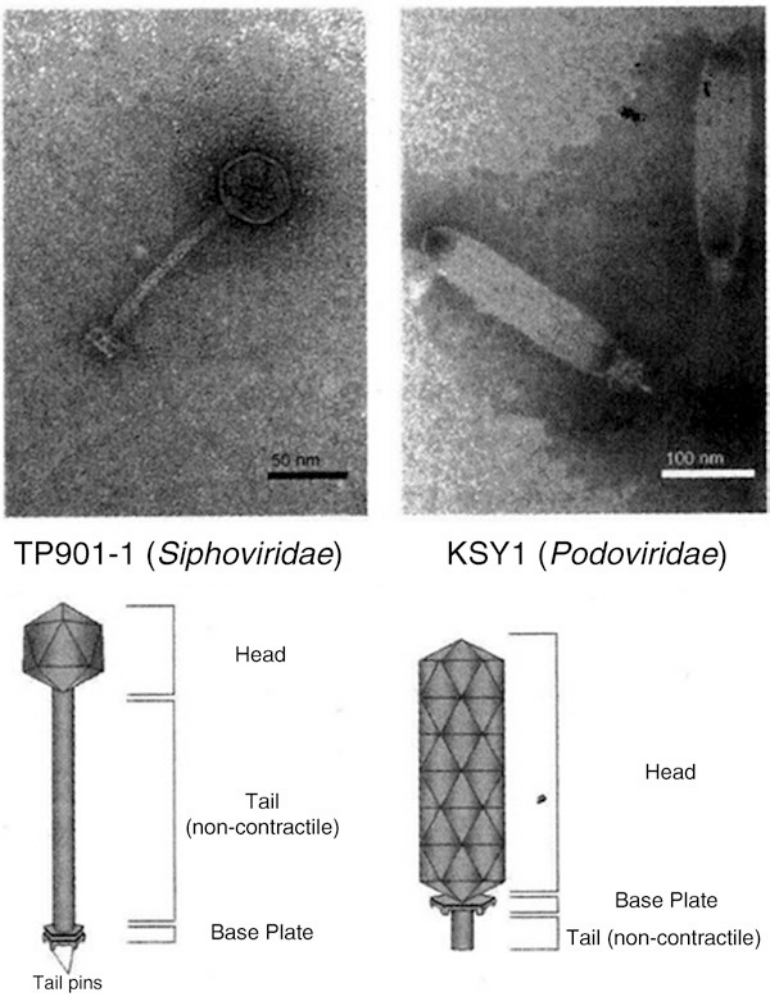


Fig. 6.15 Electron micrographs (upper panel) and schematic drawings (lower panel) of phage for *Lactococcus lactis*. The temperate phage TP901-1 is a member of the P335 species [isometric head and a long non-contractile tail (*Siphoviridae*)] while KSY1 is a virulent phage with a rare morphology [elongated head and short non-contractile tail (*Podoviridae*)]. (From Emond and Moineau 2007)

6.14.2 Detection of Phage

Phage are easily detected. They are much smaller than their hosts and are easily separated from them by filtration through a 0.45 μm filter. The host cells are retained by the filter while the phage particles are small enough to pass through the filter. A small volume (e.g., 0.1 mL) of a filter-sterilised (host-free) sample of

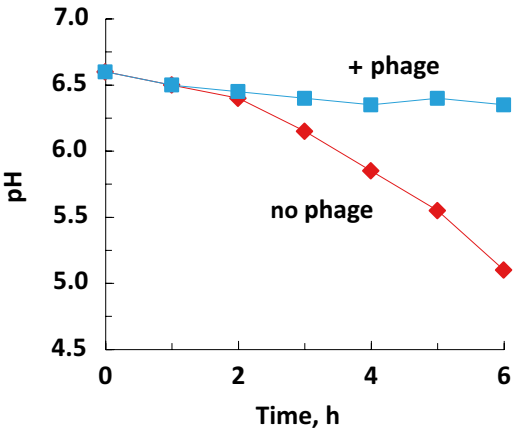


Fig. 6.16 Effect of phage on acid production by *Lc. lactis*

material suspected of containing phage, e.g., whey, is added to 10 mL of milk which has been inoculated with a susceptible host. After incubation at the optimum temperature of the host for ~6 h, the pH is measured. A difference of >0.2 units between the pH of the control, i.e., the host grown in the absence of phage, and the culture grown in the presence of the material suspected of containing phage indicates the presence of phage (Fig. 6.16). This method is qualitative and gives no indication of the number of phage present. However, it can be made semi-quantitative by testing several decimal dilutions (up to dilution 4 or 5) of the filter-sterilised sample. If inhibition is found at the higher dilutions it implies that high numbers of phage are present and *vice versa*. The decrease in pH can also be visualised by adding a suitable pH indicator (bromocresol purple) to the milk. In broths, measurement of the optical density (OD) at a suitable wavelength, e.g., 600 nm, is used. The OD increases for a short time as the cells grow but then decreases as the cells lyse.

The number of phage in a sample can be quantified relatively easily. The material suspected of containing phage is filter-sterilised and tenfold dilutions of the filtrate made. One mL of each dilution is mixed with 0.1 mL of the host culture (~10^7 cells) and 0.1 mL of 0.185 M Ca^{2+} in 2.5 mL of a suitable, molten, growth medium at 45 °C containing 0.7 % (w/v) of agar. The mixture is then poured immediately onto a pre-hardened plate of the same medium containing the normal amount of agar. After incubation at the optimum temperature of the host for several hours, clear zones, called plaques, are seen in the background lawn of bacterial growth if phage are present (Fig. 6.17). Each plaque is considered to have arisen from one phage and counting the number of plaques and multiplying by the dilution factor gives the number of plaque-forming units (pfu) per ml. Several PCR-based methods have also been developed, based on conserved regions of the DNA within a phage group or species but these methods are not used routinely in cheese factories.

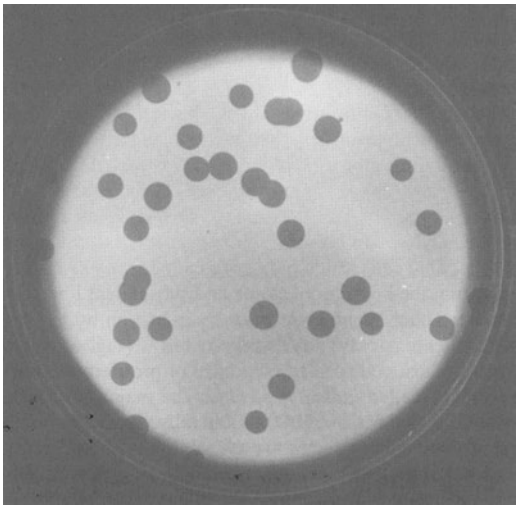


Fig. 6.17 Agar plates of a lawn of *Lactococcus lactis* cells on which clear zones (plaques) due to phage infection can be seen (*dark circles*). Each plaque is considered to result from the infection of a single cell with a single phage. The progeny phage subsequently multiply on neighbouring cells. (From Neve 1996)

6.14.3 Phage Multiplication

Phage multiplication occurs by either the lytic or lysogenic cycles. In the lytic cycle (Fig. 6.18), the first step involves adsorption of the phage onto special attachment sites, called phage receptors, on the surface of the host cell. The receptors are thought to comprise galactose-containing lipoteichoic acids, galactose/rhamose or galactose/glucuronic acid containing polysaccharides or cell wall proteins. This step normally requires Ca^{2+} to orient the base plate to face the cell but not all phage require it, e.g., the P335 phage, TP901-1 (Fig. 6.15). Chelation of Ca^{2+}, and the consequent prevention of phage adsorption, is the basis for the use of phosphate and citrate in phage-inhibitory media. Phage adsorb to the cell through their tail which may also have a base plate and spikes. Once a phage has attached to the receptors, it injects its DNA into the host cell. Immediately, phage DNA and phage proteins are produced rather than host DNA and host proteins. The phage DNA is packaged in a concentrated form in the phage head and when phage synthesis is completed, the cell lyses, releasing new phage particles, which start the process again. Lysis is caused by a lytic enzyme, called lysin, which is encoded in the phage DNA and which hydrolyses the cell wall of the host cell.

In the lysogenic cycle, adsorption and injection of DNA occur as in the lytic cycle but instead of phage multiplication, the phage DNA is inserted into the bacterial chromosome and multiplies with the chromosome (Fig. 6.18). Under these conditions, the phage is called a prophage, and the cells are considered to be lysogenised. Many mixed cultures contain lysogenic strains and have been used for decades without apparent problems. In the lysogenic state, the host cell is immune to attack by its own phage. Generally, the prophage also immunises the host cell to closely related strains of phage. This is called super-immunity. Lysogenic and lytic phage are also called temperate and virulent phage, respectively.

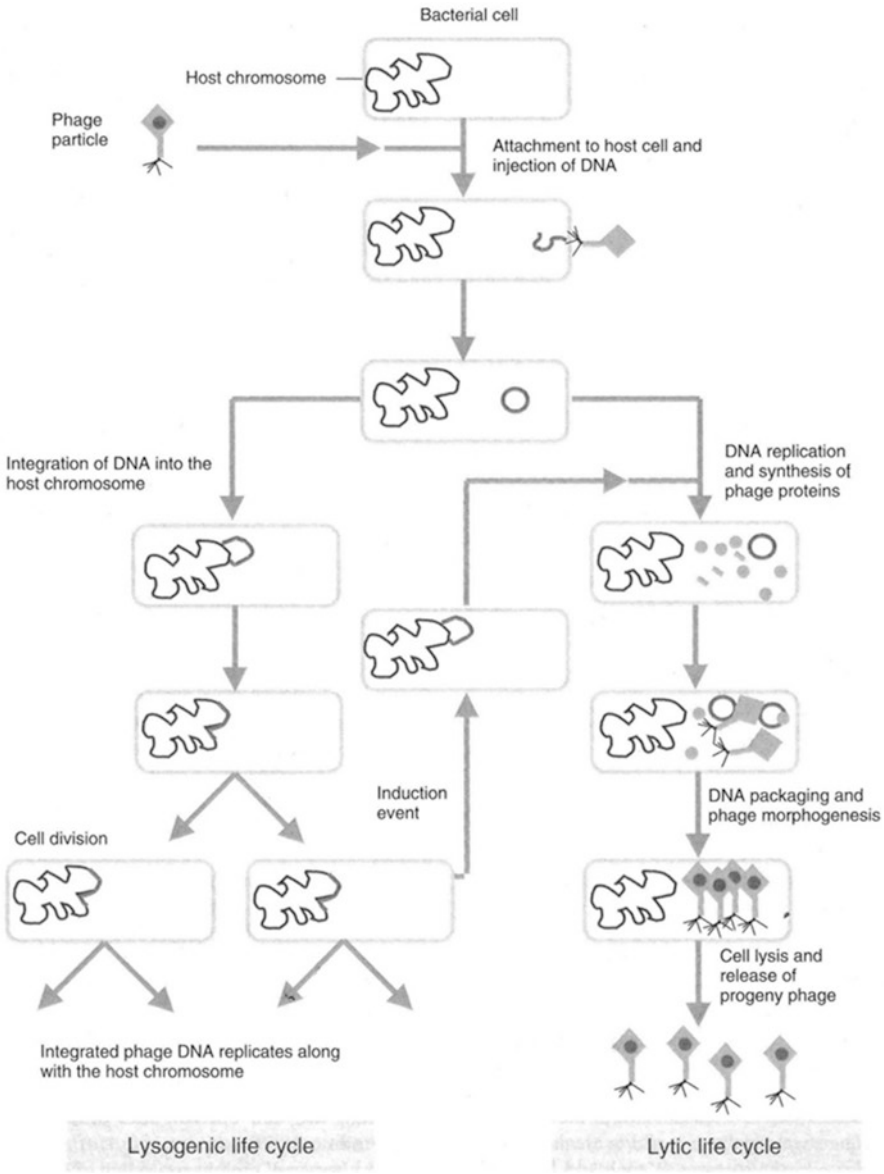


Fig. 6.18 Schematic drawing of the propagation of virulent (right branch) and temperate (left branch) bacteriophage in a host cell. The cell is shown with its chromosomal DNA. The phage DNA is indicated by dotted lines. (From McGrath and van Sinderen 2007)

Under certain circumstances, some temperate phage can be induced, become lytic and multiply. The host cells on which these phage multiply are called indicator strains. The conditions which cause induction in commercial practice are unclear but, in the laboratory, UV light, H_2O_2 and the antibiotic, mitomycin C, are used. It has been suggested that autolysis of lysogenic starters by prophage-encoded endolysins can release intracellular enzymes, which could improve the flavour of cheese during ripening.

The growth of virulent phage on a sensitive host is characterised by a latent period and a burst size, which are determined in one-step growth experiments (Fig. 6.19). For this, phage and whole cells are mixed so that the ratio of cells to phage (the multiplicity of infection) is low and the number of phage is monitored periodically during incubation. At the beginning, the number of phage remains low since new phage are being synthesised inside the cell and have not been released. This is called the latent period and spans the time from the initial adsorption of the phage to the host cell to the detection of phage progeny after cell lysis. The sudden increase in the number of phage is called the burst size and is caused by lysis of the host cells by phage lysin. For lactococcal phage, the latent period varies from 10 to 140 min and burst size from 10 to 300 phage. Compared to starters, phage multiplication is very rapid. Assuming a latent period of 1 h and a burst size 150, one phage will result in production of 22,500 phage (150×150) in a little over 2 h. In 3 h, the number of phage would increase to $3.4 \times 10^{6.}$ In 3 h, a *Lactococcus* cell would multiply three times, producing only 8 cells. Thus, the phage will vastly outnumber the bacterial cells very quickly. This clearly indicates the problems which occur following contamination with phage. Examples of phage multiplication in the vat during Cheddar cheesemaking are shown in Fig. 6.20. The initial levels were very low (10–100 pfu/mL) but these had multiplied to 100,000,000 pfu/mL within 4 h.

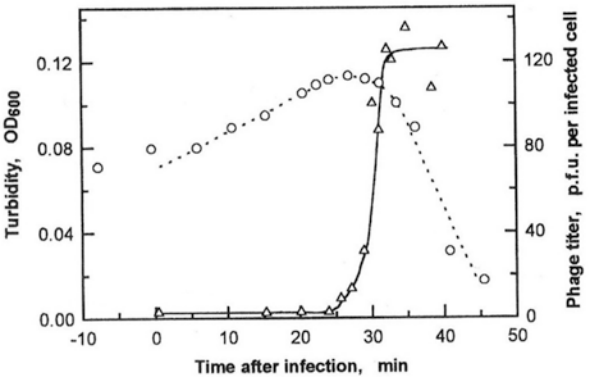


Fig. 6.19 Results of a one-step growth experiment on *Lactococcus lactis* infected with a lytic phage. The release of progeny phage (*open triangle*) begins 25 min (latent period) after infection (burst size: 124). The increase of free phage is accompanied by a decrease of culture turbidity (*open circle*) due to cell lysis. (From Neve 1996)

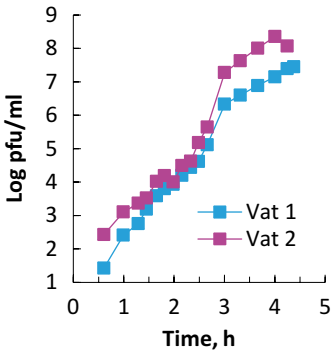


Fig. 6.20 Phage multiplication during cheese manufacture in two cheese vats. (From Pearce et al. 1970)

6.14.4 Pseudolysogeny

Many mixed-strain starters are permanently infected with a low number of virulent phage. These are called 'own' phage to distinguish them from lytic or 'disturbing' phage and they multiply on a phage-sensitive strain(s) present in the culture. Growth and acid production by the mixed culture is not affected by 'own' phage due to the presence of large numbers of acid-producing, phage-insensitive cells. This phenomenon is called pseudolysogeny and the phage insensitivity of the whole culture is stable as long as no infection with disturbing phage occurs.

6.14.5 Phage-Resistance Mechanisms

Several phage-resistance mechanisms, including inhibition of phage adsorption, restriction/modification and abortive infection mechanisms are found in LAB, all of which are commonly encoded on plasmids.

In adsorption inhibition, the receptor sites for the phage on the cell surface are masked so that the phage cannot attach to the cell and, therefore, no phage multiplication occurs. In many starters, adsorption inhibition has been shown to be plasmid-encoded.

Several distinct restriction-modification (R/M) systems have been identified in starter bacteria. They generally involve two enzymes, one of which, the restriction enzyme or endonuclease, hydrolyses the phage DNA. The other, the modification enzyme, also called methyl transferase, modifies the same DNA sequence in the host, usually by methylation of cytosine, so that the restriction enzyme cannot hydrolyse it. This mechanism operates only after adsorption and injection of phage DNA and can result in a reduction in phage numbers of 4–6 log cycles.

Abortive infection (abi) is the term used for phage-resistance mechanisms which involve neither inhibition of adsorption nor R/M systems. Generally, a total loss of the ability to form plaques or a reduction in plaque size occurs. This is generally due to a reduction in both the latent period and burst size but in some cases the effect is on only

one of these. At least 20 different types of abortive infection have been reported and their modes of action are variable with some interfering with phage DNA replication, and others with RNA transcription, or reduction in the capsid protein of the phage.

The CRISPR/Cas system, is a relatively new anti-phage defence system, which was first identified in *Escherichia coli*; it has also been found in many starter bacteria, including *Sc. thermophilus*, *Lb. helveticus* and *Lb. delbrueckii*. This is a type of immune system and is due to the presence of clustered regularly interspaced palindromic repeat (CRISPR) loci, derived mainly from extrachromosomal DNA (either phage or plasmids), situated between other palindromic repeats on the cell chromosome. The transcripts produced are cleaved by CRISPR-associated (Cas) proteins which cleave the invading DNA in a sequence specific manner. This is a highly efficient system and may be one of the main reasons for the isolation of bacteriophage-insensitive mutants (BIMs) from starters.

6.14.6 Bacteriophage-Insensitive Mutants

BIMs or phage-insensitive mutants (PIMs) of starters can be isolated relatively easily by plating the host in the presence of phage. After incubation, the phage-resistant colonies are checked for their ability to resist the particular phage used in their isolation and produce sufficient acid in milk; acid production is often reduced in BIMs compared with the parent strain, negating the use of these particular BIMs as starters. Suitable acid-producing BIMs are then used as replacement strains for their phage-sensitive parents. Isolation of BIMs forms part of starter control systems in modern cheese factories.

In addition, some strains of LAB contain phage-resistance plasmids, many of which are conjugative and can be used to improve the phage resistance of phage-sensitive, commercial cultures. The technique is relatively simple (Fig. 6.21); lac^- mutants of the strain harbouring the phage-resistance plasmid are isolated (lac^- $phage^r$) and mixed with lac^+ $phage^s$ recipients. Lac^+ $phage^r$ transconjugants are selected in the presence of excess virulent phage on lactose agar, which contains a dye to indicate acid production. The lac^- $phage^r$ cells do not grow very well on this medium and the lac^+ $phage^s$ cells are destroyed by the phage present in the agar. The lac^+ $phage^r$ transconjugants or BIMs are then isolated and checked for their ability to produce acid rapidly and for the presence of the phage-resistance plasmid. This is a totally natural method which does not involve genetic engineering techniques. It maintains the food-grade status of the recipient strains and is used to produce phage-resistant strains for commercial use.

6.14.7 Source of Phage

To control phage it is important to identify their source. The most likely source is the starters themselves. This conclusion is supported by several lines of evidence. Lysogenic phage are the source of lytic phage for the strain of *Lactobacillus casei*

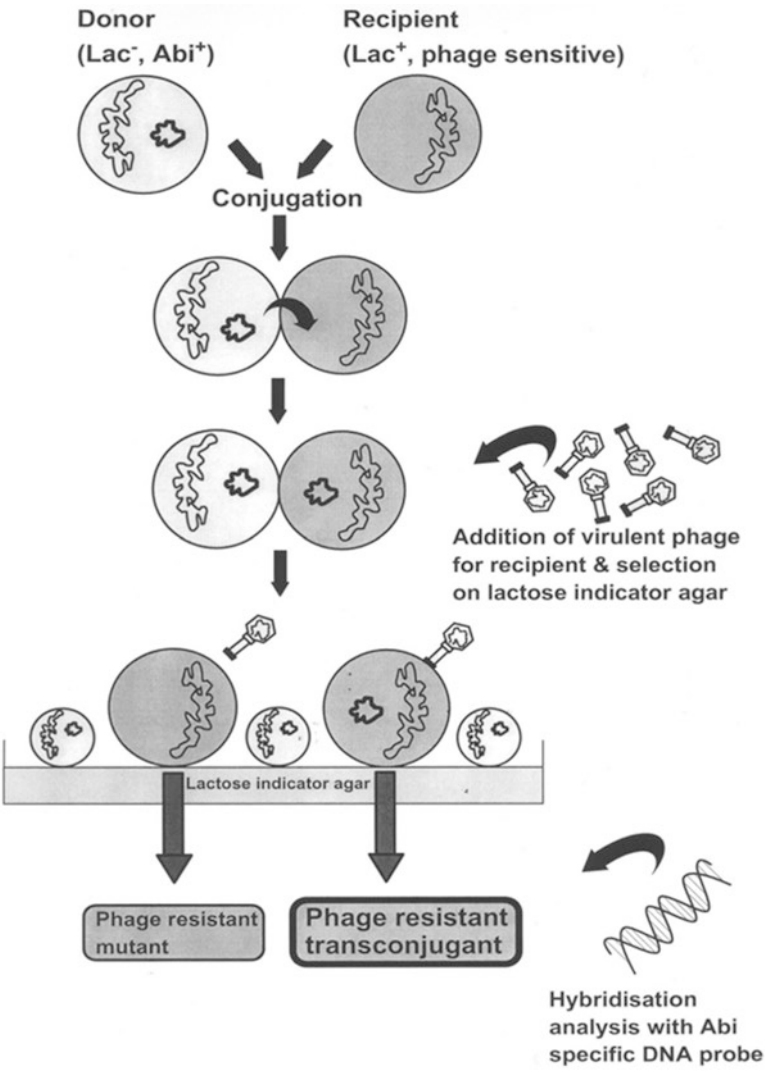


Fig. 6.21 Schematic drawing of a genetic strategy for the construction of phage-insensitive starter cultures by conjugation. A phage-resistance plasmid with an abortive infection determinant is transferred from a donor lacking the ability to metabolise lactose (Lac^-) to a recipient culture that can metabolise lactose (Lac^+). Phage-resistant transconjugants are selected on lactose indicator agar in the presence of the virulent phage. Under these conditions, the Lac^- donor cells do not grow well. Finally, a hybridization experiment is carried out to distinguish true transconjugants from phage-resistant mutants. (Modified from Klaenhammer 1989)

used in the production of Yakult (Shimizu-Kadota et al. 1983). The genome of *Lc. lactis* MG1363 contains two complete prophages and four degenerate ones and the genome of *Lc. lactis* IL1403 contains three complete prophages and three redundant ones; the latter lack most of the genes encoding the structural elements of the phage. Moreover, a P335 prophage from *Lc. lactis* can be induced from the genome and become a lytic phage (Kelly et al. 2013).

Mixed-strain starters also contain phage but they are considered to be less vulnerable to phage attack than defined-strain starters. Erkus et al. (2013) obtained direct evidence for the disappearance and reappearance of phage-sensitive strains within the different lineages of *Lc. lactis* during daily sub-culturing of a mixed culture which is used extensively in The Netherlands for Gouda cheese manufacture. This culture contained seven distinct genetic lineages of *Lc. lactis* (see above) and there was considerable phage sensitivity within and between them. The supernatant of this culture contained 10^6, 10^4 and 10^2 plaque-forming units/mL, when assayed against the sequenced representatives of lineages 1, 5 and 7, respectively. No phage was found for representatives of the other four lactococcal lineages, indicating that these lineages still function in acid production in the culture. Two to four other representatives of lineages 1 and 5 were also sensitive to their respective phage, but showed differences in levels of 4–7 orders of magnitude. The sequenced strain of lineage 7 was the only strain of its lineage tested, which was sensitive to phage. These results imply that phage sensitivity of strains in a mixed culture is a dynamic process where phage eradicate sensitive cells but do not eradicate entire lineages. The highly diverse phage resistance of the different lactococcal lineages goes a long way towards explaining the functional stability of the culture in cheese manufacture. These variations in phage sensitivity are the likely reason that mixed cultures are more resistant to phage than defined cultures.

A simplified culture that contained only a single representative of each of the lineages was made and used for cheese production. Similar aroma profiles were found in this cheese and in cheese made with the original culture, implying that mixed cultures can be constructed, provided full knowledge of the composition of the original cultures is available. However, such reconstructed cultures would probably be more sensitive to phage, since the intrinsic diversity in phage resistance of the different lineages is missing from the reconstituted culture.

In addition, raw milk, whey and air are important sources of phage in cheese factories. The phage level in raw milk can range from 0 to 30,000 pfu/mL. This implies that the host strain must also be present in the milk or the milking environment. Even numbers at the lower end of this range can quickly result in high numbers of phage, especially if the phage has a short latent period and a high burst size. Pasteurisation of milk does not inactivate phage. Many of the bacteria in NCWs contain lysogenic phage which may be a source of phage in cheese factories.

Phage multiply very rapidly during cheesemaking, quickly reaching levels of ~8 log pfu/mL (Fig. 6.20). Thus, whey is a very potent source of phage and should be handled with considerable care. Particular attention should be paid to dispersal of phage in the air through aerosol formation during centrifugation of whey. Phage levels of 10^5 pfu/m^3 of air have been reported. In addition, phage have been found on floors, walls, cleaning materials, pipes and other pieces of equipment, door handles and office tables. These findings imply that phage are ubiquitous in the factory environment.

6.14.8 Control of Phage

Starters are often produced in large volumes; a 500 L tank of starter contains ~5×10^{14} cells. One phage getting into such a tank could result in significant or total loss of the ability to produce lactic acid. Probably the most important factor in producing good quality cheese is to insure that the bulk starter is free from phage; this point cannot be over-stressed but it is often overlooked in commercial practice. Therefore, in well-run cheese factories, phage levels in starters and cheese whey are determined daily.

Phage are quite resistant to heat; some can withstand heating at 75 °C for several minutes. Hence the medium, usually 10–12 % reconstituted skim milk (RSM), used for growing starters must be heat-treated at a high temperature, e.g., 85 °C for 30 min, to inactivate any potential phage which might be present. The RSM should be heated in the same tank in which the culture is grown. Some cheese manufacturers heat-treat the milk in a pasteuriser and then fill the starter tank with the heated milk. This practice is not to be recommended because of the danger of phage being present in an otherwise clean starter tank. Because of the large burst size of phage, phage multiplication from as little as one phage in a tank is sufficient to cause significant reduction in the ability of a culture to produce lactic acid subsequently (also called its activity). The heat treatment also improves the nutritional value of the milk because it inactivates the indigenous inhibitors and causes slight hydrolysis of the proteins to peptides. Inoculation of the starter medium should be done as aseptically as possible and an aseptic inoculation device has been developed and is commonly used in the Netherlands for starter production (Fig. 6.22). During cooling, the air entering the starter tank should be filtered through a high efficiency particulate air (HEPA) filter to prevent phage from the air and whey aerosols entering the starter tank. In addition, a slight positive air pressure should be maintained in the starter tank during growth to prevent transfer of airborne phage into the starter tank during incubation.

Because of the importance of producing phage-free starter, the starter room should be physically separated from the cheesemaking and whey processing areas; the latter should be located preferably downwind of the cheese factory. The starter room should be “off limits” to all personnel except those involved in producing the starter.

Phage are quite heat resistant; D values (or time necessary to kill 90 % of them) of up to 16 min at 75 °C and z values (temperature necessary to reduce the D value tenfold) have been reported. Therefore, bulk cultures contaminated with phage should be heat-treated before discarding them to prevent the spread of phage in the factory environment. Treatment in excess of 90 °C for 45 min is recommended to ensure total destruction of every phage particle.

The greater the number of strains used in cheesemaking, the greater is the likelihood of a phage attack. Therefore, the use of a limited number of cultures and the rotation of phage-unrelated, defined-strain cultures have been advocated for a long time as useful factors in the control of phage. The idea behind this recommendation is that if a phage outbreak occurs, acid production would be continued by the other

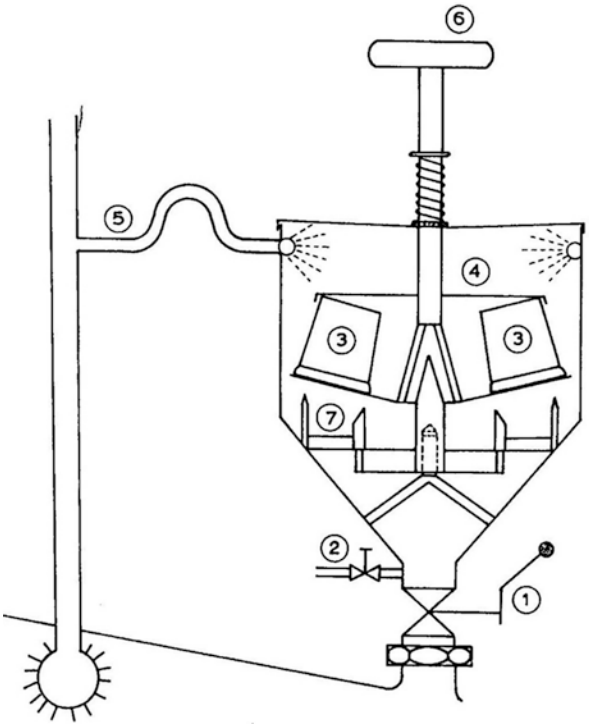


Fig. 6.22 An aseptic inoculation device for starter tanks. The device is opened using the screw (6). Cartons (3) containing the frozen inoculum are inverted and placed on the holder (4). The device is assembled and filled with chlorinated water through the pipe (5). The chlorination inactivates any contaminants on the outside of the carton and also helps to thaw the culture; as soon as this occurs, the chlorinated water is run to waste through the valve (2). The screw (6) is then turned, causing the prongs (7) to puncture the foil lid of the carton. The cartons empty and their contents (the inoculum) are added to the starter tank through the valve (1). Any residual chlorine has no effect on the starter as it will be diluted and inactivated on contact with the fluid in which the starter cells are suspended

strains in the rotation. This requires daily monitoring of the starter and the cheese whey for phage. When a phage attack occurs, the isolation of BIMs of the offending strain, after testing their acid and flavour producing abilities, is also useful.

The use of defined-strain rather than mixed cultures is generally recommended primarily because the phage relationships of defined cultures are known. Despite this, large amounts of Dutch cheese are still made with mixed cultures containing lysogenic strains, without any apparent problems. A mixed culture, TK5, was used continuously for 11 years to make Cheddar cheese in Denmark before it succumbed to attack by phage (Josephsen et al. 1999).

Addition of rennet as soon as possible after the starter has been added to the milk in the cheese vat also helps because the rennet coagulum will physically separate phage-infected cells from non-infected cells and the phage are unable to penetrate the curd to locate non-infected cells.

The use of closed vats to prevent contamination from whey aerosols is also recommended.

As little as 1 mL of residual whey in a cheese vat can be a potent source of lytic phage. Therefore, cheese vats should be cleaned routinely and sanitised between fills. Hypochlorite and peracetic acid (PAA) are effective, cheap phagicides and the final step in cleaning equipment, such as vats and filling lines, should include a sanitisation step. Exposure to 100 µg per mL of 'available' or 'active' chlorine for 10 min is usually sufficient to inactivate all phage on equipment surfaces while 0.15 % PAA has been shown to inactivate 10^6 pfu/mL of *Lb. helveticus* and *Sc. thermophilus* phage in 5 min at 40 °C (Quiberoni et al. 1999; Binetti and Reinheimer 2000). The residual sanitiser should not be rinsed from the equipment to prevent further contamination with phage from hoses or water. Any residual chlorine on the equipment is immediately inactivated when it comes in contact with organic material like milk, while PAA is converted to acetic acid and H_2O_2 in dilute solution. Care is required in the use of PAA since some lactic cultures are inhibited by 0.2 µg of PAA per ml of milk. Some phage can adapt to chemicals and it is now recommended that hypochorite and PAA should be rotated in cheese plants (Jenny Mahony, personal communication).

For further information on the various aspects of phage see the reviews of McGrath et al. (2004); Emond and Moineau (2007), Garneau and Moineau (2011); Briggiler et al. (2012) and Mahony et al. (2012, 2014).

6.14.9 Antibiotics

In the past, antibiotic residues were a major cause of slow acid production in cheese manufacture but, nowadays, with the availability of simple and sensitive tests for the detection of antibiotic residues in milk and better education of farmers, problems due to antibiotic residues in milk are rare.

Antibiotic residues occur in milk because of their use to control mastitis in dairy cows (see Chap. 5). An effective way of curing mastitis is to infuse the cow's udder with antibiotics, especially penicillin or its derivatives. This results in contamination of the milk with antibiotics. The concentration of antibiotic in the milk decreases with each milking and generally all the antibiotic will be excreted within 72 h, depending on the preparation used. Milk from cows treated with antibiotics should be withheld for the length of time prescribed for the antibiotic preparation. The effect of different antibiotics on acid production by *Sc. thermophilus* BC, which has been used to detect penicillin residues in milk, is shown in Fig. 6.23. It is obvious that each antibiotic is inhibitory over a very narrow range of concentrations and that this strain is very sensitive to penicillin and fairly resistant to streptomycin. The sensitivity of different starter cultures to several antibiotics used in mastitis treatment is summarised in Table 6.5. Generally, thermophilic cultures are much more sensitive to penicillin and more resistant to streptomycin than are mesophilic cultures.

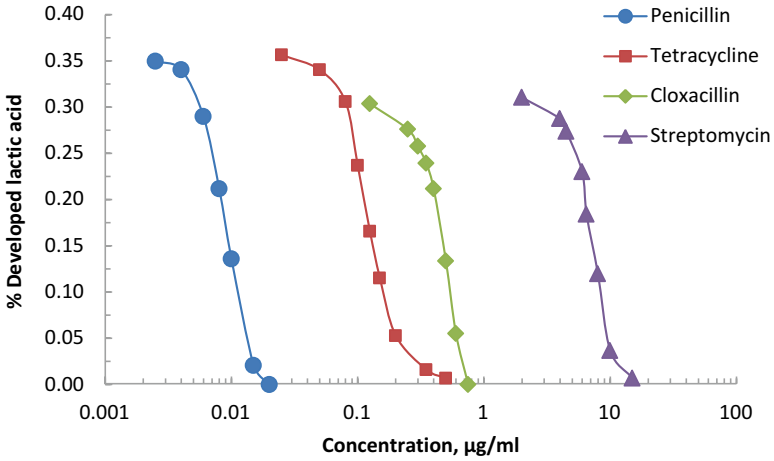


Fig. 6.23 Dose response curves of *Sc. thermophilus* BC to various antibiotics in milk (from Cogan 1972)

Table 6.5 Concentration (µg/mL ± sd) of some antibiotics that cause 50 % inhibition of growth of starter bacteria in milk[a]

		Antiboitic			
Organism	No. of strains	Penicillin	Cloxacillin	Tetracycline	Streptomycin
Lc. lactic subsp. *cremoris*	4	0.11 ± 0.028	1.69 ± 0.38	0.14 ± 0.02	0.67 ± 0.15
Lc. lactis subsp. *lactis*	4	0.12 ± 0.025	2.16 ± 0.41	0.15 ± 0.05	0.53 ± 0.18
Sc. thermophilus	3	0.01 ± 0.002	0.42 ± 0.07	0.19 ± 0.06	10.5 ± 0.29
Lb. delbrueckii subsp. *bulgaricus*	2	0.03 ± 0.006	0.29 ± 0.04	0.37 ± 0.04	3.0 ± 2.0
Lb. delbrueckii subsp. *lactis*	1	0.024	0.24	0.6	2.29

[a]From Cogan (1972)

6.14.10 Lactenins

Milk contains indigenous inhibitors, called lactenins, but they inhibit only a few strains of starter bacteria. The lactenins have been identified as immunoglobulins and lactoperoxidase (LP). The immunoglobulins cause susceptible starter bacteria to aggregate. This causes localised acid production and precipitation and, in severe cases, the aggregates settle on the bottom of the cheese vat. The starter still continues to grow but localised acid production is so great that they eventually inhibit themselves. Immunoglobulins are denatured by pasteurisation.

LP requires H_2O_2 and thiocyanate (SCN^-) for activity. All three components are required together to inhibit the growth of starters. SCN^- is normally present in milk and the concentration is higher in milk from cows which are fed *Brassica* (cabbage and kale) while the H_2O_2 can be produced by the starter bacteria during growth or through xanthine oxidase or glucose oxidase activity. The actual inhibitor has not been identified but is thought to be $OSCN^-$. LP is heat resistant and is not inactivated by HTST pasteurisation; it is inactivated by heating to 80 °C for a few seconds, i.e., so-called flash pasteurisation. Inhibition of starters by lactenins is unusual in modern cheesemaking because the strains used are selected so that they are not affected to any great extent by lactenins.

6.15 Bacteriocins

Bacteriocins are generally small peptides (20–60 amino acid residues), produced by many bacteria, which inhibit the growth of other bacteria. They have either a narrow or a wide spectrum of activity, depending on whether they inhibit only the growth of closely related bacteria or several different, unrelated bacteria, respectively. Generally, bacteriocins produced by Gram-positive bacteria do not inhibit Gram-negative bacteria and *vice versa*. Bacteriocin production is encoded either on the chromosome or on plasmids and the producing bacteria must also have a gene which encodes immunity to the bacteriocin. Their peptide nature and their relatively narrow host range distinguish them from antibiotics.

Bacteriocin production by LAB is common. In the past decade, food safety has become a major issue and a concerted effort has been made to identify bacteriocins which inhibit pathogens and food spoilage organisms. LAB are ideal for this purpose because they are generally regarded as safe (GRAS) organisms. For use in foods, the bacteriocin should have the following properties: resistance to heat, resistantce to potential proteinases which may be present in the food, active over a prolonged period, active over the pH of food (4.5–7.0), have a bactericidal rather than a bacteriostatic mode of action, and have a broad host range, inhibiting several pathogens and spoilage microorganisms.

The best known bacteriocin is nisin, which is produced by some strains of *Lc. lactis* and is commercially available as Nisaplin. It was first identified in the 1950s and is used in >50 countries to prevent the germination, growth and gas production of *Cl. tyrobutyricum* and *Cl. butyricum* in processed cheese products. These organisms produce gas and butyric acid, through their fermentation of lactate:

$$2\,\text{Lactate} \rightarrow 1\,\text{Butyrate} + 2\,CO_2 + 2\,H_2$$

The gas produces large, deformed eyes and the butyric acid gives the cheese a pronounced rancid off-flavour. Nisin has a MW of 3353 Da, contains 34 amino acids and normally occurs as dimers and tetramers. It is synthesised as a 57 amino acid peptide which is post-translationally processed to give a 34 amino acid peptide

containing several unusual amino acids, e.g., lanthionine (Ala-S-Ala) and β-methylanthionine [Ala-S-Aba (aminobutyric acid)], dehydroalanine and dehydrobutyrine. The presence of lanthionine and β-methylanthionine in its structure (Fig. 6.24) is the reason that nisin is called a lantibiotic. It is soluble only at low pH which reduces its potential use significantly. Its heat stability depends very much on pH, e.g., it remains stable to autoclaving at 115 °C at pH 2 but loses 40 % of its activity at pH 5. Nisin has a broad spectrum of activity, inhibiting *Bacillus*, *Clostridium*, *Staphylococcus*, *Listeria* and *Streptococcus* spp. Different forms of nisin are produced, e.g., Nisin Z differs from Nisin A in having asparagine instead of histidine at position 27. Because of its greater solubility, nisin Z also has a greater inhibitory activity than nisin A.

Starter bacteria also produce other bacteriocins, e.g., *Lc. lactis* produces Lacticin 3147, Lacticin 481 and Lactococcin G and Q, *Sc. thermophilus* produces Thermophilins 13, 110, 1277a and T, *Lb. delbrueckii* produces bacteriocin UO004, *Lb. helveticus* produces Helveticin J and Lactocin 27 and *Enterococcus* spp. produces various Enterocins. NSLAB also produce bacteriocins e.g., *Lb. casei* produces Lacticin 705 and *Lb. plantarum* produces several Plantaricins.

Bacteriocins of LAB are conveniently divided into three classes. Class 1 are lantibiotics, which involve post translational modification of the peptide(s) to produce the unusual amino acids, lanthionine, β-methylanthionine, dehydroalanine and/or dehydrobutyrine; they can comprise one peptide (e.g., Lactocin 481) or two peptides (Lacticin 3147). Class II bacteriocins are small, heat-stable, anti-listerial unmodified peptides, which are subdivided into Classes IIa, IIb, IIc and IId. Class IIa are anti-listerial, Pediocin-like bacteriocins, of which Pediocin PA-1, Enterocin A and Plantaricins C29 and 423 are examples; class IIb are non-modified two-peptide bacteriocins, e.g., Plantaricins, Thermophilin 13 and Lactococcins G and Q; class IIc bacteriocins consist of a small number of cyclic bacteriocins whose N- and C-termini are covalently linked, e.g., Enterocin AS-48, and Class IId bacteriocins consist of one peptide, non-cyclic bacteriocins which show no similarity to the Pediocin-like bacteriocins. Class III bacteriocins contain heat-labile, large peptides, e.g., Helveticin J. The lanthionine bacteriocins have the most complicated structures and examples of the best studied ones are shown in Fig. 6.24, where the post-translationally modified amino acids are colour-coded.

The role of bacteriocins in different aspects of cheese production has been studied. Bacteriocins increase the lysis of the starter bacteria and inhibit the growth of NSLAB during cheese ripening, resulting in better flavoured cheese (see Chap. 11). Many of them also inhibit the growth of pathogens, particularly *Listeria monocytogenes* in smear-ripened cheese (see Chap. 20). One aspect that does not appear to have been studied is the effect that bacteriocin production by starters has on other strains present in mixed cultures, especially NWCs. Presumably, they would reduce the number of strains in these cultures on sub-culture, eventually leading to a mixture of perhaps 1 or 2 strains and a culture more prone to attack by phage because of the reduction in the number of strains.

It is difficult to compare the reported host ranges of bacteriocin producers because different methods and, more importantly, different strains have been used.

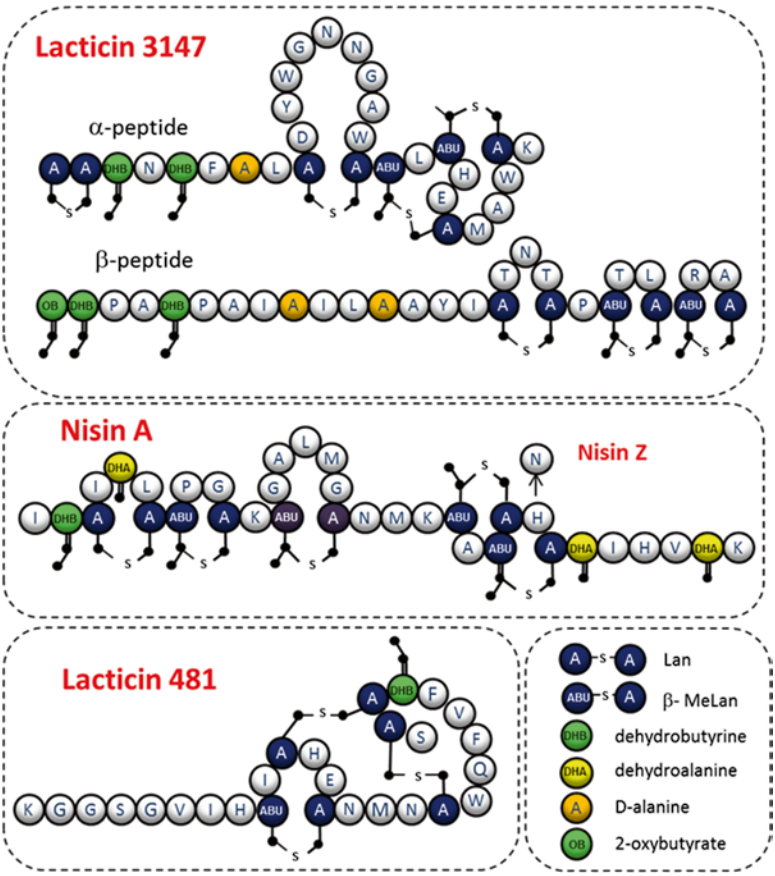


Fig. 6.24 Structures of 4 common lantibiotics. The unusual amino acids produced post-translationally are colour-coded (Colin Hill, personal communication)

Most bacteriocins produced by LAB have a bactericidal mode of action. They act by creating pores in the cell membrane of sensitive cells, destroying the proton-motive force and permitting the release of intracellular, cytoplasmic components from the cell. Lactocin 27, produced by *Lb. helveticus*, is an exception and is bacteriostatic. In the case of spores, Nisin acts by preventing spore germination.

Some useful reviews of different aspects of the bacteriocins of LAB include those of Cotter et al. (2005), mainly on Classe I and II bacteriocins, Drider et al. (2006) on the Class IIa bacteriocins, Twomey et al. (2002) and Dufour et al. (2007) on the lantibiotics, Gálvez et al. (2008), on the application of bacteriocins to control pathogens and spoilage bacteria in foods, Khan et al. (2010) on the use of enterocins in food preservation and Nissen-Meyer et al. (2010) on the structure of the Class IIb group of bacteriocins.

6.16 Production of Bulk Cultures in Cheese Plants

The inocula for bulk cultures are generally obtained from specialised laboratories where the starters are grown under optimum conditions (e.g., pH 6.3 and 28 °C in the case of the lactococci) in a proprietary medium. After growth, the cells are harvested by ultrafiltration or centrifugation, frozen in liquid N or freeze-dried in sufficient volumes to inoculate 300, 500 or 1000 L of bulk culture medium directly. Such cultures generally can contain up to 10 times more cells than a normal RSM-grown culture. Cryoprotectants, e.g., glycerol, sucrose or monosodium glutamate, are often added to protect the cells from sub-lethal stress during freezing or freeze-drying.

Until perhaps 50 years ago, milk was the medium used for bulk culture production in cheese factories. The milk was selected from cows which showed no evidence of disease, especially mastitis and so was free of antibiotics, which would inhibit growth and acid production. Milk was replaced by spray-dried skim milk powder, when it became more commonly available, at a solids level of 10 % (w/v). Today, phage-inhibitory media have replaced skim milk powder for the production of bulk culture, especially in the US. These are generally carefully formulated proprietary media which contain milk or whey solids, phosphate and/or citrate to chelate the Ca^{2+}, necessary to attach the phage tail to its host cell and yeast extract as a source of nutrients. Haem may be added to prolong the activity of the cultures during refrigerated storage (Fig. 6.13).

The medium is generally heated at ≥85 °C for 30 min in the tank in which the starter is to be grown (to inactivate any phage which might be present) and cooled to the incubation temperature of ~42 °C in the case of thermophilic cultures and 21 °C for mesophilic cultures. It is then inoculated with ~1 % (v/v) of the culture and after incubation for 8–10 h, in the case of thermophilic cultures, or overnight (16 h) in the case of mesophilic cultures, the culture is fully grown. Bulk starter cultures generally contain $\sim 1 \times 10^9$ cfu/mL. Thermophilic cultures are usually grown at their optimum temperature of ~42 °C, but mesophilic cultures are grown at 21 °C, which is about ~9 °C below their optimum temperature. The reason for the lower temperature of incubation in the case of mesophilic cultures is that if the milk is inoculated at, say, 3 pm the previous evening, the cultures are fully grown 16 h later, i.e., 7 am the following morning, which would traditionally be the beginning of the cheese-making day in small cheese plants.

In the past, the inoculum for bulk cultures was built up progressively from a small volume of mother culture to larger volumes over several days. An example for mesophilic cultures is shown in Fig. 6.25. Because of the availability of cultures which can be added to the bulk culture medium directly, this method is not commonly used to-day. Minimal sub-culturing of starters is desirable to prevent loss of plasmids and maintain the balance between strains in mixed cultures.

Fully grown cultures of *Lactococcus* and *Sc. thermophilus* generally reduce the pH of milk from its initial value of 6.6 to 4.6 and produce ~0.65 % w/v (70 mM) lactic acid while many thermophilic lactobacilli can produce up to 2 %, w/v (200 mM) lactic acid and reduce the pH to ~3.0. The actual amount of acid produced depends on the buffering capacity of the medium. However, the lactobacilli are usually grown to a pH of only ~ 4.0 (equivalent to ~1 %, w/v, lactic acid).

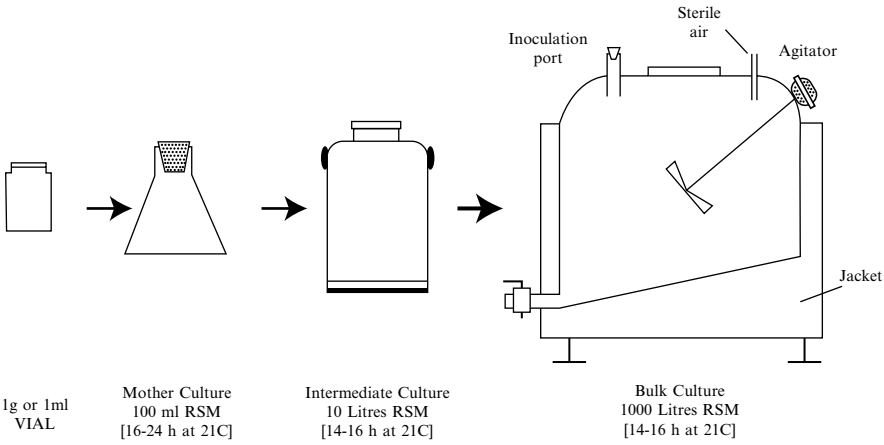


Fig. 6.25 A possible protocol for building up intermediate cultures for bulk production of starters in cheese factories

Cultures are then generally cooled to 4 or 10 °C, in the case of mesophilic and thermophilic cultures, respectively, and checked for their activity, i.e., their ability to produce lactic acid. This is normally done by measuring acid production or the decrease in pH of the culture grown under standardised conditions, e.g., in 10 % (w/v) RSM after 6 h incubation in a water bath at 30 °C for mesophiles or 5 h at 40 °C for thermophiles using a standardised inoculum, generally 1 % (v/v). For day-to-day comparisons between cultures, it is important to standardise the inoculum and the incubation conditions very carefully. Under these inoculation and incubation conditions starter cultures will reduce the pH from 6.6 to <5.3 (equivalent to ~0.5 %, w/v, lactic acid). The starter may also be checked for phage by comparing acid production in the absence and presence of microfuged and filter-sterilised starter supernatant (0.1 mL /10mL of inoculated RSM) as described in Sect. 6.14.2. Both pH and the amount of acid produced—the titratable acidity—are used to monitor growth. pH is much easier and faster to measure and automated equipment which can measure the pH of up to 24 samples continuously is commercially available. When cooled to 4 °C, mesophilic cultures will retain activity for 2–3 days while thermophilic cultures retain good activity for up to 12 days. Growth in the presence of heme will significantly reduce the loss of activity during storage of lactococci.

The pH of the medium used in the production of bulk cultures is sometimes controlled during growth, particularly in the US. Control can be external or internal. External control involves the use of a pH control unit, which maintains the pH at 6.3 by pumping in a neutraliser, e.g., NH_4OH or NH_3 on demand, while internal control involves the use of an insoluble buffer, e.g., MgO, which dissolves as lactic acid is produced, and maintains the pH above 5.3. pH control increases the number of cells per unit volume and, therefore, reduces the volume of starter required for cheese-making. Neutralisation of the pH of the bulk culture to 6.5 after growth and further incubation for a few hours will also result in an increase in the number of cells in the culture.

6.17 DVS and DVI Cultures

To-day, frozen, super-concentrated cultures are the most common form of inoculum used in Cheddar plants, although some plants still produce bulk cultures. These super-concentrated cultures are produced in specialised laboratories and the cells are sufficiently concentrated to inoculate the milk in the vat directly. They are called direct-to-vat starters (DVS) or direct-vat-inocula (DVI). Although DVS or DVI cultures are expensive, they do away with the need for producing, testing and maintaining bulk cultures in the cheese plant and the attendant costs of personnel and equipment.

Generally, proprietary media containing growth supplements are used to grow the cultures, under some form of pH control. When fully grown, the cells are harvested by centrifugation or membrane filtration and are pelleted by dropping the liquid concentrate into liquid N at −263 °C. The pellets are then collected and either frozen or freeze-dried. Cryoprotectants are generally necessary when cultures are freeze-dried to prevent sub-lethal stress. The cells are then packaged in foil pouches or cans in volumes sufficient to inoculate 3000–5000 L of milk directly. Super-concentrated cultures may contain 10^{11}–10^{12} cells/g or 100–1000 times more cells than a typical bulk starter, which generally contain 10^{9} cells/g.

In plants that produce large amounts of cheese, e.g., 60 vats, three different phage-unrelated DVS cultures are used to inoculate the milk, one for the first 20 vats, a second one for the next 20 vats and a third culture for the last 20 vats. Different phage-unrelated cultures are used on the following and on each subsequent day for 4–5 days, when the rotation is repeated. These cultures contain two or three strains of *Lc. lactis* and one strain of *Sc. thermophilus* and sometimes also a strain of *Lb. helveticus* (Mark Hurley, personal communication). Defined-strain thermophilic cultures are now also being used but less extensively than mesophilic cultures.

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Chapter 7
Enzymatic Coagulation of Milk

Summary The coagulation of milk (by proteolysis or acidification) is the key operation in cheesemaking. The enzymatic (rennet-induced) coagulation of milk can be divided into two phases: (1) hydrolysis of the micelle-stabilizing protein, κ-casein, (2) aggregation and gelation of the rennet-altered micelles, with the development of a particulate gel.

The mechanism of the primary (enzymatic) phase has been described in molecular terms and the effects of various environmental factors thereon quantified. Aggregation of the rennet-altered micelles occurs when the zeta potential of the micelles, due mainly to the surface layer of κ-casein, has been reduced to a critical level. The effects of various compositional and environmental factors on the aggregation of the altered micelles have been described. Gelation is usually regarded as a continuation of the secondary (aggregation) phase, but requires a different approach and instrumentation for its study. It is the most complex and, at present, the least well understood aspect of the enzymatic coagulation of milk. Several instruments are available with which to study the rheological properties of the gel and there is particular interest in developing methods that can be used to study/quantify gelation in the cheese vat.

An overview of the rennet-induced coagulation of milk will be presented in this chapter.

Keywords Rennet • κ-casein hydrolysis • Coagulation • Rennet substitutes

7.1 Introduction

As discussed in Chap. 2, the milk for most cheese varieties is coagulated through the action of selected proteinases, called rennets. The rennet-induced coagulation of milk is a two-stage process (Fig. 7.1). The primary phase involves the specific enzymatic modification of the casein micelles to produce *para*-casein micelles which aggregate in the presence of Ca^{2+} at a temperature $\succsim$ 20 °C; aggregation of the rennet-altered micelles is referred to as the secondary phase of coagulation. The primary phase of rennet action is well characterized but the secondary phase is less clear. The subject has been reviewed by Fox (1984), Fox and Mulvihill (1990), Dalgleish (1992, 1993), Green and Grandison (1993), Fox et al. (1996), Fox and McSweeney (1997), Hyslop (2003), Horne and Banks (2004) and Lucey (2011).

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7.2 Primary Phase of Rennet Coagulation

As discussed in Chap. 4, the caseins exist as micelles stabilized by a surface layer of κ-casein. Following its isolation in 1956, it was shown that κ-casein is the micelle-stabilizing protein and that its stabilizing properties are destroyed on renneting. Shortly afterwards, it was shown that κ-casein is the only protein hydrolyzed during the rennet-induced coagulation of milk and that it is hydrolyzed specifically at the Phe_{105}-Met_{106} bond (Fig. 7.2). The N-terminal part of the molecule, κ-CN f1-105, referred to as *para*-κ-casein, remains attached to the casein micelle while the C-terminal part, referred to as the caseinomacropeptide (CMP) or glycomacropeptide (GMP), because it contains the carbohydrate moieties of κ-casein, is lost into the surrounding aqueous medium. It has been recognised since the end of the nineteenth century that small peptides are produced on renneting. As discussed in Chap. 4, there are nine forms of κ-casein which differ in sugar content; hence, 9 CMPs are produced. All the CMPs are soluble in 2 % TCA but only the glycosylated forms are soluble at higher concentrations of TCA. Thus, TCA-soluble N, or more specifically TCA-soluble sugars, e.g., N-acetyl neuramic acid, can be used to monitor the primary phase of rennet coagulation (Fig. 7.3).

The unique sensitivity of the Phe-Met bond of κ-casein has aroused interest. The dipeptide, H.Phe-Met.OH, is not hydrolyzed, nor are tri- or tetra-peptides containing a Phe-Met bond. However, this bond is hydrolyzed in the pentapeptide, H.Ser-Leu-Phe-Met-Ala-OMe, and reversing the positions of serine and leucine, to give the correct sequence of κ-casein, increases the susceptibility of the Phe-Met bond to chymosin. Both the length of the peptide and the sequence around the Phe-Met bond are important determinants of enzyme-substrate interaction. $Serine_{104}$ appears to be particularly important and its replacement by Ala, or even L-Ser, in the above pentapeptide renders the Phe-Met bond very resistant to hydrolysis by chymosin. Extension of the pentapeptide, H.Ser.Phe.Met.Ala.Ile.OH (i.e., κ-CN f104-108), from the N- and/or C-terminal to reproduce the sequence of κ-casein around the chymosin-susceptible bond increases the efficiency with which the Phe-Met bond is hydrolyzed by chymosin (Table 7.1). The sequence κ-CN f98-111 includes all the residues necessary to render the Phe-Met bond as susceptible to hydrolysis by

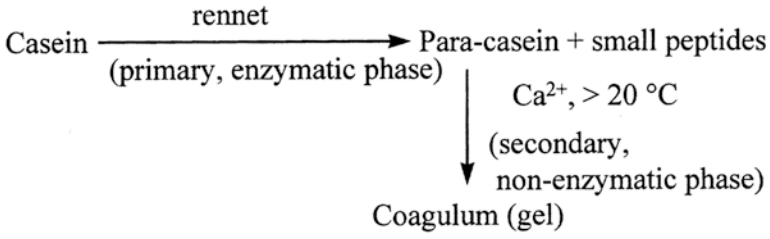


Fig. 7.1 Summary of the rennet coagulation of milk. The primary phase involves enzymatic hydrolysis of κ-casein, while the secondary phase involves aggregation of the rennet-altered *(para*-casein) micelles into a three-dimensional gel network (coagulum)

1
Pyro Glu-Glu-Gln-Asn-Gln-Glu-Gln-Pro-Ile-Arg-Cys-Glu-Lys-Asp-Glu-Arg-Phe-Phe-Ser-Asp-

21
Lys-Ile-Ala-Lys-Tyr-Ile-Pro-Ile-Gln-Tyr-Val-Leu-Ser-Arg-Tyr-Pro-Ser-Tyr-Gly-Leu-

41
Asn-Tyr-Tyr-Gln-Gln-Lys-Pro-Val-Ala-Leu-Ile-Asn-Asn-Gln-Phe-Leu-Pro-Tyr-Pro-Tyr-

61
Tyr-Ala-Lys-Pro-Ala-Ala-Val-Arg-Ser-Pro-Ala-Gln-Ile-Leu-Gln-Trp-Gln-Val-Leu-Ser-

81
Asn-Thr-Val-Pro-Ala-Lys-Ser-Cys-Gln-Ala-Gln-Pro-Thr-Thr-Met-Ala-Arg-His-Pro-His-

101 105 | 106
Pro-His-Leu-Ser-Phe↓Met-Ala-Ile-Pro-Pro-Lys-Lys-Asn-Gln-Asp-Lys-Thr-Glu-Ile-Pro-

121 Ile (Variant B)
Thr-Ile-Asn-Thr-Ile-Ala-Ser-Gly-Glu-Pro-*Thr*- Ser-*Thr* -Pro-*Thr* - -Glu-Ala-Val-Glu-
Thr (Variant A)
141 Ala (Variant B)
Ser-*Thr* -Val-Ala-Thr-Leu-Glu- -*SerP* - Pro-Glu-Val-Ile-Glu-Ser-Pro-Pro-Glu-Ile-Asn-
Asp (Variant A)
161 169
Thr-Val-Gln-Val-Thr-Ser-Thr-Ala-Val.OH

Fig. 7.2 Amino acid sequence of κ-casein showing the chymosin cleavage site (*downwards arrow*)

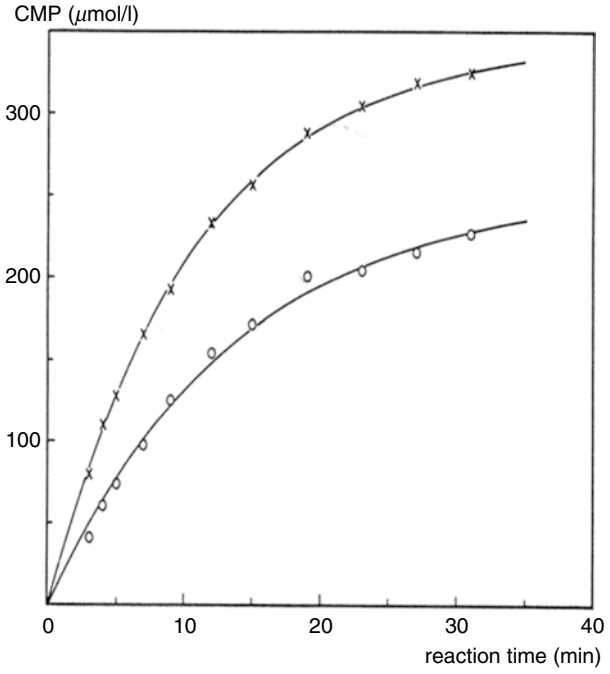


Fig. 7.3 Release of macropeptide (CMP) soluble in 2 (*cross symbol*) or 8 (*open circle*) % TCA as a function of time after rennet addition

Table 7.1 Kinetic parameters for hydrolysis of κ-casein peptides by chymosin at pH 4.7 (from Fox and McSweeney 1998)

Peptide	Sequence	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m($s^{-1}mM^{-1}$)
S.F.M.A.I.	104–108	0.33	8.50	0.038
S.F.M.A.I.P.	104–109	1.05	9.20	0.114
S.F.M.A.I.P.P.	104–110	1.57	6.80	0.231
S.F.M.A.I.P.P.K.	104–111	0.75	3.20	0.239
L.S.F.M.A.I.	103–108	18.3	0.85	21.6
L.S.F.M.A.I.P.	103–109	38.1	0.69	55.1
L.S.F.M.A.I.P.P.	103–110	43.3	0.41	105.1
L.S.F.M.A.I.P.P.K.	103–111	33.6	0.43	78.3
L.S.F.M.A.I.P.P.K.K.	103–112	30.2	0.46	65.3
H.L.S.F.M.A.I.	102–108	16.0	0.52	30.8
P.H.L.S.F.M.A.I	101–108	33.5	0.34	100.2
H.P.H.P.H.L.S.F.M.A.I.P.P.K.	98–111	66.2	0.026	2509
98–111[a]	46.2[a]	0.029[a]	1621[a]	
κ-Casein[b]		2–20	0.001–0.005	200–2000
L.S.F.(NO_2)Nle A.L.OMe		12.0	0.95	12.7

[a]pH 6.6
[b]pH 4.6

chymosin at pH 4.7 as it is in intact κ-casein; it is hydrolyzed ~66,000 times faster than the parent pentapeptide (κ-CN f104-108), with a k_{cat}/K_m of ~2 M^{-1} s^{-1}, which is similar to that for intact κ-casein (Visser 1981; Visser et al. 1987). κ-Casein and the peptide κ-CN f98-111 are also readily hydrolyzed at pH 6.6 but smaller peptides are not.

The Phe and Met residues in the chymosin-sensitive bond of κ-casein are not intrinsically essential for chymosin action. There are numerous Phe and a substantial number of Met residues in all milk proteins. In porcine and human κ-caseins, the chymosin-sensitive bond is Phe-Ile, while in rat and mouse κ-caseins, it is Phe-Leu; yet, these proteins are readily hydrolyzed by calf chymosin, although more slowly than bovine κ-casein. In contrast, porcine milk is coagulated more effectively than bovine milk by porcine chymosin, indicating that unidentified subtle structural features influence chymosin action. Camel milk, the κ-casein in which contains a Phe-Ile bond, is not coagulated by calf chymosin but bovine milk is coagulated faster by camel chymosin than by calf chymosin. Peptides in which Phe was replaced by Phe (NO_2) or cyclohexylamine are also hydrolyzed by chymosin although less effectively than those with a Phe-Met bond; oxidation of Met_{106} reduces k_{cat}/K_m ca. tenfold but substitution of Nle for Met increases it ca. threefold.

A genetically engineered mutant of κ-casein, in which Met_{106} was substituted by Phe_{106}, i.e., the chymosin-sensitive bond was changed from Phe_{105}-Met_{106} to Phe_{105}-Phe_{106}, was hydrolyzed 1.8 times faster by chymosin than natural κ-casein. These findings suggest that the sequence around the Phe-Met bond, rather than the residues in the bond itself, contains the important determinants of hydrolysis by chymosin. The particularly important residues are Ser_{104}, the hydrophobic residues Leu_{103}

and Ile_{108}, at least one of the three histidines (residues 98, 100 or 102, as indicated by the inhibitory effect of photooxidation) and Lys_{111}. Studies on chemically or enzymatically modified peptide analogues of κ-CN f98-112 indicated the relative importance of residues in the sequences of 98–102 and 111–112. It has been suggested that the sequence Leu_{103} to Ile_{108} of κ-casein, which probably exists as an extended β-structure, fits into the active site cleft of acid proteinases. The hydrophobic residues, Leu_{103}, Phe_{105}, Met_{106}, and Ile_{108}, are directed towards hydrophobic pockets along the active site cleft while the hydroxyl group of Ser_{104} forms part of a hydrogen bond with some counterpart in the enzyme. It has been proposed that the sequences 98–102 and 109–111 form β-turns around the edges of the active site cleft of the enzyme; this conformation is stabilized by Pro residues at positions 99, 101, 109 and 110. The three His residues at positions 98, 100, 102, and Lys_{111} are probably involved in electrostatic bonding between enzyme and substrate; none appears to have a predominant role. Lys_{112} appears not to be important in enzyme-substrate binding as long as Lys_{111} is present.

The significance of electrostatic interactions in chymosin-substrate complex formation is indicated by the effect of added NaCl on the rennet coagulation time (RCT) of milk: addition of NaCl up to 3 mM reduces RCT but higher concentrations have an inhibitory effect; it is claimed that the effect of NaCl is on the primary, enzymatic phase rather than on the aggregation of rennet-altered micelles. Increasing ionic strength (0.01–0.11) reduces the rate of hydrolysis of κ-CN $fHis_{98}$-$Lys_{111/112}$ in a model system; the effect becomes more marked as the reaction pH is increased but is independent of ion type.

As well as serving to elucidate the importance of certain residues in the hydrolysis of κ-casein by chymosin, small peptides that mimic or are identical to the sequence of κ-casein around the Phe-Met bond are very useful substrates for determining the activity of rennets in absolute units, i.e., independent of variations in the non-enzymatic phase of coagulation of different milks. Standard methods for such quantification have been developed and chromogenic peptide substrates are available commercially, e.g., the chromophoric heptapeptides Leu,Ser,$Phe(NO_2)$ Nle.Ala.Leu.OMe (Dunn et al. 1986) or Pro.Thr.Glu.$Phe(NO_2)$.Phe.Arg.Leu (Hurley et al. 1999). Since the specific activity of different rennets on these peptides varies, methods for quantifying the proportions of acid proteinases in commercial rennets have been proposed.

7.3 Rennet

Several proteinases will coagulate milk under suitable conditions but most are too proteolytic relative to their milk clotting activity (MCA); consequently, they hydrolyse the caseins in the coagulum too quickly, causing a reduced cheese yield (MCA is the inverse of RCT; i.e., MCA = 1/RCT). Excessive proteolysis or incorrect specificity may also lead to defects in the flavour, especially bitterness, and texture of the cheese. Although plant proteinases appear to have been used as rennets since

prehistoric times, gastric proteinases from calves, kids or lambs have been used traditionally as rennets, with very few exceptions.

Animal rennets are prepared by extracting the dried (usually) or salted gastric tissue (referred to as vells) with 10 % NaCl and activating and standardizing the extract. Standard calf rennet contains ca. 60–70 RU/ml and is preserved by making the extract to 20 % NaCl and adding sodium benzoate or sodium propionate. A rennet unit (RU) is the amount of rennet activity that will coagulate 10 ml of milk (usually low-heat skim milk powder reconstituted in 0.01 % $CaCl_2$ and perhaps adjusted to pH 6.5) in 100 s. Chymosin (an aspartyl acid proteinase, i.e., a proteinase with two aspartic acid residues at the active site and with a pH optimum of 2–4) represents >90 % of the MCA of good quality veal rennet, the remaining activity being due to pepsin. As the animal ages, especially when fed solid food, the secretion of chymosin declines while that of pepsin increases.

Like many other animal proteinases, chymosin is secreted as its zymogen, prochymosin, which is autocatalytically activated on acidification to pH 2–4 by removal of a 44-residue peptide from the N-terminal of the zymogen (see Foltmann 1993).

Chymosin, an acid proteinase, is well characterized at the molecular level (see Foltmann 1993; Chitpinityol and Crabbe 1998; Crabbe 2004). The enzyme, which was crystallized in the 1940s (Hankinson 1943; Berridge 1945), is a single-chain polypeptide containing about 323 amino acid residues with a molecular mass of 35,600 Da. Its primary structure has been established and a considerable amount of information is available on its secondary and tertiary structures (Fig. 7.4). The molecule exists as two domains separated by the active site cleft in which the two catalytically-active aspartyl residues (Asp_{32} and Asp_{215}) are located (see Plowman and Creamer 1995).

Calf rennet contains three chymosin isoenzymes, principally A and B, with lesser amounts of C. Chymosins A and B which are produced from the corresponding zymogens, prochymosins A and B, differ by only one amino acid residue at position 254 (Asp in A, Gly in B). Initially chymosin C was considered to be a degradation product of chymosin A which lacks three residues, Asp_{244}-Phe_{246}, but now it appears to be the product of a different allele. The specific activity of chymosin A, B and C is ~120, 100 and 25 RU/mg, respectively. Chymosins A and B have an optimum pH at 4.2 and 3.7, respectively. The properties of different rennets are discussed in Sect. 7.9.

7.4 Factors that Affect the Hydrolysis of κ-Casein and the Primary Phase of Rennet Coagulation

The hydrolysis of κ-casein is influenced by many factors, some of which are discussed below. While many factors influence both the primary and secondary stages, the effects on each will be discussed separately.

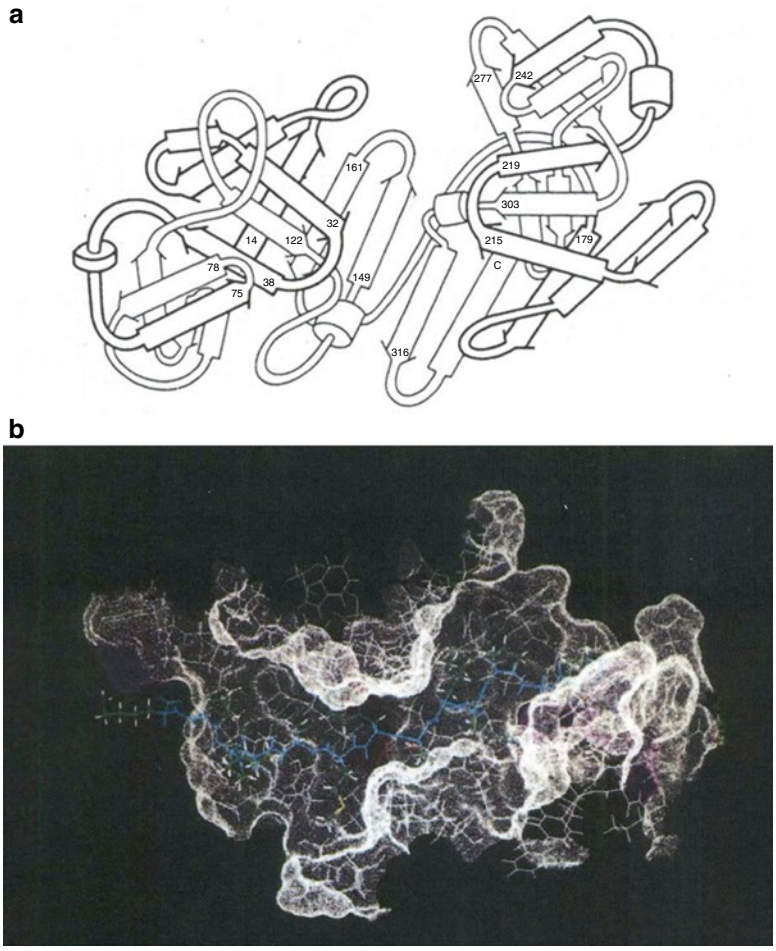


Fig. 7.4 Schematic representation of the secondary and tertiary structures of chymosin, showing the active site cleft into which the sequence comprising amino acid residues 102–108 of κ-casein fits ((**a**) from Foltmann 1987; (**b**) from Plowman and Creamer 1995)

- *pH* The pH optimum for chymosin and bovine pepsin on small synthetic peptides is ca. 4.7 but is 5.3–5.5 on κ-CN fHis$_{98}$-Lys$_{111/112}$. Chymosin hydrolyses insulin, acid-denatured haemoglobin and Na-caseinate optimally at pH 4.0, 3.5 and 3.5, respectively. The pH optimum for the first stage of rennet action in milk is ~6.0 at 4 or 30 °C.
- *Ionic strength* The influence of ionic strength on the primary phase of rennet coagulation was discussed in Sect. 7.2.
- *Temperature* The optimum temperature for the coagulation of milk by calf rennet at pH 6.6 is 45–48 °C; presumably, the optimum for the hydrolysis of κ-casein is about this value. The effect of temperature depends on the type of rennet

(Fig. 7.5). The temperature coefficient (Q_{10}) for the hydrolysis of κ-casein in solutions of Na-caseinate is ca. 1.8, the activation energy, E_a, is ~40,000 J mol^{-1}, and activation entropy, ΔS, is ~−90 J K^{-1} mol^{-1}; generally similar values have been reported for the hydrolysis of isolated κ-casein by chymosin.

- *Heat Treatment of Milk* Heat treatment of milk at a temperature >72 °C adversely affects its rennet coagulability; if the heat treatment is very severe (>90 °C for 10 min), the milk fails to coagulate on renneting. Although changes in salts equilibria are contributory factors, the principal causative factor is intermolecular disulphide bond formation between κ-casein and β-lactoglobulin and/or α-lactalbumin. Both the primary and especially the secondary phases of rennet action are inhibited in heated milk, as reflected by the marked decreases in curd firming rate and in the strength of the resulting gel. The adverse affects of heating can be reversed by acidification to pH values in the region 6.6 to 6.0, before or after heating, or by addition of $CaCl_2$ (which causes a reduction in pH); the secondary, rather than the primary, phase of rennet action probably benefits from these treatments.

7.5 Secondary (Non-enzymatic) Phase of Coagulation and Gel Assembly

Hydrolysis of κ-casein by chymosin or similar enzymes during the primary phase of rennet action releases the highly charged, hydrophilic C-terminal segment of κ-casein (glycomacropeptide), as a result of which the zeta potential of the casein micelles is reduced from −10/−20 to −5/−7 mV and the protruding peptides (hairs) are removed from their surfaces, thus destroying the principal micelle-stabilizing factors (electrostatic and steric) and their colloidal stability. When ~85 % of the total κ-casein has been hydrolyzed, the stability of the micelles is reduced to such an extent that when they collide, they remain in contact and eventually build into a three-dimensional network, referred to as a coagulum or gel (Fig. 7.6). Gel formation is accompanied by sharp increases in viscosity and elastic shear modulus, G′, which is a measure of gel firmness (Fig. 7.7; see Sect. 7.8.2). Reducing the pH or increasing the temperature from the normal values (~6.6 and ~31 °C, respectively) permits coagulation at a lower degree of κ-casein hydrolysis. Although the precise reactions involved in aggregation are not known, the kinetics of aggregation have been described.

The assembly of rennet-altered micelles into a gel has been studied using various forms of viscometry, electron microscopy and light scattering. Viscosity measurements show that the viscosity of renneted milk remains constant or decreases slightly during a period equivalent to ~60 % of the visually observed RCT (Figs. 7.6 and 7.7). It has been suggested that the initial decrease in viscosity is due to a decrease in the voluminosity of the casein micelles following release of the macropeptides which form a 'hairy layer' ~12 nm thick (de Kruif and Holt 2003; Fig. 7.6a–d). The decrease in micelle size has been confirmed by quasi-elastic light scattering.

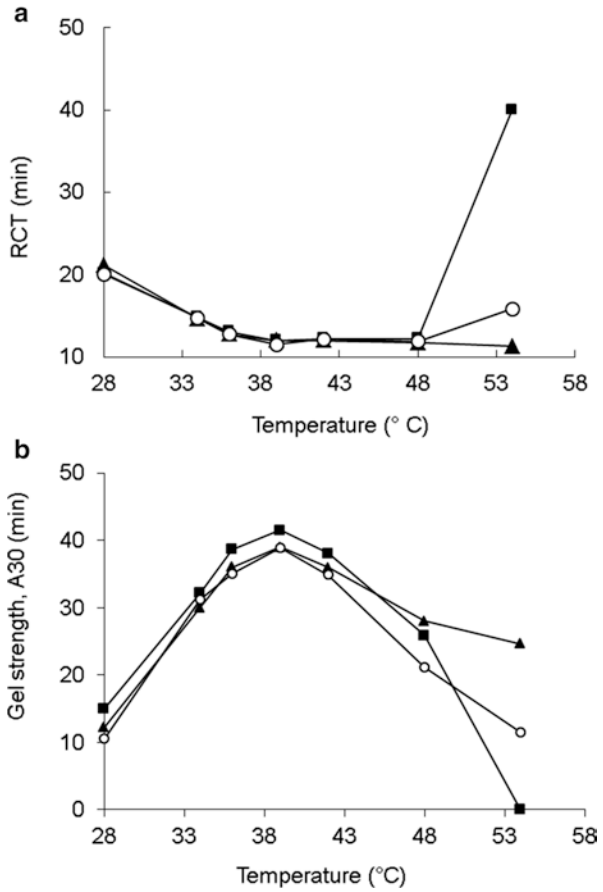


Fig. 7.5 Effect of temperature on the milk clotting activity of chymosin (*filled square*), *Rhizormucor pusillus* (*open circle*) and *Rhizomucor miehei* (*filled triangle*) proteinases, as measured by (**a**) the rennet coagulation time, RCT or (**b**) gel firmness, as measured using Formagraph

The gelation process, generally referred to as the secondary phase of rennet coagulation, involves initially the formation of chains and clumps of micelles, leading eventually to the formation of a network of partly-fused micelles. During the first 60 % of the visually observed RCT, the micelles exist as individual particles; the primary enzymatic reaction is ~85 % complete at 60 % of the visual RCT. Between 60 and 80 % of the RCT, the rennet-altered micelles begin to aggregate steadily with no sudden change in the type of extent of aggregation. Small, chain-like aggregates, rather than clumps, form initially (Fig. 7.8). At 100 % of the RCT, most of the micelles have aggregated into short chains, which then begin to aggregate with the formation of a network. Aggregation of the rennet-altered micelles can be described by the von Smoluchowski theory for diffusion-controlled aggregation of hydrophobic colloids when allowance is made for the need to produce, by

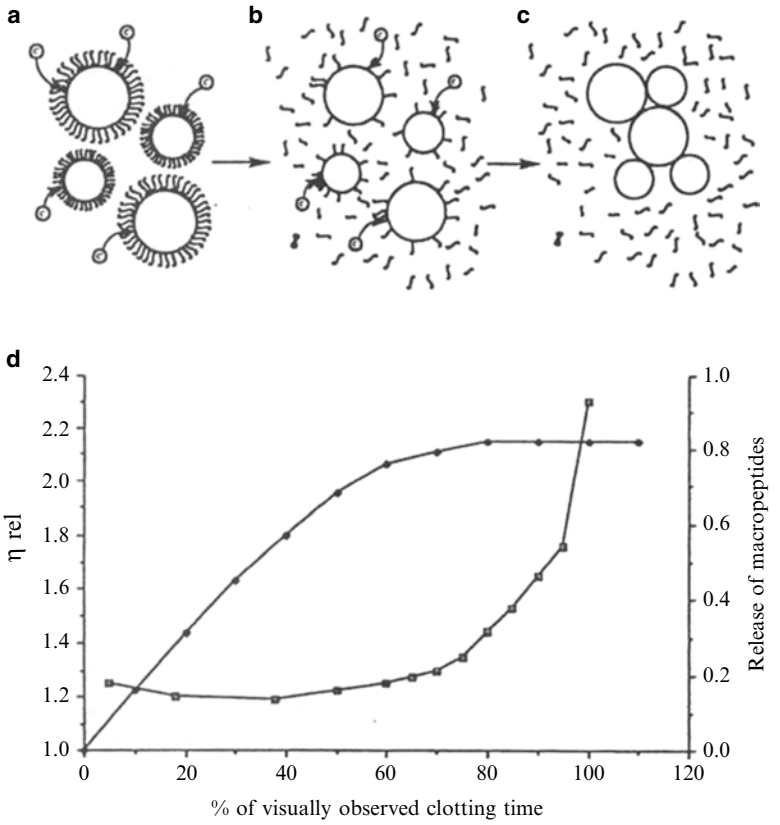


Fig. 7.6 Schematic representation of the rennet coagulation of milk. (**a**) Casein micelles with intact κ-casein layer being attacked by chymosin; (**b**) micelles denuded of κ-casein; (**c**) extensively denuded micelles in the process of aggregation; (**d**) release of macropeptides (filled diamond) and changes in relative viscosity (■) during the course of rennet coagulation

enzymatic hydrolysis, a sufficient concentration of particles capable of aggregating, i.e., casein micelles in which >97 % of the κ-casein has been hydrolysed. The diffusion of the particles is rate-limiting and is determined by the random fruitful collision of particles (rennet-altered micelles). The rate of aggregation is not consistent with a branching process model since the micellar functionality is 1.8, whereas an average functionality greater than 2 is required for network formation.

According to Dalgleish (1980), the overall rennet coagulation of milk can be described by combining three factors:

- proteolysis of κ-casein, which may be described by Michaelis-Menten kinetics;
- requirement that ~97 % of the κ-casein on a micelle be hydrolysed before it can participate in aggregation;
- aggregation of *para*-casein micelles via a von Smoluchowski process.

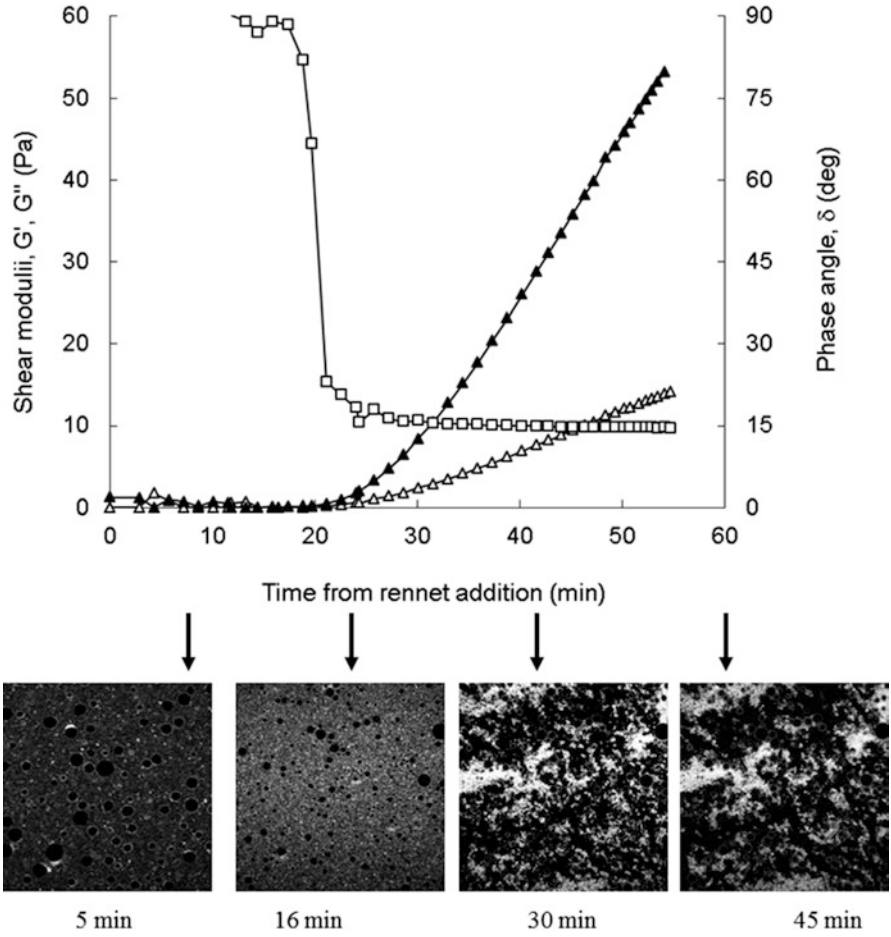


Fig. 7.7 Changes in viscoelasticity and microstructure during rennet-induced gelation of milk, showing increases in elastic shear modulus (G′, *filled triangle*), loss modulus (G″, *open triangle*), and reduction in phase angle (*open square*), and aggregation of rennet-hydrolysed casein micelles into a network of *para*-casein micelles (as indicated by the increased white area in the confocal laser scanning micrographs). Modified from Fox and Guinee 2013

The overall clotting time, t_c, is the sum of the enzymatic phase and the aggregation phase:

$$t_c = t_{prot} + t_{agg} In \frac{K_m}{V_{\max}} In\left(\frac{1}{1-\alpha_c}\right) + \frac{\alpha_c}{V_{\max}} S_o + \frac{1}{2k_s C_o}\left(\frac{M_{crit}}{M_o} - 1\right)$$

where: K_m and V_{max} are the Michaelis-Menten parameters, α_c is the extent of κ-casein hydrolysis, S_o is the initial concentration of κ-casein, k_s is the rate constant for

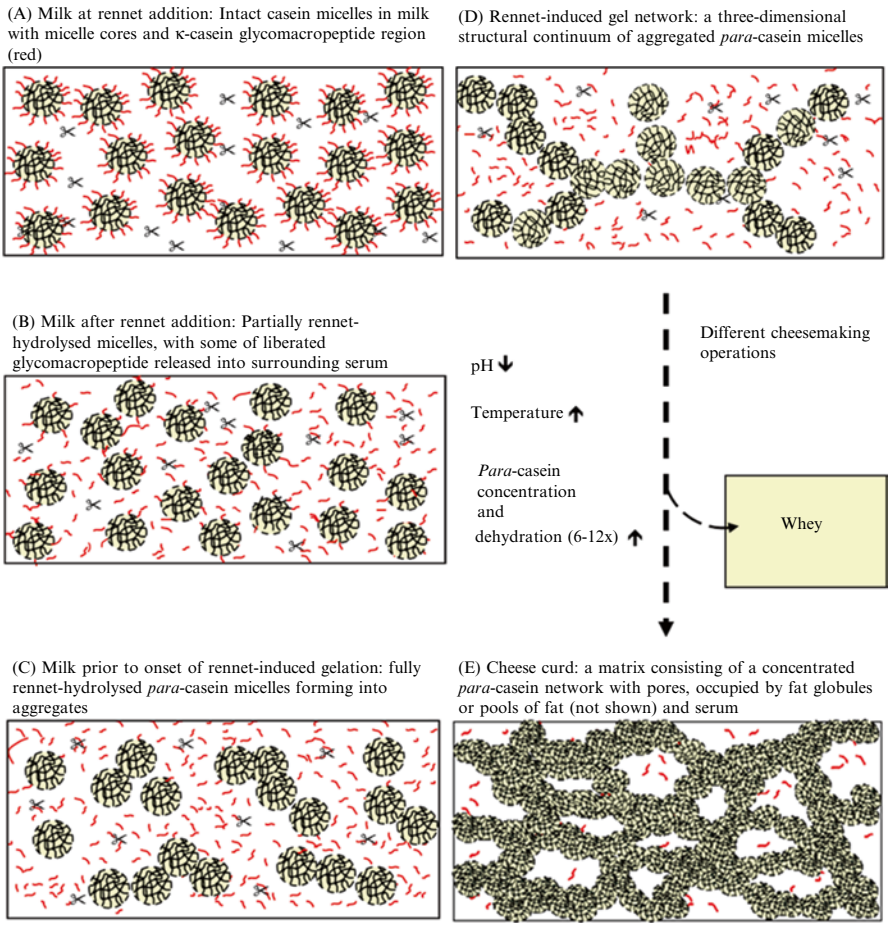


Fig. 7.8 Schematic representation of the various stages involved in the formation of cheese curd from milk, starting from the initial mixture of casein micelles and added enzyme (rennet ✂) in the milk (**A**), and proceeding through rennet-induced hydrolysis of κ-casein (**B**–**C**), aggregation of *para*-casein micelles (**C**) and formation of a *para*-casein gel network (**D**), which is dehydrated and concentrated into cheese curd (**E**) (from Fox and Guinee 2013)

aggregation, C_o concentration of aggregating material, M_{crit} = weight average molecular weight at t_c (~10 micellar units), M_o = weight average molecular weight at $t = 0$.

Darling and van Hooydonk (1981) proposed an alternative model for rennet coagulation, again by combining Michaelis-Menten enzyme kinetics with von Smoluchowski aggregation kinetics. The stability factor in von Smoluchowski's theory is considered as a variable determined by the concentration of unhydrolysed surface κ-casein. The coagulation time, t_c, is given by:

$$t_c = \frac{1}{V}\left[S_o + \frac{1}{C_m}\left(\exp\left(-C_m \times S_o\right) - 1\right)\right] + \frac{W_o \exp\left(-C_m \times S_o\right)}{k_s}\left[\frac{1}{n_c} - \frac{1}{n_o}\right]$$

where, V = velocity of enzymatic hydrolysis of κ-casein, S_o = initial concentration of κ-casein, C_m = a constant relating the stability of the casein micelle to κ-casein concentration, W_o = initial stability factor for casein micelles, n_o = initial concentration of casein micelles and n_c = concentration of casein aggregates at the observed clotting time, t_c. It is claimed that this theoretical model explains the experimentally observed influence of protein concentration, enzyme concentration and temperature on RCT, and the occurrence of a lag phase equal to 60 % of RCT.

With milk of normal concentration, ~90 % of the micelles are incorporated into the curd at 100 % of the visual RCT but only ~50 % are incorporated in a fourfold concentrate when the same level of rennet is used. The micelles that are 'free' at or after the RCT may react differently from those free prior to RCT, i.e., before visual coagulation, all micelles are freely dispersed in the serum and can aggregate randomly but once a gel matrix has started to form, free micelles may react either with the gel matrix or with other free micelles. Therefore, a gel assembly may be regarded as a two-stage process and the properties of the final gel may be affected considerably by the amount of casein 'free' at the RCT. Since this is particularly high in concentrated milks, it may explain the coarser structure of curd made from concentrated milk.

Based on viscometric data, Tuszynski (1971) suggested that gel assembly is a two-stage process: flocculation and gelation. Turbidity experiments also suggest a two-stage gel assembly process (Surkov et al. 1982):

$$E + S \underset{k_{-1}}{\overset{k_{-1}}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \xrightarrow{k_c} P^* \xrightarrow{k_s} Pn$$

where E is enzyme, S is substrate, P is the reaction product, P* is *para*-casein micelles with transformed quaternary structure and P_n is the gelled micelle aggregate. The first two steps are the Michaelis-Menten model for the primary, enzymatic, phase and are essentially as proposed by Payens et al. (1977):

$$E + S \underset{k_{-1}}{\overset{k_{-1}}{\rightleftharpoons}} ES \rightarrow E + P_1 + M$$

where P_1 is para-κ-casein and M is a macropeptide. Payens et al. (1977) suggest that the second, non-enzymatic phase may be represented by:

$$iP_1 \overset{k_s}{\rightleftharpoons} Pi$$

where *i* is any number of aggregating particles, P_1.

Surkov et al. (1982) suggested that the enzyme-altered micelles (*para*-casein micelles, P) undergo a co-operative transition in quaternary structure to yield clot-forming particles (P*) with a rate constant, k_c; the activation energy, E_a, was 191 kJ mole^{-1} and the $Q_{10\,°C}$ was 1.6. These values are very similar to those reported by Tuszynski (1971).

The sites involved in the aggregation process are not known. Following reduction of the micellar zeta potential by proteolysis of κ-casein, linkage of particles is facilitated.

Inter-particle linkage could be via calcium bridges and/or hydrophobic interactions (which the marked temperature dependence of the secondary phase indicate). Changes in the surface hydrophobicity of casein micelles during renneting have been demonstrated through changes in the binding of the fluorescent marker, 8-anilino naphthalene-1-sulphonate (Peri et al. 1990; Iametti et al. 1993). The hydrophobic amino terminal segment (residues 14–24) of α_{s1}-casein appears to be important in the establishment of a rennet-induced gel structure. It has been suggested that the matrix of young cheese curd consists of a network of α_{s1}-casein molecules linked together via hydrophobic patches which extends throughout the cheese structure. The softening of the texture during the early stages of ripening is considered to be due to breaking of the network on hydrolysis of the Phe_{23}-Phe_{24} bond of α_{s1}-casein. Modification of histidyl, lysyl and arginyl residues in κ-casein inhibits the secondary phase of rennet coagulation, suggesting that a positively-charged cluster on para-κ-casein interacts electrostatically with unidentified negative sites. In native micelles, this positive site may be masked or covered by the macropeptide segment of κ-casein but becomes exposed and reactive when this peptide is released (Hill 1970).

Normally, the rate of an enzymatic reaction increases linearly with enzyme concentration, within certain limits. In the case of rennet coagulation, RCT is inversely related to enzyme concentration, as expressed in the formula:

$$Et_c = k, \text{where } E = \text{enzyme concentration and } t_c = RCT$$

This equation, which assumes that visually observed coagulation is dependent only on the enzymatic process, has been modified to take account of the duration of the secondary, non-enzymatic phase (Foltmann 1959)

$$E\left(t_c - x\right) = k$$

where, x is time required for the coagulation of the enzymatically-altered casein micelles and (t_c-x) is the time required for the enzymatic stage. Rearrangement of this equation gives a more convenient form,

$$t_c = k\left(1/E\right) + x$$

which is valid within a certain range of rennet concentrations and under certain conditions of temperature and pH. A very good linear relationship exists between clotting time and the reciprocal of enzyme concentration (Fig. 7.9).

The coagulation equations developed by Dalgleish (1980) and by Darling and van Hooydonk (1981) might be regarded as greatly refined versions of these simpler equations and reduce to them on first approximations. Rennet clotting time, t_c, has also been expressed (Payens and Wiersma 1980) by the equation:

$$t_c = \sqrt{\frac{2}{k_s V_{max}}}$$

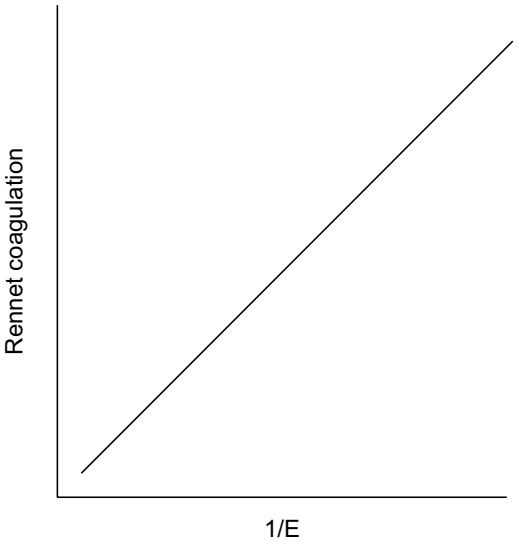


Fig. 7.9 Relationship between enzyme concentration (E) and rennet coagulation time

where k_s, the diffusion-controlled flocculation rate constant according to von Smoluchowski's theory (non-enzymatic phase), is proportional to the concentration of reactive (coagulable) particles (proteolysed micelles) and hence to enzyme concentration; V_{max} = maximum velocity in Michaelis-Menten kinetics (enzymatic phase) and is proportional to enzyme concentration.

7.6 Factors that Affect the Non-enzymatic Phase of Rennet Coagulation

The coagulation of renneted micelles is very temperature-dependent ($Q_{10} \sim 16$) and bovine milk does not coagulate <~18 °C unless Ca^{2+} concentration is increased. The marked difference between the temperature dependence of the enzymatic and non-enzymatic phases of rennet coagulation has been exploited in studies on the effects of various factors on the rennet coagulation of milk, in attempts to develop a system for the continuous coagulation of milk for cheese or rennet casein manufacture and in the application of immobilized rennets. The very high temperature dependence of rennet coagulation suggests that hydrophobic interactions are important.

Coagulation of rennet-altered micelles depends on a critical concentration of Ca^{2+} which may act by cross-linking rennet-altered micelles, possibly via serine phosphate residues, or simply by charge neutralization. Colloidal calcium phosphate is also essential for coagulation but can be replaced by increased [Ca^{2+}]. Partial enzymatic dephosphorylation of casein, which reduces micellar charge, reduces coagulability; interaction of casein micelles with various cationic species predisposes them to coagulation by rennet and may even coagulate unrenneted

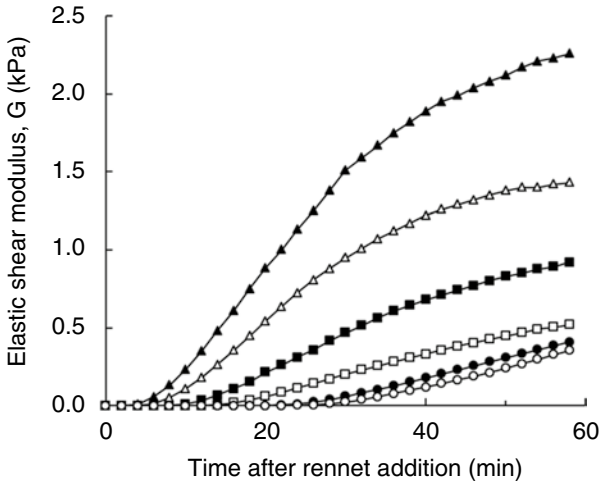


Fig. 7.10 Development of elastic shear modulus (G′, index of gel strength/firmness) in rennet-treated, high-protein (18 %, w/w) milk retentate and rennet-treated at pH 6.67 (*open circle*),6.55 (*filled circle*), 6.45 (*open square*), 6.3 (*filled square*), 6.15 (*open triangle*) or 6.0 (*filled triangle*)

micelles (Green and Marshall 1977; Marshall and Green 1980). Chemical modification of histidine, lysine or arginine residues inhibits coagulation (Hill 1970), presumably by reducing micellar positive charge.

The apparent importance of micellar charge in the coagulation of rennet-altered micelles suggests that pH should have a major influence on the secondary phase of coagulation. Reduction in pH in the range 6.6 to 6.0 is accompanied by increases in the rates of the enzymic and coagulation reactions, reductions in gelation time (time for gel onset) and the degree of κ-casein hydrolysis necessary for the onset of gelation (e.g., from ~97 to ~80 % of total κ-casein), and increases in curd firming rate and firmness after a given renneting time. Although it is claimed that pH has essentially no effect on the coagulation process, the rate of firming of the resultant gel is significantly increased on reducing the pH (Fig. 7.10).

The rate of firming of renneted milk gels is influenced by the type of rennet, especially under unfavourable conditions, e.g., high pH or low [Ca^{2+}]. Perhaps such differences reflect the effect of pH on rennet activity or perhaps some general proteolysis by rennet substitutes.

Heat treatment of milk under conditions that denature β-lactoglobulin and promote its interaction with κ-casein via sulphydryl-disulphide interaction adversely affect all aspects of rennet coagulation but especially the build-up of a gel network (Fig. 7.11) (van Hooydonk et al. 1987). Presumably, the attachment of denatured β-lactoglobulin to the surface of the casein micelles (as is evident from electron micrographs of casein micelles) prevents their aggregation in a form capable of building up a gel network.

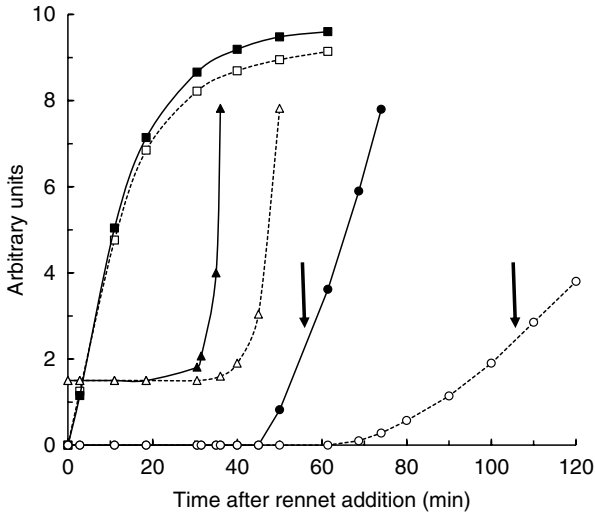


Fig. 7.11 Release of casein macropeptides (*filled square*, *open square*) and changes in the viscosity (*filled triangle*, *open triangle*) and curd firmness (*filled circle*, *open circle*) in skim milk which was unheated (*closed symbols*) or heated at 95 °C for 15 s (open symbols). *Arrows* indicate the time at which cutting is initiated during cheese manufacture (redrawn from data of van Hooydonk et al. 1987)

7.7 Measurement of Rennet Coagulation Properties

The rennet gelation of milk under quiescent conditions involves the conversion of milk from a colloidal dispersion of stable micelles to a network (gel) of aggregated *para*-casein micelles, which forms a continuous phase, entrapping moisture and fat globules in its pores; the gel becomes more elastic and firm with time (i.e., on ageing). The transformation is accompanied by a number of physico-chemical changes, e.g., hydrolysis of κ-casein with a concomitant increase in the concentration of the glycomacropeptide; aggregation of the sensitized *para*-casein micelles; increases in viscosity and elasticity; a decrease in the ratio of viscous:elastic character of the milk. Such changes may also alter some of the physical properties of the milk, e.g., light reflectance and thermal conductivity.

Numerous methods, the principles of which are based on detection of one or more of the above changes, have been developed to measure the rennet coagulation characteristics of milk or the activity of rennets. Owing to the commercial importance of gel formation from milk, as a means of recovering milk fat and casein in the form of cheese curd, most methods measure gel formation (also referred to as curd formation or rennet coagulability), i.e., combined first and second stages, but some specifically monitor the hydrolysis of κ-casein. Various terms or descriptors, some of which are used interchangeably, are used to describe the rennet coagulation of milk; these may be defined simply as follows:

- *aggregation*: the joining of particles (e.g., *para*-casein micelles) by various types of electrostatic or hydrophobic bonds; the aggregates are visible by electron microscopy.
- *coagulation or flocculation*: the collision and joining of aggregates, especially under non-quiescent conditions, to form flocs, visible to the naked eye.

- *gelation*: the aggregation of particles (e.g., micelles or aggregates of micelles) to form particulate strands, in which the particles undergo limited touching, and eventually form a gel network.
- *elasticity*: the ability of the gel to recover, instantaneously, its original shape and dimensions after removal of an applied stress; viscoelastic materials, such as a rennet-induced milk gel, are elastic at relatively small strains (e.g., 0.025; $\ll$ fracture strain); in this region of strain, known as the linear viscoelastic stress–strain region, the strain is directly proportional to the applied stress and the material (e.g., section of a gel strand which bears the applied stress and is strained) recovers its original dimensions immediately on removal of the stress.
- *viscosity*: the physical property of a gel given by the ratio between stress and strain rate.
- *Gel (curd) firmness, gel strength or gel tension*: the stress required to cause a given strain or deformation. (Curd tension is a term frequently used to express the firmness of formed gels).

Some of the methods used to measure gel-forming characteristics include:

- measurement of flocculation time under non-quiescent conditions, e.g., rennet coagulation test,
- dynamic measurement of the viscous drag created by gelling milk on a pendulum suspended in the renneted milk, by determining the tilt of the pendulum from its 'zero'-position, e.g., using instruments such as the Thrombelastrograph, Formagraph, Gelometer or Lattodinamografo.
- dynamic measurement of the ability of gelling milk to transmit a pressure, e.g., using the hydraulically-operated oscillating diaphragm apparatus,
- measurement of the apparent viscosity of gelled milk after a given time at a fixed shear rate (e.g., using various types of rotational viscometers), or alternatively measuring the firmness of the gel using various types of penetrometer,
- dynamic measurement of parameters such as viscosity, elastic shear modulus (G'), loss modulus (G'') and phase angle (δ), by applying a low-amplitude oscillating strain or stress to the milk sample, e.g., using a controlled strain or controlled stress rheometer,
- dynamic measurement of some physical properties of the gelling milk in the cheese vat using special probes, e.g., thermal conductivity (using a hot wire probe), reflectance of near infrared light (NIR diffuse reflectance probe).

Some of the more commonly used laboratory and on-line methods for monitoring the rennet-induced gelation of milk are described below.

7.7.1 Measurement of Primary Phase of Rennet Coagulation

The primary phase of rennet action may be monitored by measuring the formation of either product, i.e., *para*-κ-casein or the CMP. *Para*-κ-casein may be measured by SDS-polyacrylamide gel electrophoresis (PAGE), which is slow and

cumbersome or by ion-exchange high performance liquid chromatography (HPLC). The CMP is soluble in TCA (2–12 % depending on its carbohydrate content; Fig. 7.3) and may be quantified by the Kjeldahl method or more specifically by determining the concentration of N-acetyl neuraminic acid (Fig. 7.3) or by RP-HPLC. The activity of rennets can be determined easily using chromogenic peptide substrates, a number of which are available, e.g., the hexapeptide Pro.Thr.Glu.Phe(NO_2).Phe.Arg.Leu (Hurley et al. 1999). The latter methods are generally used as research tools to study rennet characteristics and/or the kinetics of the primary phase of rennet coagulation.

7.7.2 Methods for Assessing Coagulation, Gel Formation and/or Curd Tension

Various methods have been used to measure the coagulation and gel-forming properties of milk (O'Callaghan et al. 2002; Castillo et al. 2006). Some of these are discussed briefly.

- *Measurement of rennet coagulation time (RCT)*
  The simplest laboratory method for measuring the overall rennet coagulation process is to monitor the time elapsed between the addition of a measured amount of diluted rennet to a sample of milk in a temperature-controlled water bath at, e.g., 30 °C, and the onset of visual coagulation. If the coagulating activity of a rennet preparation is to be determined, a "reference" milk, e.g., low-heat milk powder reconstituted in 0.01 % $CaCl_2$, and perhaps adjusted to a certain pH, e.g., 6.5, should be used. A standard method has been published (IDF 1992) and a reference milk powder may be obtained from Institut National de la Recherche Agronomique, Poligny, France. If the coagulability of a particular milk is to be determined, the pH may or may not be adjusted to a standard value (e.g., 6.55) to reflect that which is typical at setting (rennet addition) during cheese manufacture. A better method [ISO 11851/IDF 157:2007], called the REMCAT [Relative Milk Clotting Activity Test] method, has been introduced for determination of the total milk clotting activity of calf rennet by comparison with a standard rennet supplied by Chr Hansen, Copenhagen, at pH 6.5. Activity is expressed International Milk Clotting Units [IMCU].
- The coagulation point may be determined by placing the milk sample in a bottle or tube which is rotated in a water bath (Fig. 7.12); the fluid milk forms a film on the inside of the rotating bottle/tube but flocs of protein form in the film on coagulation. The rennet coagulation time (RCT) provides a very good index of the gelation potential of milk; a low RCT usually indicates potentially good gel formation and high gel strength after a given renneting time. The method is simple and enables the accurate measurement of several samples simultaneously. This principle has been used to accumulate much of the extensive information reported in the scientific literature on the effects of various processing parameters

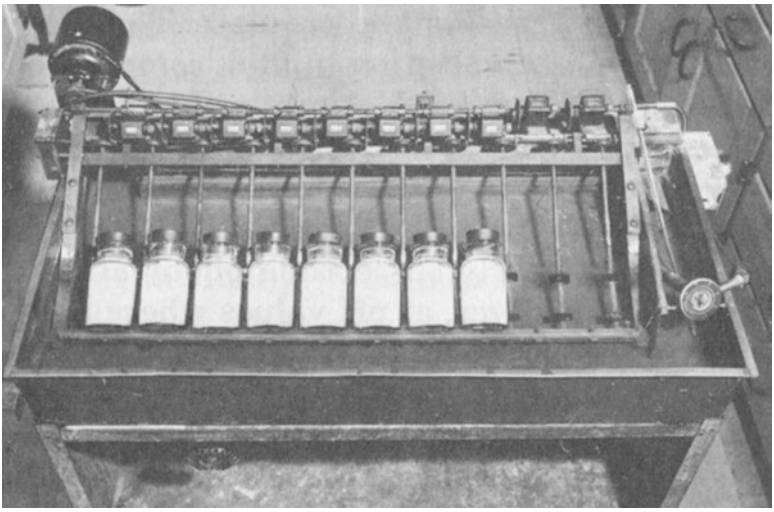


Fig. 7.12 Apparatus for measuring the rennet coagulation time of milk (from Sommer and Matsen 1935)

on the rennet coagulability of milk. However, in contrast to cheese manufacture, where milk is renneted under quiescent conditions to ensure gel formation, this method determines the time for coagulation (i.e., aggregation and flocculation) of the para-casein under agitation.

- *Nondynamic penetrometer, viscosity and gel firmness tests*
  Measuring the apparent viscosity, or firmness, of the coagulum after a fixed renneting time, using one of various types of viscometer or penetrometer, respectively, may be used to assess the coagulability of milk. However, this approach permits measurement at only a single point in time, which is a serious limitation in kinetic studies and also requires meticulous test conditions, since curd strength increases with time after renneting.
- *Dynamic gel firmness tests*
  Various instruments, involving different principles, have been developed to monitor changes continuously throughout the gelation process and are discussed below.

7.7.2.1 Lattodinamografo

The most popular of the dynamic measuring instruments, although with limited use, is the Lattodinamografo (e.g., Foss Italia S.p.A., Padova, Italy). The apparatus consists of:

- an electrically heated metal block,
- a sample rack with cavities (usually 10) into which sample cuvettes fit,

- a set of pendulums,
- the displacement of pendulum is measured by a transducer, and captured electronically. Samples of milk to be analysed are placed in the cuvettes and tempered to the desired temperature (typically 31 °C) in the heating block. Rennet is then added, the cuvettes replaced in the instrument so that a loop-shaped pendulum is suspended in each sample. The metal block is moved back and forth, creating a "drag" on, and displacing, the pendulum in the milk. The displacement is measured by a transducer and captured electronically on a personal computer, and plotted dynamically during measurement. While the milk is fluid, the viscosity is low and the drag on the pendulum is slight and it scarcely moves from its vertically suspended 'zero-time' position; hence, a single straight line is visualised on the computer screen. As the milk coagulates, its viscosity increases and the pendulum is dragged from its zero-time position, resulting in bifurcation of the trace. The rate at, and extent to, which the arms of the trace diverge are indicators of the gel-forming forming characteristics of the milk. A typical trace, shown in Fig. 7.13, may be used to calculate the following parameters:
- rennet coagulation time (r) in min, i.e., the time from rennet addition to the onset of gelation (i.e., point where the trace begins to fork);
- k20 in min, is the time from the onset of gelation until a firmness corresponding to a trace width of 20 mm is obtained; the rate of curd firming may be calculated from 1/k20.
- a_t in mm, the curd firmness at time, t, (e.g., 30 min, a_{30} or 60 min, a_{60}) after rennet addition, is the trace width at time t.

Good gel-forming properties are characterized by a relatively rapid coagulation time (low r value), high curd firming rate (low k_{20} value) and good gel firmness or strength after a given renneting time (high a_{30} value). Typical values for these parameters for a pasteurized mid-lactation milk (3.3 %, w/w, protein), renneted under normal conditions (rennet dosage, ~16 RU/L; pH 6.55, temperature, 31 °C) are: r, 5.5 min; k_{20}, 11 min and a_{30}, 48.5 mm.

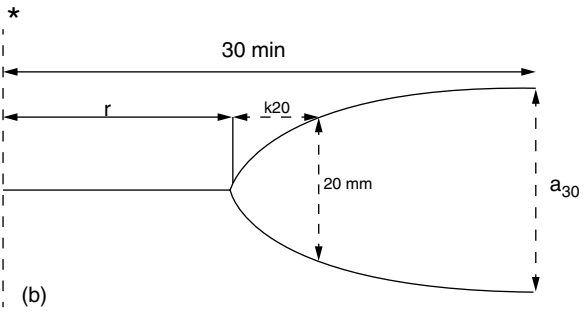


Fig. 7.13 Typical trace from the Lattodimamografo apparatus for measurement of the rennet gelation properties of milk: *Point of rennet addition, r is rennet coagulation time, k20 is the time required from coagulation for the bifurcated signal (related to the oscillation of the pendulum) to reach a width of 20 mm, and a_{30} is the extent of bifurcation 30 min after rennet addition

While the latter parameters have no precise rheological significance, the Lattodinamografo offers many advantages over the RCT test:

- the method simulates gel formation during cheesmaking;
- the results of the assay are less subjective, being independent of operator judgement;
- the test parameters provide more information on the changes in curd strength over time.

7.7.2.2 Hydraulically Oscillating Diaphragm

A hydraulically-operated oscillating diaphragm apparatus was developed by Vanderheiden (1976). In this apparatus, a sample of milk is placed between two diaphragms (Fig. 7.14) and rennet is added. One diaphram (the transmitting diaphram) is made to vibrate through the cyclical application of hydraulic pressure. When the milk is liquid, the effect of the vibration is dissipated rapidly and does not affect the receiving diaphragm. When a gel is formed, the vibrations emitted by the transmitting diaphragm reach the receiving diaphragm, causing it to vibrate. These vibrations are detected and quantified by a suitable sensing device. An output generally similar to that of the Lattodinamograffo is obtained, from which the coagulation time and a measure of gel strength is obtained. A variation of this apparatus was evaluated by Kowalchyk and Olson (1978), but has not been commercialised (Castillo et al. 2006).

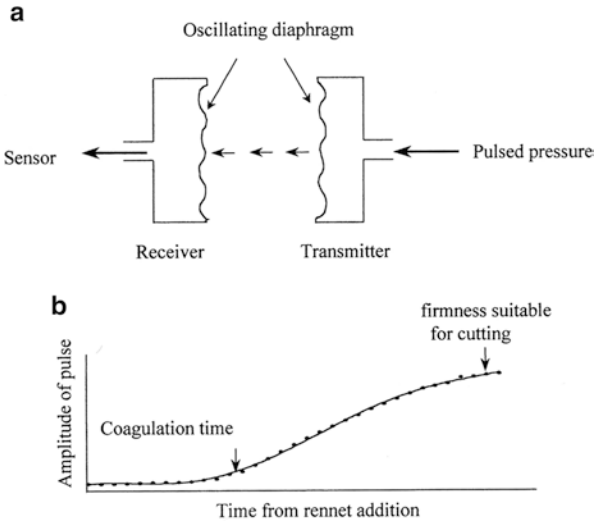


Fig. 7.14 Schematic representation of a pressure transmission apparatus for measuring the rennet coagulation time of milk and the strength of the resultant gel

7.7.2.3 Low-Amplitude Stress or Strain Rheometry

Since the 1980s, several controlled strain (e.g., Bohlin VOR, Bohlin Rheologi, Sweden; Rotovisco RV 100/CV 100, Haake Bucchler Instruments, USA; Physica MCR 501, Anton Paar GmbH, A-8054 Graz, Austria) or controlled stress rheometers (e.g., Bohlin CS, Bohlin Rheologi, Sweden; Cari-med CSL^2, TA Instruments, New Castle, DE 19720, USA; Rheometric Scientific SR5, Rheometric Scientific Inc, USA) are being used increasingly as a research tool for the continuous measurement of the viscoelastic properties of renneted milks as a function of time from rennet addition.

Dynamic measurements are performed by applying a low-amplitude oscillating shear stress (σ) or shear strain (γ), depending on the type of rheometer, to the milk sample, via oscillations of the outer cylinder. The value of σ or γ are maintained sufficiently low so as to stay within the linear viscoelastic limits of the sample (i.e., region where σ and γ are directly proportional); hence, the term low-amplitude stress or strain oscillation, where amplitude refers to the maximum displacement of any point on the oscillating cup (and hence in the milk sample or on the inner bob) from its mean, or 'zero', position. Under these conditions, the gel strands of the gelling milk are strained to a fixed displacement (within their elastic limit) and recover instantaneously when the stress is removed. The stress required to achieve a fixed strain increases as the gel strands become more elastic and rigid; hence, measurement of stress energy provides a measure of the gel strength.

When using a controlled strain rheometer, the sample of renneted milk is subjected to an harmonic, low-amplitude shear strain, γ, of angular frequency ω:

$$\gamma = \gamma_0 \cos \omega t$$

where: γ_0 = shear strain amplitude, ω = angular frequency (i.e., $2\pi f$; f = frequency of oscillation), and cos ωt is a term of the simple harmonic function. The shear strain results in an oscillating shear stress, σ, on the milk, of the same angular frequency but which is out of phase by the angle δ:

$$\sigma = \sigma_0 \cos\left(\omega t + \delta\right)$$

where: σ_0 = stress amplitude; δ = the phase angle between shear stress and shear strain oscillations, the magnitude of which depends on the viscoelasticity (ratio of viscous to elastic properties) of the gelling system (Fig. 7.7). The following rheological parameters are computed continually from the measurement of stress energy over time:

- **Storage or elastic shear modulus, G′**, which represents elastically stored stress energy and thus gel elasticity or firmness, is given by the equation:

$$G' = \left(\sigma_0 / \gamma_0\right) \cos \delta,$$

where σ_0 = stress amplitude, γ_0 = shear strain amplitude, and δ = the phase angle between shear stress and shear strain oscillations.

- **Viscous or loss modulus, G″**, which represents energy dissipated in flow, is given by the equation:

$$G'' = \left(\sigma_0 / \gamma_0\right) \sin \delta$$

- **The phase angle**, δ, the angle between the stress and strain, ranges from 0° for an ideal elastic solid to 90° for a Newtonian liquid, and between these for viscoelastic materials. Typical changes in the above rheological parameters with time after rennet addition are presented in Fig. 7.7. The onset of gelation is marked by sharp increases in G′ and G″ and a decrease in δ, which decreases abruptly from ~80° in milk to ~10° and marks the transition from a viscoelastic material which is largely viscous, i.e., milk, to a gel which is largely elastic in character.

G″ and δ are useful parameters for monitoring the viscoelastic changes in the gel during ageing, but are not directly related to gel strength. In contrast, G′, is a direct measure of curd firmness and is thus of significance in cheese manufacture. Various objective rennet coagulation parameters, which are pertinent in cheesemaking, may be derived from the G′-time curve, on modelling, as described below (Guinee et al. 1996):

- Gel time, defined as the time at which G′ reaches a threshold value, G_g, arbitrarily set at 0.2 Pa
- The firmness after a fixed renneting time, e.g. 30 or 60 min, G'_{30} or G'_{60}
- Maximum curd firming rate, defined as the maximum slope, S_{max}, of the G′-t graph
- Set-to-cut time (SCT, i.e., time between rennet addition and gel cutting) at a suitable firmness, e.g., 40Pa, SCT40Pa

7.7.2.4 On-Line Sensors for Predicting Gel Firmness and Cutting Time During Cheese Manufacture

Following the onset of gelation, there is a progressive increase in firmness and the gel eventually attains an optimum firmness (e.g., 40 Pa, after 40–50 min, depending on milk composition and renneting conditions) which, for a given vat design, allows it to withstand the mechanical action of the cutting knives without shattering. Curd shattering results from fracturing of individual curd particles (e.g., by the cheese knife, by impact with other curd particles and/or the knife and walls of the vat), especially if the gel is too soft or too rigid at cutting. Shattering results in an excessively large curd particle surface area (through which fat is lost into the cheese whey) and forms very small curd particles (i.e., curd fines, <1 mm) which are also lost in the whey. Thus, cutting at the optimum firmness and rate of curd firming are crucial in obtaining the correct particle size, minimizing the losses of fat and fines in the whey and maximizing cheese yield (Fig. 7.15).

The firmness of the gel after a given set-to-cut time is influenced by many factors, such as the concentrations of fat and casein in the milk, the stage of lactation

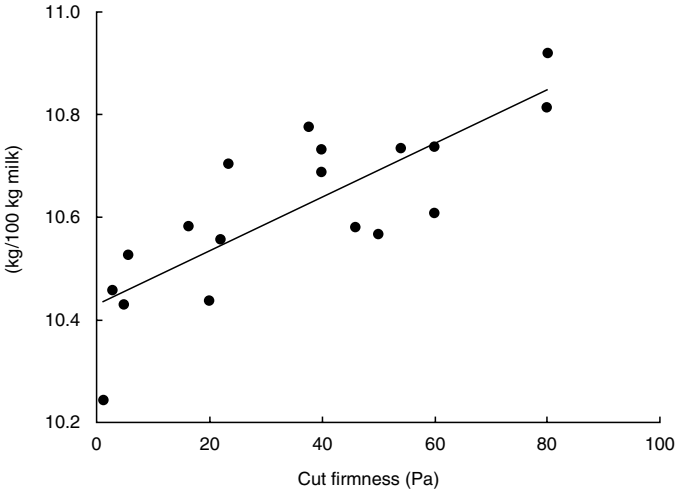


Fig. 7.15 Effect of gel firmness on the yield of Cheddar cheese (Guinee, unpublished results)

and diet of cow, milk pH, starter activity (which influences pH at setting and cutting) and rennet-to-casein ratio. Variation in the firmness at cutting can lead to variations in cheese composition (especially moisture), yield and quality. Hence, standardizing and optimizing the firmness at cutting is essential for ensuring a high cheese yield and good quality cheese.

Formerly, the time at which the gel was cut was usually determined by the cheese maker, who subjectively assessed firmness by various means, e.g., making a small cut with a knife and observing "cleanness" of the cut and the clarity of exuding whey. However, in large modern factories, conditions are not conducive to testing gel firmness in cheese vats from different milk silos because of the large scale of operation ($>2–3 \times 10^6$ L/day) and the use of pre-programmed vats with limited operator access. Hence, most of the cheesemaking operations are performed on the basis of a pre-set time schedule rather than on the basis of objective criteria, such as gel firmness at cutting, pH at whey drainage, etc. The criteria on which gel cut times are established include ongoing composition of the cheese, and/or losses of fat or curd fines in the whey.

The above methods do not enable cutting at constant gel firmness in every vat. The limitation of the current methods for assessing gel firmness in the cheese factory has led to the development of in-vat gel firmness sensors, which dynamically monitor milk coagulation and, when incorporated into an integrated system, activate the curd knives to cut the gel when it has attained the desired firmness (strength). The mechanisms used to date in designing in-vat sensors to monitor the development of curd firmness over time include monitoring related changes in:

- Convective heat transfer from a probe (a 'hot wire') to the surrounding milk, as in hot wire probe sensors (Hori 1985; Bellon et al. 1988; Lefevre and Richardson 1990).

- Turbidity (McMahon et al. 1984).
- Diffuse reflectance of visible (e.g., λ, 660 nm), NIR (e.g., λ, 820 nm) or IR light (e.g., λ, 950 nm), as in various fibre optic probes, the CoAguLite fibre optic sensor and Omron E3XA (Payne et al. 1993).
- Transmission NIR probes, e.g., TxPro, Gelograph NT (O'Callaghan et al. 1999).
- absorption and attenuation of ultrasound waves, or pulses, of different frequencies (e.g., >0.5 MHz) passed through the milk (Gunasekaran and Ay 1994; Benguigui et al. 1994).

The subject has been comprehensively reviewed by O'Callaghan et al. (2002) and O'Callaghan (2011). The hot wire and NIR reflectance probes are manufactured commercially by Stoelting Inc. (Kiel, WI) and Reflectronics, Inc (Lexington, KY) as Optiset and CoAguLite, respectively.

7.7.2.5 The Hot Wire Probe

The principle of measurement is based on changes in heat transfer from a hot wire to the milk (http://www.reflectronics.com/products.html). A constant current is passed through the wire, generating heat, which is dissipated readily, by convection currents near the wire, while the milk is liquid. As the milk coagulates, its viscosity increases and generated heat is no longer readily dissipated; the temperature of the wire increases, causing an increase in its resistance. The resistance and temperature of the wire are dynamically measured by monitoring changes in voltage across the wire, giving a continuous output signal. Equations have been developed to relate the output from the hot wire to the rheological properties of the gel.

7.7.2.6 NIR Diffuse Reflectance Fibre Optic Probe

The principle of measurement is based on changes in the light scattering properties of milk (http://www.trademarkia.com/optiset-74011453.html). Light scattered by both the fat globules and casein micelles is detected by the optical fibres and transmitted to a photodetector. As the milk coagulates, more light is reflected (due to the aggregation of the *para*-casein micelles) and transmitted to the photodetector, the output signal from which is directly proportional to the amount of light received. The output signal is related to the rheological properties of the gel, which is then related to a cut time at a given firmness, as determined by laboratory instruments.

7.8 Factors that Affect Rennet Coagulation

In addition to the actual coagulation time, the strength of the resulting gel (curd tension, CT) is equally, and perhaps more, important, especially from the point of view of cheese yield. The gel assembly process is quite slow (Fig. 7.7) and in the case of most cheese varieties a period roughly equal to the RCT is allowed from the onset

of visual coagulation for the gel to become sufficiently firm prior to cutting. If the gel is too soft or too rigid when cut, fat and casein losses may be high (Bynum and Olson 1982; Guinee et al. 1994). In general, there is an inverse relationship between RCT and CT and therefore any factor that reduces RCT increases CT and vice versa. The effect of various compositional and environmental factors on the primary and secondary phases of rennet coagulation are summarized in Table 7.2.

7.8.1 Concentration of Milk Protein

The coagulation time of milk decreases markedly with protein (and thus casein) content, in the range 2.0–3.0 % (w/w), when rennet is added on a volume basis (Figs. 7.16 and 7.17). Further increases in milk protein level (i.e., >3.0 %, w/w) result in a slight increase in gelation time, an effect attributable to the decreasing rennet-to-casein ratio, which necessitates an increase in the time required to generate sufficient hydrolysis of κ-casein to induce aggregation of *para*-casein micelles. At a constant rennet:casein ratio, the RCT decreases with increasing casein concentration, e.g., as obtained by ultrafiltration, and vice versa. From a practical viewpoint, a minimum protein level of 2.5–3.0 % (w/w) is necessary for gel formation in cheese manufacture, i.e., within 40–60 min. The maximum gel firming rate (S_{max}) and gel firmness (G′) increase more than proportionally with protein level (Fig. 7.17), with a power law dependence of the latter parameters and protein concentration, i.e., $S_{max} \propto P^{n1}$ and $G' \propto P^{n2}$, where n1 and n2 > 1.0, typically ~2.0 (Guinee et al. 1996, 1997). Hence, small variations in protein content, as can occur throughout the cheesemaking season, exert a relatively large effect on its rennet coagulation properties. The positive effects of the higher milk protein content on the rennet coagulation properties probably ensue from the higher level of gel-forming protein which increases the proximity of casein micelles and thus augments the rate of casein aggregation.

One of the economic attractions in using of UF-concentrated milk in cheesemaking is the savings that accrue from using less rennet. Cheese made from UF-concentrated milk ripens more slowly than normal, due partly to slower proteolysis, for which there may be a number of causes, including the lower ratio of rennet:casein, elevated levels of plasmin inhibitors, a higher degree of casein aggregation.

7.8.2 Concentration of Milk Fat

Increasing fat content in the range 0.1–10 %, w/w, while maintaining the protein level constant (e.g., at 3.3 %, w/w), enhances the rennet coagulation properties, as reflected by decreases in coagulation time and set-to-cut time and higher values for S_{max} and G′ (Fig. 7.17). However, the positive effects are much smaller than those obtained on increasing protein content in the same range. Indeed, in a milk in which the level of fat plus protein is maintained constant, increasing the fat content results

Table 7.2 Effects of some compositional and processing factors on various aspects of the rennet coagulation of milk

Factor	First phase	Second phase	Overall				
			RCT[a]	GT[b]	Curd firming rate	Curd firmness after a fixed renneting time	Set-to-cut time
Increasing the casein level when rennet is added on a volume basis	–	+++	ND	⇓	⇑	⇑	⇓
Increasing the fat content of milk:							
when casein level is constant	ND	+	ND	⇓	⇑	⇑	⇓
when fat plus casein levels are constant	ND	–	ND	⇑	⇓	⇓	⇑
Pasteurization temperature:							
60 °C × 15 s	++	+++	⇓	ND	ND	ND	ND
>72 °C × 15 s	––	––	⇑	⇑	⇓	⇓	⇑
Milk homogenization	ND	ND	ND	⇓	NE	⇑	⇓
Added $CaCl_2$:							
0.2–10 mM	NE	+	⇓	⇓	⇑	⇑	⇓
>10 mM	ND	–	ND		⇓	⇓	⇑
Gelation temperature (4'35 °C)	+	+++	⇓	⇓	⇑	⇑	⇓
Decreasing gelation pH (6.6'6.0)	+++	+	⇓	⇓	⇑	⇑	⇓
Rennet concentration	+++	ND	⇓	⇓	⇑	⇑	⇓

[a]RCT, rennet coagulation time, as determined by the rennet coagulation time assay
[b]GT, gelation time as determined by dynamic methods such as the Formagraph method or low-amplitude strain oscillation rheometry
NE No Effect
ND No data available
– negative effect, slight (–), moderate (––), large (–––)
+ positive effect, slight (+), moderate (++), large (+++)
⇑⇓ arrows indicate that the magnitude of the rennet coagulation parameter increases (⇑) or decreases (⇓) with the factor

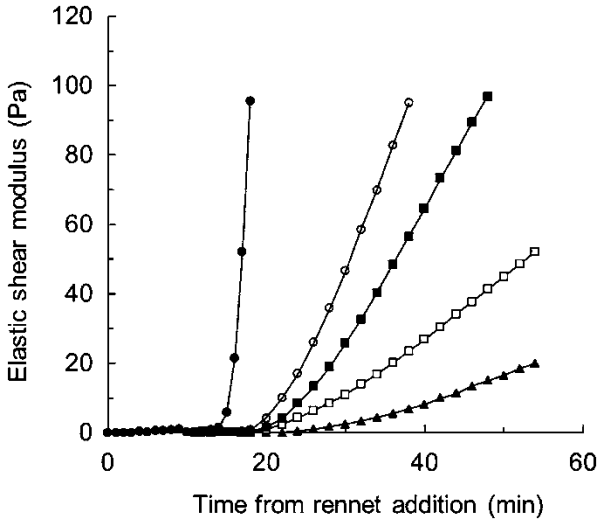


Fig. 7.16 Effect of milk protein level [3.0 (*filled triangle*), 3.5 (*open square*), 4.5 (*filled square*), 6.9 (*open circle*) or 8.2 (*filled circle*) g/kg] on the elastic modulus of rennet-coagulated milk. Milks with protein levels of 3.5–8.2 % were prepared by ultrafiltration of milk with 3.0 % protein. Coagulation parameters that may be derived from the curve are: gelation time, point at which G′ begins to increase; curd firming rate, slope of G′/time curve in the linear region; and curd firmness, the value of G′ at a given time from rennet addition (redrawn from Guinee et al. 1994)

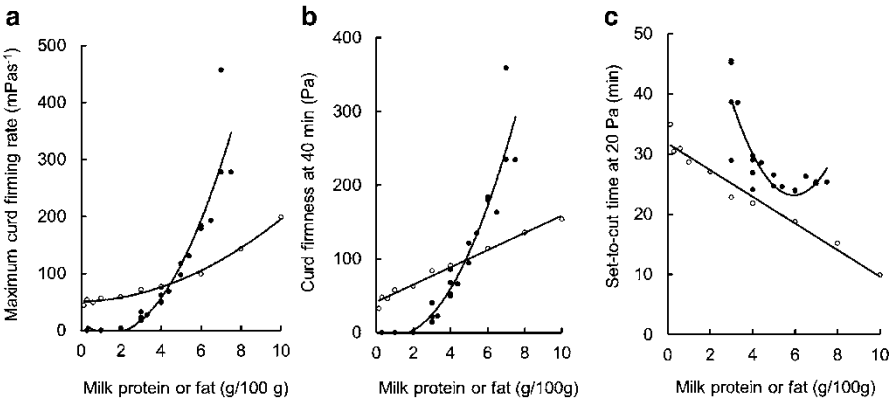


Fig. 7.17 Effect of increasing level of protein (*filled circle*) in skim milk, or fat (*open circle*) in milks containing 3.3 % protein, on rennet coagulation properties: maximum curd firming rate (**a**), curd firmness at 40 min after rennet addition (**b**) and set-to-curd time at a firmness of 20 Pa (**c**) (redrawn from Guinee et al. 1997)

in significant decreases in S_{max} and G′. In commercial cheese manufacture where standardization of milk protein to a fixed level (e.g., by ultrafiltration of skim milk) is not normally practised, S_{max} and G′ increase progressively on adding cream to give a fat content of ~4 %, w/w, and decrease thereafter. The latter effect is due to dilution of the protein, which eventually offsets the benefits of increasing fat content. The effect of increasing the fat level in milk, where the level of gel-forming protein is constant, is probably due to the concomitant increase in viscosity.

7.8.3 Pasteurization Temperature

Pre-heating milk up to ~65 °C has a beneficial effect on rennet coagulation, owing to heat-induced precipitation of calcium phosphate and a concomitant decrease in pH. These changes occur also at higher temperatures but their beneficial effects on rennet coagulation are offset and eventually overridden by the combined effects of:

- whey protein denaturation and the interaction of denatured β-lactoglobulin with micellar κ-casein, and
- the deposition of heat-induced, insoluble calcium phosphate and the reduction on subsequent cooling of the concentration of native micellar calcium phosphate, which is important for cross-linking *para*-casein micelles, and hence aggregation, during gel formation.

The complexation of denatured whey protein with κ-casein adversely affects both the enzymatic and non-enzymatic phases of rennet-indeced coagulation, but especially the latter. Increasing the extent of whey protein denaturation (as a % of total) to a level >15 % by high heat treatment of milk (e.g., >80 °C × 15 s), impairs the rennet coagulation characteristics to such an extent that the milk is unsuitable for cheese manufacture (Fig. 7.18). Very severely heated milk, e.g., 90 °C × 10 min (80–90 % total whey protein denatured), is not coagulable by rennet.

If heated milk is cooled, the RCT increases further (Fig. 7.19), a phenomenon referred to as ‘rennet hysteresis’. The effect can be explained as follows: the adverse influence of the interaction of β-lg with κ-CN on rennet-induced coagulation is offset to some extent by the beneficial effect of heat-precipitated calcium phosphate and reduced pH. However, heat-induced changes in calcium phosphate are at least partially reversible on cooling and hence the full adverse effects of the protein interaction become fully apparent on cooling. In practice, milk should be pasteurized immediately before cheesemaking and should not be cold-stored before use.

7.8.4 Cooling and Cold Storage of Milk

Cooling and cold storage of milk (raw or heated) have adverse effects on the cheesemaking properties of milk. Apart from the growth of psychrotrophs, two undesirable changes occur:

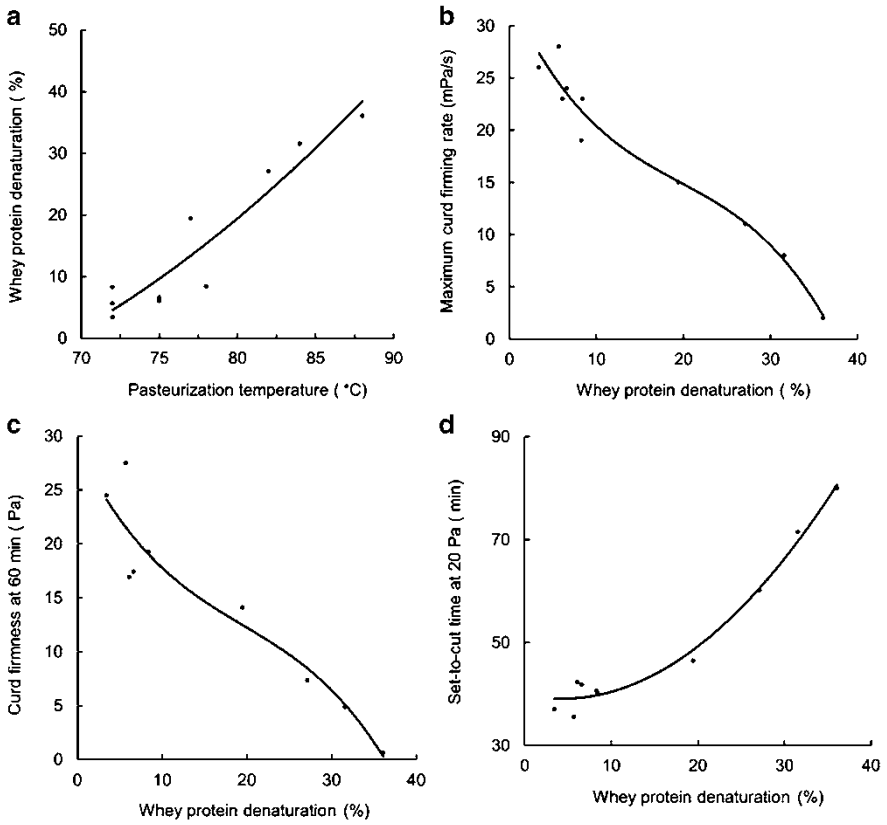


Fig. 7.18 Effect of pasteurization temperature, for 15 s, of milk on the level of whey protein denaturation (**a**), maximum firming rate (**b**), curd firmness 60 min after rennet addition (**c**) and set-to-curd time at a firmness of 20 Pa (**d**) (redrawn from Guinee et al. 1997)

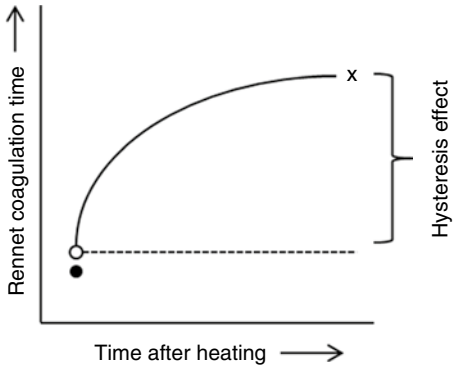


Fig. 7.19 Schematic representation of the hysteresis effect on the rennet coagulation time (RCT) of heated milk. RCT of raw milk (*filled circle*), milk immediately after pasteurization (*open circle*) and 6 h after pasteurization (X)

- Some indigenous colloidal calcium phosphate dissolves, with a concomitant increase in pH, and
- Some proteins, especially β-casein, dissociate from the micelles.

These changes are reversed by HTST pasteurization or by heating at a lower temperature, e.g., 31 °C, for a longer period.

7.8.5 Milk Homogenization

Homogenization of milk is practised in the manufacture of some cheese varieties where lipolysis is important for flavour development, e.g., blue cheese, the objective being to increase the accessibility of the fat to fungal lipases, and thus to increase the formation of fatty acids and their derivatives (e.g., methyl ketones). Homogenization is an essential part of the manufacturing process for cheeses from recombined milks. Homogenization reduces fat globule size and increases the surface area of the fat by a factor of 5–6. Simultaneously, the fat globules become coated with a protein layer consisting of casein micelles, micelle subunits and whey proteins. Hence, the newly-formed fat globules behave as pseudo-protein particles with the ability to become part of the gel network. Numerous studies have been undertaken to evaluate the effect of homogenization under different conditions of temperature, pressure and/or milk fat level. While there are some discrepancies between the results of these studies, the main trends indicate that homogenization lowers the gelation time slightly, has no effect on curd firming rate and causes a slight increase in G′. However, the higher moisture content of cheese made from homogenized milk, compared to that from unhomogenized milk, suggests that homogenization may alter the rate of casein aggregation during the later stages of cheese manufacture (i.e., after cutting).

7.8.6 Renneting (Set) Temperature

The principal effect of set temperature is on the secondary, non-enzymatic phase of coagulation, which does not occur at temperatures <~18 °C. Above this temperature, the coagulation time decreases to a broad minimum at 40–45 °C and then increases again as the enzyme becomes denatured (Fig. 7.20). In cheesemaking, rennet coagulation normally occurs at a temperature well below the optimum temperature, e.g., 31 °C for many varieties. The lower temperature is necessary to optimize the growth of mesophilic starter bacteria, which have an optimum growth temperature of ~27–28 °C and will not grow at, or perhaps not even survive, >40 °C. In addition, the structure of the coagulum is improved at the lower temperature which is therefore used even for cheeses made using thermophilic cultures.

Factor	First phase	Second phase	Overall effect, see panel
Temperature	+	++	a
pH	+++	-	b
Ca	-	+++	c
Pre-heating	++	++++	d
Rennet concentration	++++	-	e
Protein concentration	+	++++	f

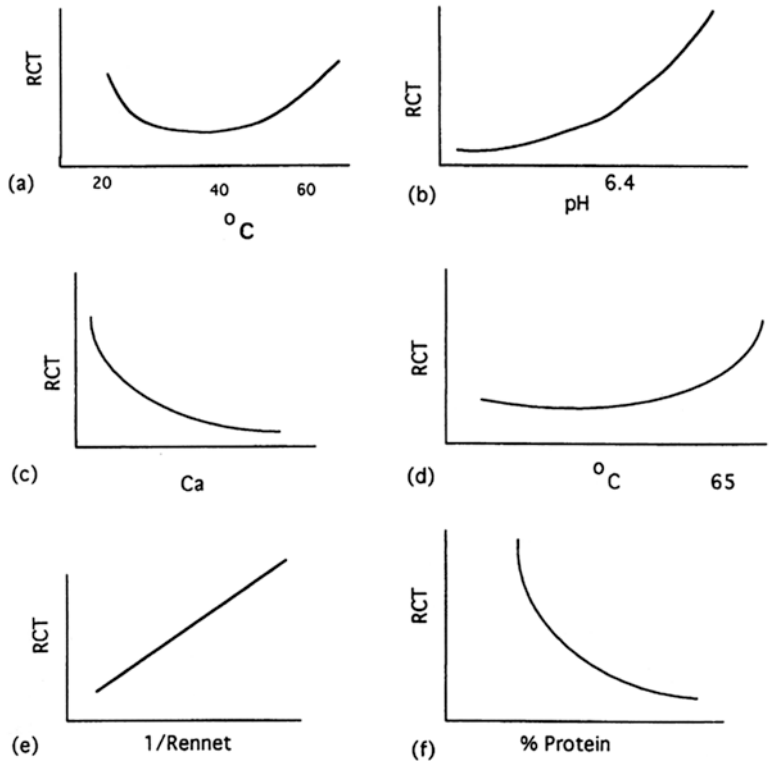


Fig. 7.20 Effect of various factors on the rennet coagulation time of milk (from Fox and McSweeney 1998)

As shown by changes in absorbance at 600 nm, sedimentability at 5000×g of 1 h, viscosity, or inelastic light scattering, micelles do aggregate on renneting at 10 °C, apparently due to hydrolysis of β-casein released from the micelles at low temperatures (Bansal et al. 2007). Aggregation of renneted micelles is promoted by adding $CaCl_2$ or reducing the pH (Bansal et al. 2008).

7.8.7 pH

Due to the effect of pH on the activity of the enzyme, the rennet coagulation time increases with increasing pH, especially >pH 6.4 (Fig. 7.20). The sensitivity to pH depends on the rennet used; porcine pepsin is particularly sensitive while the microbial rennets are relatively insensitive. Owing to the dependence of the rennet coagulation of milk on pH, factors that affect the pH of milk (amount and form of starter added, addition of $CaCl_2$, ripening of milk, pH adjustment by addition of acid or acidogen, pH of the milk itself as effected by mastitis or stage of lactation) affect rennet coagulability. Gel firmness increases markedly with decreasing pH to a maximum at pH 5.9–6.0; the decrease in CT at lower pH values may be due in part to solubilization of colloidal calcium phosphate as the pH is reduced. The pH of milk increases markedly on mastitic infection and may exceed 7.0, i.e., it approaches the pH of blood (~7.4). Mastitic milk has a longer RCT and lower CT than milk from healthy cows, due to a combination of factors, e.g., high pH, low casein content, and the high somatic cell count (e.g., $>1 \times 10^6$ cells/ml) and associated proteolytic activity (which causes extensive hydrolysis of α_s- and β-caseins). The pH of first colostrum may be as low as 6.0 but increases to the normal value (6.7) within about 1 week and then remains relatively constant for most of the lactation, before increasing substantially (to pH 7 or even higher) at the end of lactation.

7.8.8 Added $CaCl_2$

The addition of $CaCl_2$ to milk, which is common practice, promotes rennet coagulation via three beneficial changes:

- increase in $[Ca^{2+}]$,
- an increase in the concentration of colloidal calcium phosphate, and
- a decrease in pH (the addition of $CaCl_2$ to 0.02 % (0.2 g/L), i.e., 1.8 mM Ca, reduces the pH by ~0.05–0.1 units, depending on protein level).

Hence, the addition of $CaCl_2$ (to 0.2 g/L, i.e., ~1.8 mM Ca) enhances the rennet coagulation properties as reflected by a reduction in RCT and increases in curd firming rates and curd firmness (Fig. 7.20). However, on addition of >0.2 g $CaCl_2$/L, the curd firming rate and curd firmness plateau and decrease again at levels greater ≥1.0 g/L (i.e., ≥9 mM Ca). The decrease in CT at the higher Ca levels may be due to the interaction of the excess Ca^{2+} with the negatively charged carboxyl groups on *para*-casein, which increases the positive charge on the casein, making it less prone to aggregation. As expected, the addition of calcium chelators (e.g., EDTA, sodium phosphates) reduces gel firmness. Addition of NaCl increases gel firmness up to 0.35 M but markedly decreases it at higher concentrations, possibly via displacement of micellar Ca by Na^+.

7.8.9 Rennet Concentration

Obviously, the rate of the enzymatic phase of rennet-induced coagulation is directly related to the amount of rennet used; there is an inverse relationship between enzyme concentration and milk clotting activity (MCA) (Fig. 7.20). However, the results of studies differ in relation to the effect of rennet level on the curd firming rate and curd firmness, with some studies showing increases in the latter parameters and others no effect or slight decreases, depending on the stage of lactation.

In cheesemaking, the amount of rennet added is sufficient to coagulate the milk in 30–40 min [200–220 ml of standard calf rennet (~60 RU/ml) per 1000 l of milk]. This level of rennet is traditional and is presumably based on experience. However, the amount of rennet retained in the curd is proportional to the amount of rennet added to the milk (at least for calf rennet) and this has a major effect on the rate of proteolysis during ripening. The retention of gastric rennets (e.g., calf chymosin or bovine pepsin) increases with decreases in pH at gel cutting and at whey drainage. On the other hand, the retention of *R. miehei* (i.e., Rennilase) and *R. pusillus* (i.e., Emporase) proteinases is not influenced by pH at cutting or at whey drainage.

Gel strength is strongly influenced by the type of rennet used: calf chymosin gives a more rapid increase in gel strength than microbial rennets although the substrate on which the rennets were standardized for clotting activity is of some significance. The fact that rennets standardized to equal clotting activity cause different rates of curd firming and the response thereof to compositional factors, e.g., $[Ca^{2+}]$, suggests possible differences in the extent and/or specificity of proteolysis during the enzymatic phase of rennet coagulation. As far as is known, the primary phase of coagulation by all the principal coagulants involves cleavage of the Phe_{105}-Met_{106} bond except *C. parasitica*, which hydrolyses Ser_{104}-Phe_{105}; other bonds are also hydrolysed by microbial rennets (Tam and Whitaker 1972; Vanderpoorten and Weckx 1972; see Chap. 12). The amount of rennet used seems to be optional but the influence of increasing concomitantly both the level of rennet used and starter cell numbers does not seem to have been investigated as a possible means of accelerating cheese ripening.

7.8.10 Other Factors

The rennet coagulation properties of milk may be influenced by the stage of lactation and diet, which cause changes in milk composition (i.e., casein, fat, mineral level, pH), degree of casein hydrolysis (e.g., as influenced by plasmin and other proteinases) and the health of the cow. These effects tend to be more marked in countries such as Ireland, New Zealand and Australia, where milk is largely from spring-calving herds, fed predominantly on pasture. Late lactation milk, especially

when the lactose level is <4.1 % (w/w), is frequently associated with a long coagulation time and low gel firmness. These defects may be alleviated by drying-off cows at a milk yield >8 L/day, improving the quality of the feed, blending late lactation milk with early lactation milk, and standardizing the cheesemaking process, e.g., pH at set or rennet-to-casein ratio.

7.9 Rennet Substitutes

Owing to increasing world production of cheese (~2–3 % p.a. over the past 30 years), concomitant with a reduced supply of calf vells (due to a decrease in calf numbers and a tendency to slaughter calves at an older age), the supply of calf rennet has been inadequate for many years. This has led to an increase in the price of veal rennet and to a search for rennet substitutes. Despite the availability of numerous potentially useful milk coagulants, only six rennet substitutes (all aspartyl proteinases) have been found to be acceptable for cheese production: bovine, porcine and chicken pepsins and the acid proteinases from *Rhizomucor miehei*, *R. pusillus* and *Cryphonectria parasitica*. (*Rhizomucor* and *Cryphonectria* were previously known as *Mucor* and *Endothia*, respectively).

In addition to fulfilling the criteria laid down by legislative agencies regarding purity, safety and the absence of antibiotics (IDF 1990), rennet substitutes must possess the following characteristics (Guinee and Wilkinson 1992):

- A high ratio of MCA-to-proteolytic activity, as for example with calf rennet, prevents excessive non-specific proteolysis during manufacture and hence protects against a weak gel structure, high losses of protein and fat in the whey and reduced yields of cheese solids. Moreover, it avoids excessive proteolysis during maturation and thus ensures the correct balance of peptides and hence desirable flavour, body and functional characteristics in the ripened cheese, and its suitability for certain applications (e.g., processed cheese products, cheese powder). Excessive proteolysis, especially of β-casein, is associated with the development of a bitter flavour.
- A MCA which is not very pH-dependent in the region 6.5–6.9 (a sharp decrease in MCA with increasing pH may lead to slow gelation and a low gel strength at cutting, especially if the milk pH at setting is high, e.g., 6.7–6.8, as may occur in late lactation) or when the casein concentration is low (e.g., <2.4 %, w/w). These conditions are conducive to low recovery of fat and a reduced cheese yield and can occur in large factories where the duration of milk ripening is short (especially with the use of direct-to-vat starters) and the production steps (including cutting) are generally carried out according to a fixed time schedule. The addition of $CaCl_2$ or acidulants (e.g., gluconic acid-δ-lactone) may overcome the latter problems.
- Thermostability comparable to that of calf rennet at the pH values and temperatures used during cheesemaking. This can markedly influence the level of residual

rennet in high cook cheeses such as Emmental, Pecorino Romano, Provolone and low-moisture Mozzarella and hence the level of proteolysis, texture and functionality of the cheese during maturation (see Chaps. 12 and 19).

- Low thermostability of rennets during whey processing is desirable; otherwise, the rennet in the whey (~90 % of that added to the cheesemilk) may lead to coagulation of formulated milks on reconstitution, which normally include whey (e.g., infant formulae, calf milk replacer).
- Must give finished cheese with the desired flavour, body and texture characteristics. Chicken pepsin is the least suitable of the commercial rennet substitutes and was used widely only in Israel where it has now been replaced by fermentation chymosin. Owing to its high ratio of proteolytic activity-to-MCA, chicken pepsin promotes extensive degradation of both α_{s1}- and β-caseins, leading to the development of flavour (e.g., bitterness) and textural (soft body and greasiness) defects during maturation. The activity of porcine pepsin is very sensitive to pH >6.6 and it may be denatured extensively during cheesemaking and consequently proteolysis during cheese ripening may be impaired. A 50:50 mixture of porcine pepsin and calf rennet gave generally acceptable results but porcine pepsin has been withdrawn from most markets. Bovine pepsin is probably the most satisfactory rennet substitute; good quality veal rennet contains ~10 % bovine pepsin and many commercial "calf rennets" contain ~50 %. Its proteolytic specificity is similar to that of calf chymosin and it gives generally satisfactory results with respect to cheese yield and quality.

Although the proteolytic specificity of the three commonly used fungal rennets is considerably different from that of calf chymosin, they have given generally satisfactory results with most cheese varieties. However, the proteolytic activity of all the rennet substitutes is higher than that of calf chymosin, resulting in higher levels of protein in the cheese whey and lower cheese yields (Fig. 7.21). Prior to the introduction of genetically engineered chymosin, microbial rennets were used widely in the United States but not in most European countries, Australia or New Zealand. The extensive literature on rennet substitutes has been reviewed (see Guinee and Wilkinson 1992; Grag and Johri 1994; Fox and McSweeney 1997 and Jacob et al. 2011).

Like chymosin, all commercially successful rennet substitutes are acid (aspartyl) proteinases. The molecular and catalytic properties of the principal rennet substitutes are generally similar to those of chymosin (see Foltmann 1993; Chitpinityol and Crabbe 1998; Crabbe 2004). Acid proteinases have a relatively narrow specificity, with a preference for peptide bonds to which a bulky hydrophobic residue supplies the carboxyl group; this narrow specificity is significant for the success of these enzymes in cheese manufacture. The fact that the pH of cheese is far removed from their optima (ca. 2 for porcine pepsin) is probably also significant. However, not all acid proteinases are suitable as rennets because they are too active even under the prevailing relatively unfavourable conditions in milk and cheese. The specificity of porcine and bovine pepsins on α_{s1}- and β-caseins is quite similar to that of chymosin but the specificity of the fungal rennet substitutes is quite different (see Chap. 12).

Like chymosin, the Phe_{105}-Met_{106} bond of κ-casein is also preferentially hydrolyzed by pepsins and the acid proteinases of *Rhizomucor miehei* and *R. pusillus* but the acid proteinase of *Cryphonectria parasitica* preferentially cleaves the Ser_{104}-Phe_{105}. However, unlike chymosin, the *Rhizomucor* and *Cryphonectria parasitica* proteinases also cleave several other bonds in κ-casein.

The MCA of commercial rennets (calf rennet, *R. miehei*, *R. pusillus* and *C. parasitica*) increases with temperature in the range 28–36 °C. The MCA of porcine pepsin, calf rennet and bovine pepsin at pH 6.6 increases with temperature up to 44, 45 and 52 °C, respectively. The fungal enzymes lose activity at 47, 57 and 57 °C, respectively. The MCA of the pepsins, especially porcine pepsin, is more pH-dependent than that of chymosin, while that of the fungal rennets is less sensitive in the pH region 6.2–6.8 (Fig. 7.22). The coagulation of milk by *C. parasitica* proteinase is also less sensitive than calf rennet to added Ca^{2+} but coagulation by *Rhizomucor* proteinases is more sensitive. For a given MCA, the rate of gel firming depends on the rennet used; this aspect of milk coagulation should be independent of rennet type and may indicate non-specific proteolysis by the fungal enzymes.

Rennets are also produced commercially from lamb, kid and buffalo calf stomachs. Frequently, lamb rennet is commercialized as rennet paste rather than rennet extract. The extract contains a lipase, pregastric esterase, in addition to chymosin and is used for cheeses with a high level of free fatty acids, e.g., Pecorino Romano. Lamb, kid and buffalo chymosin are similar to calf chymosin (Mohanty et al. 2003; Jacob et al. 2011).

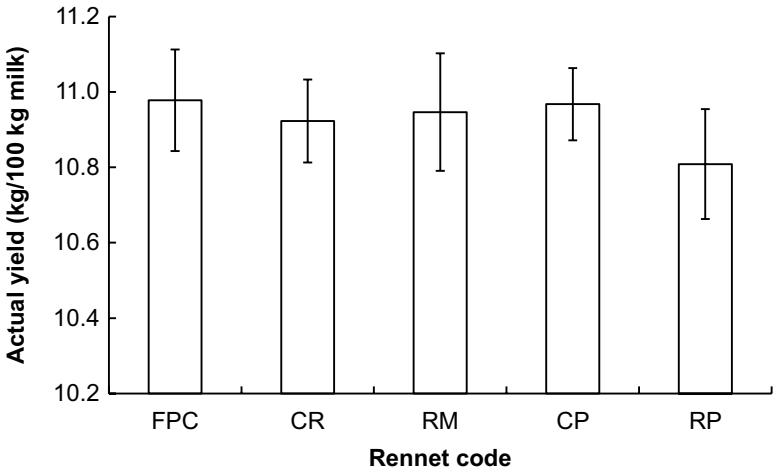


Fig. 7.21 Yield of Cheddar cheese made using different types of rennet: FPC, fermentation chymosin (Chymax plus); CR, calf rennet (Standard 190), *RM Rhizormucor miehei* (Fromase 220 TL), *CP Cryphonectria parasitica* (Suparen 600), *RP Rhizormucor pusillus* (Emporase). Cheesemaking was performed in quadruplicate under carefully-standardized conditions (rennet-to-casein ratio, pH at different stages of manufacture and firmness of gel at cut). Guinee (unpublished results)

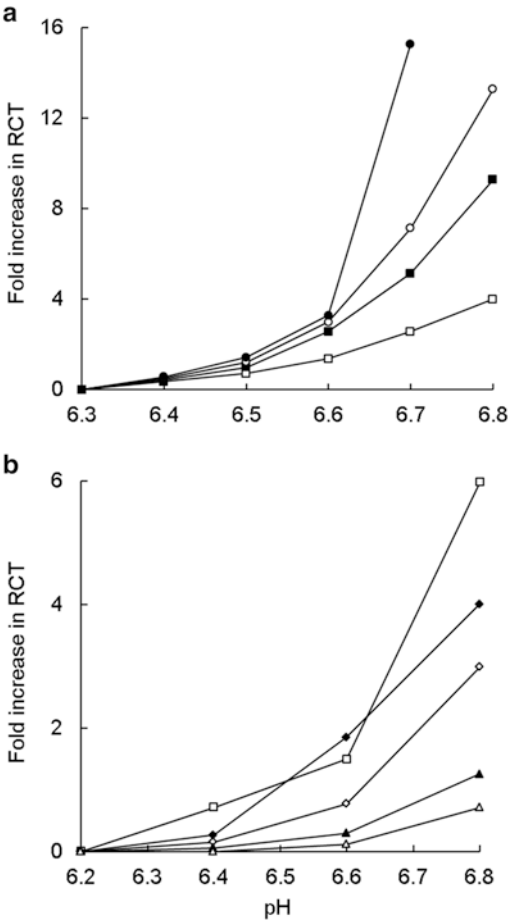


Fig. 7.22 Effect of pH on the rennet coagulation time (RCT) of milk using: (**a**) calf chymosin (*open square*), bovine pepsin (*filled square*), ovine pepsin (*open circle*) or porcine pepsin (*filled circle*); (**b**) calf rennet (*open square*), *Rhizomucor miehei* (*filled diamond*), *R. pusillus* (*open diamond*), *Cryphonectria parasitica* (*filled diamond*) or *Bacillus polymyxa* (*open triangle*) proteinases [redrawn from the data of Fox 1969 (**a**) and Phelan 1973) (**b**)]

The thermal stability of rennets which differs considerably (Fig. 7.23) is important when the whey is to be used in food processing; the early fungal rennets were considerably more thermo-stable than chymosin or pepsins but the present products have been modified (by oxidation of methionine residues in the molecule) and have thermal stability similar to that of chymosin. The thermal stability of *C. parasitica* proteinase is less than that of chymosin at pH 6.6. The thermal stability of all rennets increases markedly with decreasing pH (Fig. 7.23) (Thunell et al. 1979).

Although they are relatively cheap, rennets represent the largest single industrial application of enzymes, with a world market of ca. 40×10^6 l of standard rennet per annum (worth ~€300×10^6). Therefore, rennets have attracted the attention of industrial enzymologists and biotechnologists. The gene for prochymosin has been cloned in *E. coli*, *Saccharomyces cerevisiae*, *Kluyveromyces marxianus* var. *lactis*,

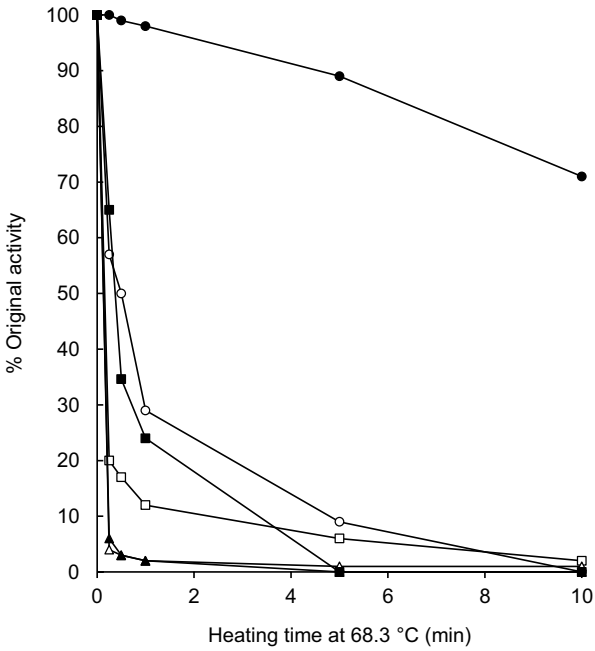


Fig. 7.23 Effect of heating time at 68.3 °C on the residual activity of various coagulants in whey at pH 5.2: calf rennet (*open square*), bovine pepsin (*filled square*), porcine pepsin (*filled triangle*), *Rhizomucor miehei* (*filled circle*), *R. pusillus* (*open circle*) or *Cryphonectria parasitica* (*open triangle*) (redrawn from Thunell et al. 1979)

Aspergillus nidulans, *A. niger* and *Tricoderma reesei* (see Pitts et al. 1992; Foltmann 1993; Crabbe 2003; Jacob et al. 2011, for references). The enzymatic properties of the recombinant enzymes are indistinguishable from those of calf chymosin although they contain only one of the isoenzymes, A or B. The cheesemaking properties of recombinant/fermentation-produced chymosins have been assessed on many cheese varieties, always with very satisfactory results (Fox and Stepaniak 1993; Jacob et al. 2011). Recombinant chymosins have been approved for commercial use in many, but not all, countries. Two fermentation-produced chymosins are now marketed commercially: Maxiren, secreted *by K. marxianus* var. *lactis* and produced by Gist Brocades (the Netherlands), ChyMax® (secreted by *A. niger*, Hansen, Denmark) and Chymax (secreted by *E. coli*, Hansen). The gene for Maxiren was isolated from calf abomasum while that used for and ChyMax® was synthesized. Fermentation chymosins have taken market share from both calf rennet and especially fungal rennets and now represent 70–80 % of the total rennet market (see Jacob et al. 2011).

An interesting recent development is the cloning of camel (*Camelus dromedaries*) chymosin in *A. niger* Camel milk is not coagulated by calf chymosin but bovine milk is coagulated readily by camel chymosin which has been characterized by Kappler et al. 2006). It has 70 % higher clotting activity per mole on bovine milk than calf chymosin but only 20 % of the general proteolytic activity, i.e., has a sevenfold higher ratio of milk clotting activity to general proteolytic activity than calf chymosin. This finding was confirmed in Cheddar cheese, in which for equal milk clotting activity,

camel chymosin produced Cheddar cheese with a much lower level of proteolysis but good flavour (Bansal et al. 2009); the higher MCA of camel chymosin has been explained by Møller et al. (2012a). The specificity of camel chymosin on bovine α_s- and β-caseins is generally similar to that of Møller et al. (2012b). Camel chymosin, as CHYMAX M, is being used commercially for cheese manufacture.

The fermentation-produced chymosins currently available are identical, or nearly so, to calf chymosin but there are several published studies on engineered chymosins (see Fox and McSweeney 1997). At present, attention is focussed on elucidating the relationship between enzyme structure and function but this work may lead to rennets with improved MCA or modified general proteolytic activity, i.e., on α_{s1}- and/or β-casein. The natural function of chymosin is to coagulate milk in the stomach of the neonate; it was not intended for cheesemaking and it is probable that the wild-type enzyme may not be the most efficient or effective proteinase to catalyse proteolysis in cheese during ripening. Therefore, it may be possible to modify chymosin so as to accelerate its action on specific bonds of casein during ripening and/or to reduce its activity on others, hydrolysis of which may have undesirable consequences, e.g., bitterness. To date, the pH optimum, thermal stability, k_{cat} and K_M on synthetic peptides have been modified through genetic engineering. We are not aware of any cheesemaking studies using engineered chymosins and approval has not been obtained for their use.

The gene for *R. miehei* proteinase has been cloned in and expressed by *A. oryzae* (Novo Nordisk A/S, Denmark). It is claimed that this new rennet (Marzyme GM) is free of other proteinase/peptidase activities present in fungal rennets and which may reduce cheese yield. Excellent cheesemaking results with Marzyme GM have been reported. Cloning of the gene for *R. miehei* proteinase has created the possibility for site-directed mutagenesis of the enzyme.

7.10 Immobilized Rennets

Most (>90 % for Cheddar) of the rennet added to cheesemilk is lost in the whey, representing an economic loss and creating potential problems for whey processors; both problems could be solved through the use of immobilized rennets. A further incentive for immobilizing rennets is the possibility of producing cheese curd continuously (using a cold-renneting technique, i.e., renneting at ~10 °C which allows the primary phase, but not the secondary phase, to occur) which should facilitate process control. The feasibility of continuous coagulation using cold-renneting principles has been demonstrated but the technique has not been commercially successful to date. However, as discussed in Chap. 12, the chymosin (or rennet substitute) retained in cheese curd plays a major role in cheese ripening; consequently if an immobilized rennet was used to coagulate milk, it would be necessary to add some chymosin (or similar proteinase) to the curd and uniform incorporation of this enzyme(s) would be problematic, as has been experienced with the use of exogenous proteinases to accelerate cheese ripening (see Chap. 12).

In modern cheesemaking, most operations are continuous or nearly so; the actual coagulation step is the only major batch operation remaining, although the use of small "batches" of milk, as in the Alpma process for Camembert, makes coagulation, in effect, a continuous process. However, in modern, large Cheddar and Gouda cheese factories very large (20–30,000 L) vats are used.

There is interest in the manufacture of rennet-free curd for studies on the contribution of enzymes from different sources to cheese ripening. A number of approaches have been used to produce rennet-free curd (see Fox et al. 1993) but a completely immobilized, effective rennet would be very useful.

Several investigators have immobilized different rennets on a range of supports and have claimed that these can coagulate milk. However, it appears that in such studies, some enzyme leached from the support and that this solubilized enzyme was responsible for coagulation. An irreversibly immobilized rennet was unable to coagulate milk although it could hydrolyse non-micellar casein; presumably, the κ-casein on the surface of casein micelles is unable to enter the active site cleft of the immobilized enzyme due to steric factors.

Even if immobilized rennets could coagulate milk, they may not be cost-competitive (rennets are relatively cheap) and would be difficult to use in factory situations. The strategy envisaged for their use involves the passage of cold milk (e.g., 10 °C) through a column of immobilized enzyme where the enzymatic phase of renneting would occur without coagulation, owing to the low temperature. The rennet-altered micelles would then be coagulated by heating the milk exiting the column to ~30 °C. Heating would have to be conducted under quiescent conditions to ensure the formation of a good gel and to minimize losses of fat and protein; quiescent heating may be difficult on a very large industrial scale (e.g., many cheese factories process $2–3 \times 10^6$ L milk/day). Hygiene and phage-related problems may present serious problems since cleaning the column by standard regimes would inactivate the enzyme. Plugging of the column and loss of activity have been problematic even on a laboratory scale, and power cuts, which would probably lead to an increase in temperature, would be disastrous as the column reactors would become plugged with cheese curd which would be difficult or impossible to remove. In short, the prospects for the use of immobilized rennets on a commercial scale are not bright and they are not being used but research in the subject continues, e.g., Pessela et al. (2004) and Sales-Gomes and Lima-Costa (2008).

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Chapter 8
Post-Coagulation Treatment of the Renneted-Milk Gel

Summary Following rennet-induced gelation, the coagulum is subjected to a series of treatments (e.g., cutting, cooking, stirring, acidification, whey drainage), the principal objective of which is to encourage syneresis (removal of whey from the gel) and effectively to concentrate the casein and fat to the degree characteristic of the variety. The principal treatments, which are characteristic of the variety of cheese, are described in this chapter. The cheddaring and *pasta filata* steps, together with moulding, pressing and packaging cheese, are also discussed.

Keywords Curd syneresis • Cheddaring • *Pasta filata* • Pressing • Packaging

8.1 Introduction

The rennet coagulation process is essentially similar for all cheese varieties and the structure of the coagula (gel) is also similar. The gel is subjected to a series of treatments (see Chaps. 2 and 3), the principal objective of which is to remove whey from the gel and to effectively concentrate the casein and fat to the degree characteristic of the variety. The principal treatments, which are characteristic of the type, and in some cases the variety, of cheese, are described in this chapter. Information on the manufacturing protocol for a number of cheeses is summarized in Chap. 3.

Rennet- or acid-coagulated milk gels are quite stable if left undisturbed but if cut or broken or subjected to external pressure, the *para*-casein matrix contracts, expressing the aqueous phase of the gel (known as whey). This process, syneresis, enables the cheesemaker to control the moisture content of the cheese and, hence, the activity of microorganisms and enzymes in the cheese and, consequently, the biochemistry of ripening and the stability and quality of the finished cheese. The higher the moisture content of cheese, the faster it will mature but the less stable it will be. High-moisture cheeses have a much greater propensity to develop off-flavours than low-moisture varieties. Although the starter and adventitious microflora of cheese have a major impact on the biochemistry of cheese ripening, they do so only in as far as the composition of the cheese curd permits. Syneresis is under the control of the cheesemaker and via syneresis, the composition and quality of the cheese.

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Many of the treatments to which rennet-coagulated milk gels are subjected may be classified generically as dehydration: cheese manufacture essentially involves concentrating the fat and casein of milk approximately tenfold, with the removal of lactose, whey proteins and soluble salts in the whey. Although there are certain common features, the factors which promote and regulate syneresis (dehydration) are characteristic of the cheese variety or perhaps more correctly, family of varieties. In the case of the Cheddar- and Swiss-type cheeses, dehydration is accomplished mainly in the cheese vat by finely cutting the coagulum, extensive 'cooking' of the curd-whey mixture (to ~40 and ~54 °C, respectively) and vigorous agitation during cooking. For the softer (high-moisture) varieties, the gel may be scooped directly into the moulds without cutting or cooking and whey explosion occurs mainly in the moulds as the pH decreases. Curds for some varieties, e.g., Cheddar and Swiss, are subjected to considerable pressure in the cheese moulds, thus aiding whey removal while curds for the softer varieties are pressed only under their own weight.

Most of the published studies on syneresis have been concerned mainly with the factors that affect it during the early stages of dehydration in Cheddar- and Dutch-type cheeses, i.e., mainly during cooking, but it is assumed that basically the same mechanisms operate in all varieties throughout the dehydration process.

Despite its accepted importance in the control of cheese moisture, the mechanism of syneresis of rennet-induced milk gels is not well understood. There is a considerable amount of empirical information on factors that influence syneresis but the actual mechanism of syneresis has received comparatively little study. Poor methodology is mainly responsible for the lack of information; the number of principles exploited in methods used to measure syneresis attests to their unsuitability. Some authors have attempted to simulate cheese manufacture, e.g., stirring, observing a cooking profile similar to that for the cheese of interest, even adding starter but the accuracy and precision of many of the methods are poor. Many of the methods have been used only by the original investigator.

The literature on the syneresis of milk gels has been reviewed by Green and Grandison (1993), Walstra (1993) and Dejmek and Walstra (2004), and is summarized here.

8.2 Methods for Measuring Syneresis

A variety of principles have been used to quantify syneresis. These include:

- measuring the volume of whey expressed from curd pieces under standard conditions, following cutting of the gel,
- changes in the moisture content, volume or density of curd pieces over time,
- use of tracers or markers to indirectly measure whey volume, or
- changes in the electrical conductivity of the curd.

Methods based on the volume of expressed whey are simple and straightforward to execute but complete recovery of whey is difficult and syneresis continues during

any separation process. Similar constraints apply to methods that depend on the volume or composition of the curd and, in addition, the actual analytical step may be difficult while avoiding continuing syneresis. Methods based on the use of a tracer or marker involve adding a small volume of a solution of some tracer (e.g., a dye) to the system at the start of syneresis; as the volume of free whey increases, the concentration of tracer in the solution decreases. The principal problems to be avoided are diffusion of the tracer into, or its adsorption, onto the curd particles. The opposite principle has also been used: a small amount of clarified whey is placed on top of the cut gel; whey expressed from the curd is turbid owing to the presence of fat globules and therefore the turbidity of the free whey increases as syneresis progresses. As the moisture content of curd decreases, its electrical conductivity decreases; as with many other methods, clean separation of curd particles from the whey without concomitant changes is a problem.

However, using these methods, and data from actual cheesemaking experiments, the influence of several factors on syneresis is now well established, at least in general terms.

8.3 Influence of Compositional Factors on Syneresis

The syneresis of rennet-induced milk gels is influenced by milk composition which, in turn, is affected by the feed, stage of lactation and health of the animals from which the milk is obtained. Fat tends to reduce syneresis and increase the water-holding capacity of cheese curd; increasing the fat content of cheese milk increases cheese yield by ~1.2 times the weight of additional fat. However, syneresis tends to be directly related to casein concentration, i.e., good syneresis occurs at high casein levels. Since the fat and casein levels in milk tend to change in parallel, they have off-setting effects on syneresis. Concentration of milk suppresses syneresis, possibly because of its effect on gel strength, although the rigidity modulus (see Chap. 14) at the time of cutting appears to have little effect on syneresis.

The rate of syneresis is directly related to the acidity, and, therefore, is inversely related to pH; it is optimal at the isoelectric point of casein (i.e., pH 4.6–4.7). The addition of $CaCl_2$ to milk promotes syneresis but the effect appears to be less than might be expected and may be negative at certain pH values and at high calcium concentrations, especially if the gel is held for a long period before cutting. The adverse effect of a high concentration of calcium has been attributed to interaction of Ca^{2+} with the aspartate and glutamate groups of proteins, leading to an increased net positive charge, swelling of the protein and suppression of syneresis. It is likely that a firmer gel, such as would be obtained on longer holding, would also be more resistant to syneresis. The influence of colloidal calcium phosphate on syneresis does not appear to have been investigated. Addition of a low level of NaCl increases the rate of syneresis but higher levels retard it.

8.4 Influence of Processing Variables on Syneresis

The extent of syneresis, and hence the moisture content of cheese, is influenced by various cheesemaking procedures; many of these factors are exploited by cheesemakers to control cheese composition, and hence its flavour and texture. The principal factors are:

8.4.1 Size of the Curd Particles

Everything else being equal, the smaller the curd pieces, the faster the rate and the greater the extent of syneresis, reflecting the greater surface area available for loss of whey. For some high-moisture cheeses, the coagulum may not be cut but scooped, unbroken, into cheese moulds. For Cheddar- and Dutch-type cheeses the coagulum is cut into cubes of about 1 cm side using knives with vertical or horizontal wires or bars (Fig. 8.1a, b). Traditionally, the coagulum for many Swiss or hard Italian varieties was cut with a harp (Fig. 8.1c) or a basket-like implement known as a spino (Figs. 8.1d and 8.2) which is used in a swirling action around the hemi-spherical or conically shaped vats used traditionally for these varieties. In the large modern vats used for Cheddar, Dutch and other varieties, the rectangular cutting knives are fixed in a staggered fashion to an axle running through the vat and serve to cut the coagulum and later to agitate the curd-whey mixture during cooking when the direction of rotation of the axle is reversed (Fig. 8.3); one side of the bars of the knives is sharpened and it is important to maintain a sharp edge to make a clean cut and minimize the loss of fat and casein into the whey.

8.4.2 Cook Temperature

Heating the curd/whey mixture (a process referred to as cooking or scalding) promotes syneresis (Fig. 8.4a). The cook temperature is characteristic of the variety, e.g., 31 °C for high-moisture varieties such as Camembert (in effect, no cooking), 36 °C for Gouda and Edam, 38–40 °C for Cheddar, 52–55 °C for Emmental and Parmesan. The cook temperature must match the thermal stability of the starter: acid production by some *Lactococcus* strains is stopped >~35 °C but others withstand cooking at 40–42 °C; cooking cheese curds to a temperature that inhibits the culture may have a negative effect on syneresis owing to the reduced rate of acidification. A cook temperature up to 55 °C may be used when a thermophilic starter is used; these starters survive but do not grow at 55 °C and hence syneresis depends on temperature rather than on pH. In fact, temperature and pH are complementary: syneresis of low-acid curds, e.g., Emmental, depends mainly on temperature while in high-acid curds, e.g., Camembert, temperature is of little consequence.

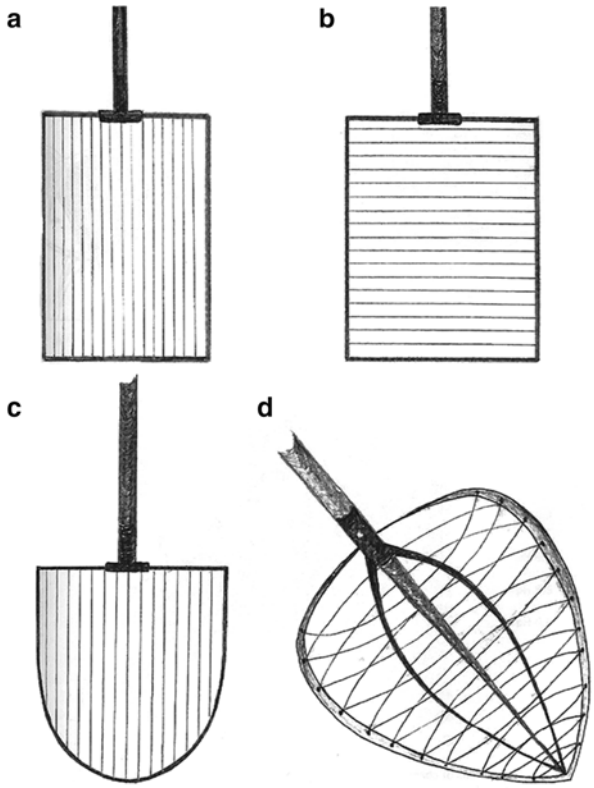


Fig. 8.1 Examples of tools used to cut rennet-induced milk gels; (**a**) vertical knife, (**b**) horizontal knife, (**c**) harp and (**d**) spino

For most varieties, cooking is performed by circulating hot water, or preferably steam, through the jacket of the cheese vat (steam is preferable because it can be shut off more readily than hot water, facilitating better control of temperature). Before the availability of hot water or steam for cooking curds and whey, cooking was performed over an open fire and would have been difficult to control precisely. Cooking over or near an open fire is still used in artisanal farmhouse cheesemaking. Illustrations of old cheese factories suggest that jacketed vats (i.e., hot water or steam) were used from the start of industrialized cheesemaking. For Dutch-type cheeses and a number of other varieties, cooking is performed by removing part (30–40 %) of the whey and replacing it by warm water to give a blend of the desired temperature. This method was probably used initially in farm-scale cheese production which lacked steam or facilities to circulate hot water or steam through a jacketed vat. It was probably used for many other varieties but its use has been discontinued for most of them except Dutch-type cheeses for which its main function now is to reduce the lactose content of the cheese curd and thereby control the pH of the cheese.



Fig. 8.2 A spino used to break the curd during the manufacture of certain Italian cheeses

The rate of cooking is characteristic of the variety (see Chap. 3). If the rate of cooking is too fast, especially during the early stages, excessive dehydration will occur at the curd surface, leading to the formation of a skin (case hardening) which will retard syneresis and the removal of whey from the interior of the curd pieces, and hence give a high-moisture cheese.

8.4.3 Rate of Acid Development

The lower the pH, the faster is the rate and the greater the extent of syneresis (Fig. 8.4b). Presumably, this reflects the reduced inter-protein repulsion by the negative charge on the casein molecules as the isoelectric point is approached.

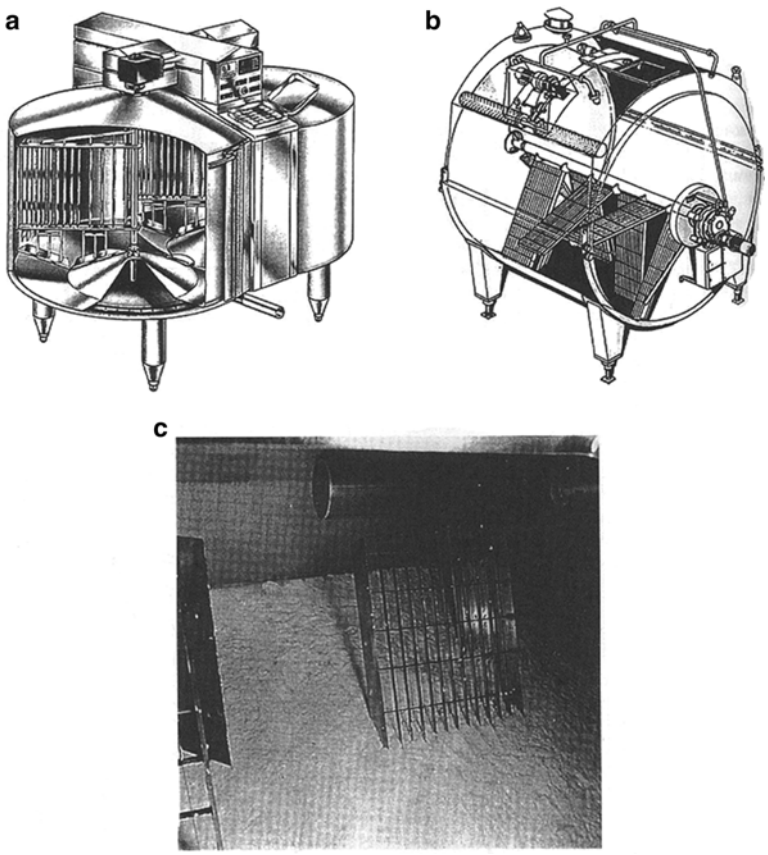


Fig. 8.3 Illustrations of cheese vats showing the curd knives/stirrer blades: (**a**) arranged vertically in a Double-O Multicurd vat, (**b**) arranged horizontally, in a staggered mode, along central horizontal shaft, in a cylindrical OST vat; (**c**) toward the end of the cutting cycle in a horizontal cheese vat. The blades are tapered and move in the direction of the sharp edge (knife) when cutting and in the reverse direction (blunt edge, stirrer) when stirring [from technical brochures by: Gadan A/S, Them, Denmark (Multicurd vat), and Tebel-MKT B.V. Leeuwarden, Holland (Ost vat)]

8.4.4 Stirring of the Curd-Whey Mixture

During cooking, the curd/whey mixture is stirred, which serves a number of functions:

- it facilitates cooking,
- it prevents the curd pieces from matting (which would have a strong negative effect on syneresis), and
- it promotes syneresis via collisions between curd pieces and between curd pieces and the vat wall.

Everything else being equal, syneresis is directly proportional to the intensity of stirring. Initially, the curd is very soft and gentle stirring should be used—a period

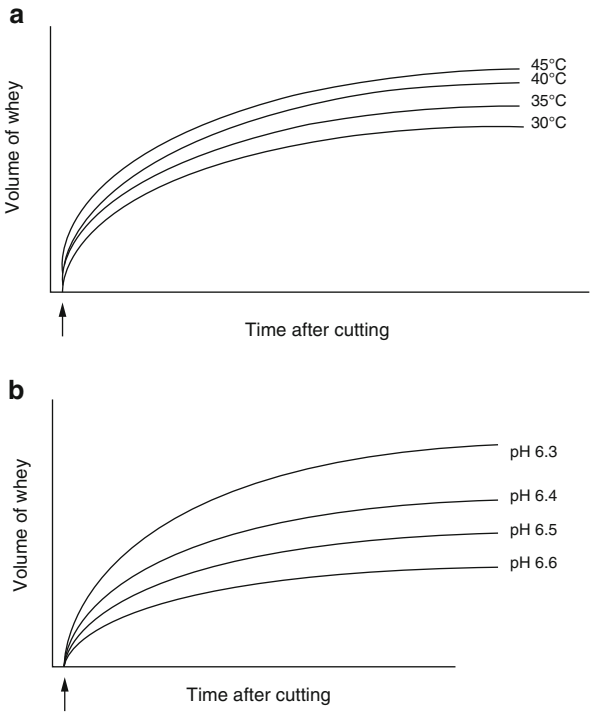


Fig. 8.4 Effect of temperature (**a**) and pH (**b**) on the rate and extent of syneresis in cut/broken renneted-milk gels (from Fox and McSweeney 1998)

of 5–10 min is allowed for the cut surfaces of the curd pieces to "heal"; vigorous agitation of the curd during this period will cause extensive losses of fat and protein into the whey and a decrease in cheese yield (see Chap. 10).

For some varieties, the curd is held in the whey until a certain pH is reached, e.g., 6.2 for Cheddar, after which the curds and whey are separated, usually using metal screens. For other varieties, e.g., Gouda, the curds and whey are separated after holding at the desired cook temperature for a defined period. For yet other varieties, e.g., Parmesan, the curds and some whey are scooped from the vat using a cheesecloth and transferred to perforated moulds where much of the whey drainage occurs. If whey separation occurs in the cheese vat, stirring of the curds during draining (referred to as dry stirring) is a useful way of promoting syneresis but is not applicable for most varieties.

8.4.5 Pressing

After removal of the whey, the curds mat to form a continuous mass. Treatment of this mass of curd is characteristic of the variety and may involve inverting the mass of curd in the moulds, turning and piling blocks of curd in the vat (traditional "cheddaring" during which period acid develops), and in many cases, pressing (see Chap. 3).

Syneresis occurs during these operations but is not easily controlled. With the exception of Cheddar-type cheese, acidification occurs mainly after moulding and this promotes considerable syneresis of the curds. For Cheddar and similar varieties, acidification occurs mainly during "cheddaring" in the vats, and relatively little syneresis occurs after moulding.

8.4.6 Salting

As discussed in Chap. 9, all cheeses are salted at the end of manufacture. Salting causes the loss of moisture from the curd (~2 kg H_2O are lost per kg of salt absorbed). However, salting should not be used as a means of controlling cheese moisture.

8.4.7 Milk Quality and Pre-Treatment

Heating milk under conditions that cause whey protein denaturation and interaction with casein micelles reduces the tendency of rennet-induced milk gels to synerese. Homogenization of whole milk has a similar effect. It has been reported that the growth of psychrotrophs in milk reduces its syneretic properties, i.e., leads to a high-moisture cheese; an increased level of rennet slightly increase the rate of syneresis, while plasmin activity in milk is reported to reduce syneresis.

8.5 Kinetics and Mechanism of Syneresis

Data from various investigations indicate that syneresis is initially a first order reaction, i.e., the rate of syneresis depends on the amount of whey remaining within the curd. It is generally assumed that syneresis is due to protein-protein interactions and may be regarded as a continuation of the gel assembly process during rennet coagulation. The inhibitory effects of high concentrations of salts ($CaCl_2$, NaCl, KCl) on syneresis imply that ionic attractions are involved. Urea promotes syneresis, suggesting that hydrogen bonds are not involved. The effectiveness of pH in promoting syneresis is probably due to a reduction of overall charge as the isoelectric point is approached. Studies on artificial milk systems implicate the ε-NH_2 group of the lysine residues in casein in syneresis; the apparent importance of ε-NH_2 groups in the second phase of rennet coagulation was discussed in Chap. 7. Lysozyme, which reacts with casein micelles, reducing their charge and rennet coagulation time, also accelerates syneresis when added to milk. Some authors have concluded that curd-firming and syneresis are different aspects of the same phenomenon but opinions are not unanimous. As discussed in Chap. 7, electron microscopic studies have

shown that the aggregation of casein micelles to form a gel is followed by increasingly closer contact between the micelles, leading to fusion. The syneretic pressure in an uncut gel is very small (~1 Pa) but when the coagulum is cut, the whey leaks out. Syneresis is initially a first order reaction because the pressure depends on the amount of whey in the curd; holding curd in whey retards syneresis owing to back pressure of the surrounding whey, while removing whey promotes syneresis. When the curd is reduced to ~70 % of its initial volume, syneresis becomes dependent on factors other than the volume of residual whey in the curd. It has been proposed that hydrophobic and ionic interactions within the casein network are probably responsible for the advanced stages of syneresis. This is in accord with the promotion of syneresis by reduced pH and low levels of $CaCl_2$, which reduce micellar charge and increase hydrophobicity, and by increased temperature which increases hydrophobic interactions.

The foregoing discussion on syneresis pertains especially to the syneresis occurring in the cheese vat, i.e., mainly to hard and semi-hard varieties. Syneresis continues after hooping (moulding) and represents the major part of syneresis in soft varieties. Presumably, the mechanism of syneresis in the moulds is the same as in the cheese vat although the range of treatments that can be applied at this stage is rather restricted. External pressure is applied to the curds for many cheese varieties after moulding and makes a significant contribution to whey removal; in general, the drier the cheese curd at hooping, the higher the pressure applied, which is probably a reflection of the greater difficulty in ensuring fusion of low-moisture curds.

8.6 Textured Cheese

The development of a recognizably fibrous texture is part of the manufacturing procedure for a small number of cheese varieties and these textural properties were traditionally regarded as an essential feature of their organoleptic properties. Texturized cheeses belong to two classes: Cheddar and some closely related varieties, in which a fibrous texture is developed prior to pressing, and *pasta filata* types, e.g., Mozzarella, Kashkaval and Provalone, in which texturization is accomplished by heating, stretching and kneading the curd.

In traditional Cheddar manufacture, the drained curds are piled along the sides of the vat during which matting (fusion) of individual curd particles occurs. To enable faster turnover of the cheese vats, it became common practice in the 1960s to transfer the curds/whey after cooking to cheaper cheddaring "sinks" where whey drainage and cheddaring occurred. The piles of curd are cut into blocks (30×10 cm) which are inverted frequently and piled over a period of ~2 h. This operation, known as cheddaring, was considered by many researchers and cheesemakers as the essential, characteristic part of the Cheddar cheese manufacturing process. During cheddaring, the curd flows under its own weight, leading to fusion and deformation of curd particles which was believed to be responsible for the 'chicken breast meat' structure of fresh Cheddar curd, and for the characteristic texture of mature Cheddar

cheese. Cheddaring promotes a number of physico-chemical conditions which are conducive to curd flow and texturization:

- solubilization of micellar calcium which is bound to the casein and acts as a cementing agent between the casein micelles/sub-micelles,
- a decrease in the concentration of micellar Ca, resulting in an increase in the ratio of soluble to casein-bound Ca; soluble Ca as a % of total Ca in the curd increases from ~5 to 40 as the pH decreases from 6.15 to 5.2.
- an increase in *para*-casein hydration which increases with decreasing pH in the range 6.6 to 5.15 (see Fig. 8.5), and
- an increase in the viscous character of the curd.

The increase in casein hydration with decreasing pH is probably a consequence of the increase the ratio of soluble to micellar Ca; it has been found in model casein systems that casein hydration is inversely related to the concentration of casein-bound Ca (Sood et al. 1980).

As a consequence of the decrease in casein-bound Ca and the increase in casein hydration, the viscoelastic casein matrix, with occluded liquid fat and moisture phases, flows if unrestricted, especially when piled and pressed under its own weight. The flow of curd gives the desired planar orientation of the strands of the *para*-casein network. The physico-chemical changes in curd during cheddaring are summarised in Fig. 8.5. However, there is little scientific support for the necessity

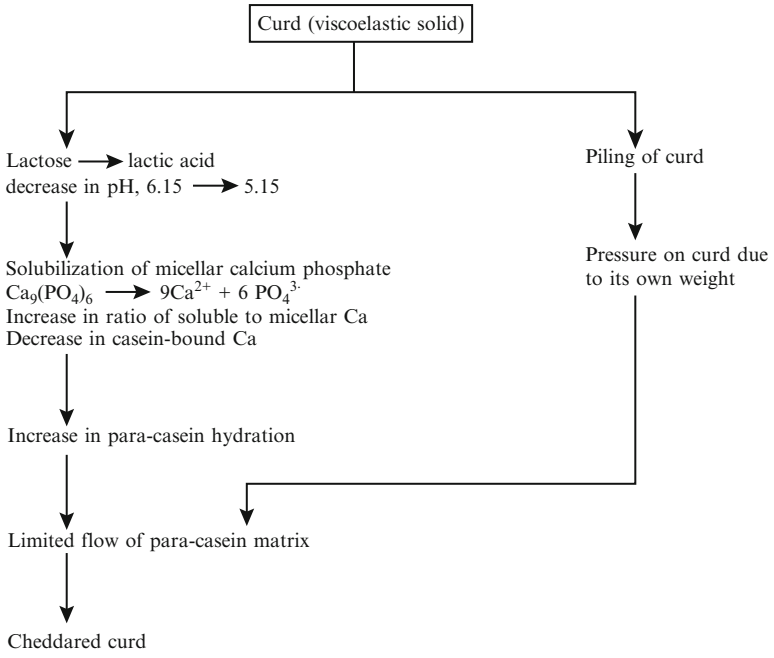


Fig. 8.5 Physico-chemical changes in cheese curd during cheddaring

of cheddaring; on the contrary, there is strong evidence that cheddaring is of no consequence to Cheddar cheese quality and serves only to allow the desired degree of acid development and syneresis to occur.

Various forms of restricted flow under different degrees of external pressure result in Cheddar cheese with a lower moisture content than curd cheddared in the traditional manner. Differences in the extent of curd deformation caused by modified "cheddaring" processes diminish during milling, salting and pressing and have little effect on the flavour and textural characteristics of the final cheese. The development of a fibrous texture results in loss of the micelle structure but this is not essential as the amount of deformation is very small and is probably altered by the subsequent and more extensive deformation during pressing.

In modern practice, most Cheddar cheese curd is manufactured in continuous, mechanical cheddaring systems in which little flow occurs in comparison with traditional methods (Fig. 8.6); indeed, matting is prevented in the manufacture of some Cheddar-type cheeses, e.g., stirred-curd Cheddar. The textural qualities of Cheddar cheese produced by these systems is acceptable, indicating that 'flow' during manufacture is not essential.

Presumably, the various interactions, ionic and/or hydrophobic, which are considered to be responsible for syneresis, continue during the cheddaring process but there appears to have been no studies on this aspect of cheesemaking.

In the manufacture of Mozzarella and other *pasta filata* (stretched-curd) cheeses, the acidified curd is heated to ~58–60 °C by kneading in hot water (~78 °C) and stretched in equipment designed to cause extension of the hot molten curd (Fig. 8.7a). This process, whereby the curd is converted into a plastic molten mass (Fig. 8.7b–d), is referred to as plasticization and was developed originally in hot climates as a means of heat-treating and hence extending the shelf-life of curd of poor microbiological status. Successful texturization of the curd requires that the viscoelastic *para*-casein matrix undergoes limited flow and stretches into hot molten sheets, without breaking, when extended. Plasticization is accompanied by microstructural changes in the cheddared curd, including further linearization of the *para*-casein matrix into fibres and coalescence of fat into elongated pools which are trapped between, and show the same orientation as, the protein fibres (Fig. 8.8).

The physico-chemical changes responsible for plasticization of the curd have not been elucidated unequivocally. However, based on information derived from studies on the behaviour of curds of different composition and pH when subjected to texturization (Guinee, unpublished results) and microstructural changes that accompany placticization (e.g., Fig. 8.8), and the viscoelastic changes in curd when heated to a temperature similar to that during the plasticization process (Guinee et al. 1998), it may be speculated that successful texturization is a consequence of:

- an adequate degree of casein hydration of the cheddared curd, which is controlled by its pH, total calcium content and ratio of soluble to casein-bound Ca
- heat-induced coalescence of free fat (formed as a consequence of shearing of the fat globule membrane) which lubricates the flow of the *para*-casein matrix, and
- extension and shear stresses applied to the curd which assist in the displacement of contiguous planes of the *para*-casein matrix.

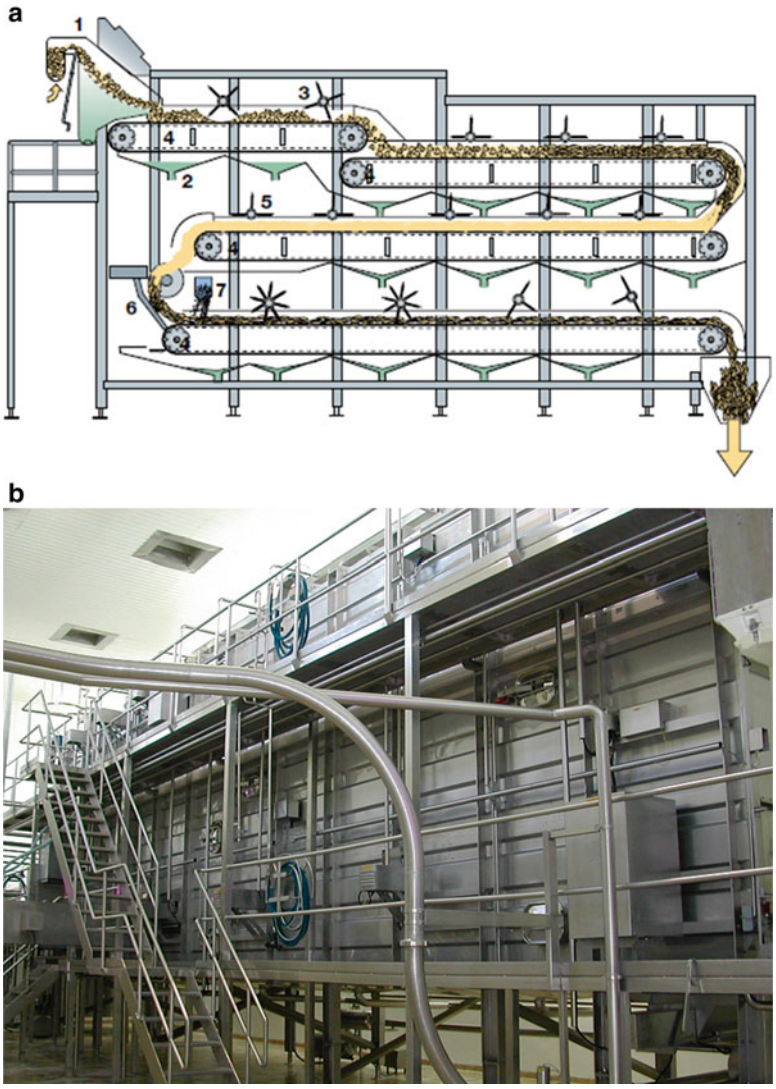


Fig. 8.6 (**a**) Schematic representation of a Tetra-Pak Alfomatic continuous system for dewheying, cheddaring, milling and salting curd for Cheddar cheese. 1. Whey strainer (screen), 2. Whey pump, 3. Agitator, 4. Conveyors with variable speed drive, 5. Agitators (optional) for production of stirred-curd Cheddar, 6. Chip mill, 7. Dry salting system (from Bylund 1997). (**b**) Photograph of the exterior of an Alfomatic continuous cheddaring system in industry. Note the walkway to the left which indicates the scale of the apparatus

The relationship between *para*-casein hydration and pH may be explained by the dominance of two opposing forces over the pH range 6.0 to 5.0, i.e.:

- those that promote neutralization of negative charges which lead to contraction of the *para*-casein thereby limiting hydration and impeding flow of the *para*-casein matrix, and

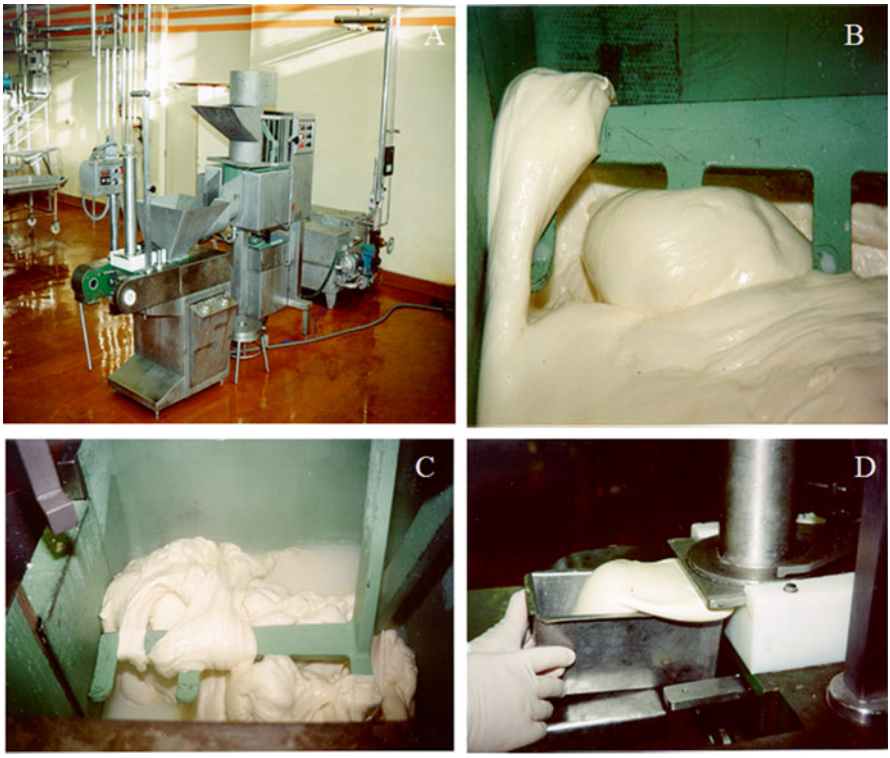


Fig. 8.7 Plasticization of low-moisture Mozzarella: (**A**) kneading-plasticizing equipment (from Costruzioni Meccaniche e Tecnologia S.p.a., Perveragno, Cuneo, Italy) consisting of hot water heating unit, cheese shredding unit, plasticization chamber where the curd is kneaded and stretched in hot water by toothed arms which oscillate backwards and forwards in opposite directions, and an auger, which conveys the plasticized cheese to the moulding unit. (**C**) mid-stage of plasticization; shredded curds have begun to fuse but plasticization is not yet complete, as shown by the presence of lumps in the curd mass; (**B**) fully plasticized molten curd mass which exhibits a long consistency and an oily surface sheen; and (**D**) moulding of the plasticized curd

- those that promote solubilization of micellar calcium which is conducive to casein hydration and promotes flow of the *para*-casein matrix.

At pH values in the range 6.0 to 5.2, solubilization of micellar calcium appears to be dominant as decreasing pH results in an increase in hydration of *para*-casein. In contrast, the reduction in pH is the dominant factor at pH 5.2–4.6, as decreasing pH results in a marked decrease in *para*-casein hydration. The total calcium content of the curd, which is controlled mainly by the pH of the milk at setting and that of the curd at whey drainage, determine the curd pH at which plasticization is possible. In the normal manufacture of Mozzarella, the milk is typically set at pH 6.55, the whey is drained at pH 6.15 and the ideal pH for plasticizing acidified curd is ~5.15. At this pH, the concentration of calcium in the curd (~27 mg/g protein) and the proportion of soluble calcium (~40 % of total) ensure that the *para*-casein is sufficiently hydrated to enable successful plasticization. At increasingly higher curd pH,

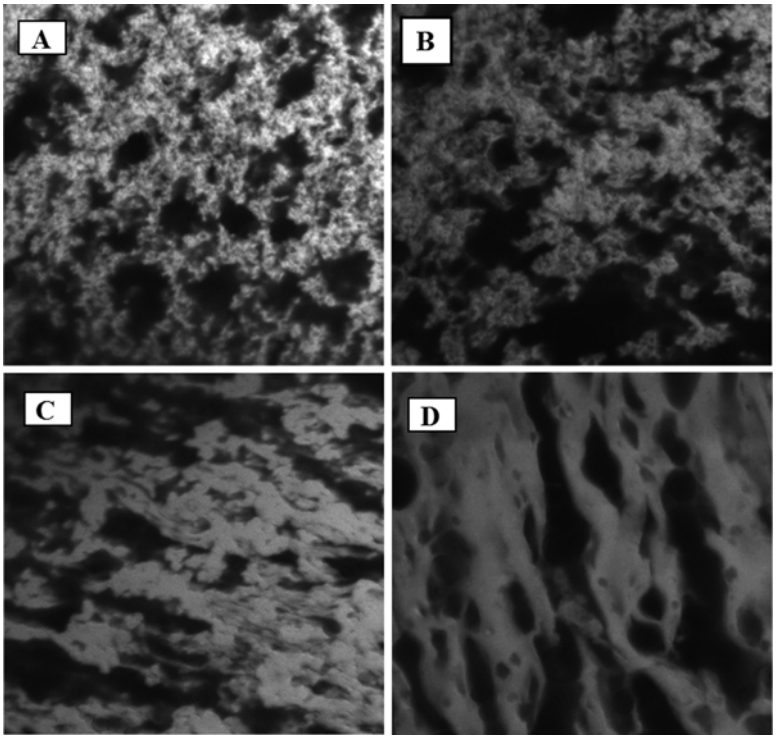


Fig. 8.8 Confocal laser scanning micrographs of Mozzarella cheese curd at various stages of manufacture: after cutting (**a**), at whey drainage (**b**), after cheddaring (**c**) and after plasticization (**d**). The *white-grey* areas represent the *para*-casein matrix, while the *black* areas represent the occluded fat and moisture phases. Bar = 25 μm (From Auty and Guinee, unpublished results)

the curd becomes progressively less smooth after plasticization, reflecting the decrease in *para*-casein hydration because of the reduced ratio of soluble to casein-bound calcium. Similarly, in processed cheese manufacture, heating of cheese is accompanied by aggregation of the protein and exudation of moisture and free fat unless emulsifying salts (e.g., sodium orthophosphates) are added to bind casein-bound Ca (see Chap. 17). At a curd pH > 5.4, the curd fails to plasticize adequately; instead a non-plastic mass, with a rough, dull, short, lumpy consistency is obtained. However, successful plasticization may be achieved at a higher curd pH, e.g. 5.6–5.8, if the Ca level in the curd is sufficiently low (e.g. <18 mg/g protein), as in the case of directly-acidified Mozzarella.

In the manufacture of directly-acidified Mozzarella, acidification is performed by the addition of a food-grade acid, rather than the conversion of lactose to lactic acid by the starter culture. The milk pH is typically adjusted to ~5.6 prior to rennet addition and no further change in pH occurs during curd manufacture, which is otherwise similar to that for conventional Mozzarella made using a starter culture. Following whey drainage, the curd, typically with a pH ~ 5.6, plasticizes readily on heating and stretching. The ability of curd made by direct

acidification to plasticize at a higher-than-normal pH can be explained on the basis of the interactive effects of total curd calcium and the ratio of soluble to casein-bound Ca (which changes with pH) on *para*-casein hydration. While soluble Ca as a % of total Ca decreases from ~40 to 20 as the pH is increased from 5.15 to 5.6, the total concentration of calcium is lower and hence the level of casein-bound Ca is probably similar to that obtained at pH 5.15 in conventional cheese manufacture. Hence, as there is an inverse relationship between casein-bound Ca and casein-bound moisture, the degree of *para*-casein hydration obtained in directly-acidified Mozzarella curd at pH 5.6 is similar to, or somewhat greater than, that in conventionally-produced Mozzarella curd at pH 5.3. Indeed, comparative studies have shown that the water-binding capacity of directly-acidified Mozzarella cheese curd (pH 5.6) is higher than that of conventionally-produced Mozzarella curd (pH 5.2) during the first three weeks of ageing (Kindstedt and Guo 1977).

8.7 Moulding and Pressing of Cheese Curd

At some stage in the manufacturing process (e.g., just after coagulation for Camembert, after cooking for Emmental or after acidification for Cheddar), the curds are transferred to moulds of characteristic shape and size. The principal purpose of moulding is to allow the curd to form a continuous mass; matting of high-moisture curds occurs readily under their own weight but pressing is required for low-moisture cheese. It is important that the curds are warm during pressing, especially for low-moisture curds.

Various pressing systems have been developed, ranging from very simple to continuous presses. In modern Cheddar cheese factories, the salted curds are formed and pressed under their own weight and under a slight vacuum in towers (Wincanton towers; Fig. 8.9) for about 30 min. On exiting the tower, 20 kg blocks are cut from the base of the column of curd by a guillotine and placed in plastic bags which are sealed under vacuum. The reader is referred to Kosikowski and Mistry (1997), Robinson and Wilbey (1998), Bennett and Johnson (2004) or other texts on cheese technology for examples of cheese pressing systems.

Cheeses are made up in characteristic shapes and sizes (see Chap. 3). At first glance, it might appear that the shape and size of a cheese are cosmetic. While this may be so in many cases, size and shape are very significant in certain varieties, e.g., surface-ripened cheese (mould or smear) are formed into small, low cylinders—these features are important since these cheeses ripen from the surface toward the centre—if large cheeses were made, the surface would become over-ripe while the centre remained unripe. For cheeses with large eyes, e.g., Emmental, a large cheese is required, as otherwise the leakage of CO_2 through the surface would be excessive and the pressure of gas within the cheese would not build-up to the partial pressure required to form eyes.

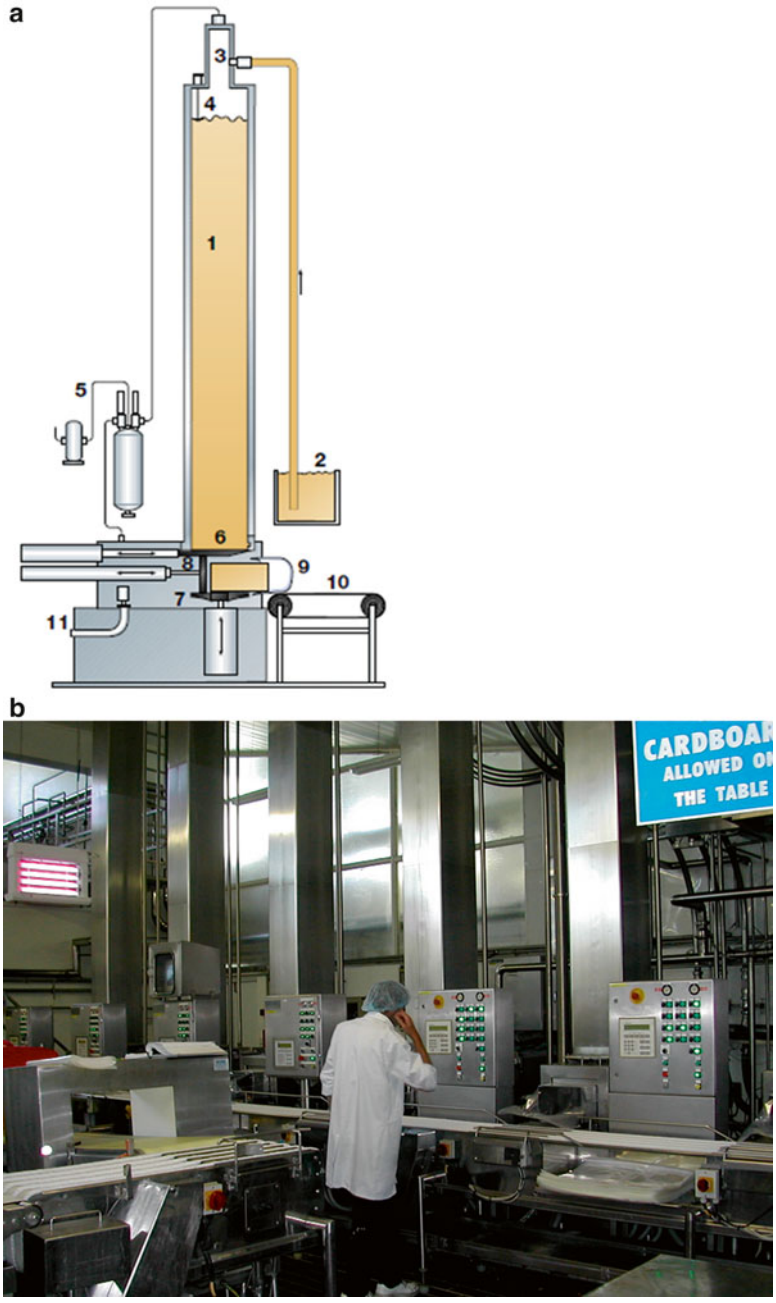


Fig. 8.9 (**a**) Schematic representation of a block former (Wincanton tower, or Block former) system for Cheddar-type cheese. 1. Column, 2. Curd feed, 3. Cyclone, 4. Level sensor, 5. Vacuum unit, 6. Combined bottom plate and guillotine, 7. Elevator platform, 8. Ejector, 9. Barrier bag, 10. Conveyor to vacuum sealing, 11. Whey drainage (from Bylund 1997). (**b**) Block formers in a commercial Cheddar cheese factory

8.8 Packaging

Like other sectors of the food industry, indeed industry in general, packaging has become a major feature of cheese production, distribution and retailing. Kosikowski and Mistry (1997) include a useful chapter on various aspects of packaging of cheese and fermented milks. Kadoya (1990) provides a more general discussion on food packaging, including a chapter on cheese and fermented milks. The science and technology of packaging are specialized subjects which will not be discussed here.

The objectives of packaging of cheese, as for any food item, are:

- To protect it against physical, chemical or microbial contamination. Mould growth is of particular concern; since moulds are aerobic, their growth can be prevented by covering the cheese with wax or plasticote or vacuum-packed in plastic film which should have low permeability to oxygen and be free of pin holes. By preventing contamination, packaging serves a public health function as well as reducing losses due to spoilage.
- To reduce the loss of moisture from the surface and therefore increase economic return; to achieve this, packaging material should have low permeability to moisture.
- To prevent physical deformation of the cheese, especially soft cheeses, and thus facilitate stacking during ripening, transport and retailing.
- Packaging provides an opportunity for product labelling and brand identification. This creates the opportunity for advertising and to provide nutritional information.

After salting (see Chap. 9), those cheeses on which the growth of moulds (surface or internal) or of a surface smear is encouraged are transferred to a room at a controlled temperature (~15 °C) and humidity (90–95 % equilibrium relative humidity). Even at this high humidity, some loss of moisture from the surface occurs but the loss is insufficient to create a rind (low-moisture surface layer). After sufficient growth of mould or smear has occurred, such cheeses may be wrapped in foil or grease-proof paper to avoid further loss of moisture.

Traditionally, the development of a rind was encouraged on internal bacterially-ripened cheese by controlled drying of the surface. If properly formed, the rind effectively seals off the interior of the cheese, preventing excessive loss of moisture and the growth of microorganisms on the surface. To stabilize further the surface of such cheeses, they were rubbed with oil (e.g., butter oil or olive oil) or coated with paraffin wax. Sometimes, wax of a particular colour is used, e.g., red for Edam, black for extra-mature Manchego, Cheddar and probably other cheeses. The colour of the wax is characteristic of the variety or of its maturity and is recognised by the consumer as an index of variety or quality.

Today, many internal bacterially-ripened cheeses are packaged in plastic bags of low gas permeability or coated with film-forming plastic material. A variety of plastic packaging materials are used for cheese, e.g., cellophane, cellophane-polyethylene,

polyvinyl chloride, polyvinylidene chloride, polystyrene, polypropylene, ethylene vinyl acetate, co-extruded polyolafin, metal foils or paper.

Gasses, e.g., CO_2 and H_2S, are produced in many cheeses during ripening; CO_2 will cause bulging of the package while H_2S has an obnoxious aroma which will render the cheese unacceptable. To avoid such problems, the package should be permeable to these gasses.

Packaging is particularly important for soft cheeses, e.g., Cottage, Quarg and Cream and processed cheeses. Metal foils are widely used for consumer or catering packages of processed cheese. Much processed cheese is commercialized as individual slices wrapped in plastic material. High-moisture fresh cheeses are commercialized in plastic tubs, plastic-, wax- or foil-lined cardboard containers or in plastic packages.

Metal cans or glass jars may be used to package natural and processed cheese to offer a novelty presentation feature or, in the case of cans, to provide extra physical protection during distribution and storage.

As for other foods, the packaging of cheese has led to the development of specialized packaging equipment, much of which is highly automated and computerized.

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Chapter 9
Salting of Cheese Curd

Summary The salt content in rennet-curd cheeses ranges from ~0.7 % (w/w) in Swiss to ~5 % (w/w) in Domiati. Salt has three major functions in cheese: it acts as a preservative; it enhances safety and contributes directly to salty flavour. Together with the desired pH, water activity and redox potential, salt assists preservation of cheese by minimization of spoilage and preventing the growth of pathogens in cheese. The dietary intake of sodium in the modern western diet is generally excessive, being two to three times the level recommended for desirable physiological function (2.4 g Na, i.e., ~6 g NaCl per day). However, cheese generally makes a relatively small contribution to dietary Na intake except when large quantities of high salt cheeses, such as Domiati and Feta, are consumed.

In addition to the above functions, salt level has a major effect on cheese composition, microbial growth, enzymatic activities, and on biochemical changes, such as glycolysis, proteolysis, lipolysis, and *para*-casein hydration, that occur during ripening. Consequently, salt level markedly influences cheese flavour and aroma, rheology and texture properties, cooking performance and, hence, overall quality. Many factors affect salt uptake and distribution in cheese and precise control of these factors is a vital part of the cheesemaking process to ensure optimum quality consistently.

Keywords Salt • Salting method • Uptake • Diffusion • Factors affecting • Cheese quality

9.1 Introduction

The use of salt (NaCl) as a food preservative dates from prehistoric times and, together with fermentation and dehydration by exposure to low humidity air, is one of the classical methods for food preservation. Until the development in the nineteenth century of modern methods for the preservation of foods, i.e., pasteurization/sterilization, chilling/freezing and "hot air" drying, salting was probably the most widely used method for the long-term preservation of many foods. Salt was a highly valued item of trade and was exchanged for goods and services. One can readily envisage how fermentation and "natural" dehydration could have been discovered by

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accident, but the use of salt as a preservative required direct intervention and was a very significant discovery at an early stage of human civilization. The three classical methods of food preservation, fermentation, salting and dehydration, and in modern times, refrigeration also, are used to preserve cheese and/or control its maturation.

The preservative action of NaCl is due to its effect on the water activity (a_w) of the medium:

$$a_w = p / p_o$$

where p and p_o are the vapour pressure of the water in a system and of pure water, respectively. If the system is at equilibrium with its gaseous atmosphere, then

$$a_w = \text{ERH} / 100, \text{where ERH is the equilibrium relative humidity.}$$

Due to the presence of various solutes in foods, the vapour pressure of water in a food system is always less than that of pure water, i.e., a_w is <1.0. The relationship between a_w and the moisture content of food is shown in Fig. 9.1. Three zones are usually evident:

- Zone I represents monolayer water that is tightly bound to polar groups in the food, e.g., the –OH group of carbohydrates, or the $-NH_3^+$ and $-COO^-$ groups of proteins
- Zone II consists of multilayer water in addition to the monolayer water.
- Zone III contains bulk phase water in addition to monolayer and multilayer water.

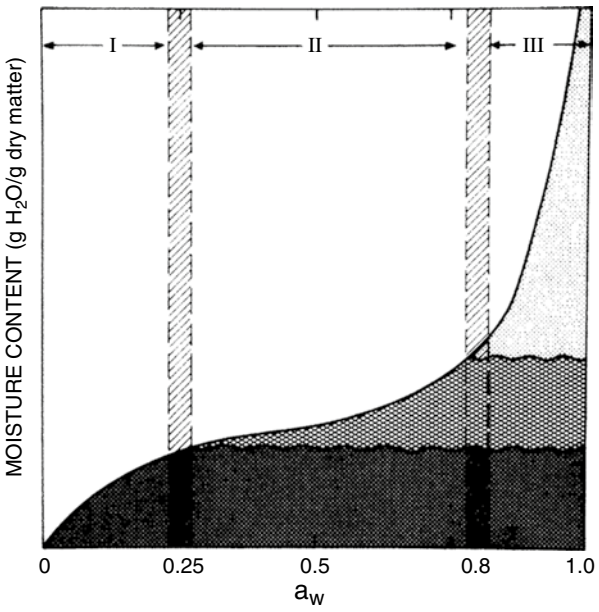


Fig. 9.1 Idealized relationship between the water activity (a_w) of food and its water content (from Fennema 1996)

General discussions on the general concept of water activity in relation to foods are provided by Rockland and Stewart (1981), Rockland and Beuchat (1987), Leistner and Russell (1991) and Fennema (1996). More specific aspects in relation to dairy products are discussed by Kinsella and Fox (1986) and Roos (1997).

The a_w of food depends on its moisture content and the concentration of low molecular mass solutes.

9.2 Salt in Different Cheese Varieties

Most of today's cheese varieties evolved independently in different regions of the world using milk from various species and different manufacturing methods, resulting in cheeses with diverse composition and sensory properties (e.g., Cheddar, Stilton, Gouda, Emmental, Mozzarella). Methods varied widely with respect to: type and composition of cheese milk, milk pre-treatments, and manufacturing protocols. The latter differed in terms of sequence and type of operations superimposed on the milk and curd, and methods and conditions of moulding, salting and pressing. Nascent cheese manufacture, especially in warm, hot climates, would have soon exploited the preserving effects of adding salt, along with moisture reduction, pH reduction and heating (cooking), in terms of prolonging the period for which the curd (cheese) from milk could be held, and used at times when milk supply or other food sources were scarce. Consequently, tradition led to a great diversity of varieties that differed significantly in salt level, other compositional characteristics, shape, maturation period and sensory characteristics (texture, flavour, usage properties). The level (%, w/w) of salt in cheese ranges from ~0.7 in Swiss to ~4.8 for Domiati (see Table 9.1).

9.3 The Major Functions of Salt in Cheese

Salt serves two main functions in cheese; it acts as a preservative, enhances safety and directly contributes to flavour. In addition, it exerts a number of important effects which impact on the overall quality of cheese (Fig. 9.2).

Its preservative effect is due mainly to its effect on a_w. Typical values for the a_w of some cheese varieties are shown in Table 9.2; they range from ~0.9 to 1.0.

The importance of salt as a determinant of a_w is evident from the findings of Marcos (1993), who showed that the a_w of young cheese varieties (rennet-curd and fresh cheeses), with moisture and salt contents ranging from 42–58 % and 0.2 to 2.5 %, respectively, can be predicted almost entirely by the concentration of NaCl in the aqueous phase according to the following equation:

$$a_w = 1 - 0.033\left[NaCl_m\right] = 1 - 0.00565\left[NaCl\right]$$

where $[NaCl_m]$ is the molality of NaCl, i.e., moles NaCl per litre of H_2O and [NaCl] is the concentration of NaCl as g/100 g cheese moisture (Marcos 1993). However,

Table 9.1 Approximate composition of selected cheese varieties[a]

Variety	Moisture (g/100 g)	Protein (g/100 g)	Fat (g/100 g)	NaCl (g/100 g)	Sat-in-moisture, S/M (g/100 g)
Brie	48.6	19.2	26.9	1.8	3.7
Camembert	50.7	20.9	23.7	2.1	4.1
Cheddar	37.2	25.4	33.1	1.8	4.8
Reduced-fat Cheddar	43.0	33.4	17.0	1.9	4.4
Cheshire	38.5	24.2	31.9	1.8	4.7
Cottage cheese (creamed)	79.1	13.8	3.9	1.0	1.3
Danish Blue	43.0	18.4	37.3	3.3	7.7
Edam	43.8	26.0	25.4	2.0	4.6
Emmental	35.7	28.7	29.7	0.7	2.0
Feta	56.5	15.6	20.2	2.8	5.0
Fromage frais	77.9	6.8	7.1		0.0
Gouda	40.1	24.0	31.0	2.1	5.2
Gruyére	33.6	27.3	34.5	1.2	3.6
Mozzarella	49.8	25.1	21.0	1.5	3.0
Parmesan	30.6	34.9	26.0	2.8	9.2
(Block) Processed cheese	49.1	18.3	23.3	3.4	6.9
Ricotta	73.2	11.3	10.3	0.4	0.5
Roquefort	41.3	19.7	32.9	3.8	9.2
Stilton	40.5	21.6	33.7	3.5	8.6
Domiati	55.0	12.0	25.0	4.8	8.7

[a]Data compiled from various sources

other water-soluble compounds, including lactic and other acids, salts such as calcium phosphate, low molecular weight peptides and free amino acids (FAA) also contribute to the depression of a_w in cheese. The a_w of cheese decreases with proteolysis and ripening, with the increase in water-soluble peptides and FAA being the preponderant factor responsible (Saurel et al. 2004; Hickey et al. 2013). Salt increases the osmotic pressure of the aqueous phase of cheese, causing dehydration of bacterial cells, killing them or, at least, preventing their growth. It is apparent from Table 9.2. that the a_w of most cheese varieties is not low enough to prevent the growth of yeasts and moulds but together with a low pH, it is quite effective in controlling bacterial growth. Enzyme activity is also affected strongly by a_w (Fig. 9.3).

Measurement of the salt content of cheese is an important quality control step in cheese production. As described above, the a_w of young cheese can be estimated from its salt and moisture levels (Marcos 1993), but can also be determined experimentally, directly by measurement of dew point, or indirectly by measurement of properties of the material effected by a change in relative humidity such as electrical resistance or capacitance.

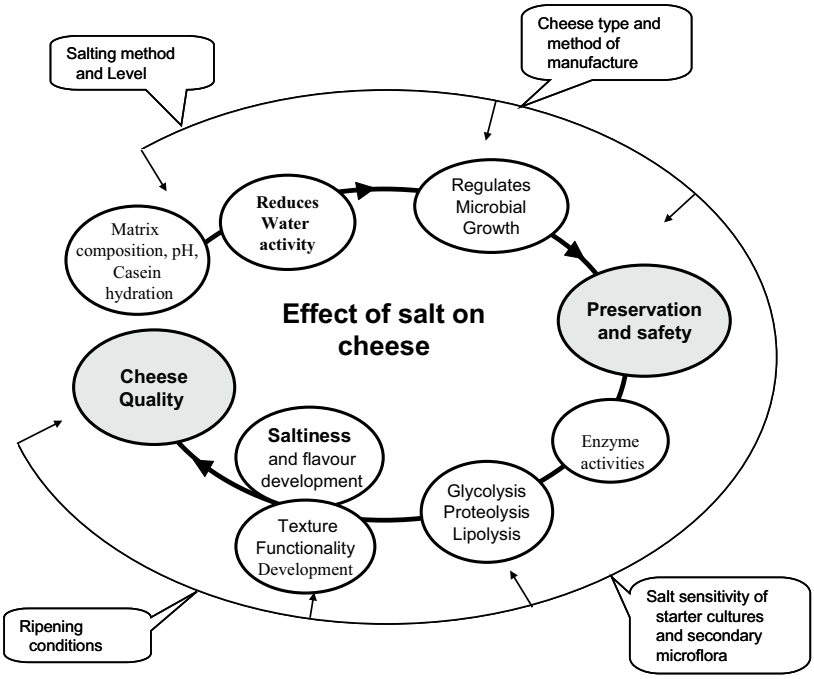


Fig. 9.2 Salt in cheese contributes to preservation, safety, saltiness and overall quality, because of its effects on water activity, microbial growth, protein hydration/confirmation, and enzymatic activities, which in turn influence biochemical changes such as glycolysis, proteolysis and lipolysis and the development of texture and flavour. The contribution of salt depends on: salting method and salt level; variety of cheese which influences make procedure, composition and sensitivity of cheese microflora to salt; and ripening conditions. Modified from Guinee and Sutherland (2011)

Table 9.2 Water activity (a_w) of some cheese varieties[a]

a_w	Cheese
1.00	Cheese curd, whey Cheese
0.99	Beaumont, Cottage, Fresh, Quarg
0.98	Belle des Champs, Münster, Pyrénées, Processed, Taleggio
0.97	Brie, Camembert, Emmental, Fontina, Limburger, Saint Paulin, Serra da Estrêla
0.96	Appenzeller, Chaumes, Edam, Fontal, Havarti, Mimolette, Norvegia, Samsø, Tilsit
0.95	Bleu de Bresse, Cheddar, Gorgonzola, Gouda, Gruyère, Manchego
0.94	Idiazábal, Majorero, Mozzarella, Norzola, Raclette, Romano, Sbrinz, Stilton
0.93	Danablu, Edelpilzkäse, Normanna, Torta del Casar
0.92	Castellano, Parmesan, Roncal, Zamorano
0.91	Provolone, Roquefort
0.90	Cabrales, Gamalost, Gudbrandsdalsost, Primost

[a]Compiled from various sources

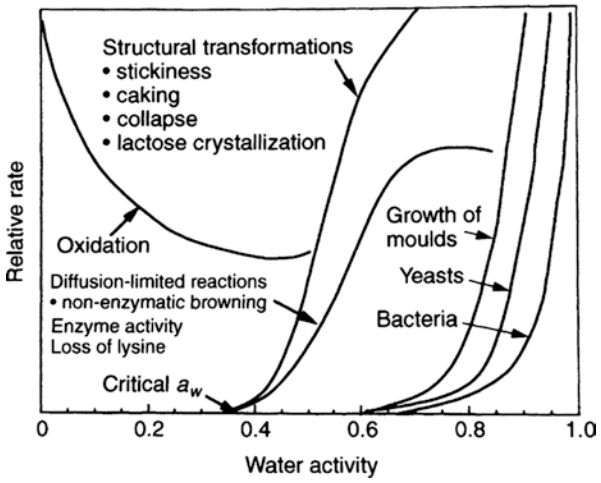


Fig. 9.3 Generalized deterioration reaction rates in food systems as a function of water activity at room temperature (from Roos 1997)

The concentration and distribution of salt in cheese have a major influence on various aspects of cheese quality; among the principal effects are:

- inhibition or retardation of microbial growth and activity, including pathogenic and food poisoning microorganisms (Table 9.3), and hence the safety of cheese (see Chap. 19).
- inhibition of the activity of various enzymes.
- affects the syneresis of cheese curd, resulting in whey expulsion and thus in a reduction in the moisture of cheese, which also influences the activity of microorganisms and enzymes.
- affects protein hydration which influences protein solubility, probably protein conformation, cheese texture and functionality.
- affects cheese flavour directly and indirectly via its influence on microorganisms and enzymes in cheese.
- high levels of salt in cheese may have undesirable nutritional effects (see Chap. 20).

Various aspects of the significance of salt in cheese have been comprehensively reviewed (Guinee and Fox 2004; IDF 2014), and are summarized below.

9.4 Salting Methods

Cheese is salted by one of four methods; the method used is characteristic of the variety:

- Dry salting, which involves addition and mixing of dry salt with small pieces of fresh cheese curd, prepared by milling or breaking larger blocks of cheese (as in Cheddar-type cheeses) prior to moulding and pressing

Table 9.3 Minimum water activity (a_w) for the growth of pathogenic bacteria in foods

Pathogen	Minimum a_w
Aeromonas hydrophila	0.970
Bacillus cereus	0.930
Campylobacter jejuni	0.990
Clostridium botulinum A	0.940
Clostridium botulinum B	0.940
Clostridium botulinum E	0.965
Clostridium botulinum G	0.965
Clostridium perfringens	0.945
Escherichia coli	0.935
Listeria monocytogenes	0.920
Salmonella spp.	0.940
Shigella spp.	0.960
Staphylococcus aureus (anaerobic)	0.910
Staphylococcus aureus (aerobic)	0.860
Vibrio parahaemolyticus	0.936
Yersinia enterocolitica	0.960

Source: Roos (1997)

- Brine-salting or brining, involving the immersion of freshly moulded cheese curds for a period, typically ranging from ~0.5 to 5 days, in brine, which is a pH-adjusted (~5.2) salt solution (~18–25 %, w/w, NaCl) containing added (0.2 %, w/w) calcium (e.g., Edam, Gouda, Camembert, Provolone).
- Surface dry salting, rubbing of dry salt or salt slurry to the surface of the moulded curds (e.g., Blue-type cheeses).
- A combination of two of these methods is used for quite a few varieties, e.g., milled (broken) Mozzarella curd may be partially dry-salted before stretching and moulding, followed by brining or surface application of dry salt.

Various modifications of brine-salting have been evaluated experimentally, e.g., brine injection under pressure, e.g., 17 MPa; high pressure (up to 500 MPa) brining; acoustic brining using a high intensity ultrasonic (30 kHz) wave, or vacuum impregnation at 3.7 kPa.

9.5 Brine-Salting

9.5.1 Mechanism of Salt Uptake

When cheese is placed in brine, there is a net movement of Na^+ and Cl^- from the brine into the cheese as a consequence of the difference in osmotic pressure between the brine and the moisture phase of the cheese. Simultaneously, water in the cheese diffuses out through the cheese matrix to establish osmotic equilibrium.

Microstructurally, cheese is essentially a matrix consisting of a network of fused *para*-casein micelles that entraps the serum phase and encases fat globules (see Chaps. 14 and 18, Fig. 14.3). The serum is solvent for various solutes, including lactic acid, soluble salts of sodium, calcium and citrate, free amino acids and peptides, and water-soluble fatty acids. The properties of the cheese serum are generally not appreciably different from those of corresponding solutions. Hence, the diffusion of salt from the brine through the moisture phase of cheese would be expected not to differ significantly from that of salt molecules through pure water (e.g., where pure water and salt solution are separated by a semi-permeable membrane). However, model experiments, designed to obey Fick's laws for one-dimensional flow (Geurts et al. 1974), have shown that the rate of diffusion of salt in cheese moisture is much lower than that in pure water. The diffusion coefficient, D, of salt in cheese moisture at 12 °C is ~0.1–0.3 cm^2/day compared to 1.0 cm^2/day in pure water; the difference is due to the structure of the cheese and the presence of dissolved solutes in the serum both of which retard and/or impede the movement of the NaCl molecules through the cheese. Specific factors include:

- the narrowness of the pores of the *para*-casein network which retard the movement of Na^+ and Cl^- passing through them;
- the impedance provided by obstructing fat globules and casein particles around which the diffusing salt molecules/ions must proceed to pass from one plane to another within the cheese increases the effective distance through which they must move;
- the relatively high viscosity of the cheese moisture compared to pure water.

Considering the impedance to the diffusion of salt and water by the structural features of the cheese matrix, the term pseudo-diffusion coefficient (D*) is used to describe the diffusivity of salt in cheese moisture, rather than the term diffusion coefficient (D), which refers to the diffusion of salt in pure water.

The mutual migrations of water and salt in opposite directions result in a decreasing salt gradient from the surface to the centre of the cheese, and a decreasing moisture gradient from the centre to the surface of the cheese on completion of brine-salting (Figure 9.4). However, these gradients gradually disappear and if the ripening/storage time is long enough, equilibrium of salt, moisture and salt-in-moisture will be established throughout the cheese (Fig. 9.5). The time required for equilibrium to be reached depends on cheese size and shape, cheese composition and storage temperature. These factors influence the distance over which salt and water molecules move, the degree of structural impedance to diffusion, and the kinetic energy of the diffusing molecules, respectively.

9.5.2 Factors that Affect Salt Uptake in Brine-Salted Cheeses and Its Diffusion Through the Curd

The absorption and diffusion of salt in cheese curd are affected by several compositional and environmental factors (Fig. 9.6).

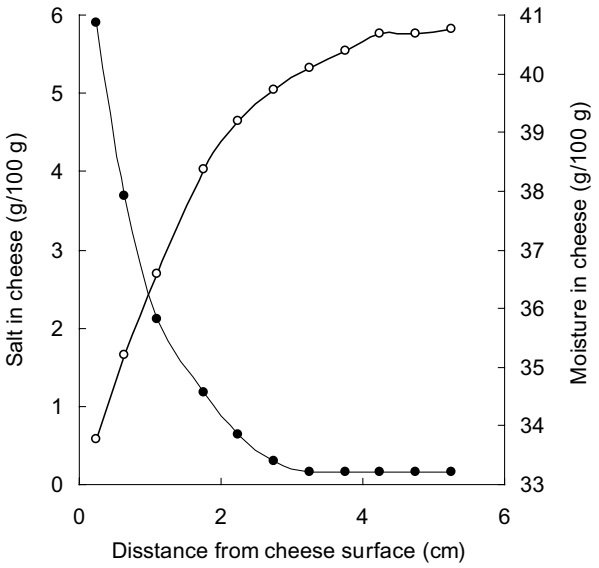


Fig. 9.4 Levels of salt and moisture in Gouda cheese as a function of distance from the salting surface following 4 days at 20 °C in 20 % NaCl brine. The data pertain to one-dimensional salting of a cylindrical (wheel)-shaped cheese, whereby the cheese was suspended over the brine bath so that only one surface was in contact the brine (Guinee 1985)

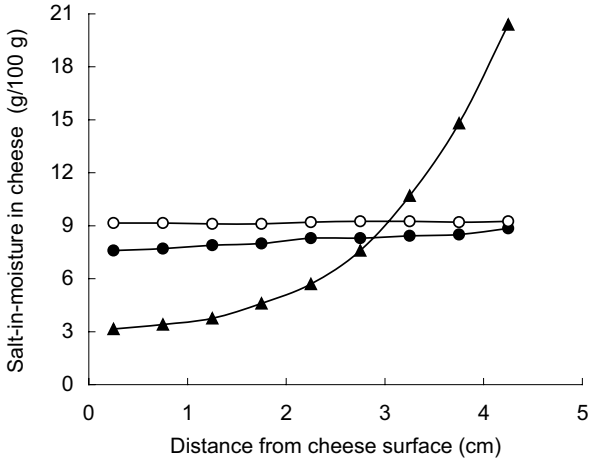


Fig. 9.5 Concentration of salt-in-moisture levels in Romano-type cheese as a function of distance from the salting surface following salting for 5 days (*filled triangle*) and after storage for 30 (*filled circle*) or 83 (*open circle*) days at 10 °C. Brining condition: 19.5 % NaCl brine at 23 °C (Guinee 1985)

9.5.2.1 Brine Concentration and Concentration Gradient

The rate of salt uptake by a cheese increases at a diminishing rate with brine concentration (Fig. 9.7). The decrease in rate may reflect a reduction in the availability of cheese moisture. Nevertheless, the rate of diffusion of absorbed salt throughout the cheese mass is essentially independent of brine concentration, as reflected by the constancy of the diffusion coefficient, D^*, for NaCl in cheese moisture.

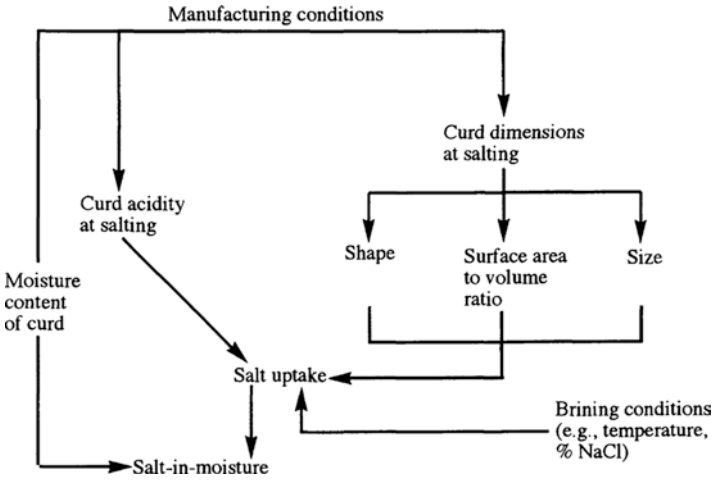


Fig. 9.6 Principal factors that affect salt uptake by brine-salted cheeses. The *arrows* indicate the interrelationships between the factors affecting salt uptake, with the factor at the base of the arrow affecting the factors at the head of the arrow. Compiled using data from various sources: Geurts et al. (1974, 1980), Guinee (1985) and Guinee and Fox (2004)

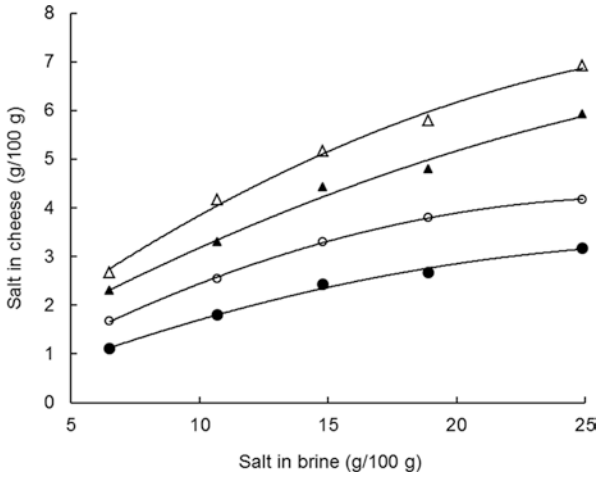


Fig. 9.7 Salt level in cheese slices (7 cm diameter, 0.5 cm thick) salted in brines of different concentration at 20 °C for 30 (*filled circle*), 50 (*open circle*), 100 (*filled triangle*) or 200 min (*open triangle*) (based on data from Guinee and Fox 1986)

9.5.2.2 Salting Time

The quantity of salt absorbed by brine-salted cheeses increases with brining time, but at a diminishing rate because of the simultaneous reduction in the NaCl concentration gradient between the cheese moisture and the brine (Fig. 9.8). Using model one-dimensional brine flow into cheese, Geurts et al. (1980) concluded that the

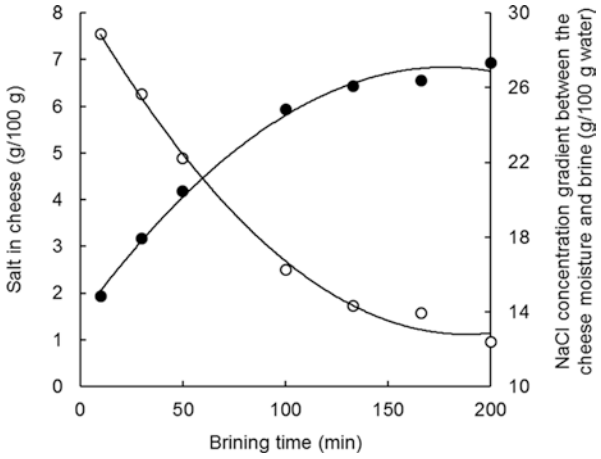


Fig. 9.8 Changes in salt concentration (*filled circle*) and the concentration gradient between the cheese moisture and the brine (*open circle*) in cheese slices (7 cm diameter, 0.5 cm thick) salted in 24.9 % NaCl brine as a function of brining time (based on data from Guinee and Fox 1986)

quantity of salt absorbed per unit surface area of flat cheese surface is proportional to the square root of brining time, according to the following relationship:

$$M_t = 2\left(C - C_o\right)\left(D^* t / p\right)^{1/2} V_w$$

where M_t = quantity of salt absorbed over time, g NaCl/cm^2
C = concentration of NaCl in brine, g NaCl/ml
C_o = original salt concentration in the cheese, g/ml cheese moisture
D^* = pseudo-diffusion coefficient, cm^2/d
t = duration of salting period, days
V_w = average water content throughout the cheese at time t, g/g

Extrapolating the relationship to a whole cheese, an estimate of the quantity of salt absorbed during brining (Q_t) can be made using the following relationship:

$$Qt = 100\,Mt\,A/G$$

Where, A = cheese surface area (cm^2), G = weight of cheese (g), and the effect of curvature is negligible.

9.5.2.3 Brine Temperature

The quantity of salt absorbed increases by 30–50 % depending on cheese composition and dimensions when the brine temperature is increased from 4 to 25 °C (Guinee and Fox 2004). These effects are attributed to an increase in true diffusion

(mobility of molecules), a temperature-related shrinkage of the casein network, and an associated increase in free water.

Model brine-salting experiments with curd cubes (1 cm^3, which may be considered as miniature cheeses) in brine (20 or 25 %, w/w, NaCl) indicate a more complex dependence of salt uptake on temperature at higher temperatures (27, 32, 38, 43 °C) (Breene et al. 1965). Salt uptake was lowest at 32 °C, highest at 43 °C and similar at 27 and 38 °C. The low salt uptake at 32 °C was attributed to a layer of exuded fat on the surface of the curd cubes which impeded salt uptake; less fat was exuded at lower temperatures while at temperatures >32 °C, exuded fat was increasingly liquefied and dispersed in the brine.

9.5.3 Cheese Size and Geometry

The quantity of salt absorbed in a given time increases with increasing surface area-to-volume (SA/V) ratio of the cheese. This is most readily observed on comparing the rate of salt uptake by milled Cheddar curd chips (each of which may be considered as a miniature cheese) and whole moulded cheeses (Brick, Emmental, Romano or Blue-type cheeses) in brine: in the former, salt absorption occurs from many surfaces simultaneously, and the time required to attain a fixed level of salt is considerably shorter than for the moulded cheeses. Shape also affects the rate of salt absorption because of its influence on the number of directions of salt penetration from the salting medium into the cheese, the distance from surface to centre of the cheese, and the ratio of planar to curved surface area of the cheese. Using model brining experiments, Geurts et al. (1980) showed that the quantity of NaCl absorbed per unit cheese surface was greater for a flat slab than for a sphere. Moreover, the reduction in the quantity of salt absorbed through a curved surface increased with brining time and with the degree of curvature, leading to a loss of the proportionality of salt uptake with $\sqrt{t}$. Thus, for cheeses with an equal volume and composition brined under the same conditions, the rate of salt absorbed per unit surface area (and hence the cheese as a whole) was in the following order: rectangular > cylindrical > spherical (Guinee and Fox 2004).

9.5.3.1 Moisture Content of Cheese Curd

The diffusion coefficient and the quantity of salt absorbed on brine-salting increases as the moisture content of the curd increases (Fig. 9.9). This has been attributed to a lower concentration of casein, a less dense and more porous casein network, and consequently, lower impedance on the salt molecules diffusing through the cheese matrix.

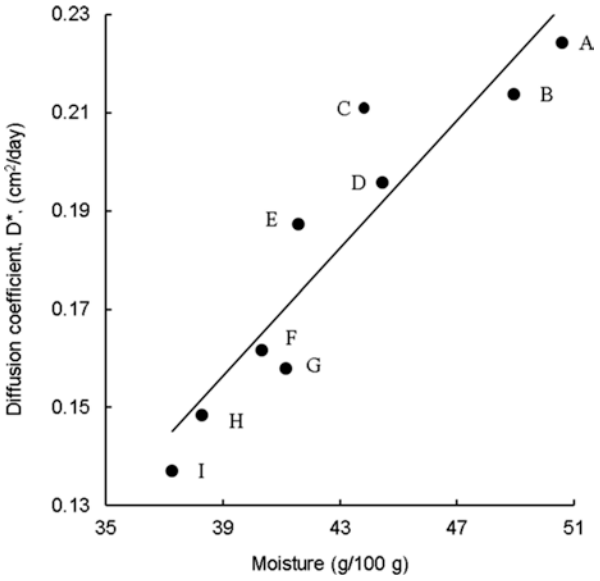


Fig. 9.9 Dependence of the pseudo-diffusion coefficient of salt in cheese moisture (D*) on the initial moisture content of cheese salted in ~20 % NaCl brine at 15–16 °C. Blue cheese (A, B), Gouda (C, D), Romano (E), Jarlsberg (F), Emmental (G, I), unsalted milled Cheddar (H) (based on Guinee 1985)

9.6 Dry Salting of Cheese

Dry salting is used mainly for British varieties (e.g., Cheddar, Cheshire, Stilton) and Cottage cheese. The curd mass is mechanically milled into small potato chip-shaped pieces (e.g., ~7 cm × 1.2 cm × 1 cm, in the case of Cheddar or Cheshire) or mechanically broken into relatively irregular shaped lumps or pieces (up to 0.25 kg) for Stilton cheese. The objective of reducing the size of the curd pieces is to maximize the surface area. Dry crystalline salt is then added to the size-reduced curd on a weight/weight basis and distributed over the surfaces by thorough mixing, which is generally performed in mechanical mixers (tumbler) and/or on rotating perforated belts where the curd bed (on the belt) is raked to facilitate mixing of curd and salt and uniform distribution of salt throughout the final cheese. Following mixing of curd and salt, the salted curd is held for a period to allow the salt on the curd surfaces to absorb, this period is frequently called "mellowing" in the case of Cheddar cheese. The milled salted curds are then moulded and pressed by application of pressure with, or without, an associated vacuum treatment.

As the surface area during dry salting is large, salt penetrates quickly throughout a curd piece. On the application of the appropriate level of dry salt, the concentration of NaCl rapidly reaches a level throughout the pieces that is sufficiently high to retard the growth of the starter culture. Hence, for dry-salted cheeses such as Cheddar, Cheshire and Stilton, the pH of the curd at salting should be close to the

desired value in the final cheese after pressing. However, although growth of the starter ceases shortly after salting, metabolism of lactose continues and the concentration of lactic acid, typically, increases, e.g., from 0.7 to 1.5 % during the first 24 h post-salting. The decrease in pH during this period is much smaller, e.g., from 5.4 to 5.2–5.1, than would be expected from the amount of lactic acid produced, because of the relatively high buffering capacity of cheese curd in the pH region 5.3–5.0 and to the fact that pH scale is logarithmic.

9.6.1 Mechanism of Salt Uptake

When salt is distributed on the surfaces of curd chips/pieces, some of the salt dissolves in the surface moisture, creating a highly concentrated brine. The salt (NaCl) from this concentrated brine layer then diffuses into the curd pieces, initiating a counter-flow of moisture (whey) from the curd to the surface. As the moisture reaches the surface it increases the volume of brine but simultaneously dilutes it. As the brine quantity increase, some of it drains away or remains on the curd surface until physically expelled during subsequent pressing. Following thorough mixing of the added dry salt with the curd, the mixture is allowed to stand for a period of 15–20 min, known as mellowing, in Cheddar manufacture, to allow the curd pieces to absorb the salt.

9.6.2 Factors Affecting Salt Uptake by Dry-Salted Cheeses

While many of the factors that influence the level of salt uptake by brine-salted cheeses apply in principle to dry-salted cheeses also, there are some differences owing to the mechanism of salt uptake. In particular, the ratio of curd to salt is much lower for dry-salted cheeses compared to brine-salted cheeses, where the brine volume is very large and the supply of salt is in effect unlimited. Moreover, the increasing loss of dry salt from the surfaces of curd chips in the form of brine that drains though the mass of curd chips, as the level of exuded moisture increases, can further reduce the quantity of salt available for uptake in the case of dry-salted cheeses.

The principal factors that affect the uptake of salt by Cheddar cheese curd are summarized in Fig. 9.10, and are discussed below.

9.6.3 Salting Rate: Level of Salt Added to the Curd

Salt uptake increases, at a diminishing rate, with the level of salt added to the curd (Fig. 9.11). The non-proportional increase in salt uptake is due to the concomitant increases in the level of whey expressed from the curd and, hence, the level of added salt lost. Consequently, higher salting rates coincide with higher levels of salt and salt-in-moisture and a lower level of moisture in the cheese after salting for a fixed time.

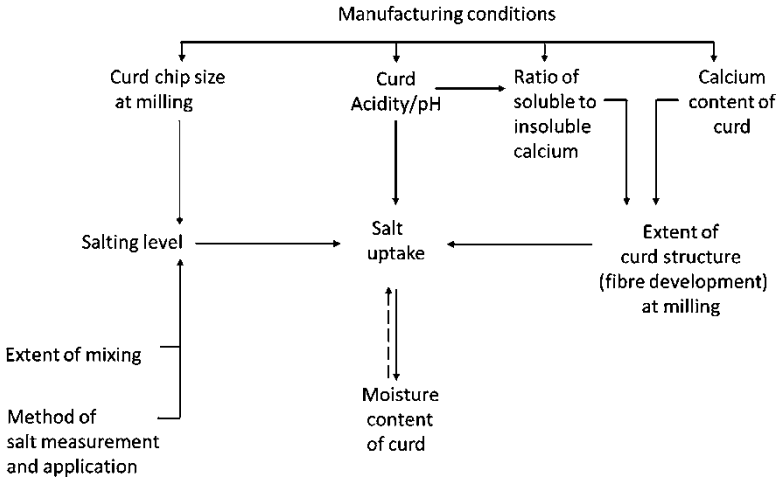


Fig. 9.10 Principal factors that affect the uptake of salt by Cheddar curd. The *arrows* indicate the interrelationships between the factors affecting salt uptake, with the factor at the *base of an arrow* affecting the factors at the *head of the arrow*. Compiled using data from various sources: Sutherland (1974), Gilles (1976) and Hou et al. (2014)

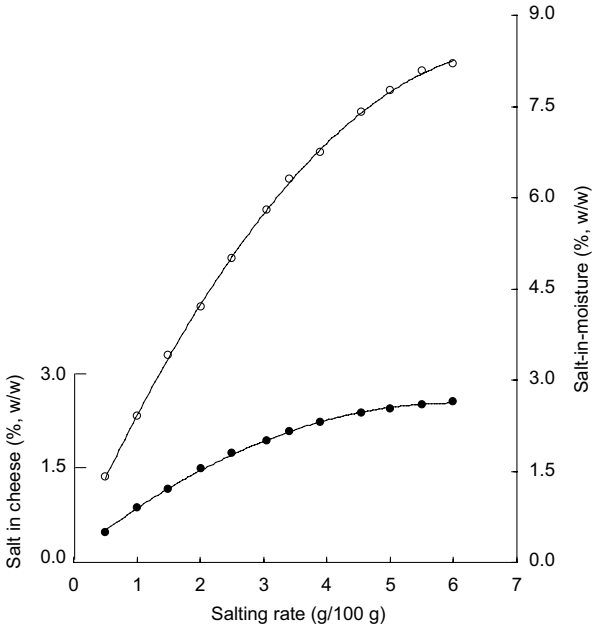


Fig. 9.11 Contents of salt (*filled circle*) and S/M level (*open circle*) of batches of curd from the same vat, salted at different levels (redrawn from Lawrence and Gilles 1982)

9.6.4 Curd Temperature

In contrast to brine-salting where an increase in brine temperature from 4 to 25 °C increases the concentration of salt in the cheese, an increase in curd temperature in the range 24–41 °C during the dry salting of Cheddar curd (chips) is paralleled by a decrease in salt and S/M in the final cheese (Sutherland 1974). This effect is due to the greater whey expulsion from the curd and a marked increase in the percentage of added salt lost during holding (mellowing). The higher outflow of moisture results in "excess brine" which percolates through the spaces between the chips, drains away and, thereby, lowers the effective amount of salt available for uptake.

9.6.5 Holding Time Between Salting and Pressing (Mellowing Period)

Increasing the duration of the curd mellowing period leads to higher salt and S/M levels in the pressed Cheddar cheese, e.g., an increase of ~0.3 % salt on increasing time from 15 to 30 min (Sutherland 1974). The increase is attributed to a higher total absorption of the added salt and hence a reduction in the physical loss of salt. During pressing, brine remaining on the surfaces of curd chips is expelled rapidly as the moulded curd chips are forced together with elimination of interstitial spaces.

9.6.6 Degree of Mixing of Salt and Curd and Curd Chip Size

Extending the duration of mixing Cheddar curd chips and salt from 20 s to 6 min caused a significant increase in salt and S/M levels, i.e., from 1.53 to 1.97 %, and 4.41 and 5.71 %, respectively (Sutherland 1974). The increases are due to more thorough mixing which maximises the proportion of the total available curd surface area (of curd chips) through which salt absorption occurs. For a given mixing period, increasing the surface area of the curd, by reducing the size of the curd chips, results in a significant increase in salt level (Gilles 1976).

9.6.7 Curd Depth During Holding

Increasing the depth of salted Cheddar curd during the mellowing period reduces the salt content of the cheese (Sutherland 1974). This is due to the increase in the pressure on the curd (under its own weight), which in effect results in pre-pressing of the curd and a reduction in the mellowing time between salting and pressing.

9.6.8 Moisture Content and pH of Curd at Salting

In contrast to brine-salting, an increase in the moisture content of curd before dry salting results in lower levels of salt and salt-in-moisture in Cheddar cheese (Sutherland 1974; Gilles 1976). This effect is due to greater losses of salt from high-moisture curds.

Cheddar curd dry-salted at low acidity (high pH) retains more salt and gives a higher salt cheese than curd salted at higher acidity (Gilles 1976). The greater salt retention is probably due to moisture being bound more tightly by the curd (casein) at the higher pH values. A higher water-binding capacity of the casein would favour less whey release, lower losses of added dry salt and greater salt absorption by the curd chips.

Owing to their impact on salt level in cheese, controlling the pH and moisture content at dry salting is critical to maintain consistent salt levels in the final cheese.

9.6.9 Uniformity of Absorbed Salt Throughout the Curd Mass

Owing to the direct mixing of small curd pieces with dry salt, it should be possible to achieve very precise and uniform control of salt concentration in dry-salted milled cheese curd. When properly executed, it is possible to get to within ±0.1 % of the desired salt concentration. However, if salt distribution in Cheddar cheese is poor initially, uniform distribution of salt throughout the cheese may not be attained during the life of the cheese (Sutherland 1977; Morris et al. 1985), as a consequence of a number of factors including:

- The lack of a continuous salt gradient within the cheese mass, as regions of high, medium or low salt are non-uniformly distributed;
- A high degree of protein dehydration and a low protein-to-fat ratio at the surfaces of the curd pieces compared to the interior, owing to contact with dry salt and ensuing high losses of moisture and fat from the surface layer.

Owing to the critical impact of salt on various aspects of cheese quality (Fig. 9.2), dry salting operations in commercial cheese plants are highly controlled to ensure the addition of the desired weight of salt and its uniform mixing with the curd chips (Bennett and Johnston 2004). Features of the salting system in large modern Cheddar manufacturing plants include:

- Uniform, consistent size distribution of the curd chips, by frequent maintenance and validation of curd milling equipment;
- Maintaining the salt dry and free-flowing, by storing in dedicated rooms with limited access, and controlled temperature and relative humidity (~35 °C, 55–65 % RH);
- Devices for accurate weighing of the curd on the rotating salting belts (e.g., load cells), metering and delivery of salt from the storage area to curd (e.g., appropriate

process control and pneumatic pumping), and uniform distribution and mixing of salt with the curd (e.g., twin stainless steel salting booms which distribute the salt across the enclosed belt of curd, mounted rotating pegged agitators that continuously mix the curd on the rotating belts or dedicated salt-curd drum mixers with side baffles).
- The use of enclosed belt systems, which are conducive to a more consistent temperature at salting and, hence, salt uptake.

In smaller artisanal cheesemaking operations, ideally the salt is stored in a dry area (to prevent moisture uptake and lumping), the curd and salt are weighed accurately and mixed thoroughly and the salted curds are allowed sufficient mellowing time, during which they are agitated frequently.

Even when properly measured and mixed, variations in salt concentration still occur, owing to variability in the composition of the unsalted curd (e.g., pH and moisture content), size distribution of curd chips at milling, and variability in salt uptake by the curds. Commercially, this can lead to variations in the salt content between cheeses from the same vat and cheeses from different vats over a day's manufacture. Variability in curd composition at salting (e.g., pH, moisture, protein-to-fat ratio) can arise from seasonal variations in milk composition, and the application of operating procedures that operate on the basis of addition of ingredients (such as starter culture, coagulant) based on milk volume, undertaking different manufacturing steps (e.g., cutting, whey drainage, milling and salting) based on time, and not washing curd to control the level of residual lactose, and hence, lactic acid in the cheese (see Chap. 15). While such an approach may be applicable where the composition of the cheesemilk is standardized with respect to casein-to-fat ratio, casein content and pH at set, it is conducive to variations when the composition of the cheese milk changes seasonally. Where milk standardization is not practiced, then the weight of starter culture and rennet should be added pro-rata with the casein content, the curd should be washed to a degree pro-rata with the level of lactose in the milk, and cheesemaking steps should be performed on the basis of pH rather than time.

9.7 Effect of Salt on Cheese Composition

For any particular variety, and everything else being equal, there is an inverse relationship between the levels of moisture and salt in cheese. Hence, a higher salt level in cheese coincides with a lower moisture level, higher dry matter content, and consequently, higher levels of fat and protein which are the main components of the dry matter.

Model salting experiments, involving one-dimensional brine-salting (salt absorption though one surface only) showed that the relationship between the quantities of salt absorbed and water lost is expressed by the following equation:

$$-\Delta W_x \approx p\Delta S_x$$

where ΔW and ΔS are the changes (from the unsalted cheese) in g H_2O and g NaCl, respectively, per 100 g cheese solids-not-salt in planes of the cheese x cm from the cheese/brine interface (Geurts et al. 1974; Guinee and Fox 1983). The experimental value of p is ~2 (Fig. 9.12), i.e., the weight of water lost is about twice that of salt taken up, but varies from 1.5 to 2.34 (or from <1 to 3.75 in another study) depending on the distance from the surface. Changes in the texture and appearance of the cheese can be seen as the salt front moves through the cheese.

However, under abnormal brining conditions (brine with <10 %, w/w, and no calcium), uptake of salt during brining can be accompanied by an increase in moisture content in the vicinity of the cheese-brine interface (Fig. 9.13). Such an effect is responsible for the "soft-rind" defect and swelling in cheese and is attributed to "salting-in" (solubilization) of the cheese protein in dilute NaCl solutions (Guinee and Fox 2004).

The concentrations of lactose and lactic acid in, and the pH of, cheese depend on the continued activity of the starter and, hence, on salt content.

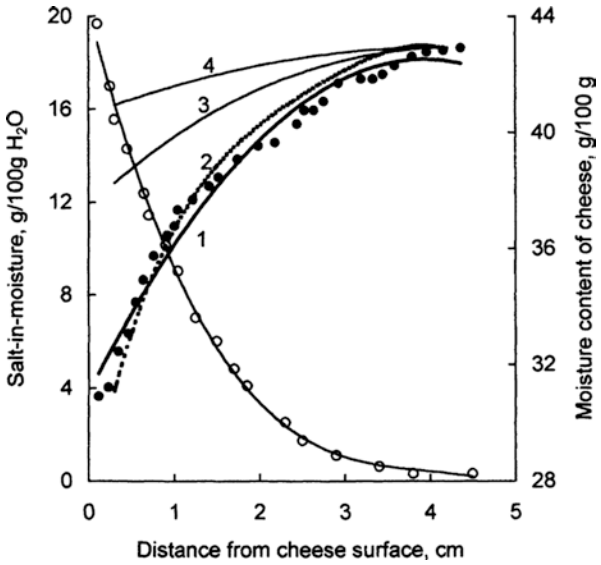


Fig. 9.12 Experimental (*filled circle*) and theoretical (1–4) moisture levels and experimental salt-in-moisture level (*open circle*) in a full-fat Gouda cheese (pH 5.64) after brining for ~8 days as a function of distance from the cheese surface in contact with the brine (20.5 g NaCl/100 g H_2O); temperature, 12.6 °C. Theoretical moisture levels were calculated using the relationship: $\Delta W_X = p\Delta S_X$, where W and S are g H_2O and g NaCl, respectively, per 100 g cheese solids-not-salt, x is the distance (cm) from the cheese surface in contact with the brine, and p is a coefficient denoted as the flux ratio. The theoretical moisture levels were calculated for: p = 2.5 (1); *p* varying from 1.7 at the salt front, i.e., maximum distance to which salt had penetrated, to 2.9 in the cheese surface (2); p = 1 (3); and p = 0 (4). Reprinted from Geurts et al. (1974)

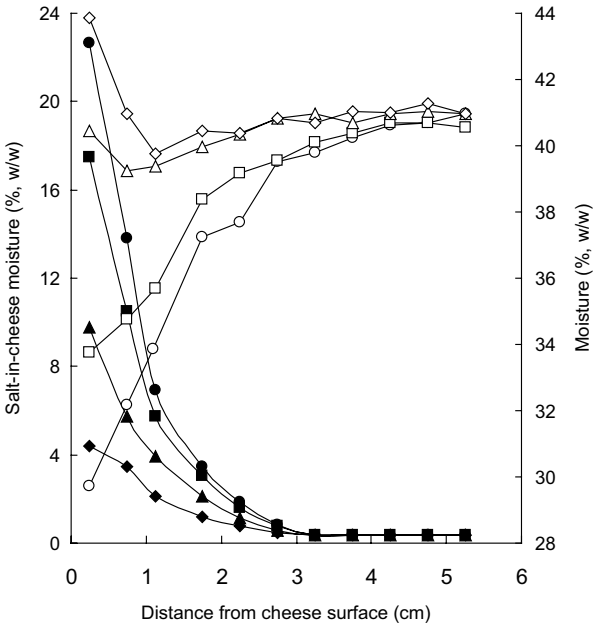


Fig. 9.13 Moisture content (*open symbols*) and salt-in-moisture concentration (*closed symbols*) in Gouda cheese as a function of distance from the salting surface after brine-salting for 4 days at 20 °C in (*filled diamond*, *open diamond*), 12 (*filled triangle*, *open triangle*), 20 (*filled square*, *open square*) or 24.8 (*filled circle*, *open circle*) % NaCl solution (without calcium) (redrawn from Guinee 1985)

9.8 Effect of NaCl on the Microbiology of Cheese

The concentration of salt in cheese moisture (S/M) has a major effect on the growth of microorganisms in, and on, the cheese (see Chap. 11; Guinee and Fox 2004; IDF 2014; Rulikowska et al. 2013). Probably the most extreme example of the use of salt to control bacterial growth is Domiati-type cheese, where 8–15 % NaCl is added to the cheese milk to inhibit bacterial growth and maintain milk quality. In the manufacture of most cheese varieties, salt is added after curd formation.

The growth of *Lactococcus* strains used as starters is stimulated by low levels of NaCl but is strongly inhibited >5 % NaCl. In dry-salted cheeses, the concentration of NaCl rapidly reaches an inhibitory level throughout the cheese. In Cheddar-type cheese, starter growth ceases shortly after salting but metabolism of lactose continues unless the level of S/M exceeds about 5 % (Figs. 9.14 and 9.15). Strains of *Lc. lactis* ssp. *lactis* are more salt tolerant than *Lc. lactis* ssp. *cremoris*: the former grow in the presence of 4 % NaCl while the latter grow in the presence of 2 % but not 4 % NaCl; however, there is considerable inter-strain variation in both supspecies. A low level of S/M may lead to high numbers of starter cells in Cheddar cheese which may lead to bitterness.

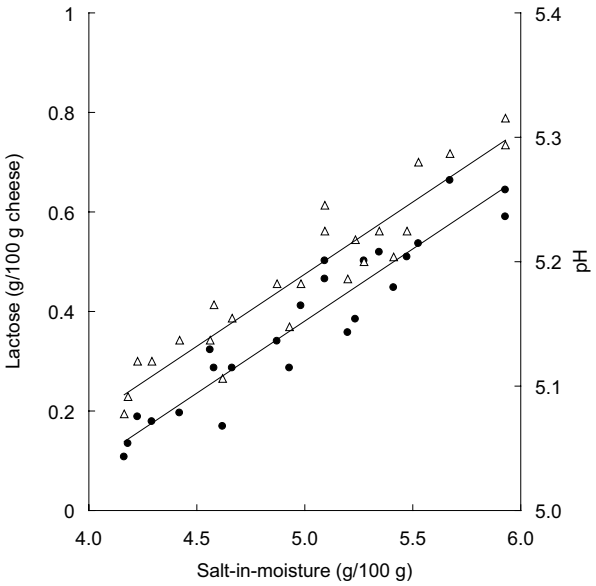


Fig. 9.14 Effect of S/M concentration on the concentration of lactose (*open triangle*) and pH (*filled circle*), within a single block of Cheddar cheese analysed 14 days after manufacture (based on data from Thomas and Pearce 1981)

If starter activity is inhibited after manufacture owing to an excessively high level of S/M, residual lactose will be metabolized relatively late in ripening when the number of non-starter lactic acid bacteria (NSLAB) is high. In modern Cheddar cheese, the number of NSLAB is low initially (<100 cfu/g) and they grow at a rate which is influenced particularly by the rate of cooling the pressed curd and the ripening temperature. NSLAB vary in their ability to grow in the presence of NaCl; most strains can grow in the presence of 6 %, but not 8 %, NaCl; those strains that can grow in the presence of 8 % NaCl are inhibited by 10 % NaCl. Normally, the number of NSLAB is too low to metabolize the residual lactose rapidly, unless it persists for several weeks (see Chap. 11).

In brine-salted or dry surface-salted cheese, NaCl diffuses slowly from the surface to the centre and even in small cheeses, salt probably does not reach an inhibitory concentration on the interior of the cheese until starter growth has ceased due to the depletion of lactose or perhaps low pH. Thus, although thermophilic starters, *Streptococcus thermophilus* and *Lactobacillus* spp. are more sensitive to NaCl than *Lactococcus* spp., this is probably not significant in cheese acidification since cheeses made using these starters are usually brine-salted.

Although data on the salt sensitivity of *Propionibacterium* spp. are variable, most studies show that they are quite salt-sensitive and can not grow in the presence of 3 % NaCl; the S/M in Emmental is only ~2 %.

Blue cheeses are among the most heavily salted varieties, at 3–5 % NaCl. Germination of *P. roqueforti* spores is stimulated by 1 % NaCl but inhibited by

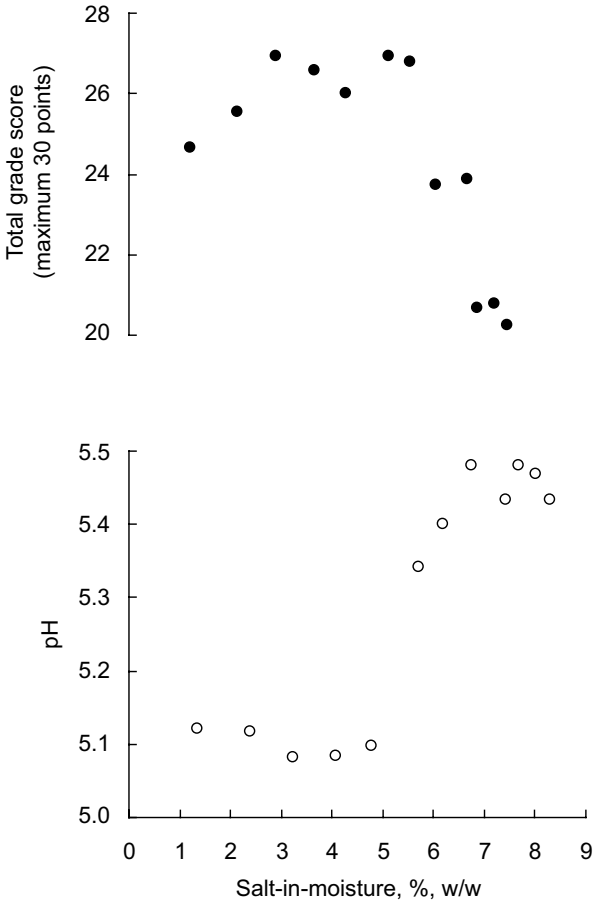


Fig. 9.15 Effect of salt-in-moisture level on the pH (*open circle*) at 8 weeks, and the total grade score (maximum 30) (*filled circle*) of Cheddar cheese made from curd from the same vat but salted at different levels (based on the data of O'Connor 1974)

3–6 %, depending on the strain. Germinated spores can grow in the presence of up to 10 % NaCl. It is fairly common commercial practice to add 1 % NaCl directly to Blue cheese curd, perhaps to stimulate spore germination although it also serves to give the cheese a more open structure, which facilitates mould growth. Since most Blue cheeses are dry surface-salted, a salt gradient from the surface to the centre exists for a considerable period after manufacture and the % S/M in the outer layer of the cheese may be high enough to inhibit spore germination during a critical period, resulting in a mould-free zone at the outside of the cheese.

Growth of *P. camemberti* is also stimulated by low levels of NaCl; mould growth on Camembert cheese is poor and patchy <0.8 % NaCl.

Since smear-ripened cheeses are brine-salted, a salt gradient from the surface to the centre exists initially. However, most of these cheeses are relatively small and

have a relatively high-moisture content; therefore, salt equilibrates throughout the cheese relatively quickly. These cheeses are also rubbed with brine occasionally to distribute the microorganisms evenly over the surface. The surface microflora of these cheeses is very complex but the principal microorganisms are yeasts and coryneform bacteria, both of which are quite salt tolerant (see Chap. 11).

9.9 Influence of NaCl on Enzymes in Cheese

9.9.1 Coagulant

With the exception of high-cooked cheeses, e.g., Emmental and Parmesan, in which the rennet is denatured extensively during cooking, primary proteolysis is catalyzed mainly by the residual coagulant (see Chap. 12). Although chymosin, pepsins and *Rhizomucor* proteinases readily hydrolyse β-casein in solution, α_{s1}-casein is the principal substrate in cheese. β-Casein is less susceptible to hydrolysis by the coagulant, probably due mainly to hydrophobic interactions between adjacent C-terminal regions, which contain the primary chymosin-susceptible bonds (see Chap. 12); these interactions are intensified at high ionic strength. The concentration of salt in cheese has a large effect on the rate of proteolysis (see Chap. 12).

9.9.2 Milk Proteinases

The principal indigenous proteinase in milk, plasmin, contributes to proteolysis in all cheese varieties that have been studied, as indicated by the formation of γ-caseins, and is a major contributor in high-cooked cheeses owing to partial or complete inactivation of the coagulant. Plasmin is associated with the casein micelles in milk and is incorporated into cheese curd. The activity of plasmin in cheese is stimulated by low levels of NaCl, up to a maximum at 2 %, but is inhibited by higher concentrations although some activity remains at 8 % NaCl. The influence of NaCl on the activity of the indigenous acid milk proteinase (cathepsin D) has not been investigated.

9.9.3 Microbial Enzymes

The effect of NaCl on the stability and activity of microbial enzymes, especially in the cheese environment, has received little attention. Gobbetti et al. (1999a) studied the interactive effects of pH (5.5–7.0) and S/M (0.0–7.5) on the activity of peptidase from various NSLAB strains, including *Lactobacillus casei* subsp. *casei*, *Lb. plantarum*, *Lb. casei* subsp. *pseudoplantarum* and *Lb. curvatus*. While the peptidases of *Lb. casei* subsp. *pseudoplantarum* and *Lb. curvatus* were markedly

inhibited at high S/M level, those of *Lb. casei* subsp. *casei* and *Lb. plantarum* were much less sensitive to NaCl. In a subsequent study, Gobbetti et al. (1999b) investigated the effects of S/M (2.5 to 7.5 %,), pH (5.0–5.7) and a_w on the proteolytic and lipolytic activities of starter and NSLAB, including strains of *Lb. delbrueckii* ssp. *bulgaricus*, *Lactococcus lactis* ssp. *lactis* and *Lb. plantarum*. The effect of S/M was both enzyme- and species-specific.

9.10 Effect of Salt on Cheese Quality

Considering that salt has a major influence on the microbiology, enzymology, pH and moisture content of cheese, it is not surprising that the concentration of salt in cheese has a major effect on its quality (Figs. 9.2 and 9.15). Ripening is retarded at high salt concentrations while defects, e.g., bitterness, are common at low concentrations. The optimum concentration for Cheddar is about 5 % S/M. Although the effect of NaCl concentration on cheese quality is well recognised, its effect(s) at the molecular level is not known. It is likely that high concentrations of NaCl retard ripening through a general inhibitory effect on several enzymes in cheese. High concentrations of NaCl (e.g., >8 % S/M in Cheddar) probably inhibit the growth of NSLAB but concentrations in the range normally encountered in Cheddar (4.6–5.6 % S/M) appear to have little or no effect. Moreover, reduction of salt content generally coincides with a higher moisture content and a lower pH, with the latter being especially the case in cheeses that are not washed during manufacture. The lower pH favours a higher ratio of proteolytic-to-peptidase activity, which promotes alterations and defects in flavour. Flavour defects encountered at low salt concentrations probably arise from excessive or the unbalanced enzyme activity, e.g., bitterness can occur in Dutch-type cheeses due to excessive proteolysis of β-casein by chymosin which releases bitter C-terminal peptides, e.g., β-CN f193-209. NaCl also makes a direct contribution to cheese flavour, as most consumers appreciate a salty taste in foods. Salt-free cheese has a rather insipid, watery taste; 0.8 % NaCl is sufficient to overcome this defect.

Apart from flavour, salt reduction also affects the rheological/deformation properties of cheese on the application of stress or strain (e.g., elasticity, fracture properties, adhesiveness, and firmness) and associated physical attributes such as shreddability, sliceability, crumbliness, springiness (see Chaps. 14 and 19; Guinee and O'Kennedy 2007; IDF 2014). Various studies have shown that an increase in S/M within the range 0.14 (unsalted cheese) to 12 % results in increased firmness, fracture stress and sensory hardness of various cheeses including Camembert, Cheddar, Feta, Gaziantep, Mozzarella and Muenster. The changes may be attributed in part to the concomitant changes in composition (e.g., reduction in moisture level and increase in protein), *para*-casein hydration/solubility and conformation, age-related effects on pH, and in the extent of proteinase/peptidase activity, and proteolysis. Likewise, salt content affects the cooking properties of cheese (IDF 2014). Unsalted Mozzarella has very poor cooking properties as reflected the

absence of flow and stretchability (Apostolopoulos et al. 1994; Paulson et al. 1998). Both these parameters were improved when the salt level was raised to 0.5 % and changed only slightly as the salt level is increased further to ~2.2 %. The increase in flowability with salt level coincided with increases in free oil content and water-binding capacity of the *para*-casein network.

9.11 Nutritional Aspects of NaCl in Cheese

High intake of salt in the diet is undesirable since it increases hypertension and the risk of osteoporosis via increased excretion of calcium; sodium rather than chloride is the responsible agent (see Chap. 20, He and MacGregor 2007; Taylor et al. 2011). Cheese, because of its many different varieties (Table 9.1), allows the consumer large choice in terms of salt content and dietary intake of sodium. Based on *per caput* consumption levels of 10–20 kg, cheese contributes only about 5–10 % of total sodium intake, depending on salt content of the cheese(s) eaten. Nevertheless, there has been considerable interest in the production of reduced-sodium cheeses (Guinee and O'Kennedy 2007; Johnson et al. 2009). Approaches used include:

- reducing the level of salt added; the degree of reduction is limited owing to the development of off-flavours in low salt cheese,
- replacing some of the NaCl by KCl, $MgCl_2$ or $CaCl_2$; about 15 % of the NaCl may be replaced by KCl without undesirable consequences, but higher levels of replacement lead to bitterness caused by KCl,
- use of flavour enhancers to mask defects,
- Altering cheese composition to counteract the adverse effects of reduced salt content, e.g., preventing low pH by reducing moisture content, increasing buffering capacity, and reducing lactic acid content through curd washing.

Processed cheese and cheese products contain much higher levels of Na than natural cheese owing to the addition of sodium-rich "emulsifying" salts (see Chap. 18).

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Chapter 10
Cheese Yield

Summary Cheese is a very important trade item in the dairy industry, accounting for ~35 % of total milk usage. In the production of commodity-type cheeses, such as Cheddar and Gouda, increasing the scale of production and cheesemaking efficiency are key factors in reducing production costs and increasing market competitiveness. Efficiency is measured by comparing the actual values of cheese yield or recoveries of milk components (fat and protein) from milk to cheese, with predicted values. Measurement of actual cheese yield requires measurement of the weights and composition (fat, protein, moisture) of inputs (milk, starter culture) and output (cheese). The actual yield of cheese (which must comply with the specified maximum moisture level and minimum fat-in-dry matter level of that variety), can be then expressed as kg cheese/100 kg milk with defined fat and proteins levels. For example, the typical yield of Cheddar cheese, for which the specified moisture content is ≤39 % and fat-in-dry matter content is ≥50 %, is 10 kg per 100 kg milk standardized to protein and fat levels of 3.3 and 3.6 %, respectively. Yield prediction equations, such as the van Slyke equation, are used to predict the quantity of a specific cheese variety expected from a given quantity of milk of a given composition. The percentage yield efficiency (% YE) expresses actual yield as a percentage of predicted yield, which is indicative of the efficiency of the cheesemaking process in converting milk to cheese. Ideally, YE = 100 %; where actual values are <100 %, corrective actions to the cheesemaking process are undertaken to redress the inefficiencies.

Cheese yield is influenced by many factors. Some of the more important include the composition and quality of the raw milk, milk handling and storage practices, milk pre-treatments (e.g., standardization of protein-to-fat and protein contents, homogenization, pasteurization temperature), firmness of the gel at cutting and the speed/duration of the cutting programme, the curd particle size, speed of stirring the curd-whey mixture, the rate of cooking of the curd-whey mixture and the temperature to which it is cooked, the duration of stirring and cooking before the physical separation of whey from the curd, design of the cheese vat and curd handling conditions following whey removal. These factors exert their effects by influencing the composition of the milk gel and the levels of moisture expulsion and fat loss from the curd particles formed on cutting the gel.

Keywords Actual yield • Measurement • Predicted yield • Factors affecting cheese yield

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10.1 Introduction

World production of cheese is about 19×10^6 tonnes per annum and utilizes ~35 % of total milk produced, and has an estimated value of US$ 6.2×10^{10}. While cheese-like products are produced in most parts of the world, the principal cheese-producing regions are Europe, North America and Oceania. Within these regions, the production and consumption of cheese varies widely with country, as does the proportion of milk used for cheese which ranges from ~<20 % (e.g., in New Zealand) to ~>70 % (e.g., Italy, Germany, France). Approximately 10 % of total cheese production is traded on the global market, the major suppliers being the EU (~38 %), New Zealand (~21 %) and Australia (~14 %), and the major importers being Russia (~21 %), Japan (~20 %) and the USA (~19 %) (ZMP 2008; IDF 2009).

Consequently, the yield of cheese and its control are of great economic importance, determining the profit of cheese plants and the price of milk accruing to farmers. Owing to its economic importance, cheese yield and the factors that affect it have been investigated extensively and several comprehensive reviews on the subject have been published (IDF 1991, 1994; Lucey and Kelly 1994).

10.2 Definition and Expression of Cheese Yield

The expression of cheese yield is important in two main applications:

- measuring the efficiency of, and determining the economic viability of, a cheesemaking operation, and
- expressing the results of experiments, which is important in evaluating the potential usefulness of a particular process or change in technology.

Yield may be expressed in various formats, as outlined by Eqs. (10.1)–(10.6) in Table 10.1 and as discussed below. These expressions assume no whey protein denaturation (or complexation with casein) and, hence, no recovery of whey proteins from milk to cheese. This is generally applicable for rennet-curd cheeses, where casein accounts for typically 99 % of the protein recovered, and whey proteins for only 1 %, depending on moisture content. However, more complex relationships between yield and milk composition are required where whey protein recovery is significantly higher, for example when milk is pasteurized at a temperature ≫72–73 °C or when whey protein powders (e.g., microparticulated whey proteins) are added to the cheese milk to enhance yield or modify the characteristics of reduced-fat cheese (Guinee 2003).

Table 10.1 Expressions of cheese yield, and equations for calculation

Abbreviation	Definition	Units	Equation for calculation
Ya	Actual cheese yield	kg cheese/100 kg milk	$Actual\ yield\,(Ya) = 100 \times \left(\frac{weight\ of\ cheese}{Weight\ of\ milk + starter\ culture}\right)$ (10.1)
Yma	Moisture-adjusted cheese yield	kg cheese with reference moisture content (Mr)/100 kg milk	$Yma = Ya \times \left(\frac{100 - Ma}{100 - Mr}\right)$, (10.2) where Ma and Mr correspond to the actual moisture and reference (target) moisture levels (%, w/w) of the cheese, respectively.
Yafpam	Milk protein plus fat-adjusted cheese yield	kg cheese/100 kg of reference milk with fat and protein levels of F_{rm} and P_{rm}	$Yafpam = Ya \times \left(\frac{F_{rm} + P_{rm}}{F_{cm} + P_{cm}}\right)$, (10.3) where F_{rm} and P_{rm} correspond to the percentages fat and protein in the reference cheese milk, and F_{cm} and P_{cm} to the percentages fat and protein in the actual cheese milk, respectively.
Ymafpam	Moisture-adjusted, milk protein plus fat-adjusted cheese yield	kg cheese with reference moisture content (Mr)/100 kg reference milk with fat and protein levels of F_{rm} and P_{rm}	$Ymafpam = Yafpam \times \left(\frac{100 - Ma}{100 - Mr}\right)$, (10.4) where Ma, Mr and Yafpam are as described for Eqs. (10.2) and (10.3) above.
Yafcam	Milk casein plus fat-adjusted cheese yield	kg cheese/100 kg of reference milk with casein and fat levels of C_{rm} and F_{rm}	$Yafcam = Ya \times \left(\frac{F_{rm} + C_{rm}}{F_{cm} + P_{cm}}\right)$, (10.5) where F_{rm} and C_{rm} correspond to the percentages fat and protein in the reference cheese milk, and F_{cm} and P_{cm} to the percentages of fat and protein in the actual cheese milk.
Ymafcam	Moisture-adjusted, milk casein plus fat-adjusted cheese yield	kg cheese with reference moisture content (Mr)/100 kg reference milk with casein and fat levels of C_{rm} and F_{rm}	$Ymafcam = Yafcam \times \left(\frac{100 - Ma}{100 - Mr}\right)$, (10.6) where Ma, Mr and Yafcam are as described in Eqs. (10.2) and (10.5) above.

Compiled from Guinee et al. (2006)

10.2.1 Actual Yield (Ya)

Frequently, actual cheese yield (Ya) is expressed simply as the 'kg of cheese per 100 kg milk' or '% yield' [Eq. (10.1), Table 10.1]. An alternative expression also used in practice is 'the number of litres of milk required to manufacture one tonne of cheese', which in the case of Cheddar cheese is ~10,000 L.

However, Ya is of limited use as it does not permit a meaningful comparison of:

- Yield of cheese differing in moisture content, e.g., variants of a given variety, or different varieties
- Yield of a given variety of cheese from milk of different composition (protein, casein and fat contents), as arise from natural seasonal variation or process intervention.

For most cheese varieties, manufacturers produce a range of variants (brands) that differ slightly in composition (e.g., moisture, fat, protein, salt, pH) and manufacturing process. While all brands share predominant characteristics (e.g., hard, mealy, sweet perception on eating in the case of Parmesan) appreciated by the generic consumer, individual brands have, in addition, specific attributes (e.g., critical levels of flavours, such as nuttiness, sweetness, picante or rancidity) that are perceived and sought after by specific groups of consumers. Moreover, the composition of milk supplied to the cheese manufacturer can vary depending on several factors, including species (e.g., cow, goat or sheep), breed of cow, stage of lactation, plane of nutrition, lactation number and animal health. Consequently, the milk is normally standardized to a specific protein-to-fat ratio (PFR), or casein-to-fat ratio (CFR) ratio, to give cheese of the required composition that complies with the levels of moisture and fat-in-dry matter (FDM) specified in legal 'Standards of Identity' (cf. Chap. 21). A further technological intervention is the standardization of the protein content of cheese milk using low concentration ultrafiltration (LCFUF). This is now practiced by most of the large cheesemaking companies to offset the effects of the naturally-occurring variation in milk protein level on cheese composition and quality, and to conform to end-product specifications (Guinee et al. 1994; Broome et al. 1998; Johnson and Lucey 2006, Guinee and O'Callaghan 2010, Govindasamay-Lucey et al. 2011). It allows standard operating procedures, which include normalization of key process factors such as starter-to-casein ratio, rennet-to-casein load, firmness and firming rate of the gel at cutting, to be followed more closely.

Consequently, normalization of yield to minimize the confounding effects of variations in milk and cheese composition is necessary.

10.2.2 Normalized Yields

10.2.2.1 Moisture-Adjusted Yield (Yma)

Moisture-adjusted cheese yield (kg cheese with a normalised (reference) moisture content/100 kg of cheese milk) normalizes the moisture content of the cheese to a fixed value, and thereby eliminates the direct effect of differences in cheese moisture

to yield and allows the yield of cheeses of a given cheese variety with different moisture contents to be compared. The moisture content on which the actual yield is normalised for the determination of Yma is chosen by consideration of the target moisture content of the variety. Using Eq. (10.2) (Table 10.1), the moisture-adjusted (to ~37 %) yield equivalent (kg/100 kg milk) of Cheddar cheese with a moisture content of 38.2 % and an actual yield of 10.3 kg is $Yma = 10.3 \times (100 - 38.2)/(100 - 37)$.

Hence, Yma facilitates comparisons of the yield of cheeses differing in moisture content, especially for variants of a given variety with the same ratio of fat-to-protein. Nevertheless, similar to Ya, it does not relate yield to the levels of protein and fat in the cheese milk.

10.2.2.2 Milk Protein Plus Fat-Adjusted Yield (Yafpam)

The use of the yield formula 'milk protein plus fat adjusted yield' (Yafpam, Eq. (10.2), Table 10.1) eliminates the effects of differences in milk composition to yield. Hence, it allows the yield of cheese from milk of different compositions to be compared, and thereby facilitates meaningful comparison of yields of a given cheese variety from different plants, and from different stages of the cheesemaking season (where milk composition changes seasonally). When modified further to normalize the moisture content of the cheese, Ymafpam also allows meaningful comparison of cheeses with a different moisture content. A related yield expression is based on casein and fat (Ymacfam), which is particularly applicable where the casein content of milk, as a percentage of total protein is increased for example by addition of microfiltered milk or micellar casein powders (Guinee et al. 2006).

10.3 Measurement of Cheese Yield and Efficiency

Determination of actual cheese yield requires measurement of the weight of all inputs (e.g., milk, starter culture, salt) and outputs (e.g., cheese, whey) during the cheesemaking process. A typical mass balance for the experimental production of Cheddar is presented in Table 10.2.

10.3.1 Actual Yield (Ya) and Related Yields

In pilot-scale cheesemaking experiments, an accurate mass balance is easily achievable because of the batch nature and small scale which facilitate weighing of all materials. The fitting of load cells to pilot-scale cheese vats further simplifies and increases the accuracy of mass balances in experimental cheesemaking. The actual cheese yield may be then calculated using Eq. (10.1) (Table 10.1). The units of actual yield are usually kg/100 kg; alternative terms include % yield or yield efficiency expressed as kg/100 kg milk + starter culture. Where the starter culture used is direct-vat starter,

Table 10.2 Typical mass balance for a full-fat Cheddar cheese

Inputs	(kg)	Outputs	(kg)
Pasteurized milk	454.8	Cheese	46.98
Starter	6.37	Bulk whey[a]	409.86
Rennet solution	1.00	White whey[b]	5.97
Salt	1.44	Fat in cheese	14.55
Fat in cheese milk + starter	16.41	Fat in bulk whey	1.71
Protein in cheese milk + starter	16.46	Fat in white whey	0.14
		Protein in cheese	12.46
		Protein in bulk whey	3.92
		Protein in white whey	0.06
Total weight of inputs	463.61	Total weight of outputs	462.81
Weight of fat + protein	32.87	Weight of fat + protein	32.84

Compiled using data from Fenelon and Guinee (1999)
[a]Bulk whey corresponds to whey removed at whey drainage and during cheddaring
[b]White whey is whey expressed from the curd during salting and pressing

DVS (see Chap. 6), the inoculum is very small ≪0.1 % and its contribution is negligible; hence, the term 'weight of milk + starter' in Eq. (10.1) can replaced by 'weight of milk'. In contrast, bulk starter culture (see Chap. 6) can make a significant contribution to yield because of relatively high level of inoculation (e.g. 0.5–1.5 %), especially if the growth medium is sterilized reconstituted skim milk powder. Once the actual yield is determined, related yields including Yama, Yamafpam and Yamacfam can be calculated from Ya using the equations in Table 10.1, to eliminate the effects of differences in milk composition and cheese moisture (cf. Sect. 10.2).

Undertaking mass balance in commercial cheesemaking operations is more difficult, especially for individual vats, because of the multibatch-continuous nature of the process, which frequently involves overlapping of curds from two or more vats in the particular curd-handing system used (e.g., Alfamatic, Casomatic). A modern manufacturing line for hard/semi-hard rennet-curd cheese in a medium to large factory processing 1.0–1.5 million L milk/day typically comprises a number of cheese vats (e.g., 10) in which the curd is manufactured and the curd/whey mixture is pumped onto continuous curd-handling systems where operations such as whey drainage, acidification, pre-pressing, milling and/or moulding take place (Bennett and Johnston 2004). Hence, at the industrial level, a mass balance tends to be performed on a day's production; quantities of milk, starter and whey are usually measured using on-line flow meters or load cells.

10.3.2 Recoveries and Losses of Milk Fat, Protein and Moisture During Cheese Manufacture

Commercially, the percentage recovery, or alternatively the percentage loss, of fat, protein (or casein) or milk solids are frequently used as indices of cheesemaking efficiency, with higher recoveries and lower losses reflecting more efficient

operations. Hence, the recoveries of milk fat, protein and/or casein are used as indirect measures of yield. Alternatively, the levels of fat and curd fines in the whey, which represent un-recovered milk solids, may also be used as an index of cheesemaking efficiency and provide indirect information on cheese yield. Moreover, information on the component losses at different points of the cheesemaking process is useful in diagnosing the cause(s) for the high losses. Hence, high fat loss to cheese whey after cutting may be indicative of incorrect gel rigidity (too low or too high) at cutting, poor knife design or inadequate knife sharpness. Similarly, a large increase in fat loss during the pumping of the curd-whey mixture onto the dewheying belts (in Cheddar) or drainage tables (e.g., in Emmental) may reflect too high a flow-rate of the curd-whey mixture through the curd pump and shearing/squeezing of the curd particles in the pump chamber or excessive back pressure and constriction on the curd-whey mixture due to poor pipeline geometry (incorrect length, bore diameter, configuration). Likewise, periodic checks on the extent of fat losses at different stages of curd handling gives information on any changes in their efficiency.

Calculation of the recovery of a particular component (e.g., fat, protein, moisture) during cheesemaking requires measurement of the weights of inputs and outputs and the concentrations of the component in these streams. The percentage recovery of the component, e.g., fat from milk to cheese, may be then calculated by expressing the weight of fat in cheese (b) as a percentage of the weight of fat in milk (a), i.e.:

$$\%\,\text{fat recovered in cheese} = (b / a) \times 100.$$

Similarly, the recovery of protein and moisture may be calculated using corresponding equations. Alternatively, the percentage of a component lost to whey may be calculated as an indirect measure of efficiency, a lower loss reflecting a more efficient cheesemaking operation.

The percentage of a component lost, e.g., fat, may be calculated by expressing the weight of fat in whey (c) as a percentage of the weight of fat in milk (a), i.e.:

$$\%\,\text{fat lost in whey} = (c / a) \times 100.$$

The recoveries of fat and protein for a particular variety of cheese are influenced by many factors, as described in Sect. 10.5. Typically, ~24–27 % of total nitrogen is lost during cheese manufacture, ~71, 14 and 14 % of which is whey proteins, casein and NPN (expressed as protein), respectively. Loss of casein (~5 % of total casein) arises mainly from the caseinomacropeptide which is soluble in whey following its release from the κ-casein by rennet. The percentage of milk fat lost to whey varies from ~9 % to 20 %, most of which is lost from the surfaces of the curd particles during the cutting and early stages of stirring in the cheese vat. However, fat loss, or fat recovery, is very dependent on the cheese variety being manufactured (which determines the number and types of operations where fat may be lost), the type of equipment/technology, and the operational protocols. In modern commercial Cheddar cheese manufacture, the loss of fat to cheese whey ranges from ~10.5 % to 16.2 % of the total fat in the milk, with 51–73 % of the loss occurring in the cheese vat, 8–21 % during curd-whey pumping, and ~22 % during salting/pressing on

cheddaring belts/tower and in the block former (Guinee et al. 2005). Experimental studies on Cheddar cheese have reported mean fat losses of ~9.8 % of total milk fat, with ~89 % of this being lost in the sweet whey (combined wheys expressed in the cheese vat and during cheddaring) and ~11 % in the white whey (combined wheys expressed during salting and pressing) (Table 10.3). Corresponding values for protein indicate that ~24 % of total milk protein (true protein + non-protein N) is lost to the cheese whey, with ~98 % of this being lost in the sweet whey (Table 10.3).

Reported values for the percentages of total fat and protein lost during commercial cheese manufacture are 11.3 and 23.2 % for Edam, and 19.9 and 24.1 %, for Emmental cheese (Antila et al. 1982). In batch pilot-scale manufacture of low-moisture Mozzarella cheese curd (LMMC), a higher percentage of fat is lost than for Cheddar (i.e., ~20 % vs. 8.5 %) due to high losses during kneading and stretching of the curd in hot (~80 °C) water; ~60 % of the total fat lost during the manufacture of LMMC occurs in the stretch water (Guinee et al. 2000).

The recovery of moisture from milk to cheese varies from ~3.5 % to 8.5 % of total (in the milk), being higher for higher moisture cheeses. Its exact value depends on milk composition, cheese variety and cheesemaking process (which determine the level of syneresis). Moisture recovery is seldom, if ever, used as an index of

Table 10.3 Effect of milk protein content on the composition of cheese wheys, and the losses of fat and protein in cheese whey during the experimental manufacture of Cheddar cheese

	CA	UF	UF
	3.3	3.6	4.0
Composition			
Bulk whey			
Fat (%, w/w)	0.35	0.33	0.38
Protein (%, w/w)	0.87	0.94	1.03
Curd fines (mg/kg)	159	210	267
White whey			
Fat (%, w/w)	3.08	2.88	2.98
Protein (%, w/w)	1.02	1.04	1.13
Curd fines (mg/kg)	1229	1173	1412
Losses of fat and protein			
Bulk whey			
Weight (kg/100 kg cheese milk)	90.0	89.0	88.2
Fat (% of total milk fat)	9.3	7.8	8.2
Protein (% of milk protein)	23.7	23.2	22.9
White whey			
Weight (kg/100 kg cheese milk)	1.29	1.33	1.33
Fat (%, w/w)	1.14	1.01	1.06
Protein (%, w/w)	0.40	0.38	0.39

Milk protein content was increased from 3.3 % (Control, CA) to 3.6 % (UF 3.6) or 4.0 % (UF 4.0) using ultrafiltration. From Guinee et al. (2006)

efficiency, probably because water, which has to be removed during cheesemaking, is rarely considered as a milk component owing to the fact that it is not included in milk pricing. Nevertheless, moisture forms a significant proportion of the mass of all rennet-curd cheeses (from ~32 % in mature Parmesan to ~57 % in Feta) and acid-curd cheeses (~82–85 % in Quark and Fromage *frais* to ~55 % in double cream cheese). While the maximum moisture content for individual cheese varieties is specified by legislation (e.g. ≤39 % for Cheddar) (cf. Chap. 21), variants (brands) within a variety are produced with different target moisture contents to ensure optimum quality, e.g., 34 to 37 % in vintage Cheddar cheese which is matured for a relatively long period to develop a strong flavour) (Guinee et al. 2008) compared to 38.5 % in mild-flavoured Cheddar which is matured for a short period (e.g., ~3–6 months) and is less likely to develop off-flavours. Instead of % moisture recovery, the attainment of the target moisture content in the cheese is considered as an index of cheesemaking efficiency, and target moisture content is almost always included as a factor in prediction equations of cheese yield (cf., Sect. 10.4). These equations are used daily in commercial manufacture to compare actual yield against predicted yield based on milk composition and the specific cheese variant. A lower than expected yield, which is undesirable in terms of operating profitability, could ensue from a number of factors, including an actual moisture content in the cheese that is lower than the target moisture content.

10.3.3 Loss of Curd Fines in Cheese Whey

A further measurement which is made routinely as an index of cheesemaking efficiency is the level of curd fines in the cheese whey. Curd fines refer to very small fragments of curd which are broken off the surface of curd particles (mainly during cutting and/or the initial phases of stirring) and which are lost in the whey as sediment or curd 'dust'. They occur as a result of a number of factors, including:

- shattering of curd particles that are too large (e.g. >1.5 cm in its largest dimension) into smaller particles with uneven surfaces with protrusions that are subsequently broken off by the frictional forces inflicted on the curd particles during the stirring of the curd-whey mixture;
- the presence of jagged-shaped curd particles resulting from poor knife design/cutting action;
- excessive gel rigidity at cutting resulting in tearing (rather than cutting) of the gel or a poor gel cutting programme/cycle.

Curd fines are measured by subjecting a sample of whey to centrifugation and filtration under defined conditions, followed by drying and weighing of the residue (Fenelon and Guinee 1999).

While the level of curd fines in whey can vary significantly, typical values reported for sweet whey from Cheddar cheese are ~250–350 mg/L; sweet whey refers to whey expressed in the cheese vat and during cheddaring (see Chap. 8).

However, in modern Cheddar manufacturing facilities, curd fines are recovered from the whey by clarification (centrifugation) and incorporated into the curd mass prior to cheddaring.

10.4 Prediction of Cheese Yield

Predictive yield equations (formulae) are used to estimate the yield of cheese from milk of a given composition. Prediction of cheese yield is useful from several perspectives:

- it allows a cheese plant to measure its efficiency by comparing actual and predicted yields;
- it facilitates production planning (i.e., capacity and technology) and inventory control;
- it assists with the optimization of product mix and milk pricing schemes.

The generation of prediction equations requires consideration of milk composition (fat and casein), cheese composition (moisture and cheese solids non-fat non-protein), component recoveries during cheesemaking (fat and protein), and the cheesemaking process in relation to its influence on the partition of the various components of milk (e.g., loss of glycomacropeptide, milk salts, fat) between the cheese curd and whey. Ideally, only historical plant data on fat recovery that simulate the current process most closely in terms of plant type (e.g., type of vats, knives, curd pumps, curd handling equipment) and manufacturing conditions (e.g., protein-to-fat ratio of milk, pasteurization temperature, gel firmness at cutting, pH at salting) should be used. Similarly, when the plant is upgraded to improve fat recovery, the fat recovery data obtained during vat commissioning and validation should be used until a new database is established.

Predictive yield formulae and their application to different types of cheese, especially Cheddar, were reviewed extensively by the IDF (1991, 1994). They may be classified into two different types based on either 'contents of fat and casein in milk' or 'percentage recovery of fat and casein in milk, and the moisture content of cheese'.

The generation of prediction equations requires consideration of milk composition (fat and casein), cheese composition (moisture, cheese solids non-fat non-protein) and the recovery of fat and protein. Compositionally, cheese is comprised of three major components, namely fat, protein and moisture, which are recovered from the cheese milk. These typically account for ~92–96 % of the total cheese weight, depending on the variety and its composition (cf. Table 9.1, Chap. 9). The minor components that make up the remaining 4–8 % of the cheese constitute cheese solids other than fat and protein (cheese solids non-fat non-protein; CSNFNP). These include:

- insoluble minerals (calcium and phosphate) associated with the *para*-casein,
- solids soluble in the moisture phase, including lactose (which is mainly converted to lactic acid), whey proteins, sodium chloride and soluble calcium and phosphate.

The level of CSNFNP increases as the moisture content of the cheese increases, and vice versa. Hence, the CSNFNP as a percentage of total cheese solids were found to be ~16.5 % for reduced-fat (7 % fat) Cheddar compared to ~9.5 % for full-fat (32 % fat) Cheddar, because of the higher moisture content of the former (~46.0 % vs. 37.5 %) (Fenelon and Guinee 1999). The contribution of CSNFNP to cheese yield (as a % of total cheese yield) was 5.3 for full-fat Cheddar and 7.6 % for reduced-fat Cheddar.

10.4.1 Predictive Yield Formulae Based on Fat and Casein in Milk

These are of the following general type : $Y = aF + bC$ or $Y = aF + bC + k$, where: Y = yield; F and C are the fat and caseins contents of the milk (with or without added starter culture as applicable; cf. Sect. 10.3.1), respectively; k is a constant, the magnitude of which depends on the loss of casein and the level of cheese solids non-fat-non-casein (CSNFNC) in the cheese; a and b are coefficients, the magnitude of which depends on the contributions of fat and casein to yield. The values of a and b have been found to range from ~1.47 to 1.6 and 1.44 to 1.9, respectively, for Cheddar cheese (IDF 1991). The data of Fenelon and Guinee (1999) showed that actual (Ya) and Yma of Cheddar cheeses with a fat content varying from 7 % to 32 % were accurately described by the following equation: Ya Cheddar = 1.56 F + 1.71 C. While the above formulae ($Y = aF + bC$ and $Y = aF + bC + k$) are empirical, they indicate that, in general, casein contributes more than its own weight to cheese yield and also contributes more than fat to Cheddar cheese yield. The relatively high contribution of casein is expected as it forms the continuous *para*-casein network, which, acting like a sponge, occludes the fat and moisture (serum) phases. Occluded moisture contributes directly to cheese yield and indirectly due to the presence of dissolved solids, which include whey proteins, κ-casein glycomacropeptide, lactate and soluble salts.

While fat on its own has little water-holding capacity, its presence in the *para*-casein network affects the degree of matrix contraction and hence moisture content and cheese yield. The occluded fat globules physically limit contraction, and hence aggregation, of the surrounding *para*-casein network and therefore reduce the extent of syneresis. Hence, as the fat content of the curd is increased, it becomes more difficult to expel moisture; consequently, the moisture-to-casein ratio generally increases unless the cheesemaking process is modified to enhance casein aggregation, e.g., by increasing the scald temperature (Gilles and Lawrence 1985; Fenelon and Guinee 1999). Owing to its negative effect on syneresis, fat contributes indirectly more than its own weight to cheese yield, i.e., Cheddar cheese yield increases by ~1.16 kg/kg milk fat (Fenelon and Guinee 1999). This greater than *pro-rata* increase is due to the increase in the level of moisture in non-fat substances (MNFS) as the content of fat in the cheese increases. However, if the content of MNFS is maintained constant (e.g., by process modifications), fat contributes less than its own weight to cheese yield (i.e., ~0.9 kg/kg), due to the fact that ~8–10 % of the milk fat is normally lost in the whey.

10.4.2 Predictive Yield Formulae Based on Percentage Recovery of Fat and Casein in Milk to Cheese, and Cheese Moisture

Probably, the simplest and most widely applied formula of this type is the van Slyke equation [Prediction Equation (10.1), Table 10.4], which was developed for Cheddar cheese in 1936. In this equation, the factor 0.93 refers to the fraction of fat recovered from milk to cheese. The value of '−0.1' is included to account for the % of casein lost to cheese whey in the form of the soluble caseinomacropeptide following rennet hydrolysis; it assumes a typical milk casein content of 2.5 % and a casein loss of 4 % of total (Thomä et al. 2006; Wang et al. 2009). The factor 1.09, which may be rewritten as '1 + 0.09', is included to account for CSNFNP which comprise ~9 % of the total solids of Cheddar cheese; CSNFNP include lactose, sodium chloride and indigenous milk salts, especially Ca and PO_4. Hence, the van Slyke equation may be rewritten more precisely in a modified form as Prediction Equation (10.2) (Table 10.4) to account for the actual values for percentage recovery from milk to cheese, casein lost to cheese whey, and cheese solids non-fat non-protein.

There is considerable inter- and intra-variety variation in the reported values of %FR/100 and the coefficient *b*: e.g., 76–80 % and 0.065–0.071 for LMMC (Guinee et al. 2000) compared to 88–91 % and 0.08–0.10 for Cheddar (Fenelon and Guinee 1999; Guinee et al. 2006, 2007a, b), respectively. The lower values of %FR/100 and *b* for LMMC compared to Cheddar reflect the kneading and stretching (plasticization) of the LMMC curd in hot water at ~80 °C, which has the effect of generating free fat, and washing out both free fat and soluble solids (which are lost in the stretch water). For commercial Finnish Edam and Emmental cheeses, the mean value of %FR/100 was 88.7–90 and 88.1–8.5, respectively (Antila et al. 1982). Variations reflect differences in milk composition (levels of casein and fat, protein-to-fat ratio, extent of whey protein denaturation and its complexation with the casein), milk quality [somatic cell count] and storage conditions, milk heat treatment, cheesemaking conditions and cheesemaking technology (cf. Sect. 10.5; Guinee and O'Brien 2010a, b). Variations in the coefficients also occur between cheese plants manufacturing similar variants of a given variety due to the above factors, which cause inter-plant differences in efficiencies.

10.4.3 Predictive Yield Formulae for Including Contribution of Denatured Whey Protein to Cheese Yield

In most rennet-curd varieties, casein is essentially the only protein which contributes to yield. At the pasteurization treatment (~72 °C for 15 s) used for rennet-curd cheeses, whey protein denaturation is low (i.e., ~5 % total) and its contribution to cheese yield is small, e.g., typically 0.07 and 0.12 kg/100 cheese milk for full- and

half-fat Cheddar, respectively, in milk with a protein content of ~3.3 % (Lau et al. 1990; Fenelon and Guinee 1999). Denatured whey proteins complex with the κ-CN (van Hooydonk et al. 1987; Jelen and Rattray 1995) and partition with the insoluble *para*-casein after rennet treatment. In contrast, native whey proteins are soluble under cheesemaking conditions (mostly, <50 °C for <2 h) and are largely lost in the cheese whey. Native whey proteins dissolved in the cheese moisture (~4 % total) contribute very little to yield, ~0.026 kg/100 kg milk. However, whey proteins contribute more to yield when milk is subjected to a high heat treatment (e.g., 90 °C × 5 min), as in acid-heat coagulated varieties (e.g., Paneer and Ricotta), or where the cheese is prepared from high concentration factor (e.g., >6×) ultrafiltered milk retentate, referred to as pre-cheese. The effect of high heat treatment on cheese yield is discussed in Sect. 10.4.4.

Prediction Equation (10.3) (Table 10.4) is a modified version of the van Slyke equation which accounts for the contribution of whey proteins denatured during pasteurization of the milk for cheese manufacture. Using the modified van Slyke equation (#3, Table 10.4), Fenelon and Guinee (1999) reported a much closer fit between actual yield and predicted yield for Cheddar cheeses made from milk with fat level ranging from ~0.5 % to 3.3 % (w/w), than between actual yield and predicted yield, calculated using the unmodified van Slyke formula (Eq. (10.1), Table 10.4).

10.4.4 Plant-Specific Predictive Formulae

The application of a generic predictive cheese yield formula for a given cheese variety may not be very accurate for predicting cheese yield in all plants. There may be several reasons for this:

- differences in plant type and design, which affect factors such as % fat recovery
- differences in plant throughput (e.g., higher than recommended milk throughput to cope with very large volumes of milk may lead to higher losses of fat and curd fines)
- differences in variety variants being manufactured at different sites within a company, which may necessitate differences in composition (e.g., protein-to-fat ratio, MNFS) which can influence factors such as % of fat lost or the level of CSNFNP
- differences in milk quality (e.g., level of free fat, somatic cell count, protein content), which could influence % FR, casein loss and cheese moisture.

As an alternative to a generic predictive formula, a plant-specific formula may be developed for each factory (Barbano and Sherbon 1984; Guinee et al. 2005). A plant-specific yield formula may be developed by statistical analysis of historical data, e.g., weekly or monthly, data accumulated on milk composition, milk quality, e.g., somatic cell count, fat and protein recoveries, and cheese moisture.

Table 10.4 Predictive cheese yield equations (formulae)

Abbreviation	Definition	Units	Prediction equation
Yp	Predicted yield	kg cheese/100 kg milk	**Prediction Equation (10.1) (van Slyke equation)** $$Yp = \left[\frac{(F_{cm} \times 0.93) + C_{cm} - 0.1}{1 - (\frac{Mt}{100})}\right] \times 1.09,$$ where, F_{cm} and C_{cm} refer to the respective levels (%, w/w) of fat and casein in the cheese milk, 0.93 is the assumed proportion of fat recovered from milk to cheese, 0.1 is the assumed quantity (%, w/w) of casein lost in the cheese whey, 1.09 is an assumed factor to account for the recovery of milk solids other than fat or protein (e.g., lactate, soluble salts) and Mt is the target moisture content of the cheese (%, w/w). Cheese milk refers to milk that has been standardized to the required protein-to-fat or casein-to-fat ratio for the variety of cheese being manufactured and pasteurized.
Yp	Predicted yield	kg cheese/100 kg milk	**Prediction Equation (10.2) (van Slyke Equation elaborated to account for actual fat recovery, casein loss and cheese solids non-fat non-protein)** (Fenelon and Guinee 1999). $$Yp = \left[\frac{\left(F_{cm} \times \frac{\%FR}{100}\right) + C_{cm} - a}{1 - \left(\frac{Mt}{100}\right)}\right] \times (1.0 + b),$$ where, F_{cm} = fat in the cheese milk (%, w/w), C_{cm} = casein in the cheese milk (%, w/w), % FR = percentage of fat recovered from milk to cheese, a = coefficient for level (%, w/w) of casein lost in the whey as caseinomacropeptide (equivalent to ~4% of total casein), b = cheese solids non-fat non-protein (CSNFNP) as a fraction of the dry matter content of the cheese, and Mt = target moisture content of the cheese (%, w/w).

Yp	Predicted yield	kg cheese/100 kg milk	**Prediction Equation (10.3) (Modified van Slyke Equation to account for contribution of denatured whey proteins to cheese yield** (Fenelon and Guinee 1999). $$Yp = \left[\frac{\left(F_{cm} \times \frac{\%FR}{100}\right) + C_{cm} - a + \left(WP_{rm} \times \frac{\%WPD_{cm}}{100}\right)}{1 - \left(\frac{Mt}{100}\right)}\right] \times (1.0 + CSNFNP),$$ where F_{cm}, $\%\ FR$, C_{cm}, a, CSNFNP and Mt are as in Prediction Equation (10.2) above; WP_{rm} = whey protein in raw (unpasteurised) milk (%, w/w), and $\%WPD_{cm}$ = denatured whey protein in the cheese milk as a percentage of total whey protein in raw milk. Raw milk refers to the milk prior to standardization and pasteurization, and cheese milk to the milk that has been treated as described in Prediction Equation (10.1) above. The values of WP_{rm} and $\%WPD_{cm}$ are obtained by measurement of total protein, casein number, CN (casein as % of total protein in milk) and non-protein nitrogen (as % of total N) in the raw milk and cheese milk. Using these measurements, the WP_{rm} and $\%WPD_{cm}$ are calculated according to Guinee et al. (1997): $$WP_{rm} = \text{Protein in raw milk} \times (100 - CN_{rm} - NPN_{rm}) / 100$$ $$WP_{cm} = \text{Protein in cheese milk} \times (100 - CN_{cm} - NPN_{cm}) / 100$$ $$\%WPD_{cm} = 100 \times (WP_{rm} - WP_{cm}) / WP_{rm}$$

A plant-specific formula tends to give very accurate prediction of cheese yield because it reflects, more than a generic prediction formula, the composition and quality of the milk, the technology used within a plant (e.g., vat type + knife deign, type of curd pumps), the actual cheesemaking conditions used in the plant, and the variant of cheese made. Depending on the number of cheese types and variants of a particular cheese type (e.g., mild or mature Cheddar), a cheese plant may have a number of specific prediction formulae.

10.4.5 Percentage Yield Efficiency

The comparison of actual (Ya) and predicted (Yp) yield of cheese from a given weight of milk may be expressed as % yield efficiency using the following formula:

$$\%YieldEfficiency = 100 \times \left(\frac{Y_a}{Y_p} \right).$$

Daily monitoring of this parameter enables the plant manager to gauge performance of the cheese plant over time and compare it with the performance of other plants (at different locations/sites) within the same company. Such information may be used to plot 'efficiency lines' (% efficiency versus day) for individual production sites across the company (Fig. 10.1). This type of construct, which is frequently displayed within plants, may have several benefits:

- allows any seasonal patterns in efficiency within individual plants to be observed (this could be related to changes in milk composition and quality between different catchment regions)
- allows the effects of differences in manufacturing technology (e.g., type of vat, curd handling equipment) and manufacturing protocol (e.g., culture form: DVS or bulk; standardisation approach) to be observed;
- allows the effects of equipment or process improvements at individual sites to be readily established;
- increases awareness of manufacturing personnel of performance and the factors that affect it.

If the *% Yield efficiency* is significantly less than 100, remedial action should be taken to redress process inefficiencies, which may occur for a number of reasons, e.g., high fat losses due to cutting the gel when it is too firm or too soft, blunt knives, or poor cut programme; high losses of curd fines; or curd losses to floor. A plant-specific yield formula should be updated regularly, e.g., annually, to reflect improvements in milk quality and cheesemaking technology, and factors which influence cheesemaking efficiency (see Sect. 10.5).

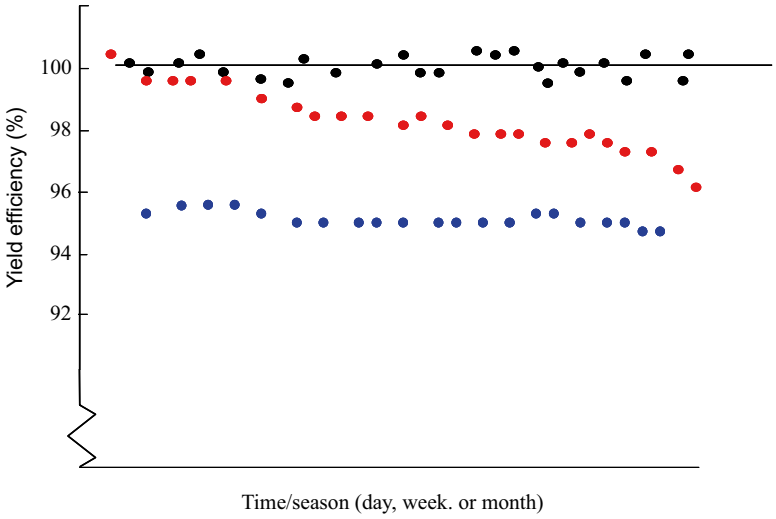


Fig. 10.1 Representation of an 100 % yield efficiency (YE) line (*solid line*) for a cheese manufacturing company and actual efficiency lines for three different manufacturing sites within the company over time (*black circle*, *red circle*, *blue circle*) where YE = (100 × actual yield)/predicted yield. The representation indicates that yield efficiency may vary with production site (high YE, *black circle*; low YE, *blue circle*) or show a seasonal trend (e.g., progressively downwards, *red circle*). Reasons for such variation and trends may include manufacturing technology, changes in milk quality over time, standard operating procedures and variant of cheese being manufactured

10.5 Factors That Affect Cheese Yield

The principal factors which influence cheese yield are discussed below.

10.5.1 Fat and Casein Levels in Milk

The single most important factor affecting cheese yield is the composition of the milk, particularly the concentrations of fat and casein, which, together, represent ~94 % of the dry matter of Cheddar cheese. This can be observed from predictive cheese yield formulae (Sects. 10.4.1 and 10.4.2), which can be generically written as: $Y = aF + bC$, where Y, F and C refer to yield, fat and casein, and *a* and *b* are coefficients. The fact that generally $b > a$ indicates that the contribution of casein exceeds that of fat, for reasons discussed in Sect. 10.4.1. Generally, cheese yield increases linearly with concentrations of fat and casein in bovine milk, with the degree of increase with casein being higher than that with fat (Fig. 10.2). The latter are influenced by many factors including breed, stage of lactation, plane of nutrition, animal health, lactation number and milking frequency (cf. Chap. 4; Guinee and O'Brien 2010b).

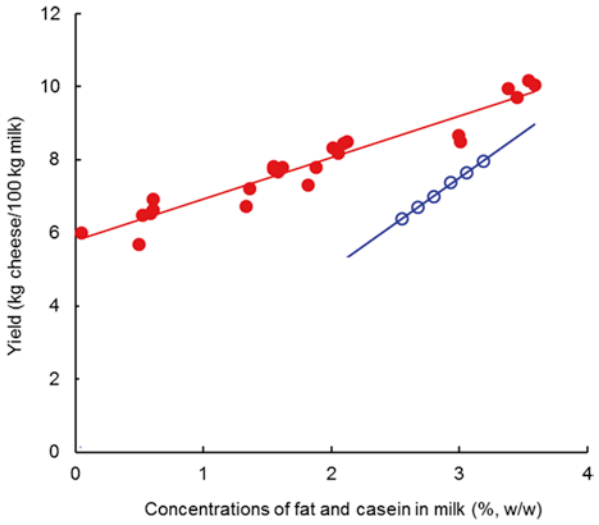


Fig. 10.2 Effect of concentrations of milk fat (red *filled circle*) and casein (*blue open circle*) on the yield of Cheddar cheese. Drawn using data for fat levels from Fenelon and Guinee (1999), and data for casein levels from Gilles and Lawrence (1985), respectively

10.5.2 Protein-to-Fat Ratio in Milk

The protein-to-fat ratio (PFR) in cheese milk is increased typically by reducing the fat content through standardization, while maintaining protein content constant through low concentration factor ultrafiltration or microfiltration. A recent study (Guinee et al. 2007b) showed that Cheddar cheese yield decreased by ~0.45 kg/100 kg cheese milk for every 0.1 increase in PFR, due mainly to the concomitant decrease in fat content of the cheese, i.e., ~0.2 % reduction in fat per 0.1 increase in PFR (Fig. 10.3). Nevertheless, the recovery of fat from milk to cheese increases with PFR while the recovery of water decreases (Fig. 10.3). Considering the casein network of the gel as a framework within which the fat globules are held, the increased fat recovery is attributable to amplification of the ability of the milk gel to retain fat on cutting and subsequent stirring of the curd (gel) particles in whey as the ratio of protein-to-fat is increased. The reduction in moisture recovery coincides with the reduction in the fat content and, hence, a greater shrinkage of the casein network and associated syneresis of the curd (cf. Sect. 10.4.1). Hence, as the PFR of the milk is increased, the MNFS content of the resultant cheese decreases.

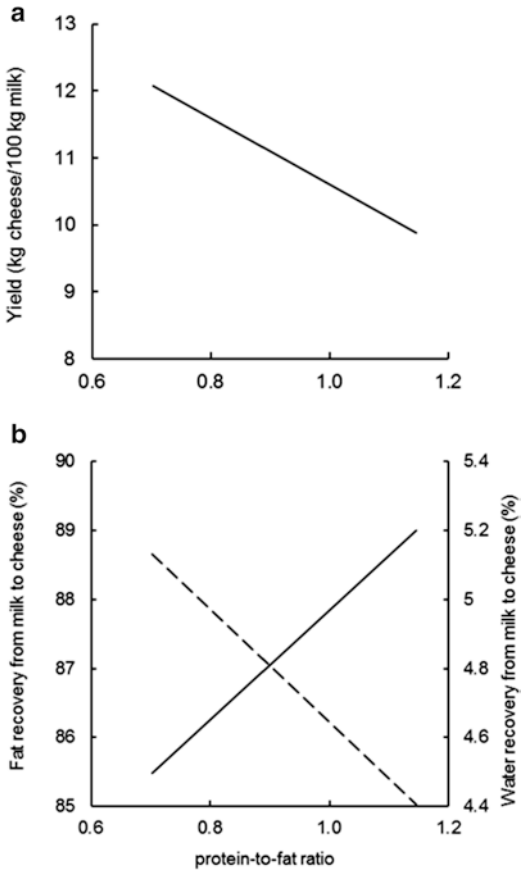


Fig. 10.3 Effect of protein-to-fat ratio in milk on: (**a**) the yield of Cheddar cheese and (**b**) the recoveries of fat (*solid line*) and water (*broken line*) from milk to cheese. Drawn from data of Guinee et al. (2007b)

10.5.3 Species and Breed

The percentage of total milk production obtained from cattle,, water buffalo, goats and sheep is ~83.0, 13.0, 2.2 and 1.3, respectively (IDF 2009; Gall 2013), the remaining milk is produced by camel, reindeer, horse and donkey. Cows' milk is the principal milk used for cheese manufacture but significant quantities of cheese are also made from goat, sheep or water buffalo milk in some EU countries such as France, Greece, Italy and Spain (IDF 2009).

The species of animal has a major influence on the concentrations of fat and casein and proportions of different caseins (Table 10.5). The predicted yield of cheese from standardized bovine milk [calculated using Prediction Equation (10.1), Table 10.4] is lower than that from standardized sheep or water buffalo milk but higher than that from goats' milk.

The breed of cow has a marked influence on milk composition and its cheese-yielding capacity, with breeds yielding higher protein and fat levels giving higher

Table 10.5 Approximate composition and predicted yield of Cheddar cheese from standardized milk from different species

Species	Composition (g/100 g)				Cheese yield (kg/100 kg milk)
	Fat_{rm}	Fat_{sm}	Protein	Lactose	
Sheep	6.5	6.2	5.7	4.4	14.3
Water buffalo	7.5	4.3	4.0	4.8	10.0
Cow	4.0	4.0	3.7	4.6	9.3
Goat	4.5	3.2	2.9	4.3	7.3

Data based on Park and Haenlein (2013)
Abbreviations: Fat_{rm} in raw milk; fat_{sm} in milk standardized to protein-to-fat ratio of 0.92. The composition of most cheeses complies with values of fat and moisture as specified in national legislation (cf., Chap. 22). This necessitates standardization of the ratio of protein-to-fat, or casein-to-fat, to a defined value for each variety according to its compositional standards; standardization is typically achieved by removal of the desired weight of fat from the milk or by the addition of skim milk to the milk. The yield was calculated according to Prediction Equation (10.2) (Table 10.2), where fat recovery was set at 90 %, casein in milk at 78 % of protein, coefficient *a* set at 4 % of casein, coefficient *b* at 0.09, and cheese moisture (Mt) at 37 % (w/w)

cheese yields. Assuming a casein number of 78 and standardization to a protein-to-fat ratio of 0.92, the estimated yield of Cheddar cheese from Jersey cow milk, containing 4.8 % fat and 3.8 % protein, is 11.8 kg/100 kg milk compared to 9.6 kg/100 kg for Holstein milk containing 3.8 % fat and 3.1 % protein.

10.5.4 Somatic Cell Count and Mastitis

The influence of somatic cells and mastitis on the composition of milk and its suitability for cheese manufacture has been studied extensively. There are three main types of somatic cells: lymphocytes (L), phagocytes and mammary gland epithelial cells (E) (Burvenich et al. 1995; Kelly et al. 2011). Lymphocytes function in humoral and cell-mediated immunity while phagocytes, of which there are two types, polymorphonuclear leucocytes (PMN) and macrophages (M_ϕ), ingest and kill pathogenic microorganisms which invade the mammary gland. Low numbers (e.g., <100,000/ml) of somatic cells are present in normal milk from healthy animals during mid lactation, with M_ϕ, L, PMN and E cells typically at a ratio of ~2.1:1.0:0.4:0.2. Somatic cells are released from the blood to combat udder infection (e.g., by *Staphylococcus aureus*) and thereby prevent or reduce inflammation (mastitis). Factors that contribute to increases in the somatic cell count (SCC) of bulk manufacturing milk include sub-clinical mastitis, advanced stage of lactation, lactation number, stress and poor nutrition (O'Brien and Guinee 2011; Kelly et al. 2011). The effects of increases in SCC on milk composition and various aspects of cheese manufacture have been studied extensively (Barbano et al. 1991; Auldist et al. 1996; Auldist and Hubble 1998; Klei et al. 1998; Politis and Ng-Kwai-Hang

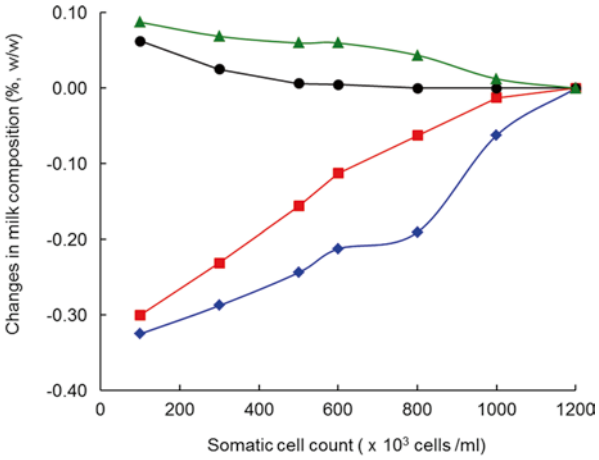


Fig. 10.4 Changes in the concentrations of fat (*black circle*), total protein (*red square*), casein (green *triangle*) and whey protein (*blue diamond*) in cows' milk on increasing somatic cell count. Drawn from data of Politis and Ng-Kwai-Hang (1988b)

1988a, b, c; Pirisi et al. 2000; Leitner et al. 2003; Somers et al. 2003; Kelly et al. 2006; O'Brien et al. 2006; Mazal et al. 2007; Rodríguez-Nogales et al. 2007; Auldist 2011). Increasing SCC in milk is generally associated with:

- higher levels of total protein, whey proteins (especially immunoglobulin) and non-protein nitrogen (NPN), and lower levels of true protein, casein and lactose (Fig. 10.4);
- elevated proteolytic activity associated higher activities of plasmin, cathepsin D and cysteine protease activities (Ismail and Nielsen 2010);
- reduction in the levels of intact β- and αs_1-caseins, and concomitant increases in the levels of serum-soluble peptides derived from these caseins, γ-caseins, λ-caseins, proteose peptones and other peptides
- poorer rennet coagulation properties, as manifested by longer gelation times and lower curd firming rates;
- higher levels of fat and protein in whey, reduced recovery of milk fat and protein in cheese
- lower moisture-adjusted cheese yield and percentage yield efficiency (Politis and Ng-Kwai-Hang 1988a, b)
- higher levels of cheese moisture, accelerated levels of cheese proteolysis (as measured by pH 4.6-soluble N and 12 % TCA-soluble N)

Politis and Ng-Kwai-Hang (1988a, b) reported that increasing SCC incrementally from 10^5 to $>10^6$ cells/ml resulted in linear increases in the percentages of milk fat (1 %) and protein (3 %) lost in whey, and reductions in Yma (1.0 kg/100 kg milk) and % yield efficiency (15 %) (Fig. 10.5) for experimental Cheddar cheese. It is noteworthy that there was a relatively large reduction in Yma (i.e., ~0.4 kg/100 kg

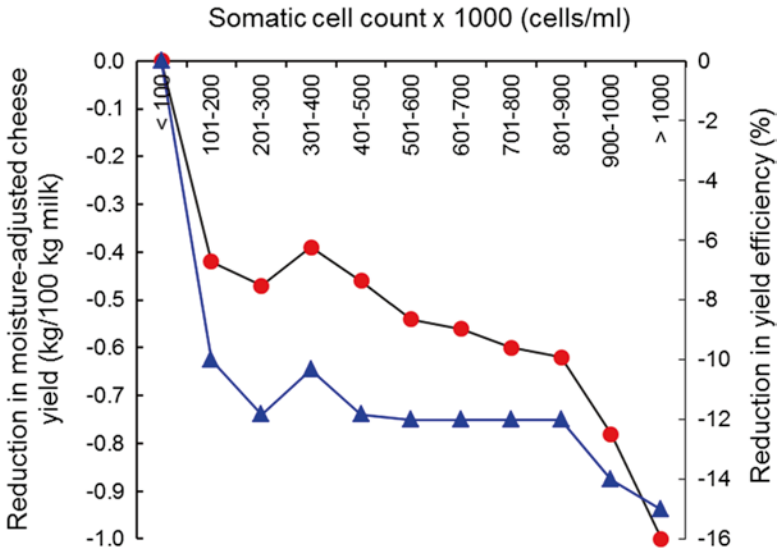


Fig. 10.5 Effect of somatic cell count (SCC) on the yield efficiency (*blue triangle*) and the yield of moisture-adjusted (to 37 %, w/w) cheese (*red circle*) from milk from individual cows. Redrawn from data of Politis and Ng-Kwai-Hang (1988b)

milk) on increasing the SCC from 100, 000–200,000 to 201,000–300,000 cells/ml, a range considered typical for bulk milk. The adverse effect of high SCC ($>5\times10^5$ cells/ml) on the rennet coagulation of milk, cheese yield and recovery of protein and fat to cheese has also been reported in other studies (Auldist and Hubble 1998; Barbano et al. 1991; Auldist et al. 1996, Klei et al. 1998; Srinivasan and Lucey 2002).

Owing to its negative impact on cheese yield and fat recovery, high SCC is detrimental to the profitability of cheesemaking. Based on the above studies, the estimated monetary loss resulting from a 2 % decrease in cheese yield on increasing the SCC from 10^5 to 5×10^5 cells/ml is ~€10,000/day in a Cheddar cheese plant processing 1million L milk/day, based on a value of €2.5/kg curd. Consequently, SCC in milk is being lowered through the use of good on-farm practices, e.g., reducing the percentage of animals with sub-clinical mastitis. The EU has set the limit of $\leq400\times10^3$ SCC/mL as the value above which milk cannot be sold by producers or used for further processing (EU 2004). The permitted SCC limit varies internationally, for example, $\leq400\times10^3$ cells/mL in New Zealand and $\leq750\times10^3$ cells/mL in the USA (Kelly et al. 2011).

In contrast to the above trends, Mazal et al. (2007) found that an increase in SCC from <200,000 to >600,000 cells/ml, while significantly reducing milk casein content and increasing cheese moisture (~2 %, w/w), did not significantly effect recoveries of milk fat or protein, or the yield of Prato cheese. Inter-study discrepancies on the effect of SCC probably arise from the confounding effects of differences in factors such as the difference in SCC between high and low SCC milks, composition of

milks, cheesemaking procedure (degree of standardization of factors such firmness of gel at cut and pH at different stages), and cheese composition (e.g., moisture content) (Guinee and O'Brien 2010a; Guinee and O'Callaghan 2010).

10.5.5 Stage of Lactation and Season

Marked changes occur in the composition of milk throughout the year, especially when milk is produced mainly from spring-calving herds fed predominantly on pasture, as in Ireland, New Zealand and parts of Australia. In addition to changes in gross composition, the relative concentrations of the individual caseins, as a percentage of total casein, show seasonal changes, especially in factory milk obtained largely from spring-calving herds (Fig. 10.6). These changes probably occur as a result of an increase in plasmin activity in milk over the course of lactation (Fig. 10.7). β- and α_{s2}-Caseins are readily cleaved by plasmin, whereas κ- and α_{s1}-are more resistant (Kelly et al. 2006).

These changes affect rennet coagulation properties, cheese composition, recovery of fat and casein, cheese yield and quality (Auldist et al. 1998; O'Brien et al. 2006; Guinee et al. 2007a, b). An Irish study (O'Brien et al. 1999) showed that milk protein increased from ~3.0 % to 3.7 %, casein from 2.3 % to ~2.8 %, and fat from ~3.3 % to 3.8 % between March and October. The yield of Cheddar cheese from the October milks, calculated using Prediction Equation (10.1) (Table 10.4) were ~9.0

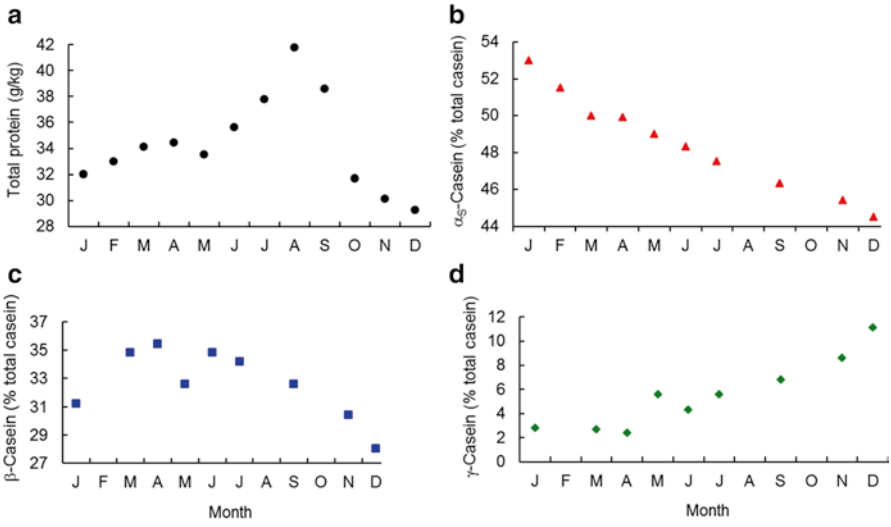


Fig. 10.6 Seasonal changes in the level of total protein in winter-spring-calving herd milk (**a**) and in the proportions of α_s-casein (**b**), β-casein (**c**) and γ-casein (**d**) in Irish bulk creamery milk, mainly from winter-spring-calving herds (**b**–**d**). Drawn using data of Donnelly and Barry (1983)

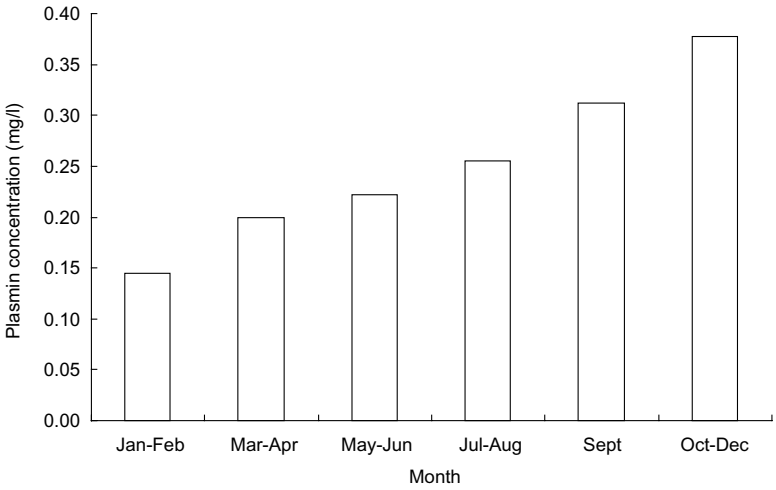


Fig. 10.7 Effect of stage of lactation on the concentration of plasmin in milk from individual cows. Drawn using data of Politis and Ng-Kwai-Hang (1988b)

to 11.2 kg/100 kg milk in March and October (assuming a protein-to-fat ratio of 0.92 in the standardized cheese milks and a fact recovery of 90 %). Similarly, the effects of seasonal variation in milk composition and quality on cheese yield and component recoveries have been demonstrated in Scotland (Banks et al. 1981), New York (Barbano and Sherbon 1984), Finland (Antila et al. 1982) and New Zealand (Kefford et al. 1995). However, the extent of seasonal variation in milk composition varies between countries owing to differences in calving pattern (e.g., spring-calving herds versus year-round calving herds), feeding regimen (e.g., pasture feeding versus in-stall feeding) and diet (supplementation/non-supplementation of pasture diets with feed concentrates) (Guinee and O'Brien 2010a).

Numerous reports have shown that the rennet coagulation and curd-syneretic properties of late lactation milk are inferior to those of mid-lactation milk. Consequently, cheese made from late lactation milk tends to have a high moisture content and to be frequently of inferior quality. Considering its high casein content, late lactation milk would be expected to have good cheesemaking properties and to give higher normalized cheese yields; however, the reverse is generally observed, possibly for a number of reasons, including high milk pH (where pH at set is not standardized), changes in the proportions of the caseins (Fig. 10.6), proteolysis by plasmin and perhaps unidentified factors. Nevertheless, seasonal changes in milk composition and its suitability for cheese manufacture can be minimized through good husbandry and milk handling practices (O'Brien et al. 2006; Guinee et al. 2007a, Guinee and O'Brien 2010a):

- maintaining a high-energy diet (e.g., dry matter intake of ≥17 kg/cow/day, with a dry matter digestibility of 820 g/kg organic matter) by good grassland management practices and concentrate supplementation of herbage-based diets, especially when grass is in short supply,

- elimination of extreme late lactation milk by drying-off cows at a suitable stage, e.g., at a milk yield ≥8 kg/cow/day,
- reducing stress and infection by good husbandry practices.

10.5.6 Genetic Polymorphism of Milk Proteins

All the major proteins in milk, i.e., α_{s1}-, α_{s2}-, β- and κ-caseins, β-lactoglobulin and α-lactalbumin, exhibit genetic polymorphism (see Chap. 4).

The genetic variants which have been investigated most thoroughly for their effects on the rennet coagulation and cheesemaking characteristics of milk are those of κ-casein and β-lactoglobulin. Compared to the AA variants, the BB genotypes of β-lactoglobulin and κ-casein are generally associated with higher concentrations of casein and superior rennet coagulation properties, as reflected by a higher gel-firming rate and gel firmness after a given renneting time. The BB variants of κ-casein and β-lactoglobulin are also associated with superior cheesemaking properties, as reflected by a higher recovery of fat, lower levels of curd fines in cheese whey, and higher actual and moisture-adjusted cheese yields for a range of varieties, including Cheddar, Svecia, Parmigiano Reggiano, Edam and Gouda and low-moisture Mozzarella (Walsh et al. 1998, Guinee and O'Brien 2010b). Reported increases in Yma with the κ-casein BB variant range from ~3 % to 8 %, depending on milk composition and cheese type. The superior rennet coagulation and cheese-yielding characteristics of κ-casein BB milk compared to the AA variant milk probably result from the higher level κ-casein as a percentage of total casein, higher overall level of casein, smaller micelles and lower negative charge. These properties are conducive to a higher degree of casein aggregation and a more compact arrangement of the sensitized *para*-casein micelles, which in turn favours more inter-micellar bonds during gel formation. Model studies on rennet coagulation have shown that for a given casein concentration, the curd firming rate of rennet-treated micelles is inversely proportional to the cube of the micelle diameter. Milk containing κ-casein AB generally exhibits rennet coagulation and cheese-yielding characteristics intermediate between those of κ-casein AA and BB (see Guinee and O'Brien 2010b).

Compared to α_{s1}-CN BB, α_{s1}-CN BC casein is associated with higher levels of α_{s1}-casein, total casein and total protein and a higher estimated yield of Parmesan cheese. However, owing to the higher yield of milk and levels of fat and protein in milk containing α_{s1}-CN BB casein over a complete lactation, cows producing this variant give a higher overall cheese yield during lactation than those producing α_{s1}-CN BC casein.

In conclusion, the BB variants of κ-casein and β-lactoglobulin enhance cheese yield and have no adverse effects on cheese quality or on functionality in the case of low-moisture Mozzarella cheese (IDF 1994; Ng-Kwai-Hang and Grosclaude 2003; Guinee and O'Brien 2010b).

10.5.7 Cold Storage of Milk

Modern farm and milk collection practices result in milk being cooled rapidly to <8 °C following milking and a relatively low frequency of milk collection from the farm, e.g., every second or third day. Moreover, cold milk is hauled over long distances and is often cold-stored at the cheese plant for 1–3 days, depending on time of year and the manufacturing schedules; hence, milk may be cold-stored for 2–5 days prior to processing. During this period, the cold milk is subjected to varying degrees of shear due to pumping, flow in pipelines and agitation.

Cold storage and shearing result in a number of physico-chemical changes which may alter the cheesemaking properties of milk, including:

- increased growth of psychrotrophic bacteria (O'Brien 2008; Guinee and O'Brien 2010b);
- solubilization of micellar caseins, especially β-casein, and colloidal calcium phosphate, leading to an increase in serum casein;
- increased susceptibility of serum casein to hydrolysis by proteinases from psychrotrophic bacteria or somatic cells and/or plasmin, and the concomitant increase in non-protein N (Yan et al. 1985; Fig. 10.8);
- damage to the milk fat globule membrane and hydrolysis of free fat by lipases from psychrotrophic bacteria and/or milk.

Cold storage impairs the rennet coagulation properties, reduces the recovery of protein and fat and reduces cheese yield (Hicks et al. 1982; Weatherhup and Mullan 1994; Fig. 10.8; Table 10.6). However, there is disagreement between reported

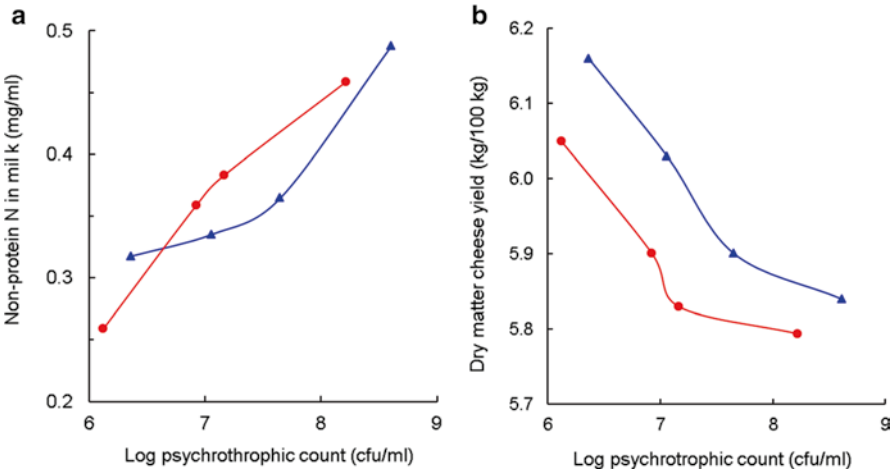


Fig. 10.8 Effect of type and population of psychrotrophic bacteria on milk composition (**a**) and dry matter yield of experimental cheese curd (**b**). Pasteurized milk was inoculated at various levels with *Bacilllus* (*red circle*) or *Psudomonas* (*blue triangle*) species isolated from raw milk (stored at 7 °C for 3 days) and incubated for 6 days (*Pseudomonas*) or 10 days (*Bacillus*) before cheese manufacture. Drawn using data from Hicks et al. (1982)

Table 10.6 Effect of cold-storing milk at 3 °C on the recovery of milk fat and solids and on actual cheese yield

	Recovery (%)		
Storage time (day)	Fat	Solids	Yield (kg/100 kg)
0	89.2	57.0	9.7
3	87.7	49.2	9.1
5	86.0	48.3	9.0

From the data of Weatherhup and Mullan (1994)

studies as to the magnitude of the effect of cold storage (cold ageing). Discrepancies between reports may be attributed to variations in experimental conditions, e.g., pre-handling and temperature history of milk prior to experimentation, milk pH, somatic cell count, bacterial count and species/strains of psychrotrophic bacteria in milk, storage temperature and time, and cheesemaking conditions. It is generally agreed that at a population $<10^6$ cfu/ml, psychrotrophic bacteria have little effect on the cheesemaking properties of milk (Fox 1989).

On storage at 4 °C for 48 h, the increase in the rennet coagulation time (RCT) of milk from individual cows ranged from 10 % to 200 % and that of bulk milk from 9 % to 60 % (Fox 1969). A direct relationship was found between the increase in the RCT of cold-aged milk and the initial RCT before ageing. The large variation in the RCT of cold-aged milk from individual cows is probably a consequence of differences in composition, microbiological status and somatic cell count. The chemical changes, i.e., increases in serum casein and ratio of soluble Ca:micellar Ca, and the increase in RCT associated with cold storage are almost complete after 24 h and are largely reversed by pasteurization (72 °C × 15 s) or milder heat treatment (e.g., 50 °C × 300 s).

The increase in RCT, and consequently the longer time required for curd formation during cheese manufacture, that generally accompanies cold storage beyond 24 h appears to be due, at least in part, to the enzymatic degradation of casein. Proteolysis reduces the concentration of gel-forming casein to an extent which depends on the proteolytic activity in the milk. Peptides, which are soluble in the serum phase (as non-protein N), do not coagulate on renneting and are largely lost in the whey. Moreover, the reduced casein level results in slow curd formation and a soft coagulum at cutting (see Chap. 7), a situation which is conducive to curd shattering, high losses of fat in the whey and reduced cheese yields. In commercial practice, the coagulum is usually cut at a fixed time after rennet addition rather than at a given firmness.

In the EU, the permitted TBC in milk for the manufacture of dairy products has been reduced from $\leq 4 \times 10^5$ cfu/ml in 1994 to $\leq 1 \times 10^5$ cfu/ml in 1998 (EU Regulation 853/2004). Improved dairy husbandry practices, combined with the more stringent standards for TBC and SCC, should reduce the degree of storage-related proteolysis and lipolysis in milk. The fact that the chemical changes that occur on cold storage are reversed by pasteurization suggests that, with modern milk production practices, cold storage of milk for several days probably has little influence on its cheesemaking properties.

10.5.8 Thermization of Milk

Prolonged holding of milk prior to processing can occur at the factory, for example over week-ends if milk is not processed, or when daily milk supply volumes are too small to justify processing each day as, for example, during early and late season from a milk supply based mainly on spring-calving herds. Extended cold storage may lead to the development of high psychrotrophic populations which produce proteolytic and lipolytic enzymes resistant to pasteurization (Yan et al. 1985; Kohlmann et al. 1991; Harwalkar et al. 1993; Fajardo-Lira et al. 2000). These enzymes can potentially reduce cheese yield (as discussed in Sect. 10.5.5) and adversely affect product quality.

Thermization refers to the heat treatment of milk at a sub-pasteurization temperature (typically 50–70 °C for 5–30 s) on reception at the dairy to reduce the viable bacterial load in the milk and minimize changes in quality and processability prior to conversion into product. This greatly reduces the development/occurrence of bacterial-associated enzymatic activities in the milk during subsequent cold storage, as reflected by lower levels of peptides and free fatty acids in the stored milk (Zalazar et al. 1993). Consequently, thermization generally improves the yield and quality of cheese prepared from milk that has been cold-stored (Girgis et al. 1999; Lalos et al. 1996). Studies on cottage cheese (Dzurec and Zall 1982) showed significantly higher yields from milk heated at 74 °C × 10 s prior to storage compared to the control (i.e., 16.85 vs. 16.0 kg/100 kg), when milk was cold-stored at 3 °C for 7 days. It has been suggested that where milk is stored for long a period at farm level, on-farm thermization (e.g., 74 °C for 10 s) may be advantageous for cheese yield (Zall and Chen 1986). A thermization temperature of 65 °C or slightly higher is recommended for optimum effects (van den Berg et al. 1984).

10.5.9 Effect of Whey Protein Addition and Denaturation

Whey proteins may be incorporated into cheese in a number of ways:

- *in situ* denaturation of the whey proteins by high heat treatment (HHT) of the cheese milk, e.g., ~65 % of total whey proteins at 100 °C × 120 s;
- high concentration factor ultrafiltration (HCF-UF), with or without HHT of the milk before UF or the rententate after UF, as in the production of pre-cheese,
- addition of partially denatured whey protein concentrates (prepared by high heat treatment and/or acidification of cheese whey) to the curd; commercial whey protein preparations of this type, e.g., Dairy Lo™ and Simplesse® 100, have been used to improve the textural characteristics of reduced-fat cheese.

10.5.9.1 Incorporation of Whey Proteins by High Heat Treatment of Milk and *In Situ* Denaturation

Pasteurization of milk (72 °C × 15 s) results in a low level (≤5 % of total) of denaturation of whey proteins, which complex with κ-casein and are retained in the cheese curd, where they contribute to a yield increase of ~0.1 to 0.4 % (Fenelon and Guinee 1999). However, most (~94–97 % depending on the cheese moisture level) of the native whey proteins, which account for 20 % of the total milk protein, are lost in the whey. Unlike casein, native whey proteins are stable to rennet treatment and acidification to pH 4.6 and thus remain soluble in whey during the manufacture of rennet- and acid-curd cheeses. Theoretically, if all whey proteins were retained, without adversely affecting cheese moisture or quality, a yield increase of ~12 % (i.e., 10.7 vs. 9.54 kg/100 kg) would be achievable for Cheddar cheese with ~38 % moisture. Indeed, since increasing the severity of milk heat treatment is paralleled by an increase in cheese moisture (Fig. 10.9), the increase in actual yield would be even higher (e.g., ~15 % following pasteurizing at 88 °C × 15 s). Hence, cheesemakers are interested in maximizing the recovery of whey protein from milk to cheese, while maintaining cheese quality.

However, inclusion of a high level of denatured whey protein (i.e., >35 % of total WP) can adversely affect rennet coagulation properties (see Chap. 7), cheese rheology (e.g., reduced elasticity and firmness), functionality (e.g., reduced flowability) and overall quality of most rennet-curd cheeses (Zoon 1994; Rynne et al. 2004). The detrimental effect of whey proteins on rennet coagulation is due to the interaction of denatured whey proteins (β-lactoglobulin and α-lactalbumin) through thiol-disulphide interchange with κ-casein at the micelle surface (Singh 1995). This interaction results in the formation of filamentous appendages which protrude from the surface of the casein micelles, render the κ-casein less susceptible to hydrolysis by rennet and limit the degree of aggregation of the *para*-casein micelles. The more finely-structured gel (compared to that from pasteurized milk) has impaired syneretic properties and consequently the moisture content (Fig. 10.9) and actual cheese yield are increased. However, rennet-induced gels from HHT milks with a level of WPD >30 % tend to be very fragile and prone to shattering on cutting and stirring, even when the milk has been acidified to pH 6.0 prior to setting (Banks et al. 1987). Hence, high losses of fat (e.g., up to 15 %) and curd fines (e.g., up to 800 mg/kg) in the whey are possible unless the gel is treated very gently (Guinee, unpublished results). The susceptibility of curd particles from HHT milk to fracture persists for a longer time than normal into the stirring period, because of the lower tendency to synerese and the slower rate of firming of the curd particles. Nevertheless, the inclusion of denatured whey proteins may sometimes be desirable in some rennet-curd cheeses. Owing to their softening effect on cheese, a high level of denatured whey proteins in cheese milk may be exploited as a means of improving the texture (reducing the firmness and elasticity) of low-fat hard cheeses, such as Cheddar, which tend to be too firm and rubbery (chewy) compared to their full-fat equivalents (Rynne et al. 2004).

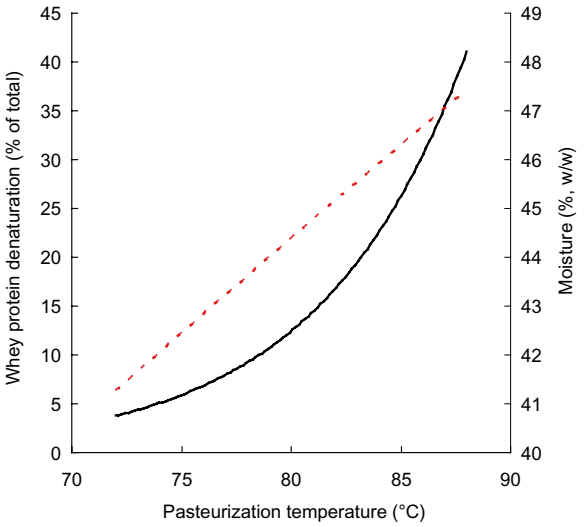


Fig. 10.9 Effect of milk pasteurisation temperature (for 15 s) on whey protein denaturation (*black solid line*) and moisture content of half-fat Cheddar cheese (*red dashed line*). Redrawn using data of Guinee et al. (1998)

Inclusion of a high level of whey proteins is desirable in many fresh, acid-curd cheeses (e.g., Quark, Cream cheese) where it promotes a finer gel structure and a higher level of gel-forming protein. These changes are conducive to a firmer or more viscous cheese which has a superior water-holding capacity at the low cheese pH (~4.6) is less prone to syneresis or to the development of undesirable granular or grainy textures during storage (see Chap. 16). In acid-heat coagulated cheese types (e.g., Thermoquark, Ricotta, Paneer, some types of Quesco Blanco), the incorporation of a high level of denatured whey protein is a central feature of the manufacturing process and is essential to impart certain desirable attributes, e.g., lack of elasticity or flow-resistance on grilling or baking.

10.5.9.2 Incorporation of Whey Proteins by Addition of Denatured Whey Proteins to Cheesemilk

An alternative to the *in situ* denaturation of whey proteins in milk is the addition of denatured whey proteins (recovered from whey) to the cheese milk. The potential advantages of this method are:

- the curd-forming properties of the casein are not (or only slightly) impaired,
- the cheese yield is increased but the texture is not altered to the same extent as in curd from HHT milk with an equivalent level of denatured whey protein.

Potential disadvantages of adding denatured whey proteins include:

- the addition of a milk protein fraction, i.e., whey protein, may not comply with the legal standard of identity of the cheese in all countries, and
- it may be expensive.

Several processes have been developed for the recovery of proteins from sweet whey, most of which involve flocculation, by heat denaturation and/or acidification, and recovery by centrifugation to yield concentrates containing ~12 % dry matter in the Centriwhey process or ~18 % dry matter in the Lactal process (Banks et al. 1994). These concentrates are added at the desired level to the cheese milk, which is then treated as in normal cheese manufacture. Commercial partly-denatured whey proteins may also be prepared by concentrating sweet whey by ultrafiltration and heat-treating to give a controlled level of denaturation. The heat-treated retentate may be supplied as a viscous, wet slurry or spray dried.

Variable results have been obtained with the addition of partially denatured whey protein concentrate (PDWPC; prepared by the Centriwhey, Lactal or ultrafiltration processes) to milk for the manufacture of hard and semi-hard cheeses, such as Cheddar or Gouda. There is general agreement that the addition of PDWPC increases the moisture content, actual yield and Yma, to an extent depending on the level of PDWPC added and the degree of whey protein denaturation of the added WPC. However, the addition of PDWPC generally leads to defective body (i.e., greasy, soft) and flavour (i.e., unclean, astringent) characteristics in Gouda and Cheddar cheese (van den Berg 1979; Brown and Ernstrom 1982; Baldwin et al. 1986).

Whey proteins prepared by concentrating sweet whey by ultrafiltration may be subjected to microparticulation. This involves a thermal treatment to induce the formation of whey protein aggregates, followed by a homogenization/shear treatment to reduce the size of aggregates/particles formed (e.g., 0.1–2.0 μm) so that they are perceived as 'creamy' and can, hence, act as a substitute for fat in reduced-fat products (Singer and Dunn 1990). The heat-treated retentate may be supplied as a viscous, wet slurry or spray dried. Commercial preparations (e.g., Simplesse® 100 or Dairy Lo™) have been used as fat mimetics to improve the texture and colour of reduced-fat cheeses, which tend to be firmer, more elastic/rubbery and more translucent than their full-fat equivalents (Guinee 2016). In contrast, conflicting results have been reported for the effects of microparticulated whey protein preparations on the properties of cooked cheese, such as degree of flow and fluidity (Guinee 2016). These protein preparations are added, typically, at a level of ~1.0 to 2.0 % to the cheese milk, resulting in an increase in protein content of 0.5–1.0 (%, w/w). Their use in half-fat Cheddar cheese gives a higher level of cheese moisture (e.g., 45.0 % vs. 42.7 %), and higher actual (e.g., 7.7 vs. 7.2 kg/100 kg) and moisture-adjusted cheese yields (IDF 1994; Fenelon and Guinee 1997). These materials also improve the texture of reduced-fat Cheddar (reduced fracture stress, fracture strain and firmness) and have little influence on flavour/aroma.

10.5.9.3 Incorporation of Whey Proteins by High Concentration Factor Ultrafiltration of Cheesemilk

HCF-UF (>6 fold) is widely used as an alternative method to traditional centrifugation for the concentration of acid-gelled milk to the final level of cheese solids in the commercial production of fresh acid-curd cheeses, such as Quark and Cream cheese.

This method gives complete recovery of whey proteins, with a higher yield of cheese of very acceptable quality. The traditional method gives a significantly lower recovery of whey protein and lower yield of these cheeses, but this depends on the degree of heat treatment of the milk prior to fermentation and the level to which the fermented, acid-gelled milk is heated prior to centrifugation and whey separation; these heat treatments affect the degree of whey protein denaturation, their binding to the casein and, hence, their recovery in the cheese curd (with the casein) (cf. Chap. 16, Schulz-Collins and Senge 2004). The estimated yield of Quark made by HCF-UF method and the conventional thermoquark method (cf. Chap. 16) are 21.3 and 19.6 kg/100 kg milk respectively.

However, in the case of HCF-UF it is essential that the recovered whey proteins in the retentate (cheese curd) are largely denatured by heated treatment, and thereby become part of the protein network (gel) of the cheese and contribute to texture of the final product. Otherwise, undenatured whey proteins that are soluble in the serum phase of the cheese do not contribute to the structure of the protein network, resulting in a product that lacks the typical stiffness and is too soft and fluid despite having the same protein content as the traditional product (cf. Chap. 16).

HCF-UF has also been used for the commercial manufacture of rennet-curd cheeses, e.g., cast Feta. Manufacture involves HCF-UF of the milk to produce a high dry matter (e.g., 40 to 50 %) retentate, or liquid pre-cheese, which has a composition close to that of the finished cheese. Rennet, starter culture or food-grade acid/acidogen are added to the pre-cheese, which is then filled into the package and gelled *in situ* under controlled time/temperature conditions to form the final product. In the case of harder cheeses with a higher dry matter content, e.g., >50 %, the liquid pre-cheese (40–50 % dry matter) is similarly treated with rennet, starter culture and/or food-grade acid, and the resultant curd may be subjected to further treatments (e.g., cutting, heating, pressing) that cause further whey expulsion and give the desired higher dry matter cheeses (cf. Guinee et al. 2009).

The attraction of HCF-UF for the manufacture of rennet-curd cheese is the increased yield owing to the high retention of whey proteins and glycomacropeptides. The exact degree of retention depends on:

- the heat treatment of the milk prior to renneting which determines the extent of whey protein denaturation and hence whey protein solubility
- the level of whey expulsion from the pre-cheese following coagulation and cutting; native whey proteins and glycomacropeptides are soluble and are lost in the whey.

When complete recovery of the latter components is achievable (as in cast Feta), the estimated saving in skim milk is ~9 %, which makes the process economically viable (IDF 1994).

10.5.10 Homogenization and Microfluidization

Homogenization of milk reduces fat globule size and increases the surface area of the fat by a factor of 5 to 6 (Huppertz and Kelly 2006). The fat globules essentially become coated with a protein layer consisting of casein micelles, sub-micelles and, to a lesser extent, whey proteins. Hence, the newly-formed fat globules behave as fat-filled protein particles, with the ability to become part of the gel network formed on renneting or acidification. Homogenization of milk or cream is not widely practiced in the manufacture of rennet-curd cheeses as it tends to give cheese with high moisture content and altered texture, e.g., lower elasticity and firmness, flavour (e.g., hydrolytic rancidity) and functionality (e.g., reduced flow on grilling/baking) to a degree dependent on milk composition and homogenization temperature and pressure. Cheese produced from homogenized milk is whiter than that from unhomogenized milk, an attribute that may be desirable, e.g., for Blue cheese or Mozzarella, or undesirable, e.g., Swiss-type cheese. The main applications of homogenization in cheese manufacture are:

- cheeses made from recombined milk, formed by homogenizing oils (butter oil and/or vegetable oils) in an aqueous dispersion of milk protein (e.g., reconstituted or reformed skim milks), in countries where the demand for cheesemaking exceeds the local supply of fresh milk;
- fresh acid-curd cheeses (e.g., cream cheese), especially when the fat content of the milk is high (e.g., 10 %, w/w). In this situation, homogenization contributes to product homogeneity (as it retards creaming of the fat globules during the relatively long gelation period, ~12 h) and enhances product texture by increasing the level of effective protein (as the casein-coated fat globules become part of the gel rather than being occluded within the gel, as with native fat globules in milk).
- Blue cheese, where the casein-based fat globule membrane allows access for lipases from the mould to the fat and thereby enhance the formation of free fatty acids which are the main substrates for the production of methyl ketones which are very important for flavour.

The effects of homogenization on composition, quality and yield have been investigated for many rennet-curd cheeses (Jana and Upadhyay 1992, Guinee and McSweeney 2006). Homogenization of milk or cream increases the yield of practically all varieties, the effect being more pronounced with:

- increasing homogenization pressure, in the range 5–25 MPa, and
- homogenization of standardized cheese milk rather than homogenization of the cream used for standardization (perhaps because of lower degree of homogenization efficiency, due to the lower protein-to-fat ratio in cream compared to milk).

The reported yield increase, which ranges from 2.8 % to 6.3 % for Cheddar, is attributed to the increased moisture content (e.g., 2–3 % for Cheddar, depending on homogenization and cheesemaking conditions) and the lower losses of fat in the whey (e.g., 2–6 % milk fat) and/or in stretch water in the case of *pasta filata* cheeses.

Table 10.7 Effects of microfluidization on the yield of Cheddar cheese

	Milk		Cream	
	0 Mpa	7 Mpa	14 MPa	69 MPa
Cheese composition				
Moisture, g/kg	350	376	384	393
Fat, g/kg	344	340	334	330
Bulk whey composition				
Fat, g/kg	5.2	2.3	1.7	1.3
Curd fines, g/kg	0.9	1.0	1.1	1.2
Cheese yield, kg/100 kg				
Actual	9.4	10.2	10.4	10.6
Moisture-adjusted	9.7	10.1	10.2	10.2

Based on data from Lemay et al. (1994a, b)

However, the efficiency of recovery of fat from the whey from homogenized milk is only ~30–40 % of that from non-homogenized milk using a centrifugal separator.

Microfluidization is a relatively new technology which has been used in the health-care industry since the late 1980s for the reduction of mean particle size in the manufacture of products such as antibiotic dispersions, parenteral emulsions and diagnostics. Microfluidization differs from homogenization in the types of forces applied to the fluid and also in size distribution and mean diameter of the resulting particles.

It is generally accepted that for the application of equivalent pressures to the milk, microfluidization gives a lower mean fat globule diameter (e.g., 0.03–0.3 μm compared to 0.5–1.0 μm) and a narrower size distribution than homogenization. Moreover, the fat globule membrane in microfluidized milk has a higher proportion of fragmented casein micelles and little, or no, whey proteins compared to that in homgenized milk. Microfluidization of milk (at 7 MPa) or cream (at 14 or 69 MPa) for Cheddar cheese was shown to give higher moisture, higher retention of milk fat, and higher actual and moisture-adjusted yields (Lemay et al. 1994a, b; Table 10.7).

10.5.11 Type of Starter Culture and Growth Medium

Starter culture is generally used as the agent for acidification during cheese manufacture, which it effects by the conversion of lactose to lactic acid. In addition to its acidifying function, the starter culture also contributes to cheese flavour development through its enzymes which contribute to glycolysis, proteolysis, and lipolysis (see Chaps. 6, 11, and 13). In addition, the starter culture ferments residual lactose in cheese curd to lactic acid, thereby removing it as a carbon source for the growth of non-starter lactic acid bacteria (NSLAB). However, direct acidification (DA) may sometimes be used as the principal or sole means of acidification in the manufacture of some cheeses, e.g., where flavour resulting from starter activity is not a major

quality attribute, e.g., low-moisture Mozzarella (Schafter and Olson 1974), Cream cheese, Quesco Blanco, Ricotta, Paneer, or where flavour can be provided by alternative means such as flavoured dressing, e.g., creamed Cottage cheese.

Casein contributes directly to cheese yield and indirectly as the casein network influences the retention of moisture and fat; the former contains dissolved substances, including native whey proteins, casein-derived peptides, lactate and soluble salts. All other conditions being equal, the recovery of casein from cheesemilk is theoretically higher when cheese is made by DA through the addition of acid and/or acidogen, e.g., gluconic acid-δ-lactone, rather than by a starter culture. Starter cultures hydrolyze casein to varying degrees, depending on their proteolytic activity (ratio of proteinase$^-$/proteinase$^+$ cells), the pH and composition of propagation medium (cf., Chap. 6). Model studies, using skim milk acidified by starter culture or DA, have shown that starter cultures give significantly higher losses (0.7 to 6.6 %) of casein than DA, depending on the proteolytic activity of the culture (Richardson 1985). However, significant inter-study variations exist on the comparative effects of starter culture versus direct acidification (e.g., with acids) on cheese yields and component recoveries (Sharma et al. 1980; Satterness et al. 1978; Demott 1983; Shakeel-Ur-Rehman et al. 2003). Such discrepancies may be attributed to confounding effects of inter-study differences in factors such as cheese type, composition of the cheese milk, proteolytic activity of the starter culture strains, composition of starter culture medium, acidifying agent, rate of acidification and cheese composition (Richardson 1984; Hicks et al. 1985; Ustunol et al. 1986; Yoon et al. 1994).

Proteinase-negative single-strain starters generally give higher dry matter yields of Cheddar cheese than the corresponding proteinase-positive starters, with the yield increase ranging from 1.4 % to 2.4 %, depending on the starter strain. Similarly, in model acidified skim milk systems, the recovery of casein with a proteinase-negative starter is significantly higher (3.1 %) than with the corresponding proteinase-positive strain and only marginally lower (–0.69 %) than that obtained with DA (Richardson 1985). Proteinase-negative strains, which rely on the indigenous amino acids and small peptides in the milk for growth, reproduce very slowly and therefore reduce the pH too slowly for cheese manufacture; moreover, their use may lead to slow proteolysis and flavour development during maturation. Hence, proteinase-negative strains are generally not used alone but rather in blends with proteinase-positive strains; such blends are commonly used as cheese cultures.

Approximately 40 % of the bulk starter solids, i.e., casein plus denatured whey proteins, are retained in Cheddar cheese when the starter medium is 10 % reconstituted low-heat skim milk powder heated at 85–90 °C for 1 h (Banks et al. 1985). Some authors have reported increases in actual yield (e.g., ~2 %) and recovery of milk solids (e.g., 49.2 % compared to 48.5 %) in Cheddar cheese when a direct-to-vat starter (DVS) culture rather than bulk starter (BS) culture was used (Salji and Kroger 1981). In contrast, a Scottish study showed that the use of BS culture grown in 10 % reconstituted skim milk powder resulted in higher actual (1.1 %) and moisture-adjusted (1.05 %) cheese yields, and higher retention of milk solids (i.e., 53.1 compared to 52.6 %) compared to DVS culture (Banks et al. 1985). Moreover,

further studies by the same group (Banks and Muir 1985) showed that, compared to a commercial casein-free starter medium, the use of reconstituted skim milk powder as starter medium resulted in a higher actual yield (1.8 %) and recovery of milk solids (1.7 %). The greater recovery of solids was attributed to the retention of casein and denatured whey protein from the starter, which are coagulable following acidification to pH 4.6 in the BS culture and re-neutralization to pH ~6.5, when the starter is added to the milk. Obviously, further studies, where factors such as starter strain(s), milk composition, set pH and gel firmness at cutting are standardized, are required to clarify the comparative effects of BS and DVS cultures on Cheddar cheese yield.

Significant increases in Cottage cheese yield have been reported when whey-based media with external pH control (i.e., pH constantly maintained at the initial value, e.g., 6.6, by addition of base) were used instead of conventional skim milk based media without pH control (Reddy and Reinbold 1983; Hicks et al. 1985). In the authors' experience, it is important that when a DVS culture is used, the pH at set should be standardized to that normally obtained with a BS culture (e.g., to 6.55) by the addition of an acid (e.g., lactic), acidogen (e.g., glucono-δ-lactone) or $CaCl_2$, or by allowing sufficient ripening time, especially when the level of casein in milk is low. Otherwise, gel firmness after a specified set-to-cut time may be low, resulting in shattering of the curds and reduction in cheese yield due to high losses of fat and curd fines. The addition of DVS starter has little, or no, immediate effect on milk pH, whereas the addition of bulk starter gives an immediate decrease in pH of ~0.1 unit; moreover, during the ripening or vat-filling, the decrease in pH is greater when a BS culture is used.

10.5.12 Addition of $CaCl_2$

The addition of $CaCl_2$ at a level of ~0.02 g L^{-1}, i.e., ~2 mM Ca, to milk is common commercial practice, especially when using late lactation milk. Addition of $CaCl_2$ generally improves the rennet coagulation properties, an effect attributable to the reduction in pH and the increase in the concentration of Ca^{2+} (see Chap. 7). While the effect of $CaCl_2$ addition on the rennet coagulation properties has been studied extensively, comparatively few studies have considered its effect on cheese yield. An investigation on the commercial manufacture of Swiss-type cheese showed that the addition of $CaCl_2$ (0.1 g L^{-1}) gave insignificant increases in the mean recovery of milk fat (85.3 % vs. 84.7 %) and non-fat milk solids (33.85 % vs. 33.75 %) and a significant increase in the mean cheese yield (0.038 kg/100 kg) (Wolfschoon-Pombo 1997). The proportion of large curd particles (i.e., 5.5–7.5 mm) was increased and the proportion of small particles (<3.5 mm) reduced. These trends suggest that the positive effects of $CaCl_2$ on the recovery of fat and protein and cheese yield probably result from an enhanced degree of casein aggregation which reduces the susceptibility of the curd to fracturing during cutting and the initial phase of stirring (see Sect. 10.3.3).

10.5.13 Rennet Type

Ideally, rennets should hydrolyze only the Phe_{105}–Met_{106} bond of κ-casein during milk coagulation, with further cleavage of caseins occurring only after complete removal of whey. In this situation, the recovery of casein is maximized and cheese yield increased. The various rennets used in cheesemaking differ in their milk clotting-to-proteolytic activity and thus hydrolyze casein to a greater or lesser degree during cheese manufacture, depending on the length of time the curd is in contact with the whey and the curd pH at whey drainage. Some breakdown products of casein are soluble in whey and are removed and lost in the whey at whey drainage. Calf chymosin is the least proteolytic of the gastric proteinases, the proteolytic activity of which decreases in the following general order: chicken pepsin > porcine pepsin > ovine pepsin > bovine pepsin > calf rennet (chymosin) ~ fermentation-produced chymosin. Microbial rennets are also more proteolytic than calf chymosin, with proteolytic activity being in the following order: *Cryphonectria parasitica* proteinase ≫ *Rhizomucor miehei* > *R. pusillus* > calf chymosin.

However, the relative proteolytic activity of different rennets is not always reflected in cheese yield. Indeed, many differences exist between reported results on the effects of coagulant on cheese yield. In a laboratory-scale (0.6 L milk) Canadian cheesemaking study (Emmons and Beckett 1990), the increase in the level of non-protein N (expressed as whey protein) in Cheddar cheese whey, compared to calf rennet, ranged from 0.006 % for bovine pepsin to 0.19 % (w/w) for *Bacillus polymyxa* proteinase. The corresponding estimated reduction in moisture-adjusted cheese yield ranged from 0.16 % to 4.5 %, respectively. In a pilot-scale study involving 9–12 replicate trials, Barbano and Rasmussen (1994) found that the losses of fat and protein in whey obtained with calf rennet (CR) or recombinant chymosin (RC) were similar, and lower than those with *R. miehei*, *R. pusillus* or bovine rennet. The moisture-adjusted Cheddar yield was highest for RC and CR and lowest for *R. pusillus* (Fig. 10.10). A similar study by Ustunol and Hicks (1990) showed that the above coagulants had no significant effect on N or fat levels in whey, or on dry matter cheese yield. However, compared to the other rennets, *C. parasitica* proteinase gave significantly higher levels of N and fat in the whey, and a lower dry matter cheese yield. The decrease in dry matter yield of Cheddar cheese on using *C. parasitica* coagulant was eliminated on the addition of $CaCl_2$ at a level of 0.02 %. The discrepancy between studies may be attributed in part to differences in curd pH at whey drainage (e.g., 5.85 vs. 6.1). At the lower pH, all coagulants are more proteolytic, especially those with a low ratio of milk clotting-to-proteolytic activity (see Chap. 7), and therefore produce more soluble peptides, which are lost in the whey; hence, cheese yield decreases as the pH at whey drainage decreases.

The gene for camel (*Camelus dromedarius*) chymosin has been expressed in *Aspergillus niger* and produced by fermentation (Kappeler et al. 2006). Camel chymosin has recently become available as a commercial milk coagulant. Compared to bovine chymosin (BC), camel chymosin (CC) has identical specificity (cleaving the Phe_{105}–Met_{106} bond of κ-CN to produce *para*-κ-CN and caseinomacropeptide) but a

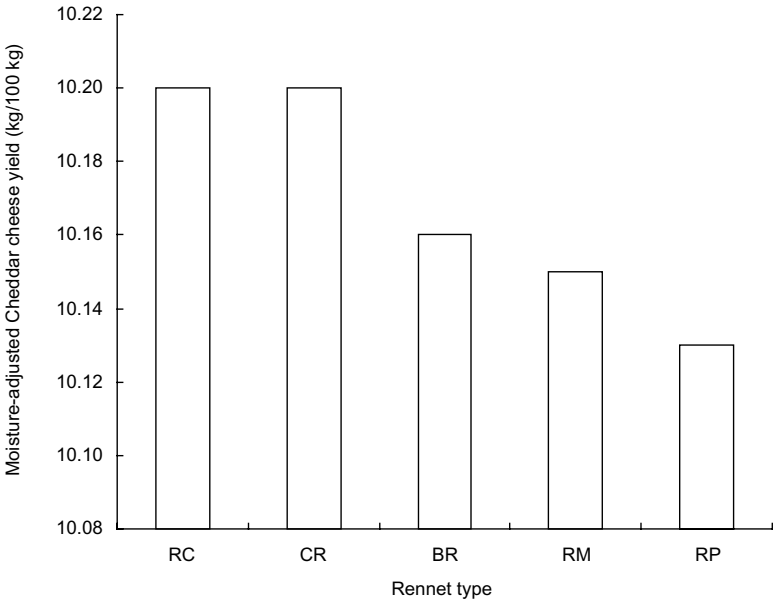


Fig. 10.10 Effect of coagulant type on moisture-adjusted Cheddar cheese yield. Coagulants: RC, recombinant chymosin (Chymax); CR, calf rennet (94 % chymosin, 6 % bovine pepsin); BR, bovine rennet (22 % chymosin, 78 % bovine pepsin); RM, *Rhizormucor miehei* proteinase (Morcurd plus); RP, *Rhizormucor pusillus* proteinase (Emporase sf 100). Drawn using data of Barbano and Rasmussen (1994)

sevenfold higher ratio of clotting to general proteolytic activity (Kappeler et al. 2006; Møller et al. 2012). Further studies comparing the performance of BC and CC at equal milk clotting activities in Cheddar cheese manufacture indicated (Bansal et al. 2009): no significant difference in cheese composition and pH in cheeses made with either coagulant, a lower level of primary proteolysis in cheeses made with CC, large differences in the peptide profiles between the cheeses, similar levels of amino acids except for isoleucine, histidine and lysine, and lower intensities of sulphur, brothy and bitter flavours in cheeses made with CC. Cheeses made with BC broke down more easily during eating, were smoother, more cohesive and adhesive, and gave better mouth-coating. The effects of BC and CC on Cheddar cheesemaking efficiency were compared under different conditions of milk clotting activity, time after rennet addition when cutting gel, and gel firmness at cutting (Guinee et al. 2013). When CC was added to the milk at 75 % of the MCA of BC and when the gel from CC-treated-milk was cut at the same time after rennet addition as the BC (with 100 % MCA), CC gave significantly higher fat recovery and actual yield, but had no effect on composition or protein recovery. Otherwise, when CC and BC were added at the same MCA and when the gels from milk treated with either enzyme were cut at similar gel strengths of 30 Pa, no significant differences in cheesemaking efficiencies were found.

In conclusion, the extent of casein hydrolysis during the manufacture of cheese curd is lowest with camel chymosin, intermediate with bovine chymosin, bovine pepsin and *Rhizomucor* rennets, and highest with *C. parasitica* and *Bacillus polymyxa* proteinases. Whether these differences in proteolytic activity impact significantly on cheese yield probably depends largely on the pH at whey drainage. Rennets with a high proteolytic activity (compared to calf rennet) probably have little effect on yield when the pH at whey drainage is high, e.g., ≥6.15, as in Cheddar, Gouda, Emmental, but reduce yield when the pH at drainage is <6.0, e.g., Blue and Camembert. The thermostability of the different rennets at the cook temperature for a given variety probably also determines how differences in proteolytic activity impact on yield.

10.5.14 Firmness at Cutting

Cutting the gel is a central part of cheese manufacture, being the first step in the dehydration process by which the colloidal constituents, i.e., fat, casein and micellar salts, of milk are concentrated to form cheese curd. During gel formation, firmness increases progressively from the onset of gelation as a consequence of ongoing aggregation of *para*-casein micelles (see Chap. 7). Eventually, the gel reaches a firmness which allows it to withstand mechanical cutting by the knives in the cheese vat without shattering. However, if the gel firmness at cutting is too low or too high, the resultant gel is too weak (under-set) or too rigid (over-set) to allow clean cutting without shattering. When the gel is too soft, the gel structure is insufficiently developed and fractures even under the small strains applied on gentle cutting. In an over-firm gel, the degree of casein aggregation is relatively high and the gel tends to be rigid (high G′) and brittle (low fracture strain) and susceptible to breakage by the deforming stress applied during normal cutting and stirring. Shattering of curd particles is undesirable as it potentially effects a higher fat loss, as more fat globules can escape from the surfaces of the curd particles, along with the outflow of whey immediately after cutting and during the early stages of stirring. Moreover, curd shattering potentially increases the level of curd fines (i.e., curd particles <1 mm), which may be lost from the curd mass to a greater or lesser degree, depending on downstream curd handling equipment (ex-vat).

Investigation of the effect of varying gel firmness at cut from 0.5 to 80 Pa (as measured using low strain oscillation) showed that increasing gel firmness significantly increased curd fines, moisture content and Cheddar cheese yield (Fig. 10.11). However, the recovery of fat and protein were not significantly affected. The main reason for the increase in yield was the increase in moisture content, as verified by the absence of a significant relationship between firmness of gel at cut and the Yma. Similarly, Banks and Muir (1984) found that variation in set-to-cut time, to give gels ranging from being under-set to over-set at cutting, did not significantly influence moisture-adjusted Cheddar cheese yield. The higher level of moisture reflects a

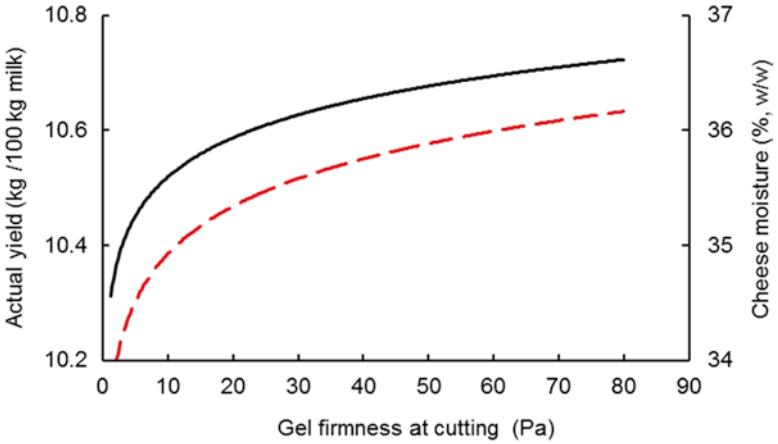


Fig. 10.11 Effect of gel firmness at cutting on the yield (*black solid line*) and moisture content (*red dashed line*) of Cheddar cheese. Drawn using data of Guinee and O'Callaghan (2010)

reduced tendency of the stiffer gel structure at cutting to rearrange (with the formation of new bonding sites) and hence, to contract and express whey.

Mayes and Sutherland (1989) investigated the effect of altering the time between rennet addition and cutting (set-to-cut time) from 100 % (control, adjudged by the cheesemaker to have optimum firmness for cutting) to as low as 60 % (adjudged to be under-set) or as high as 200 % (adjudged to be over-set). Similar to the above trends, increasing the set-cut-time resulted in a higher cheese yield and higher fat recovery. Moreover, there was an interactive effect between set-to-cut time and heal time (time between the end of cutting and starting stirring of the curd-whey mixture) on yield efficiency (Fig. 10.12). The positive effect of healing was small at set-to-cut times >85 % of the control but became increasingly greater at set-to-cut times <85 % of the control cut time. As the healing time was increased, the yield-enhancing effect of increasing set-to-cut time on cheese yield decreased significantly, especially when the gel was under-set at cutting, i.e., at set-to-cut times <95 % of control. These trends for the effect of healing time were confirmed by Riddell-Lawrence and Hicks (1988), who found that cooking curd immediately after cutting resulted in a significantly lower moisture content in cheese, a lower cheese yield, and a higher fat loss into whey. In contrast, healing cheese curd for 15 min or longer prior to stirring reduced syneresis and fat loss to whey, increased cheese moisture and cheese yield. Bynum and Olson (1982) reported that the effects of increasing set-to-cut time on fat recovery and Cheddar cheese yield depended on vat size. Increasing curd firmness had no effect on fat recovery or moisture-adjusted Cheddar yield when small experimental vats (460 L) were used and the opposite effect when using large vats (2400 L). The observed differences between large and small vats may be attributed to differences in stirrer design and to how rapidly clumps of curd particles are broken up during the initial phase of stirring. In large vats, clumps of curd particles tend to disintegrate more slowly which has the effect of increasing healing time.

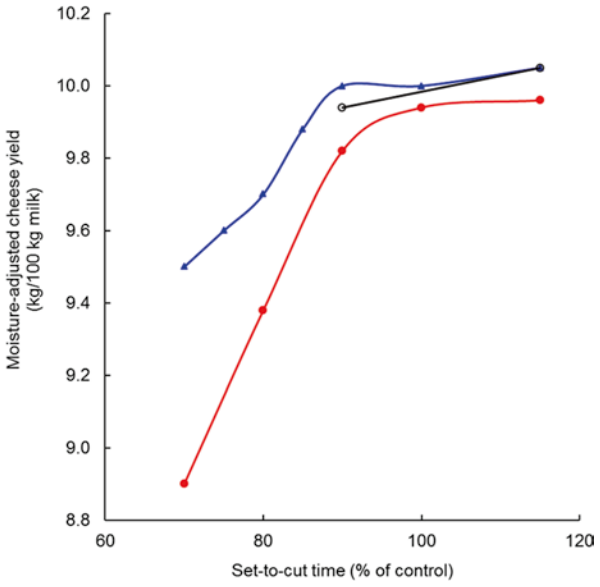


Fig. 10.12 Effect of set-to-cut time and healing time on moisture-adjusted Cheddar cheese yield. Healing times were 0 min (*red filled circle*), 5 min (*blue triangle*) or 10 min (*black open circle*). Drawn from data of Mayes and Sutherland (1989)

Factors that contribute to under-setting for a given set-to-cut time include increases in SCC and milk pH and a decrease in milk protein level, while those that contribute to over-setting include high protein content (≥4.5 %; Guinee et al. 1994, 2006), longer setting times and low milk pH (e.g., ≤6.2) (cf., Chap. 7). Apart from its effect on cheese yield, it is critical to standardise the firmness of the gel at cut to ensure consistent cheese composition and quality, especially when milk composition and quality changes significantly with season (cf., Sects. 10.5.1–10.5.4.). Commercially, this can be achieved by the use of in-vat curd firmness sensors (e.g., Optigraph) (O'Callaghan et al. 2002).

10.5.15 Particle Size

The curd particle size distribution during the initial phase of stirring affects yield efficiency as it determines the surface area through which fat escapes into the whey. However, it is not possible to measure the curd particle size distribution at this stage of cheese manufacture as the curd particles are still very fragile and would fracture during the sieving process involved in the analysis. Moreover, such a measurement is not very relevant as the curd particles fracture during the initial stages of stirring to a greater or lesser degree, depending on the size, the speed of stirring and vat

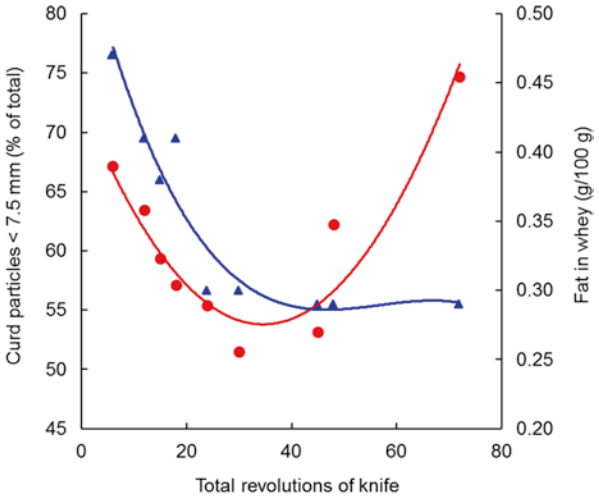


Fig. 10.13 Effect of number of cheese knife revolutions (rpm × min) on the proportion of curd particles <7.5 mm (*red circle*) and fat level in cheese whey (*blue triangle*). Drawn using data of Johnston et al. (1991)

design (Johnston et al. 1991, 1998). In practice, curd particle size is measured just prior to whey drainage, when the curd particles are sufficiently resilient to resist fracture during assay.

Johnston et al. (1991) investigated the effects of variations in the speed and duration of cutting in 20,000 L Damrow (variable speed motor) vats on the efficiency of commercial Cheddar manufacture. Perhaps contrary to expectation, cut programmes with a short duration of cutting at slow speeds produced small curd particles and high fat loss to cheese whey. These cutting conditions gave large curd particles which shattered quickly during subsequent stirring into smaller curd particles, thereby increasing the surface area for loss of fat. An optimal particle size and a minimum fat level were reached after the knives in the Damrow vat had completed ~37–40 rotations (Fig. 10.13). The time required to obtain the optimal curd particle size decreased as knife speed increased, e.g., ~18 min at 2 rpm and ~8 min at 5 rpm. Fat loss in whey decreased and cheese yield increased as the number of revolutions completed by the knives reached 37–40 (Fig. 10.13); a further increase in the number of knife revolutions had little effect on fat loss. This study indicates that for a particular vat and knife design, the curd particle size and, hence, fat loss in whey, are influenced by a combination of the speed and duration of cutting and the subsequent speed of stirring prior to cooking. Similar tends were reported by Everard et al. (2008) who found that reduced cutting intensity (number of knife revolutions) resulted in larger curd particles that shattered easily during stirring, higher levels of curd fines in the cheese whey and reduced-fat recovery from milk to cheese. For a given vat design, proper maintenance of knives, i.e., edge and knife angle, is essential to enable clean cutting and thereby reduce the risk of tearing the curds and high fat losses in cheese whey.

10.5.16 Design and Operation of the Cheese Vat

The design and operation of the cheese vat have a large influence on cheesemaking efficiency, cheese composition and, hence, quality. In all mechanized vats, the coagulum moves to a greater or lesser degree as soon as the knives are switched on, with a firm coagulum moving faster than a soft gel. Cheese vats of different design and mode of operation have been developed which enable the knives to cut the moving curd efficiently (Bennett and Johnston 2004). Design features which enable the knives to 'catch up' with the coagulum moving before it include:

- side-mounted baffles that push the curd onto the knife,
- continuously variable speed knife drives capable of speeds up to 12 rpm, and/or
- intermittent cutting cycles, followed by rest periods, which permit the knives to cut through the settling curd layers.

Surveys of commercial cheese manufacturers in the 1980–1990s indicate that vat type/design significantly influence the percentage of milk fat lost to cheese whey (Phelan 1981; Fig. 10.14). Moreover, the relative impact of vat type/design on fat loss can change during the cheesemaking season owing to changes in milk fat content. In a series of Dutch studies, 6 different vat types were compared; the level of fat in whey

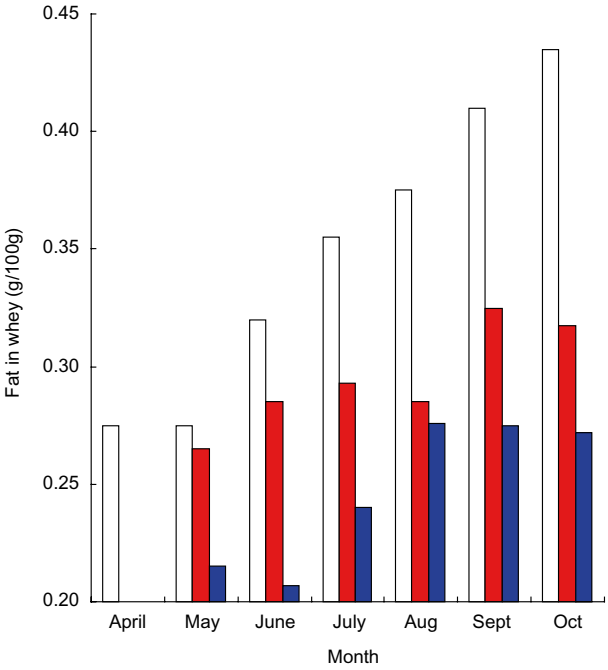


Fig. 10.14 Effect of three different types of commercial vat on the level of fat in whey from Cheddar cheese manufacture: types A (*white bar*), B (*red bar*) and C (*blue bar*).The general increase in fat content of the whey from each vat type as the year advanced coincided with an increase in the fat content of the cheese milk; the percentage of total milk fat lost to whey remained relatively constant. Redrawn from data of Phelan (1981)

ranged from 5.7 % to 7.2 % of the total milk fat and curd fines from 97 to 179 mg/kg whey during the production of Gouda cheese (Lawrence 1991). An Irish study (Palmer and Kelly 1994) showed that the mean fat level (~0.30 %, w/w) in Cheddar cheese whey from Tebel Ost IV vats and APV OCT vats was significantly lower than that (i.e., 0.47 %, w/w) in the whey from a W-vat. The above studies indicate that under normal operating conditions, some vat designs are inherently better than others in maximizing the retention of fat in cheese curd, probably as a consequence of a more favourable curd particle size distribution. However, for any vat design, maximum efficiency requires in-plant studies to optimize the interactive effects of milk composition (e.g., casein level), gel firmness, cutting programme and stirring speed so as to give the best curd particle size distribution and fat retention (Johnston et al. 1998).

10.5.17 Cooking and Stirring Conditions

Following the cut programme, the curd-whey mixture is stirred and cooked (scalded) (e.g., from typically ~30–32 °C at gelation to a temperature ranging from ~36 °C for Limburger to ~55 °C for Parmesan) to accelerate moisture expulsion (syneresis) and obtain the correct moisture content in the finished cheese. Generally, syneresis and moisture expulsion are increased by subjecting the curd-whey mixture to a higher degree of stirring, a higher cook (scald) temperature, a lower rate of heating, and a longer stir-out time before whey drainage (Whitehead and Harkness 1954; Guinee and O'Callaghan 2010; Everard et al. 2011). As is evident from cheese yield Prediction Equations (10.1)–(10.3) (Table 10.4), the yield of cheese decreases as the moisture content of the curd is reduced.

10.5.18 Curd Washing

In the manufacture of hard Dutch-type cheeses such as Gouda and Maasdammer, the whey is diluted by removal of whey from, and addition of water to, the cheese vat, typically in equal amounts (Walstra et al. 1987). The function is to control the lactose-in-moisture concentration (Hou et al. 2012) and, hence, pH of the cheese; this in turn affects several aspects of quality including texture and flavour (van den Berg et al. 2004). Increasing the quantity of added water from 30 % to 40 % reduces the yield by ~0.5 %.

10.5.19 Curd Handling Systems

Most of the losses during commercial cheese manufacture occur in the cheese vat, e.g., ~6.5 % of fat and ~4–5 % of the casein (due to loss of the caseinomacropeptides) during commercial Cheddar manufacture.

Typical fat loses in commercial Cheddar cheese factories range from ~8.5 % to 12 % of total milk fat, with ~51–73 % of the loss occurring in the cheese vat, 8–21 % during curd-whey pumping and cheddaring, and ~20–25 % during salting and pressing on Cheddaring belts/tower and block former (Guinee et al. 2005). Inter-factory variation is indicative of differences in milk composition, plant type/design, plant practices, cheese recipe/variant, frequency of equipment maintenance and revalidation, rigor of compliance to standard operating procedures, and the implementation of continuous quality improvement programmes.

Following removal of most of the whey at drainage (e.g., on drainage belts with overhead stirrers, which agitate the curd), the curd is subjected to conveying and handling processes, which are variety-specific, e.g., stirring, cheddaring, milling, salting and/or pre-pressing. During these operations, moisture and fat are lost to varying degrees, thereby affecting actual cheese yield.

Milling of cheddared curd exposes fresh surfaces from which fat is lost to an increasing extent in the whey (referred to as salty whey). The effect of cheddaring conditions in the pilot-scale manufacture of Cheddar was studied by Sutherland (1974). Increasing level of added salt reduces the moisture content of the cheese by ~2 % (w/w) per 1 % (w/w) salt added, but has little effect on fat retention. The pro-rata reduction in yield is estimated at ~1.58 % (from ~10 to 9.85 kg/100 kg milk) per 1 % (w/w) increase in added salt. An increase in curd temperature from 29 to 35 °C during cheddaring reduces moisture content and increases fat loss to cheese whey. The associated reduction in cheese yield averages 0.12 % per 1 °C over the range. The reduction in moisture content at higher temperatures is consistent with a greater hydrophobic-induced shrinkage of the *para*-casein network (cf., Chap. 18). Increasing the duration of mixing of curd chips and added salt from 20 s to 6 min significantly increases salt content of the cheese but reduces the level of fat recovery in the cheese. Apart from their effects on pH and salt and moisture contents of the cheese (cf., Chap. 9), increasing curd depth during mellowing or the mellowing time had little overall effect on fat recovery or yield.

It is also conceivable that the level of fat in whey from block formers increases with the level of vacuum. Owing to the general non-availability and high cost of downstream pilot-scale curd handling systems, little published information is available on the comparative effects of different systems. e.g., Cheddar master versus Alfamatic, or drain table (pre-pressing vat) vat versus Casomatic, under different operating conditions on cheese yield efficiency.

10.5.20 Effects of Enzymes

A relatively recent development includes the addition of specific enzymes to increase cheese yield. Lilbæk et al. (2006) reported that treatment of milk with phospolipase A1 increased moisture content, percentage of milk fat recovered, and yield of Mozzarella cheese. The study concluded that the lysophospholipids released from the fat globule membranes by the phospholipase act as surface-active agents in

the cheese curd, helping emulsification of water and fat during processing and reducing syneresis.

Transglutaminase (tgase) is an acyltransferase enzyme that catalyses the cross-linking of proteins through the formation of isopeptide bonds between glutamine and lysine residues (Griffin et al. 2002). The addition of tgase significantly influences rennet gelation properties, to a degree dependent on heat treatment of milk, inactivation or removal of an indigenous tgase inhibitor and duration of incubation time prior to renneting (Lorenzen 2000; Bönisch et al. 2008). Under optimized conditions, the addition of tgase significantly increases cheese yield, an effect due mainly to restriction of syneresis and increased moisture retention which results in higher moisture cheeses (Kumazawa et al. 2002; Radošević et al. 2007; di Pierro et al. 2010).

10.6 Conclusions

Optimizing cheesemaking efficiency is a key factor in maximizing the profit that accrues to a cheese plant and the price it pays for milk. Efficiency is generally optimized by maximizing cheese yield and minimizing the losses of milk fat and protein in the cheese whey or other by-product streams (e.g., stretch water in the case of *pasta filata* cheeses, plant rinsings). Nevertheless, the profit of a cheese plant is also influenced by the recovery of the milk components from these streams in the form of valuable by-products including whey cream, and clarified separated whey for conversion into various products such as whey powder, whey protein concentrate (WPC), whey protein isolate (WPI) and lactose or for fermentation into products such as alcohol (cf., Chap. 22).

Cheese yield is influenced by many factors, including the composition and quality of the raw milk, milk handling and storage practices, milk pre-treatments, cheesemaking process, equipment and technology and cheese composition. Maximization of cheese yield requires a comprehensive knowledge of milk composition, the factors that influence it, gelation, and the influence of the cheesemaking process on the gel. Measurement of cheesemaking efficiency is essential so that manufacturing inefficiencies can be redressed. Indices of cheesemaking efficiency include cheese yield and/or percentage recovery of components, especially casein and fat. Comparison of actual and predicted yields allows a cheese plant to monitor its efficiency over time.

In modern cheesemaking, full yield potential is not yet achievable, as fat and protein recoveries are still less than optimum. A closer realization of maximum yield is likely to follow from continued improvements in a number of areas, including:

- milk quality,
- minimization of the impact of seasonal variations in the composition of milk by improved milk production practices and/or standardization to a consistent casein level using LCF-UF or low concentration factor microfiltration,

- on-line standardization of casein and fat in milk,
- improved starter cultures which cause less hydrolysis of casein during manufacture,
- use of in-vat curd firmness sensors to optimize firmness at cutting, and/or
- the improvement in equipment and process design.

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Suggested Reading

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Chapter 11
Microbiology of Cheese Ripening

Summary This chapter considers the microbiology of cheese ripening and complements the next chapter which considers the biochemistry of cheese ripening and the development of cheese flavour. The important parameters controlling the shelf-life of cheese, viz., water activity, NaCl level, oxidation-reduction level, pH, nitrate and temperature are examined in some detail as is the growth of non-starter lactic acid bacteria, mainly lactobacilli, which grow in all cheeses during ripening. The role of the secondary cultures, e.g., brevibacteria, propionibacteria and moulds, which grow only during ripening, are considered within descriptions of the microbiology of the individual cheese varieties. The cheeses examined in detail from a microbiological view include Cheddar, Swiss-type cheese, Parmigiano Reggiano, Gouda and Edam, bacterial-, e.g., Limburger, Livarot and Tilsit, Reblochon and Gubbeen, and mould surface-ripened cheeses, e.g., Camembert and Brie, and blue cheeses. Microbial spoilage of cheese, e.g., early and late gas formation, open texture, growth of lactobacilli and propionibacteria in Dutch-type cheese, and yeast and moulds, is considered. Finally descriptions of the various genera other than starter and non-starter bacteria found in cheese, e.g., *Agrococcus, Arthrobacter, Brachybacterium, Brevibacterium, Corynebacterium, Microbacterium, Propionibacterium, Micrococcus, Kokuria, Kytococcus, Staphylococcus* and the various yeasts and moulds are given.

Keywords Control of microbial growth • Non-starter lactic acid bacteria • Secondary cultures • Cheese spoilage • Microbiology of different cheeses

11.1 Introduction

The quality of cheese is determined mainly by its flavour and texture and hence considerable effort has been devoted to elucidating the principal microbiological and biochemical changes that occur in cheese during ripening. The appearance of many varieties of cheese changes during ripening, e.g., the formation of holes, called eyes, in Swiss-type and, to a lesser extent, in Dutch-type cheese, growth of mould on the surface (e.g., Brie and Camembert) or interior (blue varieties), or the

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growth of microorganisms on the surface (smear-ripened cheeses). All these changes are caused by growth of microorganisms in or on the cheese. Therefore, an understanding of the factors involved in controlling their growth in cheese is important in trying to understand the development of cheese texture and flavour.

High numbers (at least 10^9 cfu/g) of microorganisms are present in all cheeses early in ripening and it is their lysis and the release of intracellular enzymes which mainly determine the development of flavour in the cheese (Chaps. 12 and 13).

11.2 Microbial Activity During Ripening

The factors controlling the growth of microorganisms in cheese include: water activity, concentration of salt, oxidation-reduction potential, pH, the presence of NO_3, a relatively low ripening temperature and the production of bacteriocins by some starter strains. Individually, the effect of these factors may not be very great, but the interaction of all of them, acting in concert, as so-called 'hurdles', is the real controlling factor. Other compounds produced during curd manufacture and ripening, e.g., H_2O_2 and fatty acids, also inhibit microbial growth but the concentrations of these produced in cheese are not sufficiently high to have a significant effect on the microorganisms.

11.3 Water and Water Activity

All microorganisms require water for growth but it is the availability of the water, rather than the total amount present, that is the important controlling factor. Water availability is expressed by the concept of water activity (a_w) which is defined as the ratio of the vapour pressure over the cheese, P, to the vapour pressure over pure water, P_o, at that temperature:

$$a_w = \frac{P}{P_o}$$

The value of a_w ranges from 0 to 1.0.

A reduction in moisture occurs during the manufacture of all cheeses; the lower it becomes, the harder the cheese is and the longer its keeping quality, e.g., Parmigiano Reggiano with a moisture content of ~30 % may be held for 2 years before being marketed. Cheese, unless vacuum-packed, loses moisture by evaporation during ripening. The proteins in cheese are hydrated and this 'bound' water is not available for bacterial growth. Any components dissolved in the moisture of the cheese, e.g., amino acids, peptides, short-chain fatty acids, salt and organic acids (lactate, acetate and propionate) reduce its vapour pressure and hence its a_w. Of these, the most important in practice are salt and lactate.

Table 11.1 Influence of water activity (a_w) on the growth [a]of different microorganisms[b]

	NaCl concentration (g/100 ml)				
	0	5	10	15	20
			a_w		
	0.992	0.975	0.947	0.916	0.880
Moulds					
Mucor mucedo 54	100.0[a]	47.4	11.6	–	–
Penicillium camemberti 53ll	100.0	80.9	36.4	4.1	1.1
Cladosporium herbarum 53b	82.6	100.0	62.4	13.9	3.5
Scopulariopsis fusca 53ll	100.0	78.4	76.9	65.4	14.6
Yeast					
Rhodotorula strain 44a	100.0	69.5	21.8	1.0	–
Debaryomyces strain 54k	100.0	49.7	30.2	10.5	8.2
Kluyeromyces lactis	100.0	100.0			
Geotrichum candidum 53aa	100.0	46.9	–	–	–
Bacteria					
Micrococcus saprophyticus[c] Strain 55a Strain 56b	100.0 84.7	96.3 100.0	67.2 61.2	19.1 16.7	– –
Brevibacterium linens Strain 58a Strain BL107	100.0 100.0	44.1 67.0	29.9 30.0	13.9 15.6	4.1 3.2
Arthrobacter citreus KR3 *Coliforms* Strain 54i Strain SL	100.0 100.0 100.0	19.4 23.4 19.9	7.0 – –	– – –	– – –

[a]The results are expressed as % of maximum development after 10 days incubation in Nutrient Broth, pH 6.6 at 25 °C; —no growth
[b]From Stadhouders and Langeveld (1966)
[c]These are likely to be strains of *Staphylococcus saprophyticus*

Most bacteria require a minimum a_w of 0.92 for growth. Yeasts grow at a lower value of a_w than bacteria, and moulds at a still lower value. The limit for most yeast is ~0.83 but osmophilic yeast grow at a_w values <0.60, while moulds have a lower a_w limit of ~0.75. Growth of microorganisms at low a_w is characterised by a long lag phase, a slow rate of growth (i.e., long generation time) and a reduction in the maximum number of cells produced, each of which helps to limit the growth of the microorganisms. Starter bacteria generally have a higher minimum a_w value than other bacteria. The minimum a_w for the growth of *Lc. lactis, Sc. thermophilus*, *Lb. helveticus* and *P. freudenreichii* are 0.93, >0.98, >0.96 and 0.96, respectively. The influence of a_w on the growth of some other microorganisms associated with cheese is shown in Table 11.1. *Penicillium camemberti* is the mould responsible for the white coating on Camembert and Brie cheese while *Brevibacterium linens* and *Debaryomyces hansenii* are important microorganisms in the surface flora of smear-ripened cheeses. *P. camemberti, B. linens* and *D. hansenii* can grow slowly in the

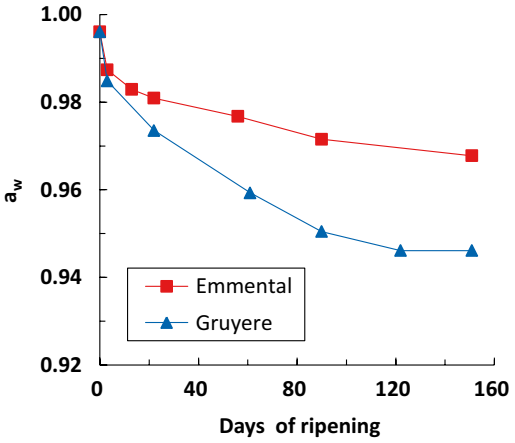


Fig. 11.1 Decrease in the a_w of Emmental and Gruyère cheese during ripening. The a_w at time zero, 0.995, corresponds to that of milk (From Ruegg and Blanc 1981)

presence of 10, 12 and 15 % NaCl, respectively. *Staphylococcus aureus* and micrococci can grow quite well in the presence of 6.5 % NaCl, which is equivalent to an a_w of 0.96. Compared with other fungi, *Geotrichum candidum* is very sensitive to a_w while *B. linens* is quite resistant. Propionibacteria are also particularly sensitive to a_w. Facultative anaerobes have different minimum a_w values depending on whether the organisms are growing aerobically or anaerobically, e.g., in the presence of O_2, *S. aureus* has a minimum a_w of 0.86 but in the absence of O_2, the minimum is 0.91.

Evaporation of water from the cheese surface during ripening also contributes to the reduction of the a_w of cheese; examples for Emmental and Gruyère are shown in Fig. 11.1. The reason for the faster rate of decrease in the a_w of Gruyère is probably due to the surface salting of Gruyère during the early stages of ripening. In addition, the a_w of cheese can vary throughout its mass (Fig. 11.2). Variations are much greater in large cheeses, like Emmental (50–60 kg), than in a small cheese, like Appenzeller (6–8 kg). This is due to several factors, including the temperature gradient in the cheese during the early stages of the fermentation, the loss of moisture during ripening, the NaCl gradient in the cheese and microbial activity on the rind. These factors must be taken into account in determining the significance of a_w, especially in large cheeses. Typical a_w values for cheese are listed in Table 11.2. As a comparison, the a_w of milk is 0.995. Since the a_w of cheese decreases during ripening, some of these values must be interpreted with care; however, they are useful as a guide. Except for the soft cheeses like Brie and Camembert, most of these values are close to the minima for starter growth.

11.4 Salt

The use of NaCl to prevent microbial spoilage of food is probably as old as food production itself. The concentration required depends on the nature of the food, its pH and moisture content but, generally, less than 10 % is sufficient. Salt and a_w are

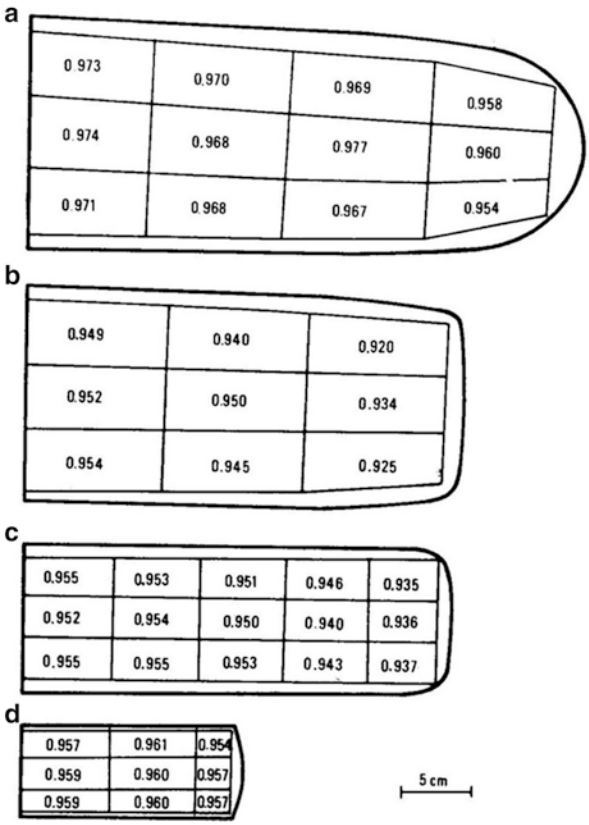


Fig. 11.2 Typical variations in the a_w of slices, from the centre to the surface, of (**a**) Emmental (**b**) Sbrinz, (**c**) Gruyère and (**d**) Appenzeller cheese. The cheeses were ~5 months old and the a_w of the rinds was (**a**) 0.90–0.95, (**b**) 0.80–0.90, (**c**) and (**d**) 0.92–0.98 (From Ruegg and Blanc 1981)

intimately associated and the major inhibitory factor is the reduction in a_w which occurs when salt (or any solute) is dissolved in water. The relationship between salt concentration and a_w is shown in Fig. 11.3 and is almost but not quite linear. The linear equation is:

$$a_w = -0.0007x + 1.0042$$

where x is the amount of salt, in g/1000 g of water, and describes the relationship very well since the r^2 value is 0.997. It is generally considered that an a_w of <0.92 is necessary to prevent bacterial growth; this is equivalent to a salt concentration of ~12 %. In cheese, the salt concentration varies from 0.7 to 7 %. The type of ion is also important, e.g., Na^+ is a much more effective inhibitor than K^+. In calculating the inhibitory effect of salt in cheese, the concentration of salt dissolved in the water of the cheese, rather than the actual concentration of salt, is the important parameter, e.g., in a Cheddar cheese with 38 g moisture/100 g and 1.9 g salt/100 g, the

Table 11.2 Typical water activity (a_w) values for different cheeses

Type	Typical a_w[a]	sd	a_w range in rind	Typical moisture, %	Salt, %
Appenzeller	0.960	0.011	0.97–0.98		
Brie	0.980	0.006	0.98–0.99	48.4	1.91
Camembert	0.982	0.008	0.98–0.99	51.8	2.5
Cheddar	0.950	0.010	0.94–0.95	36.8	1.5
Cottage cheese	0.988	0.006	–	82.5	1.0
Edam	0.960	0.008	0.92–0.94	41.5	2.0
Emmentaler[b]	0.972	0.007	0.90–0.95	37.2	1.2
Fontal	0.962	0.010	0.93–0.96		
Gorgonzola	0.970	0.017	0.97–0.99		3.5
Gouda	0.950	0.009	0.94–0.95	41.4	2.0
Gruyere[b]	0.948	0.012	0.92–0.98	34.5	1.06
Limburger	0.974	0.015	0.96–0.98	48.4	2.74
Munster	0.977	0.011	0.96–0.98	41.8	1.8
St. Paulin	0.968	0.007	0.96–0.97		
Parmesan	0.917	0.012	0.85–0.88	29.2	2.67
Quarg	0.990	0.005	–	79.0	0.70
Sbrinz[b]	0.940	0.011	0.80–0.90	42.9	1.90
Tilsiter	0.962	0.014	0.92–0.96		2.63
Processed cheese	0.975	0.010	–		

[a]Measured at 25 °C

[b]Values for Emmentaler, Gruyere and Sbrinz were measured after a ripening period of 4–5, 6–7 and 10–11 months, respectively. The other values were determined in commercial samples, of unknown age (From Ruegg and Blanc 1981)

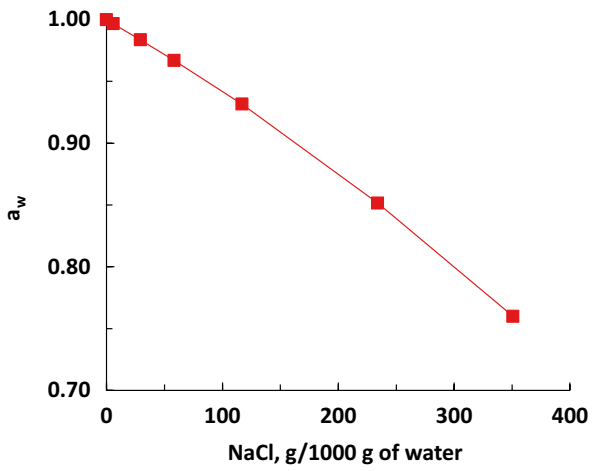


Fig. 11.3 The relationship between salt concentration and a_w (Redrawn from Hardy 1986)

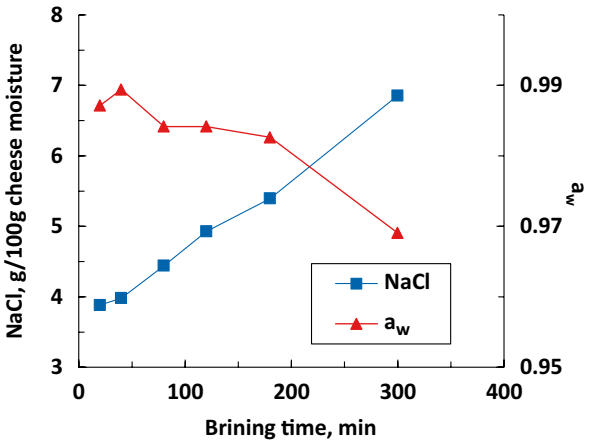


Fig. 11.4 Influence of the duration of brining at 14 ° C in a 20 % NaCl brine on the a_w of Camembert cheese. The NaCl concentration and a_w level were determined 15 days after manufacture (From Ruegg and Blanc 1981)

salt-in-water (S:M) is 5 %. Generally, the S:M in Cheddar cheese varies from 4 to 6 %. Most starter bacteria grow in the presence of 3 % but not 4 % NaCl (Chap. 6) and one of the reasons why most cheeses are brine-salted may be allow starter growth and the consequent reduction in lactose level to continue unimpeded in the early days of ripening.

Cheese is either dry-salted (e.g., Cheddar) or brine-salted (most cheeses). In brine-salted cheeses, the salt concentration is influenced directly by the size of the cheese, the concentration of salt in the brine, the temperature of the brine and the length of time for which the cheese is immersed in the brine (see Chap. 9). This will also affect the a_w of the cheese. Data for the effect of brining time on the salt concentration and the a_w of Camembert cheese are shown in Fig. 11.4. The brine normally used contains ~20 % NaCl, has a pH of ~5.2 (adjusted with lactic acid) and a Ca^{2+} content of 0.2 % (adjusted with $CaCl_2$). The pH and Ca concentration simulate the levels in cheese and help to prevent the efflux of lactate and Ca^{2+} from the cheese. The tolerance of starter lactic acid bacteria (SLAB) and non-starter lactic acid bacteria (NSLAB) to salt are discussed in Chap. 6 and below, respectively.

11.5 Oxidation-Reduction Potential

Oxidation-reduction potential (E_h) is a measure of the ability of chemical/biochemical systems to oxidise (lose electrons) or reduce (gain electrons). E_h is generally measured using a platinum electrode coupled with a calomel reference electrode and is expressed in mV. It can also be estimated using indicator dyes which change colour at different redox potentials. A positive value indicates an oxidised state while a negative value indicates a reduced state.

The E_h of milk is about +150 mV, while that of cheese is about −250 mV. The reduction in the E_h of cheese is directly related to the fermentation of lactose to lactic acid by the starter during growth. The exact mechanism by which the E_h is reduced is unclear but is probably connected to the reduction of the small amount of O_2 in the milk to H_2O (or H_2O_2 and then to H_2O) and the reduction of NAD^+ to NADH. Because of these reactions, cheese is essentially an anaerobic system, in which only facultatively or obligately anaerobic microorganisms can grow. Obligate aerobes, like *Pseudomonas*, *Brevibacterium* and *Micrococcus* spp., will not grow within the cheese, even when other conditions for growth are favourable. The bacteria which develop on the surface of cheese are mainly obligate aerobes and are unable to grow within the anaerobic cheese environment.

11.6 pH and Organic Acids

Most bacteria require a neutral pH value for optimum growth and grow poorly at pH values <5.0. The pH of cheese curd after manufacture generally lies within the range 4.5 to 5.3 so that pH is also a significant factor in controlling bacterial growth in cheese. Lactic acid bacteria, especially lactobacilli, generally have pH optima below 7 and *Lactobacillus* spp. can grow at pH 4.0; most yeast and moulds can grow at a pH <3.0, although their optimum ranges from 5 to 7. *B. linens*, which is found on the surface of smear-ripened cheese, cannot grow below pH 6.0. *Micrococcus* sp., which is also commonly found on the surface of soft cheeses, cannot grow at pH 5 and only slowly at pH 5.5.

The efficacy of organic acids as inhibitors of microbial growth is thought to depend on the amount of undissociated acid present and therefore on the dissociation constant (pK_a) and pH. The pK_a values for propionic, acetic and lactic acids, the principal acids found in cheese, are 4.87, 4.75 and 3.08, respectively. The undissociated form of the acid is more inhibitory than the ionised form, so that, at the same pH, lactic acid is the least and propionic the most effective inhibitor. However, the concentration of the acid is also important and, in cheese, lactate is invariably present in cheese curd at much greater concentrations than either of the other two acids. Sometimes, it is thought that the difference between pH 5.2, the pH of a well-made Cheddar cheese, and pH 5.4, the pH of a poorly made Cheddar, is not very great. However, this is not so; pH is a log scale and a difference of 1 pH unit is equivalent to a tenfold difference in the H^+ concentration. The difference in $[H^+]$ between 5.2 and 5.4 is twofold.

11.7 Nitrate

NO_3^-, as KNO_3 (saltpetre) or $NaNO_3$, is added to the milk (20 g/100 L) for some cheeses, especially Dutch-type cheeses, like Gouda and Edam, to prevent the production of early and late gas by coliforms and *Clostridium tyrobutyricum*,

respectively. Much of the NO_3^- is lost in the whey. The maximum amount of NO_3^- permitted in cheese is 50 mg/kg, calculated as $NaNO_3$. The real inhibitor is NO_2^- which is formed from NO_3^- by the xanthine oxidase present in the milk or curd. How NO_2^- acts in preventing microbial growth is not clear. NO_2^- can also react with aromatic amino acids in cheese to produce nitrosamines, many of which are carcinogenic (Chap. 20).

Nitrate does not inhibit the growth of coliforms but changes their metabolism so that less H_2 is produced from formate, a product of sugar metabolism, by the formate dehydrogenase/hydrogenase enzyme system. Nitrate represses the formation of this enzyme system and induces a formate dehydrogenase/nitrate reductase which reduces NO_3 to NO_2, without the formation of H_2.

11.8 Temperature

Generally, the optimum temperature for the growth of bacteria is ~35 °C for mesophiles and ~55 °C for thermophiles. However, thermophilic starters have an optimum temperature of ~42 °C. Psychrophilic bacteria have an optimum temperature below 20 °C but true psychrophiles are not found in cheese. At temperatures below the optimum, growth is retarded.

The temperature of cooking varies for different cheeses and the time the temperature is maintained at higher values will affect the survival of different organisms in the cheese. A comparison of the temperature profiles of Cheddar, Emmental, and a soft cheese is shown in Fig. 11.5. Emmental is heated to 54 °C during manufacture and the temperature is retained above 40 °C for a considerable time, while Cheddar is cooked to 39 °C and soft cheeses are heated at ~35 °C. Other cheeses cooked at a high temperature include Comte, Parmigiano Reggiano and Grana. Little acid production occurs at the maximum cooking temperature for cheeses cooked to 54 °C but the thermophilic starters withstand the temperature and begin to produce acid when the temperature falls below 48 °C. Traditional Emmental cheese is made from raw milk and because of the relatively high temperature of ripening of this cheese [18–24 °C for several weeks to promote the growth of propionic acid bacteria (PAB)], great attention must be paid to the microbial quality of the raw milk.

The temperature of ripening of cheese is also important and is dictated by two opposing forces—on the one hand, the need to control the growth of potential spoilage and pathogenic bacteria and, on the other, the need to promote the ripening reactions and the growth of the secondary microflora in the case of soft and Swiss-type cheeses. Higher temperatures promote faster ripening by the starter and non-starter microorganisms but also allow the growth of spoilage and pathogenic bacteria. Generally, Cheddar cheese is ripened at 6–8 °C while surface-ripened cheeses, like Camembert and bacterial smear-ripened cheeses, are ripened at 10–15 °C. Emmental cheese is ripened initially for 2–3 weeks at a low temperature (~12 °C), after which the temperature is increased to 20–24 °C for 2–4 weeks to promote the growth of propionic acid bacteria and the fermentation of lactate to propionate

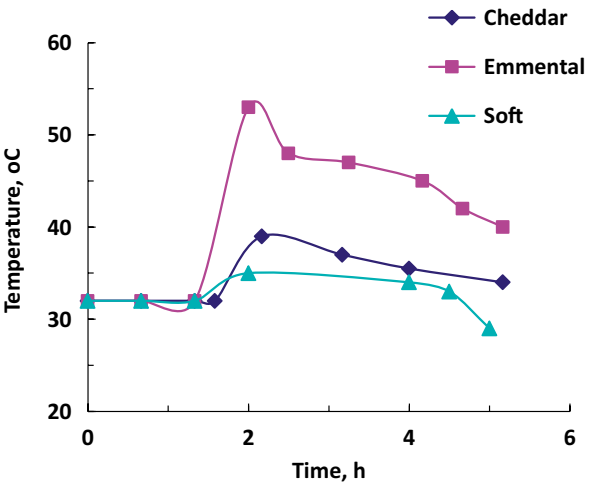


Fig. 11.5 Time-temperature profile of Cheddar, Emmental and a soft cheese during manufacture

and acetate; the temperature is then reduced to ~4 °C. For soft cheeses, the humidity of the environment is also controlled to prevent excess evaporation of moisture from the cheese surface.

Increasing the temperature of ripening is probably the simplest and most cost-effective way of accelerating the ripening of cheese (Chap. 12). This will also increase the rate of growth of other bacteria which may be present.

11.9 Growth of Starter Bacteria in Cheese

The initial number of SLAB in cheese milk ranges from about 10^5 to 10^7 cfu/ml, depending on the level of inoculation of the starter. Growth of the starter during cheese manufacture results in final numbers of ~10^9 cfu/g of cheese within one day. During ripening, starter organisms dominate the microflora of cheese but most die off and lyse relatively rapidly (Fig. 11.6). In the case of Cheddar cheese, the rate of lysis depends on the strain, and in the case of Comté cheese, the rate of lysis of *Sc. thermophilus* is faster than that of *Lb. helevticus*. Many artisanal cheeses, especially Spanish varieties, are made without the intentional addition of a starter. In these cheeses, lactococci also comprise the major part of the microflora and, except for La Serena, also show significant rates of lysis during ripening (Fig. 11.7). The reason for the slow rate of lysis in La Serena cheese may be due to the relatively low salt level in that cheese during the early weeks of ripening.

Once lysis occurs, intracellular enzymes, particularly peptidases, are released, which hydrolyse the caseins and fat to amino acids and fatty acids, which are the precursors of the flavour compounds in cheese (see Chaps. 12 and 13). Starters vary in their ability to lyse—some strains lyse relatively quickly while others lyse slowly.

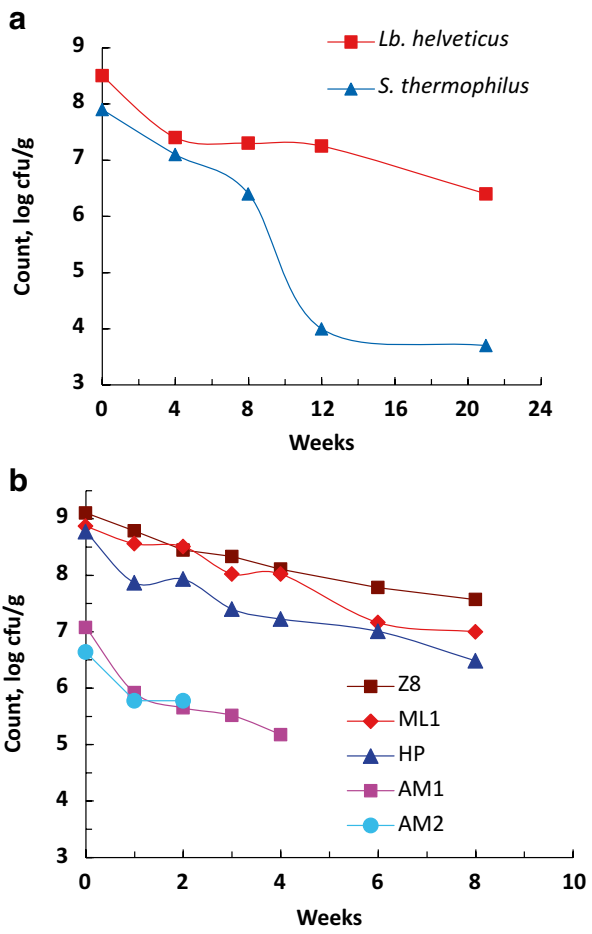


Fig. 11.6 Changes in the numbers of (**a**) *Streptococcus thermophilus* and *Lactobacillus helveticus* during the ripening of Comté cheese and (**b**) different strains of *Lactococcus lactis* during the ripening of Cheddar cheese (From Beuvier, personal communication and Martley and Lawrence 1972)

Lysis is caused by an intracellular muraminidase which hydrolyses the bacterial cell wall peptidoglycan. This enzyme is under stringent regulation, otherwise the cells would not grow. Generally, *Lc. lactis* subsp. *cremoris* strains lyse faster than *Lc. lactis* subsp. *lactis* strains which may partly explain why the former is thought to produce a better flavoured cheese than the latter. Lysis is influenced by several factors, including the level of salt and the presence of prophage, which can be induced by cooking. The presence of small numbers of lytic phage may also have a role in lysis. Cheese made with a fast-lysing starter will ripen more rapidly than one made with a slowly-lysing strain.

There is some evidence (Ganesan et al. 2006, 2007) that lactococci enter a metabolically active but non-culturable state for long periods (up to 3.5 years), after the carbohydrate in the medium is used up. During this period, they can metabolise amino acids to fatty acids, some of which, e.g., 2-methyl butyric acid production

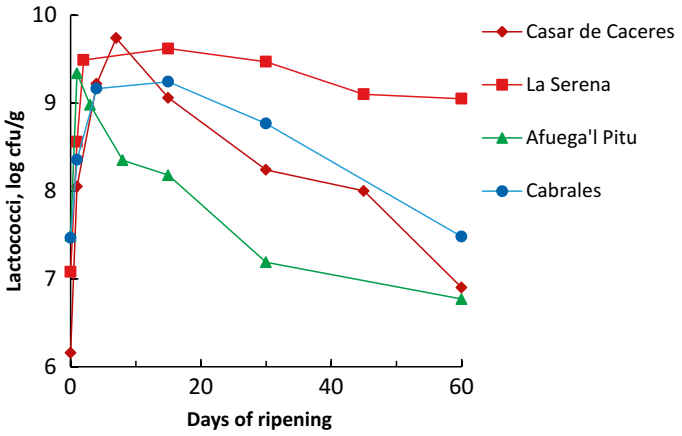


Fig. 11.7 Changes in the numbers of lactococci during the ripening of several artisanal Spanish cheeses (From Nunez 1978; del Pozo et al. 1985; Poullet et al. 1991; Cuesta et al. 1996)

from leucine, are important in cheese flavour formation. A population of non-culturable but metabolically active cells also occurs in Emmental cheese during ripening (Falentin 2012).The contribution that such cells make to cheese ripening is not clear and should be investigated.

11.10 Growth of Non-Starter Lactic Acid Bacteria in Cheese

Most, if not all, cheeses, whether made from raw or pasteurised milk, contain adventitious, NSLAB. These are mainly facultatively heterofermentative lactobacilli (FHL), especially *Lb. casei*, *Lb. curvatus, Lb. paracasei*, *Lb. plantarum* and *Lb. rhamnosus,* which ferment hexoses homofermentatively to lactic acid and pentoses heterofermentatively to lactate and acetate. NSLAB are also called mesophilic lactobacilli to distinguish them from the thermophilic lactobacilli used as starters. Obligately heterofermentative lactobacilli, e.g., *Lb. brevis* and *Lb. fermentum,* and obligately homofermentative pediococci, e.g., *Pediococcus pentosaceus* and *P. acidilactici* are found occasionally as NSLAB in cheese. Many of these species are also present in natural whey cultures (see Chap. 6). The dominant species of FHL found in most cheeses are *Lb. paracasei* and *Lb. plantarum* and generally several strains of each species are present. In Cheddar cheese, an average of 7 strains are present, and, in addition, there is some evidence that a succession of strains occurs during ripening (Fitzsimons et al. 2001). *Lb. rhamnosus* is an important component of NSLAB in New Zealand Cheddar cheese (Crow et al. 2001).

The taxonomy of *Lb. casei* and *Lb. paracasei* is controversial. The type strain of *Lb. casei*, ATCC 393, is actually a strain of *Lb. zeae* and many strains identified as *Lb. casei* do not hybridise with it, The Judicial Commission of the International

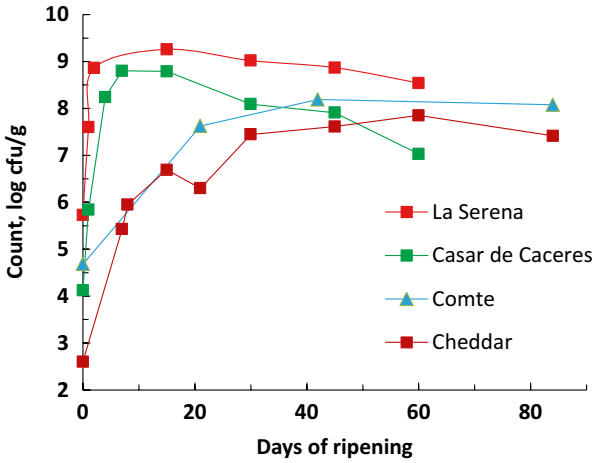


Fig. 11.8 Growth of mesophilic lactobacilli (mainly facultatively heterofermentative) in four different cheeses during ripening (From del Pozo et al. 1985; Poullet et al. 1991; Demarigny et al. 1996; Jordan and Cogan 1993)

Committee on Systematics of Bacteria (Anon 2008) ruled that this was still correct from a nomenclature viewpoint and rejected the proposal of Dellaglio et al. (2002) that *Lb. casei* ATCC 334 be considered as the new type strain of *Lb. casei*. *Lb. paracasei* was created by Collins et al. (1989) for strains of *Lb. casei* which did not hybridise with *Lb. casei* ATCC 393 but did hybridise with *Lb. casei* NCDO 151, a strain which is closely related to *Lb. casei* ATCC 334. The Judicial Commission also found that the name, *Lb. parcasei*, was legitimately published. This is quite confusing and many cheese isolates identified as *Lb. casei* are probably strains of *Lb. paracasei*. The genomes of 34 dairy, plant, human and animal strains of *Lb. paracasei* have been determined (Smokvina et al. 2013). The core genome consists of the cell envelope proteinase, the capacity to produce branched chain fatty acids and factors associated with host-microbe interactions, e.g., pili, while the variome consists of hypothetical proteins, phages, plasmids, cell-surface proteins, transporters and enzymes involved in EPS biosynthesis.

SLAB are found in high numbers (>10^8 cfu/g) in all freshly made cheese. In contrast, the initial number of NSLAB varies considerably from about 100 cfu/g in Cheddar cheese to 10^6 cfu/g in Casar de Cáceres (Fig. 11.8) and, within the first few week of ripening, they grow relatively quickly to high numbers (~10^8 cfu/g) in all cheeses at a rate which depends on the ripening temperature, the moisture content and the availability of a suitable energy source. Cheddar is the only cheese in Fig. 11.8 which is made from pasteurised milk, which partly explains the low initial number of NSLAB in this cheese, since NSLAB are partially but not completely inactivated by pasteurisation. In raw milk cheese, the number of NSLAB in the curd is higher, their growth is faster and the population is more heterogeneous. NSLAB grow much more rapidly in Casar de Cáceres and La Serena cheese than in Comté

or Cheddar cheese due to the higher moisture content of the first two cheeses compared to Comté and Cheddar cheese. The higher rate of growth of NSLAB in Comté cheese compared with Cheddar is due to the higher ripening temperature of Comté (3 weeks at 14 °C, followed by 9 weeks at 18 °C, before the temperature is reduced to 7 °C) compared to Cheddar (6–8 °C throughout ripening). The higher temperature used in ripening Comté cheese is to promote the growth of PAB, which are responsible for eye formation in this cheese.

The ultimate source of NSLAB in cheese is not clear. Small numbers survive pasteurisation and the high cooking temperature (52–54 °C) used in producing hard cheeses, like Emmental, which is traditionally made from raw milk, suggesting that raw milk is the source. There is also some evidence that biofilms formed on processing equipment, e.g., raw milk silos, ultrafiltration units, if present, cheddaring belts and cheese towers, can be potent sources, with numbers ranging from 100 to 10,000 cfu/cm^2 (Agarwal et al. 2006).

NSLAB are acid-tolerant and most of them are also salt tolerant, e.g., 90 % of the strains of *Lb. casei, Lb. plantarum* and *Lb. curvatus* isolated from Cheddar cheese grow in the presence of 6 % NaCl (Jordan and Cogan 1993). In contrast, SLAB are sensitive to this level of salt. The tolerance of NSLAB to salt and acid and their ability to grow in the absence of oxygen imply that they should grow well in cheese, provided an energy source is present. The energy source used by them in cheese is thought not to be lactose, since at the time of exponential growth of NSLAB, lactose is not present, unless the salt level is very high. However, the amount of lactose required to sustain 10^6 cfu/g is small (~1 mg/g) and so trace amounts of lactose in the cheese could be a potential source of energy. Other possible sources have also been suggested, including citrate, amino acids, and the sugars present in the glycoproteins of the milk fat globule membrane or in RNA (ribose) or DNA (deoxyribose), produced from lysis of the SLAB. Diaz-Muniz and Steele (2006) have shown that *Lb. casei* ATCC 334, which was isolated originally from cheese, can use citrate as an energy source in the presence of limiting, but not excessive, concentrations of galactose in a chemically defined medium and in the presence of both limiting and excess concentrations in a cheese extract medium. Neither lactose nor glucose could replace galactose. However, free galactose is unlikely to be present in cheese unless *Sc. thermophilus* is used as a starter. In another study, extracts of 2-, 4- and 6-month-old Cheddar cheese supported the growth of this organism to final cell densities of 10^7–10^8 cfu/ml implying that adequate energy source(s) are present in the ripening cheese (Budinich et al. 2011). Recently, Lazzi et al. (2014) showed that *Lb. rhamnosus*, growing in a cheese broth, could oxidise pyruvate, which can be produced from lactate, citrate or amino acids, to acetate with the concomitant production of ATP and therefore growth.

Despite extensive study, the role of NSLAB in the development of cheese flavour is unclear; some studies have shown positive effects, others negative effects and others no effect. The catabolism of amino acids, particularly the aromatic amino acids (phenylalanine, tyrosine and tryptophan), the branched chain amino acids (leucine, isoleucine and valine), and the S-containing amino acid, methionine, is important in the development of cheese flavour (see Chaps. 12 and 13). A prerequisite for this is lysis of the cell to release the intracellular enzymes responsible for flavour develop-

ment. In contrast to SLAB, NSLAB lyse very slowly in cheese (Fig. 11.8) and so their intracellular enzymes are released only slowly into the cheese matrix. Evidence for the lysis of NSLAB can be inferred from the finding that a progression of different strains occurs during cheese ripening (Fitzsimons et al. 2001). It should also be remembered that the high numbers of NSLAB found in cheese would have considerable metabolic activity in their own right without having to lyse.

In Cheddar cheese, NSLAB transform the L-lactate, produced by the SLAB, to D-lactate. This is also likely to occur in other cheeses. A racemic mixture of both isomers is eventually formed. Some NSLAB can also transform lactate to acetate on the cheese surface in the presence of O_2. This will result in a sharper taste of the cut surfaces of the cheese, especially if the cut surfaces remain uncovered for several hours. Pediococci are much more active than lactobacilli in forming acetate from lactate but are found only in small numbers in cheese.

Bacteriocins (see Chap. 6) can prevent the growth of NSLAB, e.g., Lacticin 3147, a bacteriocin produced by a strain of *Lc. lactis*, isolated from a kefir grain, prevented the growth of NSLAB in Cheddar cheese ripened for 6 months at 8 °C (Ryan et al. 1996, 2001). The bacteriocin-producing strain was not very useful as a starter culture as it produced an off-flavour in milk. Although the cheeses were not evaluated for flavour, there were no differences in the usual indices of protein breakdown in the cheese, viz., water-soluble N, phosphotunstic acid-soluble N and free amino acids. Lacticin 3147 is a two peptide bacteriocin, containing the unusual amino acids, D-alanine and lanthionine; its production is encoded on a conjugative plasmid, which also encodes phage resistance. The plasmid was transferred by conjugation to several *Lc. lactis* strains. Cheese made with the transconjugants had 100-times (2 log cycles) less NSLAB than the control cheese over a 6 month ripening period (Ryan et al. 2001). This was correlated with the presence of the bacteriocin in the cheese and the cheese graded slightly better than the control cheese, which was described as somewhat bitter, inferring that some NSLAB produce off-flavours in cheese. In another study, cheese was made using *Lc. lactis* CNRZ 481, which produces the bacteriocin, pediocin PA-1, as an adjunct to the starter culture *Lc. lactis* HP. The adjunct did not affect acid production by the starter strain; instead it increased lysis of the starter and inhibited the growth of NSLAB (O'Sullivan et al. 2003). Both the experimental (made with strain CNRZ 481 and HP) and control cheeses (made with HP only) graded well but the experimental cheese had a nicer flavour than the control. For more information on NSLAB in cheese see the reviews of Beresford et al. (2001), Broadbent et al. (2003), Beresford and Williams (2004) and Steele et al. (2006).

11.11 Spatial Development of Bacteria in Cheese

Generally, about 90 % of the bacteria present in the milk are retained in the curd during cheesemaking; the remaining 10 % are lost in the whey. These bacteria are immobilised or entrapped in the curd when it coagulates and grow as colonies in the three-dimensional cheese matrix. Until recently, little study of colony formation in

cheese has been undertaken because of the lack of suitable techniques. Jeanson et al. (2011), using confocal microscopy, a starter, which produced a green fluorescent protein, and a model cheese in gel cassettes, studied colony formation in cheese and showed that larger colonies were produced at lower inoculation levels. Colonies were also shown to have a Poisson distribution and microgradients in pH did not occur around them. These workers also showed that, in a cube of cheese, there will be only a short distance between colonies at high inoculation rates and greater distances at lower inoculation levels, and that final cell numbers were not affected by the inoculation level. The normal inoculation rate is about 1×10^7 cfu/ml milk which gives a theoretical inter-colonial distance of 26 μm, which compares quite well with the experimental value of 34 μm found at an inoculation rate of 9.6×10^6 cfu/ml.

Cheese ripening is the result of the action of enzymes on fat and protein, ultimately forming the compounds which cause flavour to develop in the cheese (see Chap. 12). As the starter bacteria are immobilised in the cheese, it follows that diffusion of substrates to (and products from) the colonies must occur for flavour to develop. Diffusion of dextrans of different molecular masses (4.4, 70 and 155 kDa) in cheese and agar has been studied in model systems and the results showed that the larger molecular mass dextrans were able to penetrate colonies immobilised in both systems (Floury et al. 2013). Diffusion was faster in cheese than in agar. The shape of the colony was also different in cheese (spherical) than in agar (lenticular) indicating that the physical pressure exerted on the colony in cheese was similar in all directions (isotropous).

11.12 Non-Starter Lactic Acid Bacteria as Adjunct Cultures

Considerable effort has been expended in New Zealand in selecting and developing NSLAB, particularly *Lb. paracasei* and *Lb. rhamnosus*, which improve the flavour of Cheddar cheese (Crow et al. 2001; Coolbear et al. 2008). These are added to the cheese milk as a mixture of 2–4 strains at an initial level of 300–1000 cfu/ml of milk. At the same time, factory hygiene was improved so that the initial count of NSLAB in the cheese was <10 cfu/g. The idea behind this development is that the deliberately added NSLAB would dominate the 'wild' NSLAB microflora during ripening. Selection of strains that would dominate the 'wild' NSLAB may be the key to using NSLAB to improve cheese flavour, since some studies have shown that added NSLAB do not dominate the 'wild' NSLAB during ripening (Broadbent et al. 2003). Another point worth considering is the ability of the added NSLAB to lyse.

Nowadays, it is also common to add strains of *Lb. helveticus* or *Sc. thermophilus* as adjuncts to mesophilic starters to improve the flavour of Cheddar cheese. They give a more "rounded" flavour to the cheese and are also able to continue to produce acid if the mesophilic culture is infected with phage. There is one caveat: galactose may be present at significant levels in the cheese since most strains of *Sc. thermophilus* are unable to utilise galactose and excrete it into the curd during growth.

The addition of yeasts, particularly *Debaryomyces hansenii* and *Yarrowia lipolytica*, with known proteolytic and lipolytic activities, has also improved the flavour of South African Cheddar cheese (Ferreira and Viljoen 2003); this finding does not appear to have been studied in other countries.

11.13 Enterococci

Enterococci can be found at levels in excess of 10^7/g in many cheeses, particularly those made around the Mediterranean, and are considered to be essential for flavour development. Many of these are artisanal, raw milk cheeses, made at farm-house level, without the deliberate use of starters. Enterococci can metabolise lactose and their tolerance to salt and heat make them ideal candidates as starters.

There is considerable debate on whether enterococci should be considered to be pathogens (Franz et al. 2003; Foulquie Moreno et al. 2006; Fisher and Phillips 2009). During the past few decades, they have been incriminated as the cause of several diseases, including bacteremia, urinary tract infections and endocarditis. Many strains are promiscuous and easily pick up plasmids encoding antibiotic resistance, e.g., vancomycin. Many of these plasmids are also conjugative and are easily transferred naturally from cell to cell. Vancomycin is a glycopeptide antibiotic which acts by inhibiting cell wall biosynthesis, the incidence of vancomycin-resistant enterococci (VRE) in hospitals has increased dramatically. The use of avoparcin, which is also a glycopeptide antibiotic, as a growth promoter in animal feed has been incriminated in the increased occurrence of VREs in farm animals, including pigs and poultry. Because of this, the use of avoparcin has been banned in several European countries. Many VREs are difficult to deal with because they are also resistant to other therapeutic antibiotics, implying that alternative antibiotic therapy may not be available. In this context it is interesting that some SLAB, e.g., *Leuconostoc* spp. and NSLAB, e.g., *Lactobacillus* and *Pedicoccus* spp., are intrinsically resistant to vancomycin.

There is little information on how rapidly *Enterococcus* spp. grow in milk but in Cheddar cheese they grow very well during manufacture and remain fairly constant during ripening (Fig. 11.9). These trials involved the separate evaluation of three strains of *Ec. faecalis* and one strain each of *Ec. faecium, Ec. durans* and *Ec. casseliflavus* in duplicate trials. There was little difference in the rate of growth of either the strains or the species and the data for all strains tested was amalgamated. In addition, there was no statistical difference in the grading of control and enterococci-containing cheese. In Trial 2, a small number of enterococci were present in the control but the levels were too low to have any effect on flavour development.

Enterococci also remain fairly constant in other cheeses, e.g., artisanal Spanish and Italian cheeses (Fig. 11.10). Casar de Cáceres and La Serena are made from raw ewes' milk and Afuega'l Pitu from raw cows' milk; no deliberate inoculation with starters occurs in any of these cheeses. Pecorino Umbro is made from pasteurised ewes' milk and a mesophilic starter is deliberately added. A surface microflora

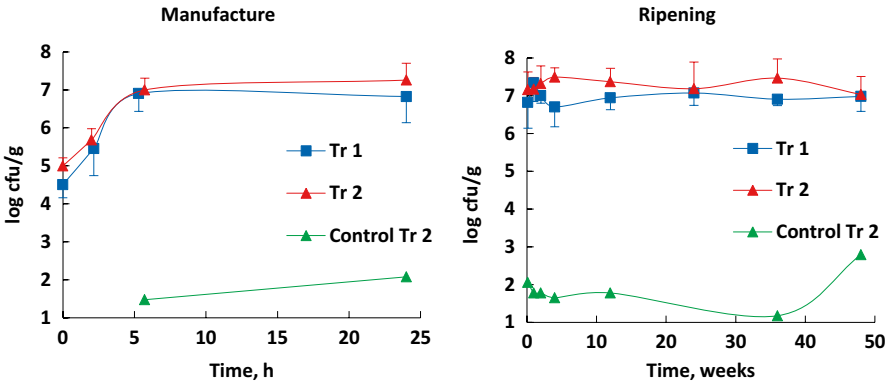


Fig. 11.9 Growth of a 6-strain cocktail of enterococci (3 strains of *Ec. faecalis* and 1 strain each of *Ec. faecium, Ec. durans* and *Ec. casseliflavus*) during the manufacture and ripening of Cheddar cheese. Two trials were conducted and the data are plotted as the average ±s.d. No enterococci were found in the control of Trial 1 at any stage during manufacture or ripening; small numbers were found in the control of Trial 2 (From Rea et al. 2004)

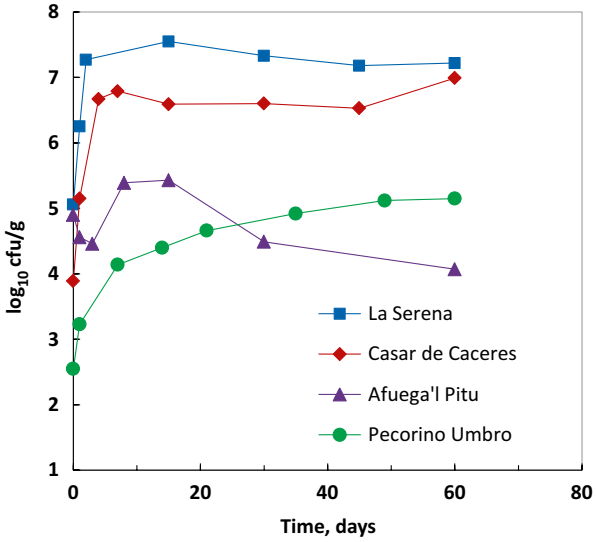


Fig. 11.10 Growth of enterococci in La Serena, Casar de Cáceres, Afuega'l Pitu and Pecorino Umbro cheeses during ripening. The first point on each line is the count in the milk (Redrawn from del Pozo et al. 1985; Poullet et al. 1991; Cuesta et al. 1996; Gobbetti et al. 1997)

develops on some of these cheeses but the counts in Fig. 11.10 are from the internal part of each cheese. The first point on each line in the graph is the number of enterococci present in the milk at the beginning of manufacture. The data show that considerable growth occurs during manufacture and during the first days of ripening, after which they remain constant, except for Afuega'l Pitu cheese, in which the

numbers decreased. The numbers of *Enterococcus* in Casar de Cácares and La Serena cheese were well in excess of 10^6/g and may have contributed to flavour development. It is sometimes difficult to distinguish between lactococci and enterococci. However, the numbers of enterococci shown in Fig. 11.10 are reliable as media selective for enterococci were used to enumerate them.

11.14 Secondary Microorganisms in Ripening Cheese

Many cheese varieties contain a secondary, non-lactic microflora, the function of which is to produce some specific characteristic change in the cheese, e.g., surface growth in the case of surface-ripened cheeses or the production of CO_2, propionate and acetate in the case of some Swiss varieties, e.g., Emmental and Comté. CO_2 is responsible for eye formation in the latter cheeses and propionate gives them a sweet flavour. In all of these cheeses, flavour development is dominated by the metabolic activity of the secondary flora during ripening.

Several microorganisms are involved as secondary starters, including bacteria (*Agrococcus*, *Arthrobacter, Brevibacterium, Brachybacterium, Corynebacterium, Microbacterium, Propionibacterium, Staphyloccocus* and *Micrococcus* spp.), yeast (*Kluyveromyces marxianus* and *Debaryomyces hansenii*) and moulds (*Geotrichum candidum*, *P. camemberti* and *P. roqueforti*). All of these microorganisms are not present in every cheese and, except for *Propionibacterium* spp. and *P. roqueforti*, all of them develop only on the cheese surface. All the bacteria are Gram-positive although small numbers of Gram-negative organisms are isolated occasionally (see below). These are considered more fully in the description of the microbiology of the various cheeses below.

11.15 Molecular Methods of Identification

Cheese is a very dynamic environment for the growth of microorganisms and up to 10^9 LAB/g can be found in them during ripening. In many cheeses, particularly surface-ripened ones, numerous different species are involved, some of which are very difficult to identify. Considerable efforts have been made to understand the microbiology of cheese and categorically identify all the microorganisms present. Traditionally, selective and non-selective media were used to isolate the different microorganisms in cheese and the colonies were then purified and identified. In recent times, molecular methods have been developed and applied to either organisms isolated from the cheese, in culture-dependent methods or to DNA (and RNA) extracted directly from the cheese, in culture-independent methods. Most workers agree that the results obtained from culture-dependent and culture-independent methods complement each other. The common culture-dependent methods are pulsed field gel electrophoresis (PFGE) and randomly amplified polymorphic DNA (RAPD) and the common culture

independent methods involve direct extraction of DNA and RNA from various cheeses, amplification of 16S rRNA genes by PCR and their separation by denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE) or single strand conformational polymorphism (SSCP). These techniques have been applied to the milk for cheesemaking, whey starters and cheese during ripening, including NSLAB and the surface microflora, and have been reviewed by Ogier et al. (2004), Randozzo et al. (2009) and Quigley et al. (2011).

More recently, so-called next generation, high-throughput, sequencing analysis has been used. This technique can give useful information on the microbial composition of the cheese, on strain composition, or on the distribution of particular genes within the cheese, depending on the amplicons used as primers for the DNA or RNA extracted from the cheese (for reviews see Bokulich and Mills 2012; Ercolini 2013). These techniques have resulted in the identification of genera not previously found in cheese, e.g., *Marinilactibacillus* and *Stenotrophomonas* (Delbès et al 2007), *Prevotella* and *Faecalibacteria* (Quigley et al. 2012) and the existence of "house"-specific microbes on washed-rind cheeses, which may have a role in determining site-specific products (Bokulich and Mills 2013). Marinilactibacilli are slightly halophilic, alkaliphilic LAB, which are found in marine environments and have also been isolated from spoiled dry-cured hams while *Stenotrophomonas maltophilia* is an emerging multidrug-resistant global opportunistic pathogen. Both *Prevotella* and *Faecalibacteria* are strict anaerobes; *Prevotella* are commensals of the rumen and hind gut of animals while *Faecalibacterium* are the dominant organisms in the human gut. In addition, they also form butyrate, D-lactate and formate, which are commonly found in cheese. The influence of such bacteria on the flavour of cheese has not been studied. This technique has also been used to study the growth of various pathogens, starters and the indigenous microflora in raw milk cheese (Masoud et al. 2012).

11.16 Development of Microorganisms in Different Cheeses

Cheeses are commonly divided into hard, semi-hard and soft cheeses, which primarily reflects the moisture content of the cheese, with hard cheeses containing ~38 % moisture, semi-hard containing 42 % moisture and soft cheeses containing 50 %. The higher the moisture content, the faster is the growth of different microorganisms in the cheese and consequently the quicker the flavour develops. The development of different microorganisms in several, well-known examples of these cheeses is examined below.

11.16.1 Cheddar Cheese

Cheddar is a hard, dry-salted cheese made with a mesophilic starters, which grows rapidly in the cheese from an initial level of ~10^7 to 10^8 or 10^9/g at salting (about 5.5 h after inoculation). Nowadays, thermophilic cultures are also added at low levels

(see Chap. 6). The cheese evolved in the village of Cheddar in Somerset, England, but is now made all over the world. In the past, Cheddar was generally ripened at 6–8 °C but the tendency now is to ripen at a slightly higher temperature, which results in faster proteolysis and lipolysis and consequently faster flavour production. The manufacture of Cheddar cheese was a very labour intensive process but nowadays the process is highly mechanized and automated. Vacuum packing in rectangular blocks and rapid cooling of the blocks before the beginning of ripening is a common feature of commercial Cheddar production today.

Normally, the fermentation of lactose by starter LAB in cheese is rapid and is complete within 1 day of manufacture. However, in dry-salted cheeses, like Cheddar, a relatively large amount of lactose (~10 g/kg of cheese) is present in the curd after overnight pressing. This is due to inhibition of the metabolism of the starter cultures by the salt and the relatively low pH. The S:M in Cheddar cheese determines the subsequent rate of lactose fermentation by the starters; a high S:M level reduces the rate while a low level increases it, e.g., at a S:M of 4.1, the fermentation is virtually complete in 7 days while at a S:M of 6, it takes > 50 days (Fig. 11.11). There is little difference in the rate of lactose utilisation at intermediate S:M levels, around 5. These values are only indicative and vary depending on the sensitivity of the particular culture to salt. *Lc. lactis* subsp. *cremoris* is much more sensitive to salt than *Lc. lactis* subsp. *lactis* strains. The former cannot grow in the presence of 4 % salt while the latter can. Salt may also uncouple acid production from growth.

The development of starter and NSLAB in a Cheddar cheese during ripening at 6 °C is shown in Fig. 11.12. After 2 months, the number of lactococci was not measured because NSLAB grow on the medium (LM17) used to estimate the numbers of lactococci. Mesophilic starters generally die out relatively rapidly during the first

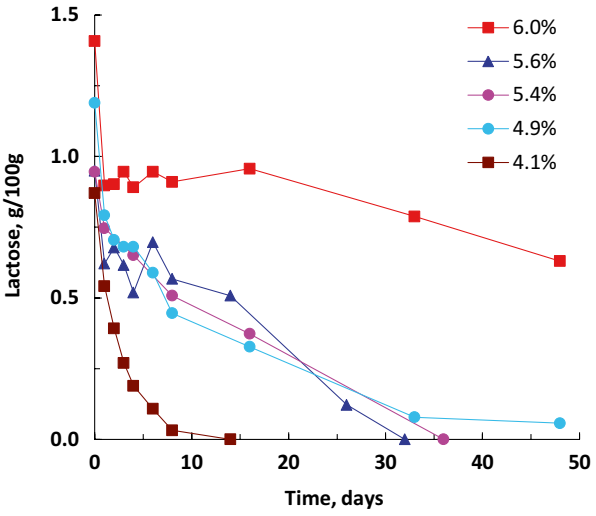


Fig. 11.11 Effect of salt-in-moisture (S/M%) on lactose metabolism in Cheddar cheese, made with *Lc. lactis* ssp. *cremoris* C13 and 266, ripened at 12 °C (From Turner and Thomas 1980)

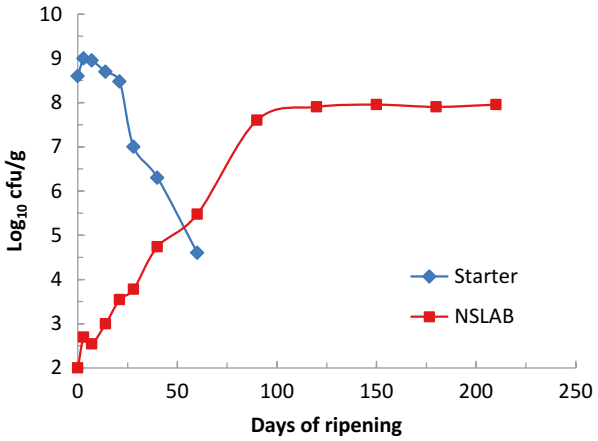


Fig. 11.12 Development of starter and non-starter lactic acid bacteria in Cheddar cheese ripened at 6 °C (Cogan, unpublished data)

few weeks of ripening (Fig. 11.6) but the rate is strain-dependent and probably reflects the ability of the strain to withstand the cooking temperature of the cheese and its ability to lyse. Phage may also be involved in reducing the number of cells. NSLAB grow relatively rapidly in Cheddar cheese during ripening from a low initial number (~10^2/g) to a final number of 10^7–10^8/g after 15 weeks, with a generation time of 8.3 days at 6 °C.

NSLAB also transform L-lactate to D-lactate, eventually producing a racemic mixture. This transformation has no effect on the flavour of the cheese but Ca D-lactate is insoluble and can precipitate as small, white crystals throughout the cheese late in ripening. Some consumers consider this a defect in the cheese. Strains of *Lb. curvatus* and *Lb. fermentum* are important in forming crystals of Ca D-lactate in ripening cheese (Somers et al. 2001). An example of the growth of NSLAB and the racemisation of L- to D-lactate during Cheddar cheese ripening is shown in Fig. 11.13. Metabolism of residual lactose by the starter lactococci is also shown in Fig. 11.13.

11.16.2 Swiss-Type Cheeses

Swiss-type cheeses, of which Emmental and Comté are important examples, are hard cheeses that are characterised by the development of eyes in the cheese during ripening due to the fermentation of lactate to propionate, acetate and CO_2 by propionic acid bacteria (PAB). In the past, milk itself was the source of the PAB but nowadays carefully selected strains of PAB are deliberately added to the milk with the starter culture. The inoculum is usually small (only a few hundred cells per ml of milk) but this number is sufficient for the development of a more regular propionic fermentation. The most common species of PAB used is *Propionbacterium*

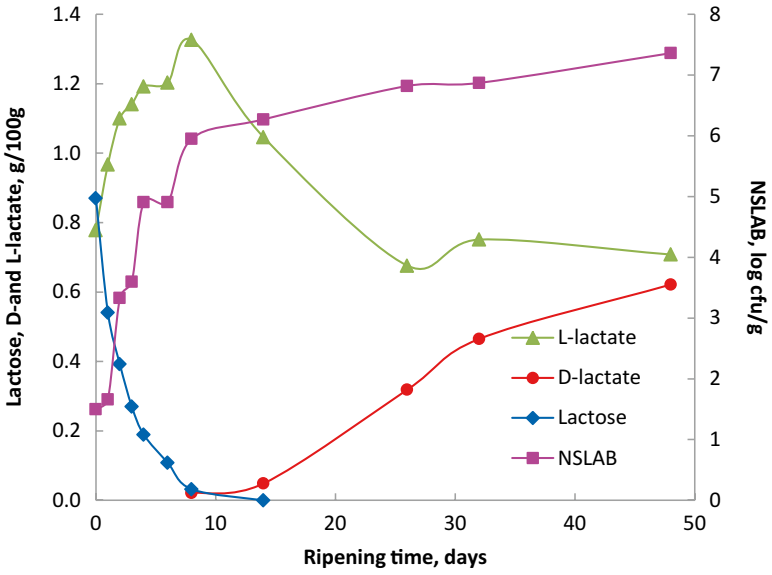


Fig. 11.13 Relationships between non-starter lactic acid bacteria (NSLAB), production of L- and D-lactate and metabolism of lactose in Cheddar cheese during ripening at 12 ° C. The S:M% was 4.1 (From Turner and Thomas 1980)

freudenreichii and the strains used are commonly grown in sodium lactate broth at 30 °C; the grown culture survives for several weeks when stored at 4 °C.

The eyes are larger in Emmental than in Comté because the former is ripened at a higher temperature. Traditionally, both of these cheeses are made from raw milk using thermophilic cultures consisting of *Sc. thermophilus* and *Lb. helveticus* and little acid is produced in the vat during their manufacture. Nowadays, much Swiss-type cheese is made from pasteurised or thermised milk and *Lb. delbrueckii* subsp. *lactis* has replaced *Lb. helveticus* as the rod starter in Emmental cheese because of its lower peptidolytic activity and less propensity of the resulting cheese to late fermentation. The latter is thought to be due to a more intense propionic fermentation and additional production of CO_2 from decarboxylation of amino acids late in ripening. This is seen mainly as cracks or splits in the cheese because the body of the cheese has become short and crumbly and cannot retain the excessive CO_2 production.

During the initial hours in the press, the lactic acid is produced by *Sc. thermophilus* but, as the temperature and pH decrease, *Lb. helveticus* begins to grow, reaching maximum numbers 12–20 h after the addition of starter. Counts of both *Sc. thermophilus* and *Lb. helveticus* in Comté cheese, and presumably Emmental also, are higher at the periphery than at the centre (Fig. 11.14). In both cheeses, growth of the starter is limited by the high cooking temperature (52–54°C) but growth begins again as soon as the temperature decreases. The temperature falls more rapidly at the periphery than in the centre of the cheese and hence greater bacterial growth (and acid production) occurs at the periphery.

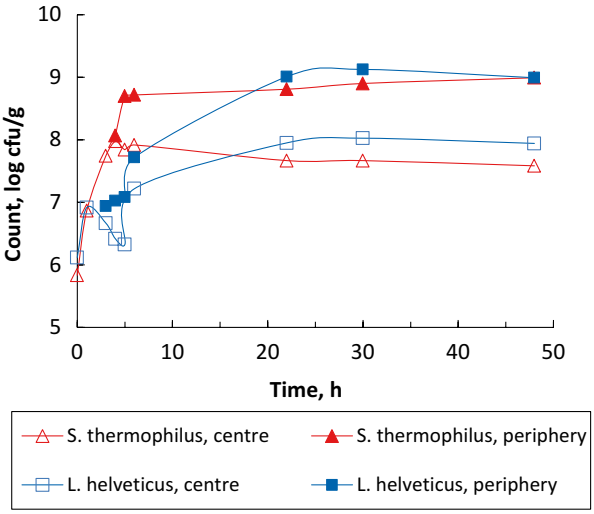


Fig. 11.14 Growth of *Streptococcus thermophilus* (*open triangle, filled triangle*) and *Lactobacillus helveticus* (*open square*, *filled square*) at the centre (*open symbols*) and periphery (*closed symbols*) of Gruyerè cheese during manufacture (From Accolas et al. 1978)

Sc. thermophilus, Lb. delbrueckii subsp. *bulgaricus* and some strains of *Lb. delbrueckii* subsp. *lactis* metabolise only the glucose moiety of lactose and excrete galactose, which, along with any residual lactose, can then be metabolised by *Lb. helveticus* and galactose-utilising strains of *Lb. delbrueckii* subsp. *lactis*, if present. All the lactose is fermented during the first 10 or 12 h of manufacture. The L isomer of lactate is produced by both *Sc. thermophilus* and *Lb. helveticus*, while the D isomer is produced only by the latter organism.

After several weeks ripening at a low temperature (4–14 °C), the cheese is placed in a 'warm room' at 18–24 °C, during which *P. freundreichii* grows and transforms the lactate to propionate, acetate and CO_2, which is responsible for eye formation. Traditionally, natural contamination of the milk was relied upon as the source of the PAB in traditionally made Comté and Emmental but nowadays, selected strains of *P. freundreichii* are deliberately added to the milk with the starter cultures to give an initial count of a few hundred cells per ml of milk. The eyes in Emmental cheese are much larger than in Comté cheese because Emmental is ripened at 22 °C and Comté at 18 °C. PAB are also stimulated by unidentified low molecular mass products of growth of some strains of *Sc. thermophilus* and *Lb. helevticus* (Piveteau et al. 1995).

The pathway of lactate fermentation by PAB is complicated (Fig. 11.15). The classical fermentation involves two separate pathways, in one of which propionate is produced and the other in which acetate is produced. The lactate is first oxidised to pyruvate, two moles of which are reduced to propionate and one mole oxidised to acetate and CO_2. ATP is generated only in the production of acetate. The overall stoichiometry is:

$$3\,\text{Lactate} \rightarrow 2\,\text{Propionate} + 1\,\text{Acetate} + 1\,CO_2$$

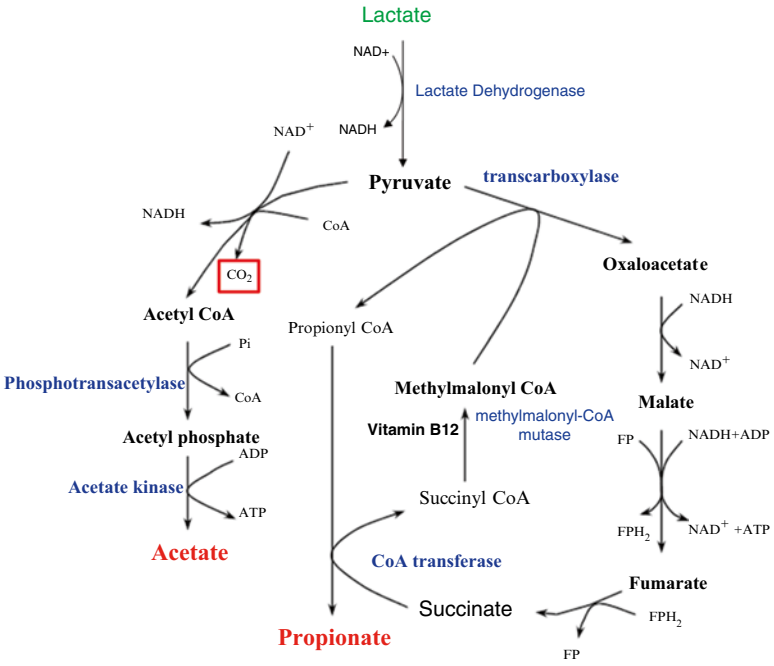


Fig. 11.15 Pathway for the production of propionate and acetate from lactate by *Propionibacterium* spp

Transcarboxylase is the key enzyme in the production of propionate and requires biotin for activity. Generally, PAB are able to metabolise both isomers of lactate but, in a mixture of the two, preferentially metabolise the L rather than the D isomer.

The theoretical ratio of propionate:acetate is 2:1 but the ratio in cheese is often less, averaging 1.4:1 (Crow 1986). PAB co-metabolise lactate and aspartate, which can be produced from casein by proteolysis. Aspartate is metabolised to fumarate and NH_3 by aspartase activity, and the fumarate is then reduced to succinate. In the presence of aspartate, more lactate is metabolised to acetate than to propionate to maintain the redox balance in the cells; the overall effect is to reduce the ratio of propionate:acetate. Strains of PAB with a high level of aspartase produce higher levels of propionate, acetate and CO_2 in cheese than those with a low level. PAB also have a prominent role in lipolysis but not proteolysis in Swiss cheese. For more information on the physiology and metabolism of PAB, see Thierry et al. (2011).

The complex interrelationships between lactose and lactate utilisation and production of propionate and acetate by the PAB in Emmental cheese are shown in Fig. 11.16—lactate production occurs early and is due mainly to the growth of *Sc. thermophilus;* essentially, D-lactate production does not begin until the cheese is in the press and propionate and acetate are not detected until the numbers of PAB have

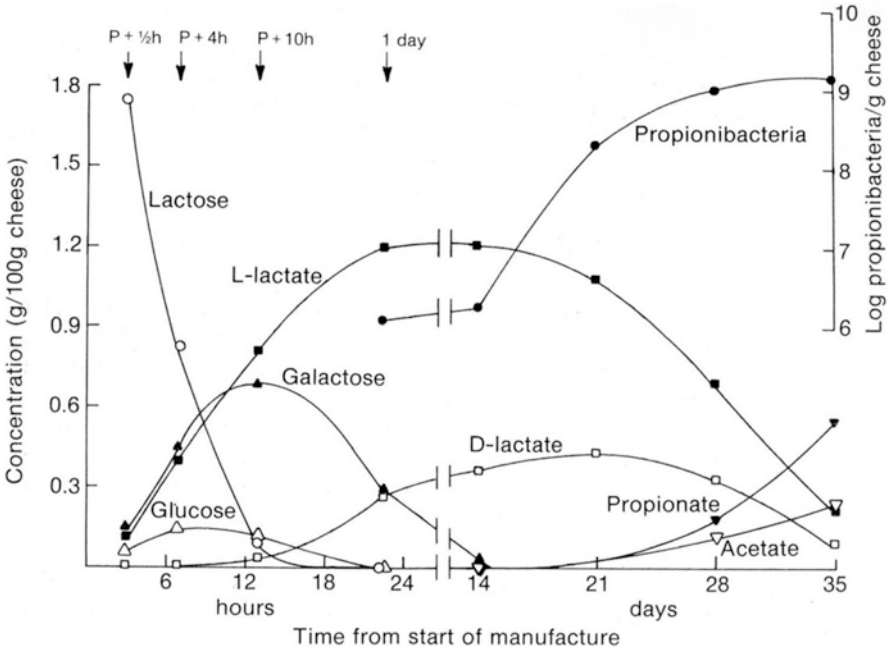


Fig. 11.16 Relationships between the degradation of lactose, production of lactate, growth of propionibacteria and production of propionate and acetate in Swiss-type cheese. The starters used were 0.4 % *Sc. thermophilus* MC and 0.005 % *Lb. helveticus* 5001 (From Turner et al. 1983)

increased significantly. Galactose accumulates early in manufacture, during the growth of *Sc. thermophilus*, but is subsequently used when *Lb. helveticus* begins to grow.

NSLAB, particularly, *Lb. casei* and *Lb. rhamnosus*, slow down the propionic acid fermentation in Emmental cheese (Froehlich-Wyder and Bachmann 2004). This is thought to be due to citrate metabolism since Cit^- mutants of the NSLAB showed much less inhibition. The exact mechanism of this reaction remains unclear. NSLAB are also important in the production of flavour compounds, particularly esters and alcohols, in Swiss-type cheese (Bouton et al. 2009). This was shown by adding *Lb. paracasei* and *Lb. rhamnosus* to microfiltered milk and following their subsequent development and the production of various flavour compounds during ripening. Microfiltration is a membrane filtration technique which can be applied to raw skim milk. It retains the bacteria but allows the casein micelles and the rest of the milk constituents to pass through. Pasteurised cream is added subsequently to the filtrate to bring the fat up to the normal level before the cheese is made. This technique was also useful in studying the effect of the raw milk flora on cheese flavour. Swiss-type cheese was made from raw, microfiltered, pasteurised or pasteurised milk to which the microfiltered retenate was added, the latter effectively simulating a raw milk cheese. Cheese made from milk containing the raw milk flora, i.e., the raw milk cheese and the milk containing the

retentate, had the better overall aroma, which correlated with higher levels of NSLAB, PAB (which were not added deliberately to the milk), and enterococci, implying that the raw milk microflora is important in determining the flavour of this cheese (Beauvier et al. 1997).

Comté cheese is covered by an orange-coloured smear, called the morge, composed mainly of corynebacteria, micrococci and yeast. Levels of ~10^{10}/cm^2 are present in the ripened cheese and it has been calculated that the total number of bacteria in the smear of Comté cheese is the same as the total number in the cheese mass; the species involved do not appear to have been identified.

11.16.3 Parmigiano Reggiano Cheese

This is a very hard PDO (Protected Designation of Origin) Italian cheese made from partly skimmed raw cows' milk using natural whey starters, and is made in the Emilia-Romagna region of Northern Italy. Grana Padano is a similar PDO protected cheese made over a wider area of Northern Italy. The cooking temperature is 55–56 °C and the curd is left in the whey for 40–60 min before being placed in a circular mould. The cheese is brine-salted (27 % NaCl at 16 °C for 20–24 h), before ripening it at 16–18 °C and 85 %RH for a minimum of 2 years. It should not be confused with Parmesan cheese which is a similar cheese made mainly in North America.

The microbiology of Parmigiano Reggiano is shown in Fig. 11.17. The natural whey starter used contained *Lb. helveticus*, small numbers of *Sc. thermophilus* (<100/ml) and, unusually, *Lb. delbrueckii* subsp. *bulgaricus*, which is a starter for

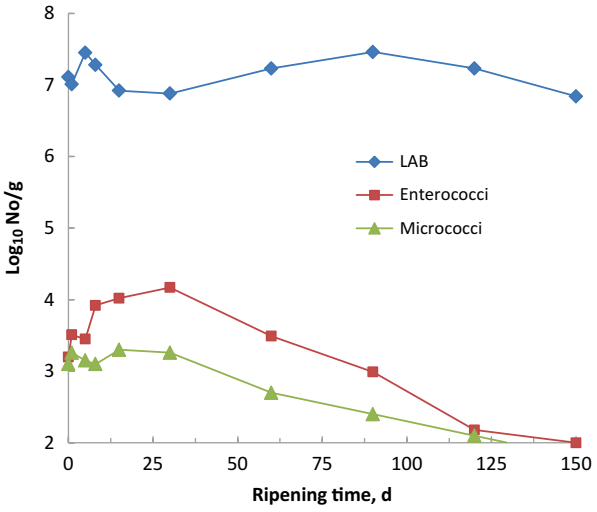


Fig. 11.17 Changes in the numbers of lactic acid bacteria, enterococci and micrococci in Parmigiano Reggiano cheese during ripening. (From Coppola et al. 2000)

yoghurt production, but no lactococci or enterococci (Coppola et al. 2000). The LAB isolated during the first days of ripening were similar to those in the starter; however, after 8 days of ripening they were then supplanted by *Lb. paracase*i subsp. *paracasei, Lb. rhamnosus* and *Pd. acidilactici*. Small numbers of lactococci were identified but only in the early days of ripening. Relatively high numbers (~10^4 cfu/g) of enterococci, identified as *Ec. faecalis* and *Ec. faecium, Kocuria kristinae, K. rosea, Kytococcus sedentarius* and *Arthrobacter agilis*, were present but all of these decreased throughout ripening. *Kocuria* and *Kytococcus* spp. are essentially aerobic organisms and why they were present in an essentially anaerobic system is not clear.

11.16.4 Gouda and Edam Cheeses

Gouda and Edam are the most important semi-hard cheeses and are of Dutch origin. Both are made in similar ways using DL mixed-starter cultures and are brine-salted. The shapes are very different with Gouda being flat and cylindrical and weighing upto 14 kg, usually covered in a yellow wax while Edam is spherical and covered in red wax and weighs 1–2 kg. Both have small eyes due to CO_2 production from citrate by the Cit^+ lactococci and leuconostocs in the DL starter culture. Whey is removed during manufacture and replaced with warm water to reduce the level of lactose in the curd. This means that lactose disappears from the cheese very early in ripening. The milk is usually bactofuged to eliminate spores of *Clostridium tyrobutyricum* and *Cl. butyricum* which cause late gas formation and off-flavour production in the ripening cheese, due to CO_2, H_2 and butyric acid production from lactate. The sediment or sludge from the bactofugation is sterilised and added back to the milk to increase the cheese yield. If the milk is not bactofuged, 0.015 % $NaNO_3$ is added to the milk to prevent the growth of clostridia. NO_3^- is not the actual inhibitor but NO_2^- produced from it by xanthine oxidase, naturally present in the milk. The NO_2^- slows down the germination of the spores until the NaCl has diffused sufficiently into the cheese to serve the same purpose. The changes in pH and the levels of lactate and lactose occurring during manufacture and the early stages of ripening of Gouda cheese are shown in Fig. 11.18. The decrease in pH and the increase in lactate are rapid and the lactose has been completely utilised in 9–10 h. The pH in the ripening cheese continues to rise due to the utilisation of citrate and the increased level of proteolysis by the rennet and starters.

A high-resolution AFLP technique has been used to determine the complex composition of a DL mixed culture, commonly used in Gouda cheese manufacture in the Netherlands (see Chap. 6). One leuconostoc and seven lactococcal lineages were found. This technique was also used to follow what happened to the different lactococcal lineages during cheese manufacture and ripening. The Prt^- *Lc. lactis* subsp. *cremoris* lineage dominated the microbial community during the first phase of manufacture in which fast acid production is the key process. This is in agreement with the notion that 10 % or less Prt^+ strains are sufficient to supply peptides and free

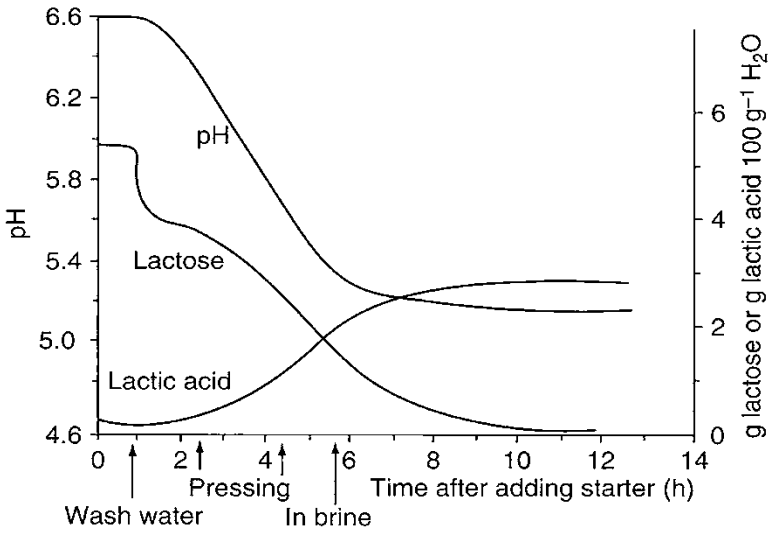


Fig. 11.18 Changes in the levels of lactose and lactate and the decrease in pH in Gouda cheese during manufacture. Note the large decrease in lactose coinciding with washing the curd (From van den Berg et al. 2004)

amino acids to the Prt⁻ component in an actively growing culture (Erkus et al. 2013; Smid et al. 2014). Brining the cheese triggered a sixfold decrease in the Prt⁻ lineage during the first 2 weeks of ripening. Assuming that loss of viability coincides with cell lysis, the Prt⁻ lineage is likely to be the primary supplier of intracellular lipases and proteinases for the initial production of fatty acids, peptides and amino acids in the cheese matrix. The two citrate-utilising *Lc. lactis* subsp. *lactis* lineages displayed much better survival characteristics then the non-citrate utilising components of the culture. This was linked to the capacity of these cells to metabilise arginine by the arginine deiminase pathway and to cheese flavour production. The *Leuc. mesenteroides* lineage was responsible for 37 % *ara*T transcription, encoding aromatic amino tranferase, during the first 24 h of cheese manufacture, even though it comprised only 1.8 % of the culture.

11.16.5 Surface-Ripened Cheeses

Surface-ripened cheeses are subdivided into bacterial- and mould-ripened cheeses, depending on the major microorganisms involved. Bacterial surface-ripened cheeses include Comté, Livarot, Reblochon, Limburger and Tilsit, and are characterised by the development of a red to orange-coloured, smear on the surface. Mould surface-ripened cheeses include the well-known French varieties, Brie, Camembert,

Coulommier and Carre de l'Est and are characterised by a white, felt-like growth on the cheese surface. Bacterial surface-ripened cheeses are also called smear-ripened cheeses, because of the glistening appearance of the cheese surface; they are also called washed-rind cheeses, because their rind is washed several times with brine during ripening, or red-smear cheeses, because of the red-orange colour which characteristically develops on the surface of these cheeses. The ripened cheeses generally have a strong, pungent smell, reminiscent of smelly socks.

Typically, hard, surface-ripened cheeses, e.g., Comté, are made with thermophilic starter cultures and semi-hard, e.g., Tilsit and Pont l'Evéque, and soft surface-ripened cheeses, e.g., Reblochon, are made with mesophilic cultures. Cheeses made with thermophilic cultures are cooked to temperatures around 54 °C whereas only limited cooking (~35 °C) is given to washed-rind cheeses made with mesophilic cultures which consequently have a relatively high moisture content. After light pressing, sometimes overnight, the cheeses are brined (usually saturated brine, pH 5.2; 0.2 % Ca) for 4–18 h depending on their size, small cheeses are brined for shorter times than larger ones. Sometimes, the only pressing received is that of the weight of the curd itself. The cheeses are then drained for a few hours after which they are smeared. Both bacterial- and mould-ripened cheese are ripened at 10–15 °C at a high relative humidity to prevent loss of moisture and consequent drying out of the cheese surface.

11.16.5.1 Deacidification

Environmental factors, particularly the temperature of ripening, and the composition of the cheese, e.g., high moisture (in most cheeses of this type), low initial pH, and high lactate levels, determine the succession of microorganisms that grow on the surface of surface-ripened cheese. The pH of a young cheese after acidification of the cheese curd by the starter lactic acid bacteria is ~5.0. This low pH selects microorganisms (yeast and moulds) which grow on lactate metabolising it to CO_2 and H_2O and deaminating amino acids producing NH_3 and keto acids, causing the pH on the surface to rise and permit the development of bacteria, particularly coryneforms. This is called deacidification and occurs in both mould- and smear-ripened cheeses. The presence of moulds and yeast on the surface of cheese is to be expected since the cheese has a relatively low pH (both can grow at a pH value <3), a ready substrate, lactate, for energy production and a relatively low a_w. Once the surface pH increases to >5.8, salt-tolerant bacteria (STB) begin to grow. The interrelationship between the increase in pH and the numbers of STB and yeast in Tilsit cheese is shown in Fig. 11.19. The STB were counted on Plate Count Agar containing 8 % salt. The pH increases steadily from about 5.5 to 7.5 in the first 2 weeks of ripening after which it remains more or less constant. Simultaneously, the numbers of yeast and STB also increase with the STB increasing more rapidly than the yeast.

Deacidification also enhances the action of enzymes, including lipases, proteinases and peptidases, many of which have optima close to neutrality. The lipases and proteinases hydrolyse the fat and protein to fatty acids and peptides and amino

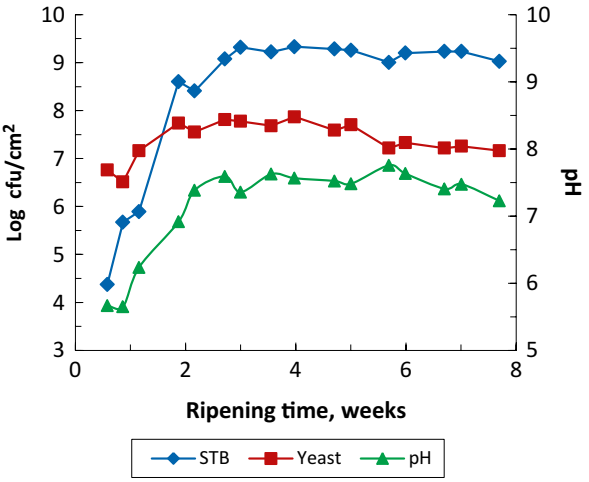


Fig. 11.19 Growth of yeast and salt-tolerant bacteria (STB) (on plate count agar containing 8 % salt) and the change in the pH of Tilsit cheese during ripening (Redrawn from Eliskases-Lechner and Ginzinger 1995a,b)

acids, while the peptidases hydrolyse the smaller peptides to amino acids. Both the fatty acids and amino acids are the precursors of many of the flavour compounds in surface-ripened cheese (see Chaps. 12 and 13).

11.16.5.2 Bacterial Surface-Ripened Cheeses

Bacterial surface-ripened cheeses can be classified as hard, e.g., Gruyère and Comté, semi-hard, e.g., Tilsit, Brick and Limburger or soft, e.g., Münster, Livarot and Reblochon. Most bacterial surface-ripened cheese is brine-salted. However, Comté is an exception and is dry-salted by rubbing salt and smear on to its surface several times a week during the first 3 weeks of ripening.

Two types of smearing are used, either the "old-young" method, which is traditionally practiced in Germany, or dipping or washing the surface of the cheese with a solution containing various combinations of yeast and bacteria, traditionally different combinations of *Geotrichum candidum, Debaryomyces hansenii* or *Brevibacterium linens*, obtained from commercial sources (used in most other countries). In the "old-young" method, a smear from ripened (old) cheese is washed off the surface of the cheese and is then used to inoculate the surface of the young cheese. This ensures that the surface microorganisms that contributed to the ripening of the old, ripened cheese are transferred to the young, fresh cheese. Then, the cheese is ripened at 10–15 °C at an RH > 90 % for several weeks to allow the surface microflora to develop and produce the red or orange colour. The cheese is smeared at the beginning of ripening and then once or twice at 2–4 days intervals to give a

uniform distribution of the organisms on the cheese surface. This reduces the risk of unwanted contaminants like moulds colonizing the cheese surface. Generally, visible growth on the surface is apparent within a few days of the beginning of ripening. After 2–3 weeks, the desired microflora has developed and soft and semi-soft cheese are then wrapped or transferred to another ripening room at a lower temperature for further maturation.

Growth of *Listeria monocytogenes* is a considerable problem on smear cheeses. This organism can grow at 0 °C, pH 4.4 and in 12 % salt and causes listeriosis in humans (Chap. 19). The "old-young" method of smearing can also result in contamination of the young cheese by this and other pathogenic bacteria, which is totally undesirable in a cheese.

Until recently, the bacteria involved were not clear and many were misnamed. The major reason for this is that coryneforms, the dominant bacteria in the smear, are quite difficult to identify accurately unless molecular techniques are used. For a long time, *Brevibacterium linens* was thought to be the major bacterium on the surface of smear-ripened cheese. Nowadays, it is known to constitute only a minor portion of the flora of a mature cheese. *B. linens* does not grow below pH 5.5 or 6 and, in fact, has been shown to be a mixture of two different species, *B. linens* and, a new species, *B. aurantiacum* (Gavrish et al. 2004).

The microflora of five smear-ripened cheeses, viz. Limburger from Germany, Reblochon and Livarot from France, Tilsit from Austria and Gubbeen from Ireland, has been examined in detail recently, using both traditional and molecular techniques to identify the microorganisms (Cogan et al. 2014). Limburger cheese had the simplest microflora, containing two yeasts, *D. hansenii* and *G. candidum,* and two bacteria, *Arthrobacter arilaitensis* and *B. aurantiacum*. Livarot was the most complicated, comprising 10 yeasts and 38 bacteria, including many Gram-negatives. Reblochon also had a very diverse microflora containing 8 yeast and 13 bacteria (excluding Gram-negatives which were not identified) while Gubbeen comprised 7 yeast and 18 bacteria and Tilsit 5 yeasts and 9 bacteria. *D. hansenii* was by far the dominant yeast and was found in all cheeses, followed in order by *G. candidum,* which was found in all cheeses except Gubbeen, *Candida catenulata*, which was found only in Livarot and Gubbeen, *K. lactis*, which was found only in Reblochon and Livarot and *C. lusitaniae* which was found only in Tilsit and Gubbeen.

B. aurantiacum was the dominant bacterium and was found in each batch of cheese. The next most common bacteria in order were *Staphylococcus saprophyticus*, which was found in all cheese except Limburger*, Arthrobacter arilaitensis,* which was found in all cheeses, *Corynebacterium casei*, which was found only in Reblochon, Tilsit and Gubbeen, *C. variabile,* which was found only in Reblochon, Tilsit and Gubbeen and *Microbacterium gubbeenense*, which was found in all cheeses except Limburger. All of these are coryneform bacteria, except *S. saprophyticus*. Micrococci and staphylococci dominated the bacterial flora early in ripening but later they were outgrown by corynebacteria. Other bacteria were isolated in low numbers, suggesting that each of the five cheeses has a unique microflora. The smear bacteria are all Gram-positive, salt-tolerant (the surface layer of surface-ripened cheese can contain up to 15 % NaCl), aerobic or facultatively anaerobic

microorganisms and hence grow easily at the high salt level in the surface layer of these brine-salted cheeses. Gram-negative bacteria were isolated from the two French cheeses, Reblochon and Livarot, but only those from Livarot were identified and included *Hafnia alvei, Proteus vulgaris, Alcaligenes faecalis* and *Psychrobacter* spp. (Cogan et al. 2014). *Halomonas venusta*, *H. variabile* and an unidentified *Halomonas* sp. have been found in several other smear cheeses (Maoz et al. 2003; Mounier et al. 2005). *Halomonas* and *Vibrio* are salt tolerant. *Halomonas* are considered to indicate hygiene problems and are normally associated with seawater and salterns and, therefore, it is likely that the salt used in the brining process is the source of this organism. *Hafnia, Proteus, Alcaligenes, Psychrobacter, Halomonas* and *Vibrio* spp. are Gram-negative and the effect of such bacteria on the flavour of smear cheeses is unclear.

Several new species were identified during the above study including *Agrococcus casei* (Bora et al. 2007), *C. casei* (Brennan et al. 2001a) *Mb. gubbeenense* (Brennan et al. 2001b) and *Mycetocola reblochoni* (Bora et al. 2008). New species have also been isolated from other smear cheeses, e.g., *S. succinus* subsp. *casei* and *S. equorum* subsp. *linens* from a Swiss smear-ripened cheese (Place et al. 2003a, b), *Brachybacterium tyrofermentans* and *Brach. alimentarius* (Schubert et al. 1996) from the smear of hard cheese and *Arthrobacter bergerii* and *Arthrobacter arilaitensis* from Camembert and Reblochon cheese, respectively (Irlinger et al. 2005). The genomes of *Arth. arilaitensis* Re117 (Monnet et al. 2010), *C. casei* UCMA 3821 and LMG S-19264 (Monnet et al. 2012b; Walter et al. 2014) and *C. variabile* DSM 44702, which was isolated from Gubbeen cheese as *C. mooreparkensis,* (Schroder et al. 2011), have been sequenced and ranged in size from 3.11 to 3.85 Mb. In addition, strains Re117 and LMG S-19264 contained two plasmids and strain DSM 44702 a putative phage. Genes of importance for the growth of these bacteria and their putative role in cheese ripening were detected, including the uptake of iron, osmoprotection and catabolism of lactate, citrate, protein and fat, but whether these genes are transcribed in the cheese during ripening has not been studied, except for those involved in the metabolism of iron (Monnet et al. 2012a). Iron is essential in the respiration of lactose, lactate and citrate, where it functions as part of the cytochrome system. The cheese surface is surrounded by oxygen implying that the organisms on the surface can respire, if they have the necessary enzymes. Bovine milk is low in iron (0.2–0.4 mg/L) and the main mechanisms used by these bacteria to transport iron are siderophores which are strong chelators of Fe^{3+}. Thirty genes involved in chelation and transport of iron by siderophores were identified in strain Re117 (Monnet et al. 2010) and 29 in strain DSM 44702 (Schröder et al. 2011). Further studies (Monnet et al. 2012a) showed that the availability of iron on the cheese surface is limiting for the growth of these bacteria on the cheese surface. The osmoprotectant genes detected included the transport of ectoine, a derivative of proline, proline itself, and glycine betaine, a derivative of glycine where the H atoms on the amino group are replaced by methyl groups. These are all highly soluble compounds which allow these organisms to tolerate and grow at high concentrations of salt. *C. variabile* DSM 44702, contained the genes for transport of lactate and citrate and the conversion of lactate to pyruvate, which can then be oxidised

through the TCA cycle. The genes encoding enzymes involved in proteolysis included a proteinase, and a proline iminopeptidase, which releases proline from the amino terminal end of peptides, while the genes encoding enzymes involved in lipolysis include esterases, lipases and the enzymes involved in β-oxidation of fatty acids. The different species have also been shown to develop different colours on the cheese surface.

Arthrobacter, Brevibacterium, Brachybacterium, Corynebacterium and *Microbacterium* are often called coryneform bacteria. All of them are Gram-positive, catalase-positive, non-sporeforming and are generally non-motile. A major feature of their growth is that exponential phase cells are pleomorphic, showing the presence of irregularly shaped rods, including wedge, club, V and curved shapes. In addition, *Arthrobacter*, *Brevibacterium* and *Brachybacterium* spp. go through a marked rod/coccus cycle during growth, with rod forms dominating the exponential phase of growth (1–2 days) and coccal forms dominating the stationary phase (5–7 days). All belong to the Actinomycete (high GC) branch of the Gram-positive bacteria.

A study of the surface microflora of five Italian washed-rind cheeses, Taleggio, Gorgonzola, Casera, Scimudin and Formaggio di Fossa, has also been conducted using molecular techniques (Fontana et al. 2010). Most of the bacteria were cocci including *S. saprophyticus, S. equorum, S. vitulinus, S. caprae, Micrococcus luteus* and *M. caseolyticus* and only two coryneforms, *B. linens* or more likely *B. aurantiacum,* since the reference strain used was actually the type strain of *B. aurantiacum*, and *C. flavescens*. These data suggest that the microflora of Italian smear-ripened cheeses differ significantly from others similar European cheeses.

Bokulich and Mills (2013) used high-throughput sequencing technology to study the microbial ecosystems in two US artisanal cheesemaking plants, producing fresh and smear- and mould-ripened cheeses, in great detail. Fermentation-associated microorganisms, especially *Lactococcus* and *Debaryomyces* dominated most surfaces, suggesting that these microorganisms establish biofilms on equipment surfaces and may play an important role in transferring microorganisms to the cheese. In addition, environmental microorganisms from the processing environment dominated the surface microflora of smear cheeses in both plants, demonstrating the importance of the environment in populating the cheese surface, even when it is deliberately inoculated with smear bacteria. Gram-positive bacteria dominated the cheese surface. However, Gram-negative bacteria were also found on the surfaces of mature cheeses, many of which were halotolerant, e.g., *Pseudoalteromonas* spp., *Halomonas* spp. and *Vibrio casei* and which may have originated in the salt used in cheesemaking.

More recently, Wolfe et al. (2014) examined the ecology of the rinds of 137 cheeses, including washed-rind, mould-ripened and cheeses with natural, undisturbed rinds, from 10 countries (England, France, Germany, Ireland, Italy, Portugal, Spain, Sweden, Switzerland and the US) using molecular techniques. Bacteria from 14 genera (*Arthrobacter, Brachybacterium, Brevibacterium, Corynebacterium, Nocardiopsis, Yaniella, Staphylococcus, Halomonas, Pseudomonas, Psychrobacter, Pseudoalteromonas, Vibrio, Hafnia/Serratia* and *Sphingobacterium*) and fungi from 10 genera (*Debaryomyces, Galactomyces, Candida, Scopulariopsis, Fusarium, Acremonium, Peniillium, Aspergillus, Chrysosporium* and *Sporendoema*)

were found at more than 1 % abundance but not in all cheeses. The average number of bacterial and fungal genera were 6.5 (range 1–13) and 3.2 (range 1–7), respectively. More than 60 % of the bacteria and 25 % of the fungi were environmental contaminants. This is the first report of the presence of the actinobacteria, *Nocardiopsis* and *Yaniella*, on cheese rinds. The data also showed that *Pseualteromonas* spp contain methionine-γ-lyase which converts methionine to methanthiol, a key component of the flavour of washed-rind cheeses and which to date has only been found in *B. linens*.

The finding of staphylococci in cheese raises issues regarding their pathogenicity even though the strains isolated were coagulase negative. A French study (Coton et al. 2010) has shown that *S. equorum S. xylosus, S. saprophyticus* and *S. epidermidis* were the dominant species in numerous French cheeses examined over a 16 year period from 1990; 11 other coagulase-negative species were also identified. *S. epidermidis* and *S. saprophyticus* were also found in clinical samples but PFGE analysis showed no relationship between the clinical and the food strains.

11.16.5.3 Sources of the Bacteria on Surface-Ripened Cheese

Commercially, only *B. linens, D. hansenii* and *G. candidum* are used as secondary cultures to deliberately inoculate the cheese surface. However, in several studies, very few of these cultures were re-isolated from the cheese and, when they were, it was only from the initial stages of ripening. These results imply that smear cheese production units must have an adventitious 'house' flora and that the use of commercial secondary cultures in the production of smear-ripened cheeses is questionable. One way around this problem is to identify the dominant organism present in a particular cheese and then give them back to the cheesemaker and this has been shown to be effective in practice. Brines, many of which can be several years old, were shown to be an important source of *S. saprophyticus* and *D. hansenii* and the skin of the arms and hands of workers were important sources of *C. casei* and *C. variabile* (Mounier et al. 2006). This raises interesting questions concerning the ecology of surface-ripened cheese and human skin since the dominant genera on both skin and the cheese surface are *Staphylococcus* and *Corynebacterium* species. In addition, micrococci, coryneforms, yeast and moulds are present as a biofilm on the shelves (Mariani et al. 2007) and wooden vats ('gerles' in French) (Didienne et al. 2012) used in ripening the French smear-ripened cheeses, Reblochon and Salers, respectively. An example from Reblochon shelves is shown in Fig. 11.20. Therefore, other wooden tools used in cheesemaking should also be considered likely sources of microorganisms.

Defined-strain secondary cultures for bacterial surface-ripened cheese are also being developed and the successful use of a defined-strain culture containing, *D. hansenii, B. linens, A. nicotianae* (probably *Mb. gubbeenense*), *C. ammoniagenes* (probably *C. casei*) and *S. sciuri* has been shown on a pilot scale; such cultures are not yet available commercially (Bockelmann et al. 2005) The fact that commercial cultures are not subsequently recovered from cheese may militate against the use of

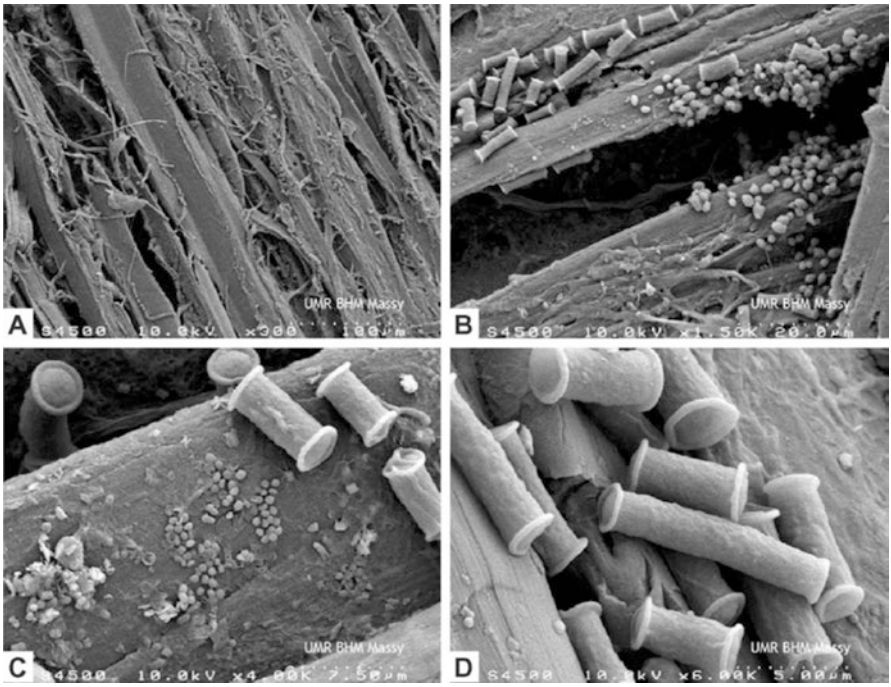


Fig. 11.20 Scanning electron microscopy of the surface of a ripening shelf. A (×300), ripening biofilm does not cover all the surface of the shelf. B (×1500), bacteria are located in the cracks. C (×4000) and D (×6000), details of *Geotrichum candidum* (*cylinders*) and bacteria (From Mariani et al. 2007)

defined cultures but a better understanding of the microbiology, ecology and interactions which occur between bacteria on the cheese surface will help considerably in developing them.

11.16.5.4 Mould Surface-Ripened Cheeses

Camembert is a mould-ripened cheese with a relatively high moisture content (~50 %) and short ripening time. It is probably the most common mould-ripened cheese and is produced in two forms, traditional and non-traditional Camembert. Traditional Camembert, more commonly known as Camembert de Normandie, is made from raw milk and is dry-salted whereas non-traditional Camembert is made worldwide, generally from pasteurised milk and is brine-salted. Traditional Camembert is also a PDO (Protected Designation of Origin) cheese, made only in designated areas of Normandy in France. DL mixed cultures are used in production of both types and no pressing of the curd occurs. Instead the moulds, containing the curd, are inverted a few times to help syneresis of whey from the curd. Generally,

P. camemberti, K. lactis, G. candidum and *B. linens* are necessary to develop the optimum flavour of Camembert cheese. These microorganisms are obligate aerobes and grow only on the cheese surface and are either added to the milk with the starter cultures (in the case of non-traditional Camembert) or sprayed on the cheese surface after manufacture (traditional Camembert). A new type of Camembert is also made with a mixture of mesophilic and thermophilic cultures. Part of the whey is drawn off during manufacture, ensuring that a less acidic cheese is produced. These are called stabilised or solubilised cheeses and are often sold under trade names. The manufacture and ripening of Brie, another very common mould surface-ripened, is similar to that of Camembert except that Brie has a larger diameter and a thinner wheel.

A detailed study (Leclercq-Perlat et al. 2004) of some of the microbial changes which occur on the surface of experimental Camembert cheese made under aseptic conditions but with the deliberate addition of both starter and the common ripening organisms, *P. camemberti*, *K. lactis, G. candidium* and *B. linens*, is shown in Fig. 11.21 and gives some understanding of the complex microbiological and biochemical changes occurring in the cheese during ripening. The ripening conditions for the cheese were as follows: 24 h after moulding, the cheeses were brined at 14 °C for 25 min: they were then transferred to a sterile ripening chamber, held at 14 °C, 85 % RH for 24 h, after which they were ripened at 12 °C and 95 % RH for 14 days. On day 6 the cheeses were turned, and on day 14, the temperature and RH were changed to 14 °C and 85 % RH for 1 day, after which the cheeses were wrapped and stored at 4 °C until day 45.

Deacidification was much more rapid at the cheese surface than in the core. The surface pH increased slowly initially from ~4.6 on day 1 to 5.1 on day 6 (Fig. 11.21). This slow change in pH reflects two opposing conditions, consumption of lactate by the rapidly growing *K. lactis* and *G. candidum* and continuing production of lactic acid from the residual lactose, by the starter, which occurred, even though a significant amount of salt was present on the surface, from the brining. The rind was completely devoid of lactose by day 7 and the pH reached 7.7 on the surface on day 11. The number of lactococci probably reached ~10^9/g at the beginning of ripening and remained at this level in both the interior and the surface throughout ripening.

Numbers of *K. lactis* and *G. candidum* increased rapidly during the first 2 days of ripening and then, more slowly, until day 7 after which growth of *G. candidum* continued and that of *D. hansenii* ceased; both organisms reached final cell densities of ~8 log cfu/g DM. In contrast, numbers of *B. linens* remained static at 2 log cfu/g DM for the first 5 days of ripening, after which they increased to 4 log cfu/g DM from day 5 to day 9 and then more slowly reaching 9 log cfu/g DM by day 40. *P. camemberti* is an obligate aerobe and grows only on the cheese surface. Its numbers were also estimated but gave no real idea of how rapid growth was as the mycelium was destroyed during the analysis. However, growth of *P. camemberti* was visible from day 7 and completely covered the cheese surface on day 12.

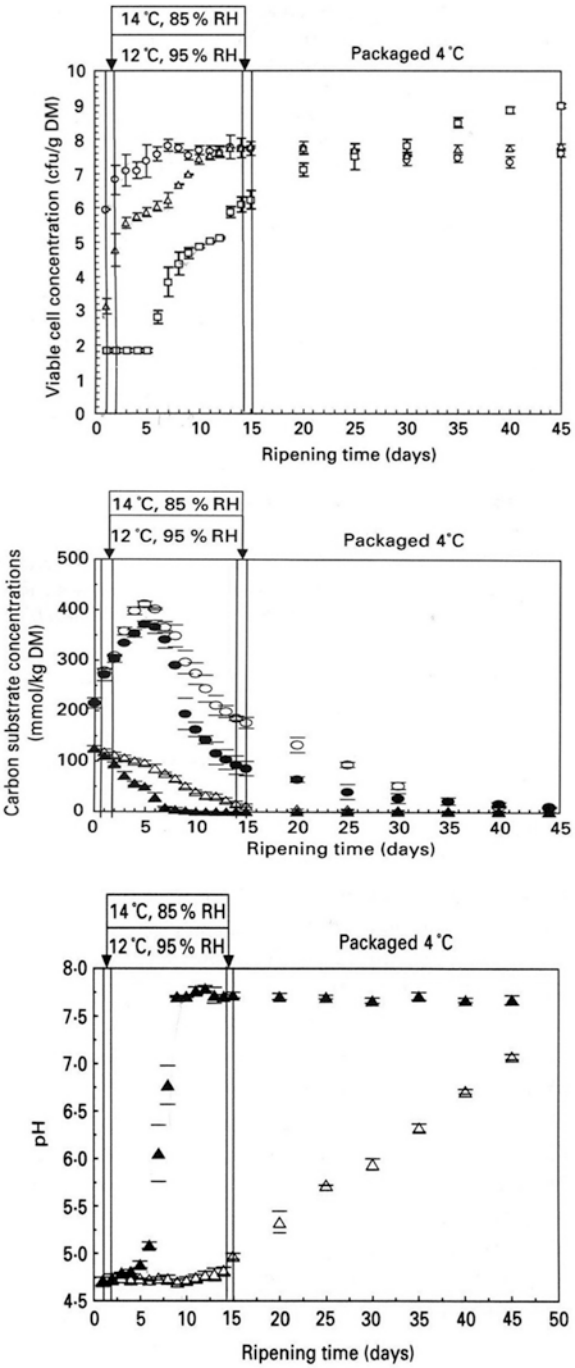


Fig. 11.21 *Top* figure, changes in the numbers of *K. lactis* (*open circles*), *G. candidum* (*open triangles*) and *Brevibacterium linens* (*open squares*) on the rind; middle figure, lactose in the rind (*closed triangles*) and core (*open triangles*) and lactate in the rind (*closed circles*) and core (*open circles*) and *bottom* figure, pH in the rind (*solid triangles*) and core (*open triangles*) in Camembert cheeses during ripening for 45 days. The temperature and RH maintained during the ripening are also indicated (From Leclercq-Perlat et al. 2004)

11.16.6 Blue Cheeses

Blue cheeses are characterised by blue veins of the mould, *Penicillium roqueforti*, running through the cheese and include some famous varieties, e.g., Gorgonzola, Stilton, Danablu and Roquefort. Gorgonzola and Stilton are made from pasteurised bovine milk, Danablu from thermised bovine milk while Roquefort is made from raw ovine milk. The colour can vary from brown to green to blue depending on the age of the cheese. In many blue cheeses no starters are used but mesophilic cultures are used for Stilton, Danablau and Roquefort while a mixture of both mesophilic and thermophilic starters are used for Gorgonzola. Generally, spores of *P. roqueforti* are added with the starter. Salting is either by brining or dry salting and the cheese is pierced to allow limited entry of O_2 to promote the growth of *P. roqueforti*. Essentially cheese is an anaerobic system but *P. roqueforti* is unique among moulds in being able to grow at low levels of O_2 which the piercing process allows to diffuse into the cheese from the air.

There is little published information on the microbial changes which occur in blue cheese during ripening except for Cabrales, a Spanish blue cheese, made from raw milk without the deliberate addition of starters or *P. roqueforti* (Nunez 1978; Nunez et al. 1981). Adventitious LAB in the milk are responsible for acid production during manufacture and ripening. The coagulum is cut 2 h after addition of farm-made goat rennet and is then scooped into moulds which are held at 16–18 °C for 48 h to allow whey drainage to occur. Then, the curd is removed from the mould, covered with coarse salt, held for a further 48 h at 16–18 °C and then ripened at 10–12 °C for 10–15 days and transferred to caves for further ripening at 9–12 °C at 90–95 % RH.

The microbiological changes which occur in Cabrales cheese during ripening are shown in Fig. 11.22. In each graph, the first and second points refer to the counts in the milk and the curd on days 1 and 2, respectively. Growth of lactococci is relatively rapid during the first few days after which their numbers decrease but more rapidly on the surface than in the interior of the cheese. Numbers of mesophilic lactobacilli remain more or less constant at 10^6 cfu/g on the surface but increase to 10^8 cfu/g in the interior, after which they decrease. *Micrococcus* spp. show the opposite trend, being higher on the surface than in the interior while yeast show significant growth on the surface of the cheese early in ripening after which they decrease. Coliforms grow during cheesemaking but their numbers decrease rapidly over the next 2 weeks. This is probably due to the very rapid decrease in pH which reaches ~5.0 in 48 h due to the growth of the lactococci after which it increases to 6.5 in the interior and to 7 on the surface due to metabolism of lactate to CO_2 by the yeast and moulds.

Pronounced pH, NaCl and a_w gradients occur in blue cheese. Deacidification also occurs since *P. roqueforti* utilizes the lactate in the cheese and produces NH_3 from deamination of amino acids, causing the pH to increase. Blue cheeses also contain large numbers of yeasts which also metabolise lactate and produce NH_3. However, their role has not been studied extensively.

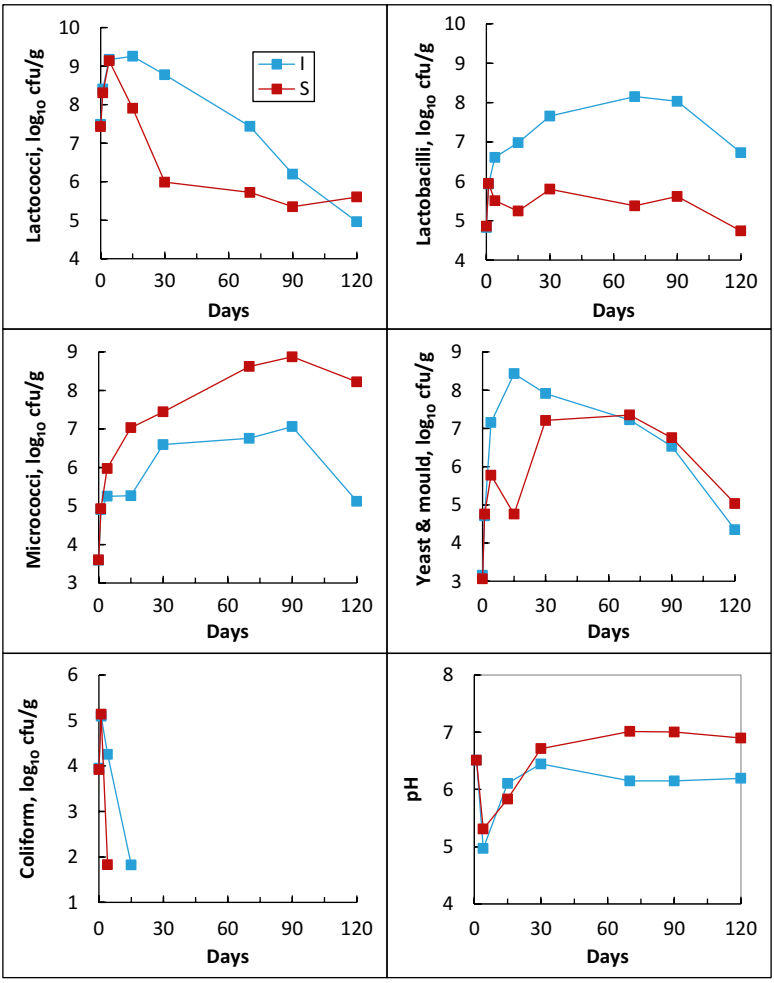


Fig. 11.22 Growth of different organisms and changes in the pH at the surface (S) and in the interior (I) of Cabrales cheese during ripening (From Nunez 1978)

The most important organisms in flavour development of Blue cheese are the SLAB and *P. roqueforti* and *G. candidum* but yeast, particularly *D. hansenii, Kluveromyces marxianus* and *Yarrowia lipolytica*, and NSLAB, particularly *Lb. paracasei, Lb. casei* and *Lb. plantarum*, also probably play a role in flavour development. Interactions can occur between yeast and *P. roqueforti*; certain strains of *D. hansenii* have been shown to stimulate and strains of *Y. lipolytica* to inhibit the growth of *P. roqueforti*. Brines are an important source of yeast and their composition and the conditions of ripening vary from country to country. In France, the brine used for Blue cheese production contains 19–20 % NaCl, has a pH of 4–6 and

a temperature of 13–16 °C while in Denmark, the concentration of salt and the temperature are higher (22–23 % and 19 °C, respectively), and the pH somewhat lower (4.5) (Cantor et al. 2004). The higher salt and temperature will significantly increase the rate of salt diffusion into the cheese. Yeast numbers in brine can reach 10^6 cfu/ml with *D. hansenii* being the predominant one while numbers on the surface of Roquefort cheese reach ~10^9 cfu/g of dry matter (Besancon et al. 1992).

11.17 Microbial Spoilage of Cheese

The most common microbial defects of cheese are early and late gas. They are relatively uncommon in cheese to-day, due to improved hygienic quality of the milk and better quality control in cheese plants.

Early gas generally occurs within 1 or 2 days after manufacture. It is characterised by the appearance of many small holes throughout the cheese and is caused by coliform bacteria. The gas is mainly H_2 which is produced from formate, a product of lactose metabolism, by formic hydrogenylase. It is more problematic in soft and semi-hard cheese than in hard cheese because of the higher a_w in the former cheeses. An effective way of controlling early gas is to add KNO_3 or $NaNO_3$ at a low level (0.2 %) to the milk. NO_3^- does not prevent the growth of coliform but acts as an alternative electron acceptor, allowing complete oxidation of lactose to CO_2 and H_2O, rather than fermentation to formate, thus effectively reducing the production of H_2 from formate. Early gas production can occasionally be caused by yeast due to CO_2 production from lactose or lactate.

Late gas formation, or late blowing, does not occur until late in ripening and is due to the fermentation of lactate to butyrate and H_2 by *Clostridium tyrobutyricum* and *Cl. butyricum*. The butyrate gives the cheese a pronounced off-flavour and the H_2 is responsible for large, deformed holes or eyes. Late gas can be particularly prevalent in Dutch- and Swiss-type cheese; in the latter cheese clostridia grow with the PAB, during the "hot" room ripening period. Silage is a potent source of these bacteria and, for this reason, it is forbidden to feed it to cows, whose milk is intended for cheesemaking, in Switzerland. Clostridial spores survive passage through the cows' digestive system and appear in the dung. The degree of contamination of the milk with dung depends on the hygienic conditions during milking. The spores are not inactivated to any extent by pasteurisation of the milk but germinate in the cheese during ripening. Late gas production can also be controlled by removal of the spores from the milk by bactofugation but this often results in inferior quality cheese, or by the addition of 0.15 % KNO_3 to the milk before renneting. In addition, many thermophilic cultures are thought to stimulate the growth of clostridia, through the production of peptides and amino acids. Strains of *Cl. tyrobutyricum* vary widely in their abilities to grow under different conditions of pH, salt concentration and temperature; 7 out of 10 strains grew at pH 5 (at 37 °C), 5 strains grew in the presence of 3 % NaCl (at pH 7 and 37 °C) and no strain grew in 3.5 % NaCl or at 10 °C (at pH 7 and 37 °C) (Ruusunen et al. 2012). This data suggests that the low ripening

temperatures, low pH and relatively high concentrations of most hard cheeses during ripening prevent the growth of many strains of *Cl. tyrobuyricum*.

The bacteriocin, nisin, produced by some strains of *Lc. lactis* subsp. *lactis,* is effective in controlling the growth of clostridia and is used for this purpose in processed cheese. However, it is not suitable for use in natural cheese because many strains of starter are sensitive to it. Defined-strain starters that produce nisin are now being marketed to a limited extent for use in Dutch-type cheeses. Other bacteriocin-producing starters, e.g., lacticin 3147, have also been shown to control late gas formation in cheese (Martinez-Cuesta et al. 2010). Increasing the level of salt, lowering the pH of the cheese rapidly through the use of an active starter and addition of lysozyme can also be effective in preventing late gas production.

Lysozyme, which is found in milk, saliva, tears and other body fluids, hydrolyses the cell walls of sensitive bacteria, like *Cl. tyrobutyricum*, causing them to lyse. It is commonly used in Italy and is added to the milk with the starter at a level of 25 mg/L. It is generally considered to have no effect on the growth of starters, although some strains in Italian natural whey cultures are inhibited by it.

Other microorganisms have occasionally been implicated as spoilage organisms. Citrate-metabolising lactobacilli have been incriminated as the cause of open texture or slit openness in Cheddar cheese, due to the production of CO_2 from citrate. The optimum pH for uptake of citrate ranges from 4 to 5 and significant metabolism of citrate occurs in the absence of an energy source at pH 5.2, the pH of many semi-hard and hard cheeses. Rapid refrigeration of the cheese blocks after manufacture and good hygiene reduce this defect. If the permeability of the wrapping material allows O_2 into the cheese during storage, some NSLAB, particularly pediococci, can oxidise lactate to acetate and CO_2 on the cheese surface. Open texture in cheese has been reviewed by Martley and Crow (1996).

Growth of NSLAB (*Lb. casei, Lb. plantarum* and *Lb. brevis*) in Dutch-type cheese to more than 10^7 cfu/g in 4–6 weeks causes the production of gas and putrid off-flavour development. Brines are considered to be a major source of these organisms and it is recommended that they should contain $<10^3$ lactocbacilli/ml (van den Berg et al. 2004). The increased levels of NSLAB in brines is due to growth of yeast on deposits which naturally occur just above the brine surface, and on racks and other equipment. Growth of the yeast decreases the local pH in the curd allowing the NSLAB to grow. Entry of the NSLAB from the brine is facilitated by poor pressing of the cheese and consequent lack of closing of the rind. Weak brines also support the growth of these NSLAB, and it is important that the brine strength should be maintained at >16 % NaCl, pH < 4.5 and 13 °C.

Ec. malodoratus, as its name implies, causes the production of bad flavours, and has been found in Gouda cheese.

Growth of thermo-resistant streptococci, particularly strains of *Sc. thermophilus*, occurs in the regeneration section of pasteurisers during long pasteurisation runs and reaches high numbers (10^6/ml) in the pasteurised milk. Their number may increase to 10^8/g of cheese early in ripening and cause unclean yeasty flavours to develop in the cheese. Thus, in many cheese plants, pasteurisers are routinely cleaned after continuous runs of 8 h.

PAB are also prone to grow in cheese, particularly Dutch cheese, during prolonged ripening, causing development of a sweet taste and very open texture, due to excessive gas production. Under normal conditions their growth is significantly slowed down by the relatively high salt level (PAB are very sensitive to salt) and the ripening temperature of <12 °C; some cheeses may be ripened at 5–6 °C to prevent growth of PAB.

Yeasts and moulds are occasionally incriminated as spoilage organisms in cheese. The surface of cheese, especially when it is moist, e.g., an unwrapped soft or semi-soft cheese, is an ideal environment for their growth. These cause little damage to the cheese but are unsightly. They can be washed off the cheese surface with a dilute brine solution. Sometimes hard cheeses are dipped in a dilute solution of the antifungal antibiotic,natamycin (pimaricin), to prevent the growth of yeast and moulds on their surfaces Sorbic acid is allowed in Italy as a preservative for hard, fresh and processed cheese. Sorbate-resistant moulds, *Paecilomyces variotti*, and yeast, *D. hansenii* from Crescenza and Provolone cheese are able to grow in the presence of 3 mg of sorbic acid/g and are also able to transform sorbic acid to *trans*-1,3-pentadiene, which has a taste and odour like kerosene (Sensidini et al. 1994).

Cladosporium cladosporioides, Penicillium commune, *C. herbarum, P. glabrum* and a *Phoma* spp. are responsible for the "thread mould" defect of Cheddar cheese (Hocking and Faedo 1992). This defect occurs as a black, dark brown, or dark green spot or thread (hence the term 'thread mould') in the folds, creases and gusset ends of the plastic bags used to wrap Cheddar cheese during ripening. It can occur on the cheese surface but is more often associated with free whey drawn from the fresh cheese block during vacuum packaging. These moulds are obviously able to grow in the presence of low levels of O_2.

Growth of *P. commune*, which is closely related to *P. camemberti*, can result in discoloration of cheese surfaces and the production of off-flavours (Lund et al. 1995).

11.18 Probiotics

These are live cultures, generally of *Lactobacillus* or *Bifidobacterium* spp., which, when administered in adequate amounts, confer a health benefit on the host. They have been exploited extensively in the dairy industry in the development of novel functional foods. More than 40 different strains are being used but, with the exception of *Lb. casei* Shirota, *Lb, rhamnosus* GG and *B. animalis* Bb12, very few of them have been clinically proven to have a positive effect on human health. To realize their health benefits, the probiotic strain must be viable at the point of sale and present at concentrations of ~10^6 cfu/g. The health benefits include alleviation of lactose intolerance, prevention and reduction of diarrhoea, prevention of allergies, reduction of the risk associated with mutagenecity and carcinogenicity, reduction in cholesterol, inhibition of *Helicobacter pylori*, the cause of some stomach ulcers, and other intestinal pathogens, prevention of inflammatory bowel disease and

modulation of the immune system. Yoghurt and fermented milks are the main vehicles for the delivery of probiotics but cheese is also useful since it has a higher pH, more solid consistency and relatively higher fat, which help to protect probiotic bacteria**.** When cheese is used it is important to determine what effect the survival of the probiotic culture has on the various ripening parameters and on the development of cheese flavour. In addition, particularly where probiotic lactobacilli are added, suitable media to distinguish between the probiotic and NSLAB strains must be developed. For a review of probiotics see Vasiljevic and Shah (2008).

11.19 Non-Lactic Genera of Bacteria Found in Cheese

A brief description of the genera of bacteria other than starter and NSLAB found in cheese is given below. Many, especially the corynebacteria, are difficult to identify by simple tests and generally chemical analysis of cells for the types of polar lipids, menaquinones, fatty acids, the presence or absence of mycolic acid and amino acid composition of the cell wall, are required. More recently, molecular methods, e.g., *rep*-PCR with different primers, PFGE or 16S rRNA sequencing are being used but these techniques require sophisticated equipment.

11.19.1 Agrococcus

These are Gram-positive, catalase-positive, non-motile, aerobic ovoid to short rods which contain diamino butyric acid in their cell wall. The cells occur singly, in pairs, in short flexible chains, or in small irregular clusters. The phospholipids are phosphatidylglycerol, diphosphatidylglycerol. The main menaquinones are MK-12 and MK-11. No mycolic acids are present. The type species is *Agrococcus jenensis.* They do not grow at 42 °C and have been isolated from air, soil and medieval wall paintings as well as smear cheese.

11.19.2 Arthrobacter

These are Gram-positive, catalase-positive, non-motile, strictly aerobic rods which go through a marked rod/coccus cycle during growth, with the rod forms dominating in exponentially growing cultures and the coccus forms dominating stationary phase cultures. Their cell wall contains lysine. Little or no acid is produced from glucose or other sugars. They have non-exacting nutritional requirements; generally, biotin is the only vitamin required. Their habitat is soil and they do not withstand pasteurisation. They can be confused with micrococci (see below). *A. agilis* has been isolated from cheese (Coppola et al. 2000).

11.19.3 *Brachybacterium*

These are Gram-positive, facultatively anaerobic short rods which exhibit a rod-coccus growth cycle. Their optimum temperature is ~30 °C. Their cell wall contains *meso*-diaminopimelic acid and glucose, galactose and rhamnose but not mycolic acids. Five species are recognised and two of these, *Br. alimentarium* and *Br. tyrofermentans* have been isolated from Comté and Beaufort cheese, respectively. Their nutritional characteristics do not appear to have been studied but *Br. alimentarium* and *Br. tyrofermentans* can grow in the presence of 14 and 16 % NaCl, respectively.

11.19.4 *Brevibacterium*

Brevibacteria are Gram-positive, catalase-positive, non-motile, strictly aerobic rods which go through a marked rod/coccus cycle during growth. Their cell wall contains *meso*-diaminopimelic acid and they metabolise sugars by respiration. There are five species, *B. linens, B. aurantiacum, B. casei*, *B. iodinum* and *B. epidermidis*. The first three species have been isolated from cheese, the fourth from milk and the fifth from skin. *B. linens* has recently been shown to be a mixture of two species, *B. linens* and *B. aurantiacum* both of which produce yellow- or orange-coloured colonies (Gavrish et al. 2004) while those of *B. iodinum* are purple, due to the production of a phenazine derivative; the other two species produce gray-white colonies. Brevibacteria grow poorly, if at all, at 5 °C, have an optimum temperature of 20–25 °C and grow in the presence of a high concentration of NaCl; *B. linens* and *B. iodinum* grow in the presence of 8–10 % NaCl and *B. casei* and *B. epidermidis* in the presence of 15 % NaCl. Nutritionally, they have not been studied very well but most strains of *B. linens* require amino acids and vitamins for growth. Their metabolism is respiratory and they do not produce acid from glucose. They are easily confused with *Arthrobacter* spp. *B. linens* metabolises methionine to methional, which is thought to be responsible from the characteristic “dirty sock” odour of smear-ripened cheeses. *B. linens* can be determined specifically through the production of a stable pink colour within 2 min on treatment of a small amount of a colony with a drop of 5 M KOH or 5 M NaOH or a salmon pink colour within 1 min on treatment with glacial acetic acid. Brevibacteria are acid-sensitive and will not grow a at pH values <6.0. Their major habitats are dairy products, especially cheese, activated sludge and human skin.

11.19.5 *Corynebacterium*

These are Gram-positive, catalase-positive, non-motile, slightly curved rods with tapered ends; club-shaped forms may be found also. Some species are strict aerobes and others are facultative anaerobes. A rod-coccus cycle does not occur. Methylene

blue stains show the presence of deep blue coloured metachromatic granules. *Meso*-diaminopimelic acid and short-chain (22–36 C atoms) mycolic acids are found in their cell wall. They are nutritionally exacting, requiring several vitamins, amino acids, purines and pyrimidines for growth. Two bacteria, *Microbacterium flavum* and *Caseobacter polymorphus,* which were isolated from cheese, have been reclassified as *C. flavescens* and *C. variabile*, respectively. *C. polymorphus* was isolated originally from the surface of Dutch smear-ripened cheese and produces grey-white, slightly pink or slightly red colonies.

11.19.6 Microbacterium

Microbacteria are small, Gram-positive, non-motile or motile, obligately aerobic rods which do not go through a rod/coccus cycle; however, in older cultures (3–7 days), the rods are short and a proportion may be coccoid. Currently, there are 13 species only one of which, *M. lacticum*, has been found in milk. Their optimum temperature is 30 °C. Colonies vary in colour from gray-white to pale green or yellow. Their cell wall peptidoglycan contains lysine. Generally, their metabolism is respiratory but acid is produced from glucose and some other sugars in peptone-containing media. Most strains require biotin, pantothenic acid and thiamine for growth. The main species found in milk is *M. lacticum*, which is thermoduric, surviving heating at 63 °C for 30 min. The organism is not found in aseptically-drawn milk and there is strong evidence that the major source of contamination of milk with this organism is improperly cleaned dairy equipment.

11.19.7 Propionibacterium

These are Gram-positive, non-motile, pleomorphic rods, which may be coccoid, bifid or, sometimes, branched in shape. They occur singly, in pairs, in short chains or in clumps with 'Chinese lettering' arrangements. Colonies vary in colour and can be white, grey, pink, red, yellow or orange. Although these organisms are catalase positive, they are essentially anaerobic or microaerophilic bacteria. Propionibacteria are divided into the 'classical' and 'acnes' groups. The classical group are found mostly in dairy products, particularly cheese, and also in silage and olive fermentations while the acnes group are found mainly on human skin. The classical group is divided into four species: *P. freudenreichii,* the most common one, *P. jensenii*, *P. thoenii* and *P. acidipropionici*. The peptidoglycan of *P. freudenreichii* contains *meso*-diaminopimilic acid (DAP) while the L isomer is found in the other three species. *P. freudenreichii* was considered to exist as two subspecies, *P. freudenreichii* subsp. *freudenreichii* and *P. freudenreichii* subsp. *shermanii* but genetic analyses have shown that both subspecies are identical; the only phenotypic difference is that *P. freudenreichii* subsp. *freudenreichii* is able to ferment lactose while the other

subspecies cannot. The colour of *P. freudenreichii* colonies changes during incubation from grey to tan or pink. *P. freudenreichii* and *P. jensenii* are non-hemolytic while *P. thoenii* and *P. acidipropionici* are β-haemolytic. PAB generally have relatively simple nutritional requirements although they generally require pantothenic acid, biotin or thiamine for growth and many of them can use NO_3^- as the sole source of N.

11.19.8 Pediococcus

These are Gram-positive, catalase-negative cocci which occur as tetrads. Tetrad formation is due to cell division in two directions in a single plane and is characteristic of this genus. There are eight species. They are homofermentative, producing either DL- or L-lactate from sugars and most strains can grow in the presence of 6.5 % NaCl. They generally have complex nutritional requirements. Most pediococci do not ferment lactose and therefore do not grow well in milk. Those strains which metabolise lactose may also lack a proteinase to hydrolyse milk protein to the amino acids and peptides, required for their growth. Some of them can grow at pH 8.5 and 4.2. Some pediococci can metabolise citrate but the products are acetate and formate rather than diacetyl and acetoin. They are found occasionally as a minor part of the NSLAB flora in some hard cheeses. Their influence on the production of cheese flavour is not clear.

11.19.9 Micrococcus

Micrococci are Gram-positive, catalase-positive, strictly aerobic, non-motile cocci (0.2–2.0 μm in diameter) which occur in pairs, clusters or tetrads. The cell wall contains L-lysine. Division occurs in several planes resulting in the formation of regular and irregular clusters. Their natural habitat is skin and currently 17 species are recognised. All grow in the presence of 5 % NaCl and many in the presence of 10–15 % NaCl. Many species produce yellow, orange or red colonies. Their nutritional requirements are variable. *M. luteus*, the type species, produces yellow colonies and grows on glutamate as the sole source of C and N, in the presence of thiamin and/or biotin. Some species can utilise ammonium phosphate as the N source but many species have complex nutritional requirements. They are commonly found on the surface of smear-ripened cheese but the species found are not clear; they dominate the surface microflora of Comté and blue cheese.

Based on 16S rRNA sequencing, the *Micrococcus* and *Arthrobacter* genera overlap. A major taxonomic reassessment of the *Micrococcus* genus has been carried out and a further four new genera have been proposed, *Dermacoccus, Kocuria, Kytococcus* and *Nesterenkonia* (Stackebrandt et al. 1995). *Kocuria* and *Kytococcus* spp. are found in cheese.

11.19.10 Kocuria

These are Gram-positive, catalase-positive, strictly aerobic cocci, although strains of *K. kristinae* are slightly facultative anaerobes. Their cell wall contains lysine and alanine and they can grow in 7.5 % NaCl with some species growing in the presence of 10–15 %. The GC content varies between 66 and 75 mol %. This genus now contains *Micrococcus varians, Mc. roseus and Mc. kristinae* which are described as *K. varians, K. rosea and K. kristinae*, respectively. The latter two species have been found in significant numbers in Parmigiano Reggiano cheese (Coppola et al. 2000).

11.19.11 Kytococcus

These are Gram-positive, catalase-positive, strictly aerobic cocci which can grow in the presence of 10 % NaCl. Their cell wall contains lysine and their GC content is 68–69 mol %. *Ky. sedentarius* has been found in significant numbers in Parmigiano Reggiano cheese (Coppola et al. 2000).

11.19.12 Staphylococcus

These are Gram-positive, catalase-positive, facultatively anaerobic, non-motile cocci (0.5–1.5 μm in diameter), which characteristically divide in more than one plane to form clusters. They can also occur in pairs and tetrads. Currently, 19 species of staphylococci are recognised and many produce yellow or orange colonies. They are facultative anaerobes and grow better aerobically than anaerobically. Most strains grow in the presence of 10 % NaCl and between 10 and 40 °C. Acid is produced anaerobically from several sugars, including glucose and lactose. They are fastidious, requiring from 5 to 12 amino acids and several B vitamins for growth. Major habitats of staphylococci include the nasal membranes, skin and the GI and genital tracts of warm-blooded animals. *S. aureus* causes mastitis in cows and boils and carbuncles in humans and is considered to be a pathogen. Many strains of *S. aureus* produce a heat-stable enterotoxin which causes food poisoning; growth to about 10^6 cfu/g in food is necessary to produce sufficient toxin (0.1–1.0 μg/kg) to cause food intoxication. Coagulase activity is accepted as the indicator of pathogenicity in staphylococci and *S. aureus*, *S. intermedius* and *S. hyicus* produce it. *S. intermedius* has been found in the nasal passage of horses, dogs, mink and foxes and *S. hyicus* on the skin of pigs and less frequently on the skin and in the milk of cows.

Micrococcus and *Staphylococcus* appear as regular and irregular clusters of cells when examined microscopically and traditionally both genera have been placed in the family *Micrococcaceae,* indicating that they are closely related. However, phylogenetic studies show that they are quite distant from each other, *Staphylococcus* spp. belong to the *Clostridium* branch of the Gram-positive bacteria and contain

30–39 mol % GC while *Micrococcus* belong to the *Actinomycete* branch and contain 63–73 mol % GC.

It is relatively easy to distinguish micrococci from staphylococci. The simplest way is to check for acid production from glucose under aerobic and anaerobic conditions. Staphylococci produce acid from glucose aerobically and anaerobically while micrococci either do not produce acid or produce it only aerobically. In addition, micrococci are resistant to lysostaphin, a cell wall degrading enzyme, and are sensitive to erythromycin (0.04 mg/ml) and bacitracin (0.04 U) while staplylococci give the opposite reactions.

11.20 Yeast and Moulds

Yeast and moulds are generally not nutritionally demanding, and are larger and grow more slowly than bacteria. Therefore, they do not compete with bacteria in environments in which bacteria grow, e.g., at pH values around 7. However, they grow quite well at pH values of 2–4 where bacteria either do not grow or grow very poorly. The low pH of freshly made cheese is therefore partially selective for their growth. Yeast and moulds are eukaryotes, i.e., they contain a clearly identifiable nucleus, and most of them also contain chitin, a β-1,4 polymer of *N*-acetylglucosamine, which is responsible for their rigid structure.

Colonies of yeast are generally soft in consistency while those of moulds are hard and large and often show different colourations. In addition, they look quite different under the microscope; yeast are generally round or pear-shaped while moulds show a mycelial network of filamentous hyphae. Some fungi are dimorphic producing hyphae under one set of circumstances and yeast-like cells under another. The human pathogen, *Candida albicans*, is the best example of dimorphism and grows like a yeast in body fluids but develops hyphae to invade tissue.

Both yeast and moulds are classified as fungi and are divided into 3 major groups, *Ascomycetes, Zygomycetes* and *Deuteromycetes*. Classification of fungi is complex and only a few important features are described here. These include determining whether cells in the mycelium are septate (showing the presence of a cross-wall) or non-septate (absence of a cross-wall), the types and ways spores are produced and whether reproduction is sexual or asexual. *Ascomycetes* and *Zygomycetes* are septate, while *Deuteromycetes* are non-septate. The spores produced by *Ascomycetes* are formed in a sac called the ascus (for this reason these spores are called ascospores) and are involved in sexual reproduction. The spores produced by *Deuteromycetes* and by *Zygomycetes* are called conidia (see below) and sporangiophores, respectively and are not involved in sexual reproduction. Yeast generally multiply by budding in which a protuberance is formed on the wall of the cell which eventually breaks off to form a new cell in which further budding occurs. Sometimes, several buds are produced by the same cell and remain attached to it. Some yeast (*Schizosaccharomyces* spp.) multiply by binary fission. Sexual reproduction is given the generic name, teleomorph, while asexual reproduction is called the anamorph. The same fungus has often been given different names depending on the

type of reproduction and some examples of this are shown in Table 11.3. Taxonomically, the teleomorphic name is normally used but there are exceptions, e.g. the anamorphic name, *Geotrichum candidum,* is more commonly used than the teleomorphic one, *Galactomyces candidum.*

The species of yeast found on the surface of different cheeses show considerable diversity (Table 11.3). The dominant species in all cheeses, except Romadour from one plant, is *D. hansenii. Kluvyeromyces* spp. are also dominant in the French (Roquefort, Camembert and St. Nectaire) cheeses and in the Spanish (Cabrales) cheese but appears to be absent from the German and Austrian cheeses (Weinkase, Limburg, Romadour and Tilsit). *S. cerevisiae* and *Pichia* spp. are also important in Camembert and Cabrales cheese. All these yeasts are members of the *Ascomycetes* group. *S. cerevisiae* is also involved in wine, beer and bread-making. Very few yeast are capable of fermenting lactose but *K. lactis* is an exception. This may be one reason for its dominance in some surface-ripened cheese. Whether variation occurs within the same cheese has not been studied to any great extent. The evidence in Table 11.3 suggests that it does occur, at least in Limburg; both cheeses examined contained *D. hansenii* and *G. candidum* in significant numbers but, in addition, *T. delbrueckii* was found in one cheese and *Y. lipolytica* in the other.

The most important moulds in cheese are *P. camemberti, P. roqueforti* and *G. candidum* and all are members of the *Deuteromyces* group. *P. camemberti* is responsible for the white growth on the surface of Camembert and Brie while *P. roqueforti* is responsible for the blue veins found in Roquefort and other blue cheeses. It is generally thought that *G. candidum* is present on the surface of most mould and bacterial-ripened cheese. The results in Table 11.3 would suggest that it is found only in Weinkase, Romadour, Limburg and Tilsit. Scanning electron micrographs of Camembert cheese show the presence of *G. candidum* and it is likely that the reason it was not reported to be present in the other cheeses in Table 11.3 is that the various workers involved considered it to be a mould rather than a yeast.

Microscopic observation is very important in classifying fungi because their various structures can be seen clearly. Both *P. camemberti* and *P. roqueforti* reproduce asexually from conidia (spores) which are extruded from a flask-shaped cell called a phialide which is borne on the conidiophore or spore-bearing hyphae (Fig. 11.23). The multiplication of *G. candidum* is quite different. The hyphae grow to a considerable extent, then stop and septa are formed transversely, separating the hyphae into short compartments which eventually fragment into separate conidia, which start the reproductive process again. *G. candidum* has characteristics of both yeast and moulds and, in the past, was often called a yeast-like fungus. When it was first isolated, it was called *Oidium lactis,* which was later changed to *Oospora lactis*, and later still to its current name; it is commonly known as the dairy mould. Its natural habitat is soil where it is involved in the decay of organic matter (Boutrou and Gueguen 2005).

Many moulds produce toxins, which are carcinogenic, e.g., the aflatoxins produced by *Aspergillus flavus.* However, the strains involved in cheese do not produce toxins. Physiological conditions for the production of toxins by microorganisms are generally much narrower than those for growth.

Most fungi grow quite well at the pH of cheese and most of those found in cheese are also quite tolerant to salt, e.g., the growth of *P. camemberti* is largely unaffected

Table 11.3 Species of yeast found in different smear-ripened cheeses

Teleomorph	Anamorph	Weinkase[1a]		Romadour[1]		Limburg[1]			Tilsit[2]	Roquefort[3]	Cabrales[4]	Camembert[5]	St. Nectaire[6]
		Factory		Factory		Factory							
		A	B	C	D	A	A	C					
Candida catenulata			2	3				12	2				
Candida intermedia			2		10								
Candida mogii												6	
Candida rugosa											4		
Candida saitoana										11			
Debaryomyces hansenii	*Candida famata*	86	95	69		55	64	85	79	16	30	6	86
Dipodascus capitatus	*Geotrichum capitatum*										15		
Galactomyces geotrichum	*Geotrichum candidum*	4	1	6	3	21	17	2	5				
Kluyveromyces lactis	*Candida sphaerica*									35	7	52	10
Kluyveromyces marxianus	*Candida kefyr*									6		9	1
Pichia anomala	*Candida pelliculosa*									15			
Pichia fermentans	*Candida lambica*										16		
Pichia kluyveri											6		
Pichia membranifaciens	*Candida valida*									7	21		
Rhodotorula spp.										5			
Saccharomyces cerevisiae	*Candida robusta*										1	3	

(continued)

Table 11.3 (continued)

Teleomorph	Anamorph	Weinkase[1a]		Romadour[1]		Limburg[1]			Tilsit[2]	Roquefort[3]	Cabrales[4]	Camembert[5]	St. Nectaire[6]
		Factory		Factory		Factory							
		A	B	C	D	A	A	C					
Saccharomyces unisporus											3		
Torulaspora delbrueckii	*Candida colliculosa*					24					1		
Trichosporon beigelii		3		22									
Yarrowia lipolytica	*Candida lipolytica*				87		19		7	5			
Zygosaccharomyces rouxii											1	8	

1.Valdes-Stauber et al. 1997; 2. Eliskases-Lechner and Ginzinger 1995b; 3. Devoyod and Sponem 1970; 4. Nunez et al. 1981; 5. Baroiller and Schmidt 1980; 6. Vergeade et al. 1976

[a]Results from 1 are as a percentage of the surface yeast microflora; other results are as a percentage of the number of strains isolated and/or identified

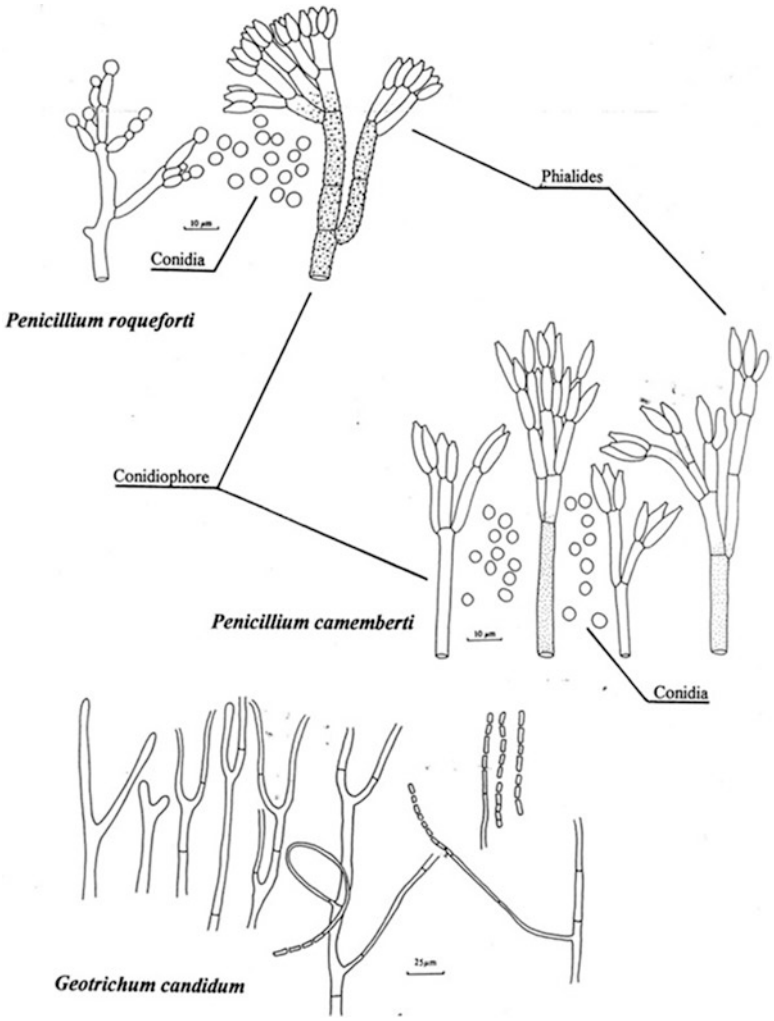


Fig. 11.23 Major features of *Penicillum roqueforti, Penicillum camemberti* and *Geotrichum candidum* (From Samson et al. 1995)

by 10 % NaCl (Table 11.1) and some strains of *P. roqueforti* can tolerate 20 % NaCl. *G. candidum* is an exception and is quite sensitive to salt. A slight reduction in growth occurs in the presence of 1 % NaCl and it is completely inhibited by ~6 % NaCl. Therefore, too much brining will prevent its growth on the cheese surface. Perhaps its intolerance to salt explains why it is sometimes deliberately added in the manufacture of some surface-ripened cheeses.

Generally, yeast are facultative anaerobes while moulds are considered to be obligate aerobes. However, *P. roqueforti* is an exception and can grow in the presence of limited levels of O_2, which is exemplified by its growth throughout the mass of blue cheese. Yeast and moulds are generally heat sensitive and are killed by pasteurisation.

Occasionally yeast have been incriminated in the spoilage of cheese, either through the production of gas (CO_2) or the development of off-flavours. Unripened cheeses, e.g., Cottage, Quarg, etc., especially if they contain sucrose, as a sweetener, are particularly prone to spoilage by yeast.

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Chapter 12
Biochemistry of Cheese Ripening

Summary It is during ripening that the flavour and texture characteristic of the cheese variety develop. Three major pathways constitute the biochemistry of cheese ripening: (1) metabolism of residual lactose and of lactate and citrate, (2) lipolysis and fatty acid metabolism and (3) proteolysis and amino acid catabolism. Cheese ripening is mediated by metabolically active cells from the starter, non-starter and adjunct starter microbiotas and enzymes that contribute to ripening come from the coagulant (principally chymosin but sometimes other proteinases, and in cheeses made using rennet paste, also pregastric esterase), milk (plasmin, somatic cell enzymes and lipoprotein lipase), starter and non-starter lactic acid bacteria (cell envelope-associated proteinases and a wide range of intracellular peptidases, esterases and amino acid catabolic enzymes) and adjunct starters (proteinases, peptidases and lipases). The primary products of cheese ripening (peptides, amino acids and fatty acids) are metabolized further to volatile flavour compounds through metabolism of fatty acids and amino acid catabolism.

Keywords Cheese ripening • Metabolism of lactose and lactate • Lipolysis and fatty acid metabolism • Proteolysis and amino acid catabolism

12.1 Introduction

As discussed in Chap. 1, the original objective of cheese production was to conserve the principal constituents of milk, i.e., the lipids and caseins. However, although well-made hard cheese is quite a hostile environment for microbial growth, with several preservative hurdles, these hurdles are not sufficient to prevent the growth of certain microorganisms (see Chap. 11) and the activity of enzymes from various sources that may be present. These microorganisms and enzymes catalyse a complex series of biochemical reactions, which, if unbalanced, cause off-flavours and textural defects but if properly controlled and balanced, lead to the desirable and

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characteristic flavour and texture of the numerous cheese varieties. Although the biochemistry of cheese ripening is not yet fully characterized, a considerable body of information is now available. The objective of this chapter is to describe the principal biochemical reactions that occur in cheese during ripening.

Cheese curd is a relatively simple mixture of casein (and very little whey proteins except in cheese made from milk concentrated by ultrafiltration), lipids, a little lactose (~1 % at pressing), lactic acid (~1 %), citric acid (0.2 %), NaCl (0.7–~6 %) and water. Not surprisingly, the primary features of ripening involve the two principal organic constituents, i.e., proteins and lipids. However, the metabolism of lactose and citrate, although present at low concentrations, is important in all varieties and critical in some types. Most of the primary reactions are well characterized. Many of the products of the primary reactions undergo further modifications, which are largely responsible for the characteristic flavour of cheese.

12.2 Ripening Agents in Cheese

The ripening of cheese is catalysed by the metabolic activity of living organisms and enzymes from these organisms or from other sources:

- *Coagulant*: usually contributes chymosin or other suitable proteinase (see Chap. 7) but rennet paste used in some Italian varieties contributes both chymosin and lipase, pregastric esterase. In high-cooked cheeses, e.g., Emmental and Parmesan, and *pasta filata* varieties, e.g., Mozzarella, enzymes in the coagulant are extensively denatured by the high temperature used during curd preparation.
- *Milk*: As discussed in Chap. 4, milk contains about 60 indigenous enzymes, at least some of which are significant in cheese ripening, e.g., proteinases, especially plasmin, lipoprotein lipase (in raw milk cheese), acid phosphatase and xanthine oxidase. Most of the indigenous enzymes are quite heat stable and fully or partially survive pasteurization. Furthermore, they are either associated with the casein micelles or present in the fat globule membrane and are, therefore, more likely to be incorporated into the cheese curd than lost in the whey. Enzymes which are present in the serum phase are largely lost in the whey and are therefore of little importance in cheese ripening.
- *Starter culture*: These have been discussed in Chap. 6. Live starter cells probably contribute little to cheese ripening but they possess a diversity of enzymes (see Chap. 11 and Sect. 12.7.4) which are located mainly intracellularly and are released on cell death and lysis. These starter enzymes are major contributors to ripening.
- *Secondary microflora*: Many cheese varieties contain a secondary (non-starter) microflora (see Chap. 11), the function of which is not acid production but rather some specific secondary function; in many cases, flavour development is

dominated by the metabolic activity of the secondary culture. The microorganisms involved include propionic acid bacteria, coryneform bacteria, yeasts and moulds. In addition to these, the growth of which is characteristic and encouraged, cheeses contain adventitious non-starter lactic acid bacteria (NSLAB) which originate from the milk or environment. Owing to the selective nature of the interior of cheese (see Chap. 11), this adventitious microflora is comprised mainly of mesophilic lactobacilli and, to a lesser extent, pediococci.

- *Exogenous enzymes*: With the objective of accelerating ripening, there has been interest in adding exogenous enzymes, usually proteinases and perhaps peptidases and lipases, to the cheese curd.

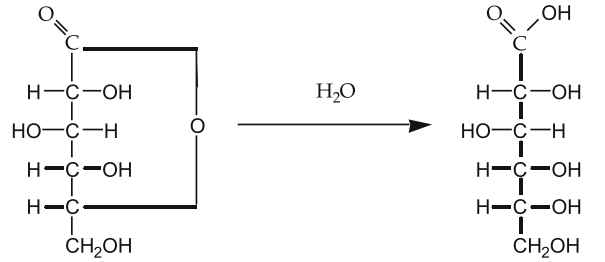
12.3 Contribution of Individual Agents to Ripening

The role of the individual ripening agents in cheese has been studied using model cheese systems in which the action of one or more of the ripening agents is eliminated (see Fox et al. 1993; McSweeney 2004). Pioneering studies on this subject used milk obtained from selected cows by aseptic milking techniques. However, in our experience, bulk herd milk with a total bacterial count (TBC) $< 10^3$ cfu/ml can be obtained from a healthy commercial herd using good, but not special, milking practices.

Heat treatment of aseptically-drawn milk is necessary to further reduce bacterial counts. Batch pasteurization (68 °C × 5 min or 63 °C × 30 min), HTST pasteurization (72 × 15 s) or UHT treatment have been used. A heat treatment of 83 °C × 15 s or 72 °C × 58 s is necessary to ensure an 8-log reduction in the bacterial population which is deemed necessary to produce cheese with a non-starter count < 10 cfu/kg cheese from milk with an initial TBC of 10^3 cfu/ml; HTST pasteurization (72 °C × 15 s) is sufficient for milk with an initial count of 10 cfu/ml.

To avoid contamination from the environment, cheese may be manufactured under aseptic conditions which can be achieved using enclosed cheese vats, a sterile room with a filtered air supply or a laminar air-flow unit; the latter is probably the simplest of these techniques. Using aseptic conditions, it is relatively easy to produce curd free of NSLAB but, in our experience, NSLAB always grow in such cheese, sometimes only after a long (e.g., ~100 days) lag period. A cocktail of antibiotics (penicillin, streptomycin and nisin) extends the lag period and reduces the final number of NSLAB. The growth of NSLAB is strongly retarded, essentially prevented, by ripening at ~1 °C, although the whole ripening process is also retarded.

The acidifying role of starter can be simulated closely using an acidogen, usually gluconic acid-δ-lactone (GDL), although the rate of acidification is faster than occurs in biologically-acidified cheese; incremental additions of lactic acid and GDL give best results, although precise control of pH is difficult.



To study the role of the coagulant in cheese ripening, it is necessary to inactivate the rennet after coagulation, for which four techniques have been developed. One approach involves separating the first and second stages of rennet action: milk depleted of Ca^{2+} and Mg^{2+} by treatment with an ion-exchange resin is renneted (but does not coagulate), heated (72 °C × 20 s) to inactivate the rennet, cooled to <15 °C and $CaCl_2$ added; the renneted milk is then heated dielectrically to induce coagulation. Porcine pepsin may be inactivated after coagulation of the milk by adjusting the pH of the curd-whey mixture to 7.0. Piglet gastric proteinase, which hydrolyses bovine κ-casein but has little activity on α_{s1}- or β-casein, has been used to prepare rennet-free curd in small-scale cheesemaking trials. Chymosin and all commercial rennet substitutes are aspartyl proteinases and are inhibited by pepstatin. The effectiveness of adding pepstatin to Cheddar cheese curd at salting has been demonstrated on a small scale.

6-Aminohexanoic acid (AHA), a non-competitive inhibitor of plasmin, has been used to study the significance of plasmin in cheese ripening. It is necessary to use a high concentration of AHA which affects curd syneresis and the moisture content of the cheese; also, since AHA contains N, the background level of soluble N is increased greatly. Plasmin is inhibited by several proteins, including soytoo high, the concentration of Ca bean trypsin inhibitor, which may be suitable for the inhibition of plasmin in cheese. It is also specifically inhibited by dichloroisocoumarin but neither it nor the inhibitory proteins has been investigated in cheesemaking. Plasmin activity is increased by a high cooking temperature, probably due to inactivation of the indigenous inhibitors of plasmin or of plasminogen activators. A number of plasminogen activators have been used in cheesemaking to increase the plasmin activity of the curd and plasmin itself may also be added to cheesemilk to increase activity. The high heat stability of plasmin suggests that it may also be possible to develop a model system based on aseptic curd in which the rennet is denatured by a suitable cook temperature and acidified by GDL, in which to assess plasmin activity alone.

Some or all of these techniques, in various combinations, have been used to study the contribution of various agents to cheese ripening, especially proteolysis and flavour development.

The biochemistry of cheese ripening will be considered in three principal sections based on the principal biochemical events, i.e., (1) metabolism of residual lactose and of lactate and citrate, (2) lipolysis and metabolism of fatty acids and (3) proteolysis and amino acid catabolism.

12.4 Metabolism of Residual Lactose and of Lactate and Citrate

The primary glycolytic event, the conversion of lactose to lactate, is normally mediated by the starter culture during curd preparation or in the early stages of ripening. In cases where glycolysis has not been completed by the starter, non-starter lactic acid bacteria may contribute. The metabolism of lactose by lactic acid bacteria was discussed in Chap. 6.

Approximately ~96 % of the lactose in milk is removed in the whey as lactose or, after fermentation, as lactate. However, fresh curd contains a considerable amount of lactose, the fermentation of which has a significant effect on cheese quality. Obviously, the concentration of lactose in fresh curd depends on its moisture content, the extent of fermentation prior to moulding and on whether the curd is washed with water or not. Cheddar curd, which is extensively drained and has reached a pH of ~5.4 at milling, contains 0.8–1.0 % lactose. In the manufacture of Dutch-type cheese (Edam and Gouda), part of the whey is removed and replaced by water but the curd is subjected to less syneresis and the pH is high, ~6.2–6.3, at moulding; hence Gouda cheese curd contains ~3.0 % lactose at moulding. Although Emmental cheese curd is cooked to a high temperature (52–55 °C), and hence undergoes extensive syneresis in the vat, curds are transferred with the whey to moulds at ~ pH 6.4 and contains ~2 % lactose at moulding.

Compared with other varieties, the residual lactose in Cheddar is fermented relatively slowly at a rate and to an extent dependent on the percentage salt-in-moisture (S/M) in the curd. At a low S/M concentration and a low population of NSLAB, residual lactose is converted mainly to L-lactate by the starter. At a high population of NSLAB, e.g., at a high storage temperature, a considerable amount of D-lactate is formed, partly by fermentation of residual lactose and partly by isomerization of L- to D-lactate. At a high S/M level (e.g., 6 %) and a low NSLAB population, the concentration of lactose decreases slowly and changes in lactate are slight. The quality of Cheddar cheese is strongly influenced by the fermentation of residual lactose: the pH decreases after salting at a S/M level <5 %, due primarily to the continued action of the starter, but at a higher level of S/M, starter activity decreases abruptly, as indicated by a high level of residual lactose and a high pH, accompanied by a sharp decrease in cheese quality.

Dutch-type cheese contains ~3.0 % lactose at pressing but this decreases to undetectable levels within about 12 h.

Typically, Emmental cheese curd contains ~1.7 % lactose 30 min after moulding. Neither *Streptococcus thermophilus* nor starter *Lactobacillus* spp. grow in Emmental curd during cooking owing to the high cook temperature (52–55 °C). The curds are transferred to moulds while still hot but as they cool in the moulds, *Sc. thermophilus* begins to grow and metabolize lactose. Only the glucose moiety of lactose is metabolized by *Sc. thermophilus* and consequently galactose accumulates to a maximum of ~0.7 % at ~10 h, when the galactose-positive lactobacilli begin to multiply.

These metabolize galactose and residual lactose to a mixture of D- and L-lactate, which reach ~0.35 and 1.2 %, respectively, at 14 day, when all the sugars have been metabolized (see Chap. 11).

12.4.1 Effect of Lactose Concentration of Cheese Quality

Although the concentration of lactose in milk decreases with advancing lactation (see Chap. 4), its concentration in bulked factory supplies from cows on a staggered calving pattern is essentially constant. However, when synchronized calving is practiced, as in e.g., New Zealand, Ireland and Australia, substantial seasonal changes occur in the concentration of lactose in milk and, consequently, in fresh cheese curd. Variations in the concentration of lactose in cheese curd probably affect the final pH of the cheese, which, in turn, affects cheese texture, enzyme activity, and perhaps the non-starter microflora. Cheese flavour is likely to vary owing to variations in the concentrations of lactic and acetic acids, and to variations in the metabolic activity of the cheese microflora.

The concentration of lactose in cheese curd is affected by some features of the manufacturing process. As far as is known, the concentration of lactose in cheese curd is not increased intentionally for any variety of rennet-coagulated cheese. However, curd made from milk concentrated to a high factor by ultrafiltration (i.e., pre-cheese) contains a high level of lactose owing to the lack of syneresis and lactose may have to be reduced to an appropriate level by diafiltration. If the concentration of lactose in cheese curd is too high, the concentration of Ca D-lactate will exceed its solubility and crystallize in the cheese. The concentration of lactose in the curd of several varieties, including Gouda and Edam, is reduced by replacing part of the whey by warm water. This process, which was probably introduced as a simple method for cooking the curds on farms lacking jacketed cheese vats, effectively controls the pH of the cheese. In these cheeses, the level of wash water added is based on the concentrations of lactose and casein in the milk. This washing protocol minimizes variations in the pH of cheese curd ex-press which might otherwise occur due to seasonal variations in lactate-to-casein ratio. Curds are washed in the washed-curd variants of Cheddar cheese and perhaps in the production of low-fat cheese to increase its moisture content.

The effect of variations in the concentration of lactose in cheese curd on the quality of the mature cheese has received very little attention. In an attempt to vary the concentration of lactose in Cheddar cheese curd, Huffman and Kristoffersen (1984) added lactose to the curds/whey after cutting the coagulum but owing to the strong outflow of whey from the curd at that stage, due to syneresis, the increase achieved in the concentration of lactose within the curd was quite small. Waldron (1997) reduced the lactose content of Cheddar cheese curd by replacing 35–45 % of the whey shortly after cutting the coagulum by an equal volume of warm water; the curd contained 0.25 % lactose compared with ~1 % in the control. A curd containing only 0.03 % lactose was obtained by repeating the whey removal/replacement treatment.

The lactose level was also reduced by washing the curd, prior to salting, with water but this was less effective than whey replacement, possibly because little syneresis occurs at this late stage of curd production. The lactose content of other batches of curd was increased by using lactose-supplemented milk (6.4 or 8.4 % lactose) for curd manufacture. Overall, the concentration of lactose in the 1-day-old cheese ranged from 0.03 % to 2.5 %. Changes in the concentration of lactose in, and the pH of, the cheese during ripening are shown in Fig. 12.1a, b. The lactose in both types

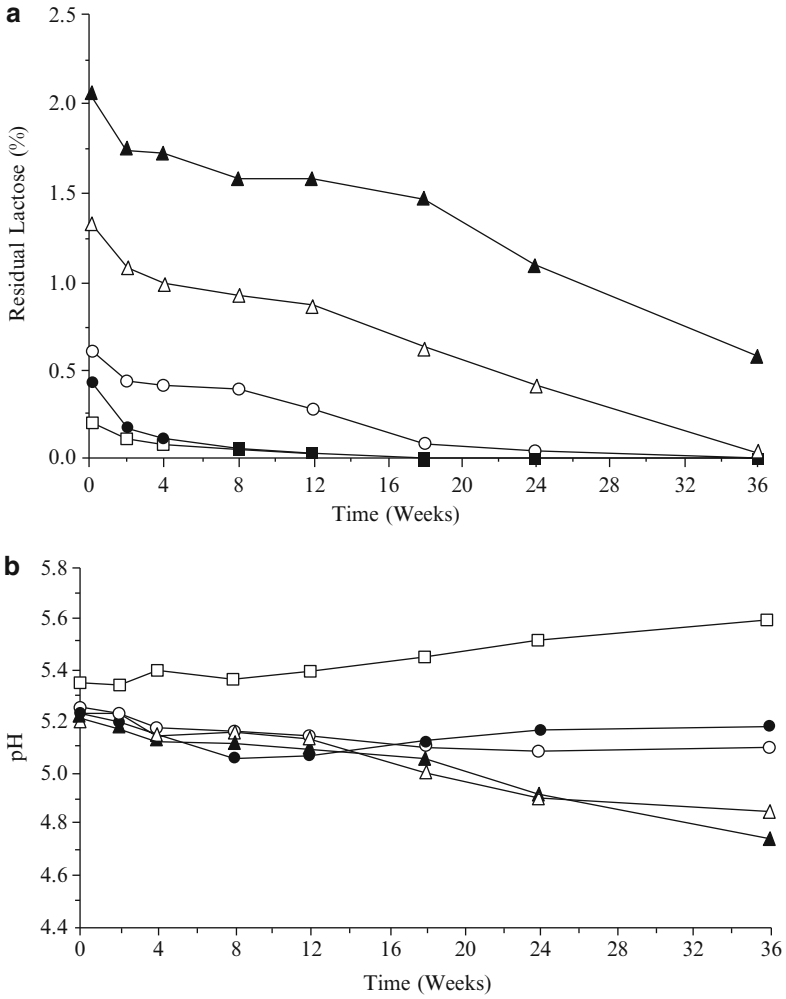


Fig. 12.1 Changes in the concentration of lactose (**a**) and in the pH (**b**) of cheese made from curd with modified levels of lactose during ripening; control: (*open circle*); 35 % whey-replaced: (*open square*); washed-curd: (*filled circle*) and lactose-enriched (6.4, *open triangle* or 8.4, *filled triangle*) cheeses (Waldron and Fox unpublished)

of washed-curd cheese was completely metabolized within ~2 weeks but it persisted in the high-lactose cheeses throughout ripening. Not surprisingly, the pH of the cheeses was inversely proportional to the concentration of lactose in the curd; the pH of high-lactose cheeses continued to decrease (to ~4.8) throughout ripening whereas in the washed-curd cheeses, the pH increased once the lactose had been exhausted. This increase in pH is common in many varieties of cheese, probably due to proteolysis and the production of NH_3 from amino acids. Flavour development was substantially faster in the high-lactose cheese than in the washed-curd cheeses, although it was considered to be rather harsh, perhaps due to the low pH; the flavour of the low-lactose cheeses was clean and mild. The rate of growth and the final number of NSLAB were not affected by the concentration of lactose, suggesting that NSLAB do not depend on lactose as a growth substrate. The results of Waldron (1997) were confirmed by Shakeel-ur-Rehman et al. (2004). (Factors affecting the growth of NSLAB in cheese are discussed in Chap. 11 and by Fox et al. 1998).

The results of this study suggest that the concentration of lactose in cheese curd has a substantial effect on the quality of Cheddar and probably other cheeses. Replacing some of the whey by water or washing the cheese curd might be considered when a mild, clean flavour is desired. Normal variations in the lactose content of milk from mixed-calving herds are probably not significant but may have a substantial effect when synchronized calving is practiced. Under such circumstances, the pH of the cheese may vary with undesirable consequences. The use of low concentration factor (LCF)-UF milk for cheese manufacture is not expected to influence cheese pH, as the lactose content of cheese is increased only very slightly (by ~0.25 % when milk is concentrated 1.5-fold). The problem is more serious when high concentration UF retentate is used and diafiltration of the cheesemilk or washing of the curd would appear to be desirable or essential. Cheese made from milk with a high content of fat and casein may have a reduced lactose content.

The presence of residual lactose or its component monosaccharides in cheese may lead to Maillard (non-enzymatic) browning if the cheese is heated, e.g., as a food ingredient. Lactose *per se* is unlikely to be a problem except when very young cheese curd is used in processed cheese. Browning is most likely to be problematic in cheeses made with thermophilic cultures; as discussed in Chaps. 6 and 11, *Sc. thermophilus* is unable to metabolize the galactose moiety of lactose, which it excretes. If a galactose-positive strain of *Lactobacillus* is used, the galactose will be metabolized to L- or DL-lactate but most strains of *Lactobacillus delbrueckii* and *Lb. lactis* are galactose-negative and therefore galactose accumulates. The residual galactose may cause undesirable browning in many cheeses but is a particularly serious problem in Mozzarella which is subjected to considerable heating during the cooking of pizza. Browning may also be problematic in grated cheese, e.g., Parmiagiano Reggiano and Grana Padano, which have a low moisture content and approach a water activity which is optimal for Maillard browning (~0.6). Since grating cheeses are extensively ripened, other carbonyls, e.g., diacetyl or glyoxals, which are very active in Maillard browning, may contribute to browning. The presence of a fermentable carbohydrate during ripening can also lead of undesirable secondary fermentations leading to off-flavour, gas production and/or reduced quality.

12.4.2 Modification and Catabolism of Lactate

The fate of lactic acid during cheese ripening has some significance in all varieties and is of major consequence in some types. Lactic acid has a direct effect on the taste of cheese, especially young cheese which lacks other flavour compounds. Obviously, lactic acid affects the pH of cheese and consequently its texture (see Chap. 14). pH affects the solubility of $Ca_3(PO_4)_2$ and hence also indirectly affects cheese texture. Perhaps most importantly, lactic acid is an important substrate for microbial growth in many cheese varieties and its catabolism has major effects on cheese flavour (see Fox et al. 1990; McSweeney and Fox 2004).

Typical concentrations of lactate in Camembert, Swiss, Romano and Cheddar are 1.0, 1.4, 1.0 and 1.5 %, respectively. The fate of lactic acid in cheese depends on the variety. Initially, Cheddar contains only L(+) lactic acid but as the cheese matures, the concentration of D-lactate increases and eventually a racemic mixture is formed (see Chap. 11). D-Lactate could be formed from residual lactose by lactobacilli or by racemization of L-lactate by NSLAB, including pediococci. Except in cases where the post-milling activity of the starter is suppressed (e.g., by S/M >6 %), racemization is probably the principal mechanism. Racemization of L-lactate appears to occur in several cheese varieties (Thomas and Crow 1983; McSweeney and Fox 2004) and a racemic mixture will be formed if the duration of ripening is long enough (Table 12.1). Racemization is not significant from the flavour viewpoint but calcium-D-lactate, which is less soluble than L-lactate, may crystallize in cheese, especially on the surface, causing undesirable white specks of calcium lactate pentahydrate crystals.

Table 12.1 Concentration of L(+)-and D(−)-lactate in various cheeses (modified from Thomas and Crow 1983)

		Lactate (g/100 g cheese)	
Cheese type	Age (weeks)	L(+)	D(−)
Cheshire	15	0.75	0.66
	23	0.74	0.73
Colby	16	0.71	0.68
	–	0.57	0.51
Egmont	20	0.62	0.37
Gouda	10	0.84	0.31
	15	0.66	0.55
	–	0.69	0.42
Blue	8	0.65	0.61
	–	0.74	0.43
Camembert	6	0.17	0.02
	8	0.04	0.01
	3	0.57	0.02
Feta	–	0.97	0.88

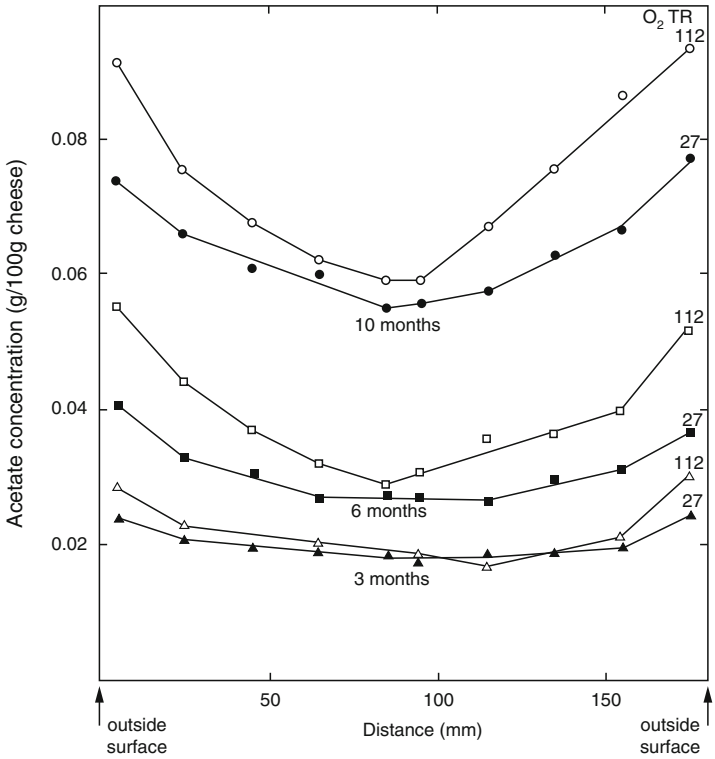


Fig. 12.2 Acetate concentration gradients in 20 kg blocks of Cheddar cheese, inoculated with *P. pentosaceus* 1220, and ripened at 12 °C and 85–90 % RH for 3, 6 and 10 months. The cheeses were wrapped in plastic film with O_2TRs of 27 ml $O_2\,m^{-2}$ 24 h^{-1} (*closed symbols*) or 112 ml $O_2\,m^{-2}$ 24 h^{-1} (*open symbols*) (from Thomas 1987)

Lactate in cheese may be oxidized to acetate. Pediococci produce 1 mol of acetate and 1 mol CO_2 and consume 1 mol of O_2 per mole of lactate utilized; lactate is not oxidized until all sugars have been exhausted. The oxidation of lactate to acetate in cheese depends on the NSLAB population and on the availability of O_2, which is determined by the size of the block and the oxygen permeability of the packaging material (Fig. 12.2); since cheese is very anaerobic, it is not clear if this pathway is of significance to cheese ripening. However, acetate, which may also be produced by starter bacteria from lactose or citrate or from amino acids by starter bacteria and lactobacilli, is usually present at a fairly high concentration in Cheddar cheese and is considered to contribute to cheese flavour, although high concentrations may cause off-flavours.

In Romano cheese, L-lactate predominates initially, reaching a maximum of~1.9 % at 1 day. The concentration of L-lactate begins to decrease after 10 day, reaching 0.2 to 0.6 % after 150–240 day of ripening. Some of the decrease is due to racemization to D-lactate, which reaches a maximum (up to 0.6 % in some cheeses)

at ~90 day and then declines somewhat. In some cheeses, acetate reaches a very high level (1.2 %) at ~30 day, but decreases thereafter; the agents responsible for the metabolism of acetate have not been identified, but yeasts (e.g., *Debaryomyces hansenii*) may be involved. It is possible that the oxidation of lactate to acetate also occurs in other hard and semi-hard cheeses but studies in this area are lacking.

The metabolism of lactate is very extensive in surface mould-ripened varieties, e.g., Camembert and Brie. The concentration of lactic acid in these cheeses is ~1.0 % at 1 day, produced exclusively by the mesophilic starter, and hence is L-lactate. Secondary organisms quickly colonize and dominate the surface of these cheeses, initially *Geotrichum candidum* and yeasts, followed by *Penicillium camemberti,* and, in traditional manufacture, by *Brevibacterium linens* and other coryneform bacteria (see Chap. 11). *G. candidum* and *P. camemberti* rapidly metabolize lactate to CO_2 and H_2O, causing an increase in pH (Fig. 12.3). Deacidification occurs initially at the surface, resulting in a pH gradient from the surface to the centre and causing lactate to diffuse outwards. When the lactate has been exhausted, *P. camemberti* metabolizes proteins, producing NH_3, which diffuses inwards, further increasing the pH. The concentration of calcium phosphate at the surface exceeds its solubility at the increased pH and it precipitates as a layer of $Ca_3(PO_4)_2$ at the surface, thereby causing a calcium phosphate gradient within the cheese (Fig. 12.4). The elevated pH stimulates the action of plasmin, which contributes significantly to proteolysis, along with residual coagulant. Although surface microorganisms secrete very potent proteinases, they diffuse into the cheese to only a very limited extent; however, peptides and other compounds produced at the surface may diffuse into the cheese. The combined action of increased pH, loss of calcium (necessary for the integrity of the protein network) and proteolysis are necessary for the very extensive softening of the body of Brie and Camembert (Lenoir 1984; Karahadian and Lindsay 1987; McSweeney and Fox 2004).

The pH of blue cheese also increases substantially during ripening (Fig. 12.5); however, in contrast to surface mould-ripened cheeses, the extent of the increase is greater at the centre than at the surface. One would expect that catabolism of lactic acid would be responsible for the increase in pH but the only published data available suggest that blue cheese contains a high concentration of lactic acid (~1.2 %, see Table 12.1). Perhaps the increase in pH is due to the production of NH_3 on the catabolism of amino acids. Among blue cheeses, the increase in pH appears to be least in Danablu, in which a low level of NH_3 is produced.

Large changes in pH also occur in surface smear-ripened cheeses, especially at the surface (Fig. 12.6). In these cheeses, lactate in the surface layer is catabolised by yeasts, which are the first microorganisms to colonize the surface. The increase in pH at the surface is a critical factor in the ripening of these cheeses. The characteristic Gram-positive pigmented microorganisms in the smear, do not grow at a pH < 5.8 (see Chap. 11).

The catabolism of lactic acid is also critical in Swiss-type cheeses but the causative agents and effects are different from those in surface mould and smear-ripened cheeses. On transfer to the warm room, *Propionibacterium freudenreichii*, the characteristic microorganism in Swiss-type cheeses, multiplies by 2–3 log

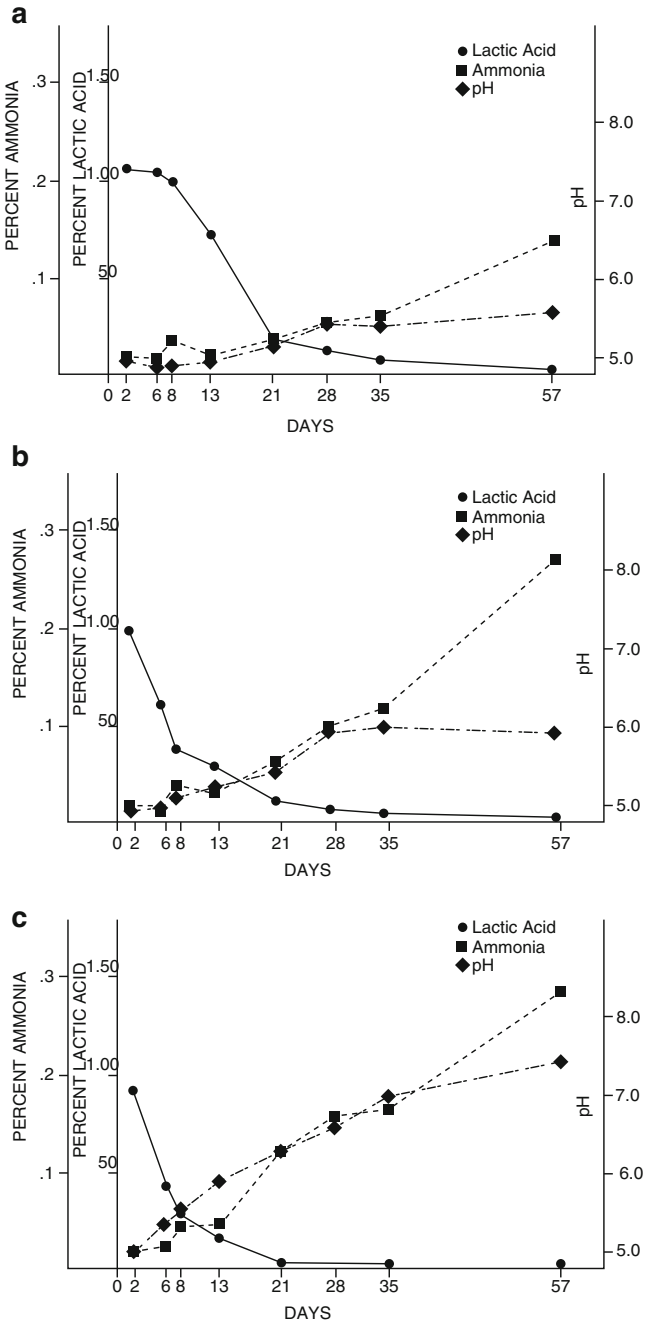


Fig. 12.3 Rate of lactic acid metabolism (*filled circle*), ammonia production (*filled square*), and pH changes (*filled diamond*) in Brie cheese sampled at the centre (**a**), surface (**b**), and corner (**c**) during ripening (from Karahadian and Lindsay 1987)

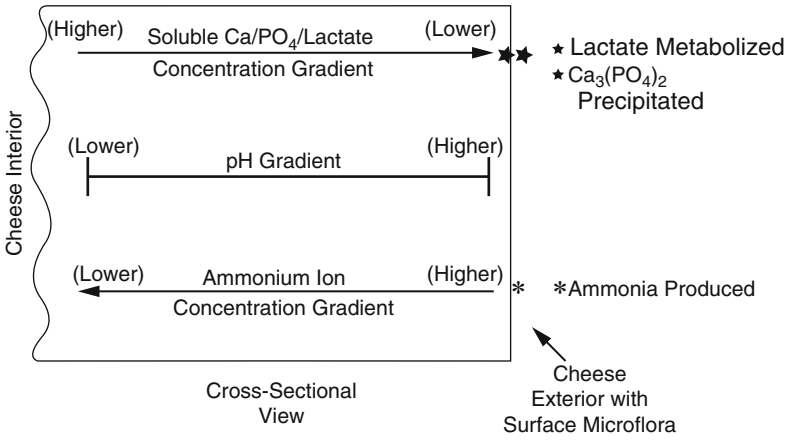


Fig. 12.4 Schematic representation of the gradients of calcium, phosphate, lactic acid, pH and ammonia in ripening of Camembert cheese (from Karahadian and Lindsay 1987)

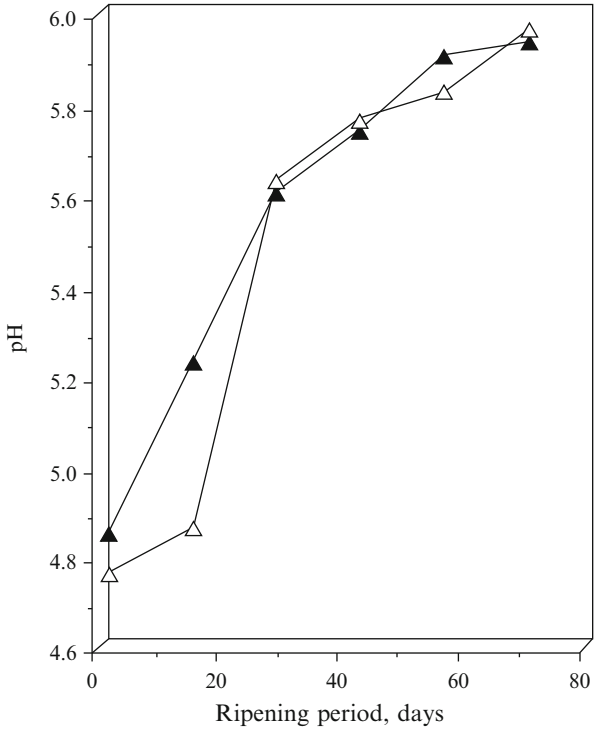


Fig. 12.5 Changes in the pH of two batches (*filled triangle*, *open triangle*) of an Irish blue cheese during ripening (from Zarmpoutis 1995)

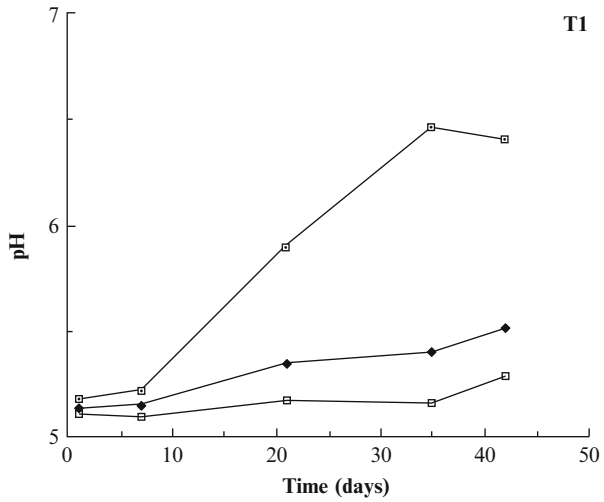
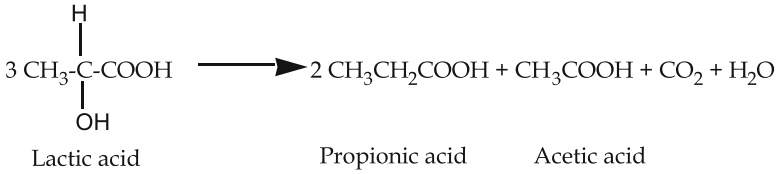


Fig. 12.6 Changes in the pH in the surface (*open aquare*), middle (*filled diamond*) and core (*square with center dot*) layers of Taleggio cheese during ripening (from Lowney 1997)

cycles and metabolizes lactate, preferentially the L isomer, to propionate, H_2O acetate and CO_2 (see Chap. 11):



The CO_2 generated is responsible for eye development, a characteristic feature of these varieties. Most of the CO_2 produced diffuses through the curd and is lost but if the growth of *Propionibacterium freudenreichii* is adequate, sufficient CO_2 is produced to induce good eye formation. The acetic acid and especially the propionic acid produced in this fermentation contribute to the flavour of Swiss-type cheeses.

A common defect in many cheeses arises from the metabolism of lactate (or glucose) by *Clostridium* spp. to butyrate, H_2 and CO_2 (Fig. 12.7). This reaction leads to late gas blowing and off-flavours in many cheese varieties unless precautions, e.g., good hygiene, addition of $NaNO_3$ or lysozyme, bactofugation or microfiltration, are taken.

The significance of the primary fermentation of lactose to L-lactate in cheese manufacture is well recognized (see Chaps. 6, 7 and 11). The foregoing discussion indicates that the metabolism of lactose and lactate in cheese during ripening is well understood. In quantitative terms, these changes are among the principal metabolic events in most cheese varieties. However, in comparison with other biochemical changes during cheese ripening, the conversion of lactose to lactate may have relatively little direct effect on the flavour of mature cheese but since it determines the

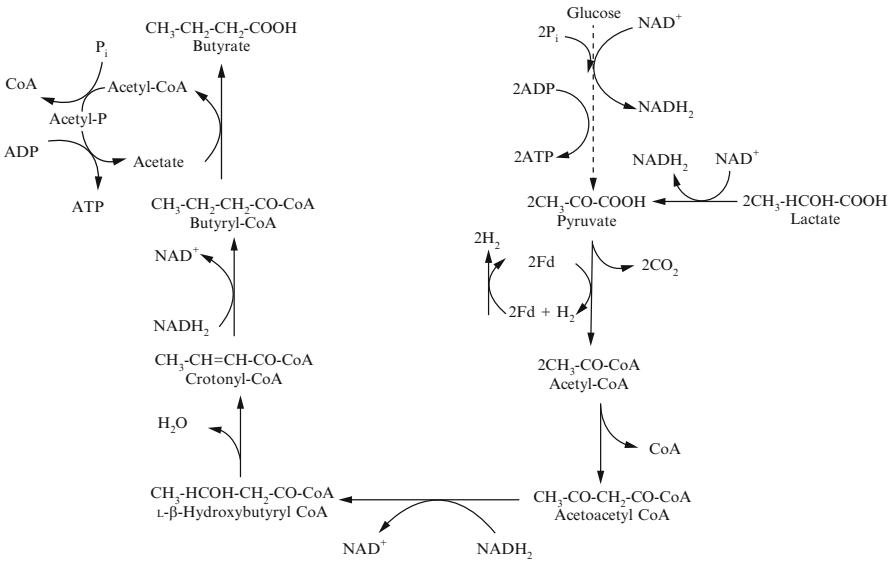


Fig. 12.7 Metabolism of glucose or lactic acid by *Clostridium tyrobutyricum* with the production of butyric acid, CO_2 and H_2 (from Fox and McSweeney 1998)

pH of cheese, it is of major indirect significance by regulating the various biochemical reactions that occur in cheese during ripening. The isomerization of lactate probably has little impact on cheese flavour, but its conversion to propionate and/or acetate is probably significant and, when it occurs, the metabolism of lactate to butyrate has a major adverse effect on cheese quality.

12.5 Citrate Metabolism

The relatively low concentration of citrate in milk (~8 mM) belies the importance of its metabolism in some cheeses made using mesophilic cultures (for reviews see Cogan and Hill 1993; Parente and Cogan 2004). Most of the starters used in cheese production, i.e., *Lc. lactis* subsp. *lactis, Lc. lactis* ssp. *cremoris, Lactobacillus* spp. and *Sc. thermophilus,* do not metabolize citrate but a minor component of mixed-strain mesophilic starters, e.g., those used for Dutch-type cheeses contain strains of *Lc. lactis* ssp. *lactis* and *Leuconostoc* spp which metabolize citrate to diacetyl in the presence of a fermentable sugar during manufacture and early ripening (see Chap. 6). The CO_2 produced is responsible for the small eyes characteristic of Dutch-type cheeses.

Diacetyl is a very significant compound in the aroma/flavour of unripened cheeses, e.g., Cottage cheese, Quarg and many fermented milks. It contributes to the flavour of Dutch-type cheeses and possibly Cheddar also. Acetate may also contribute to the flavour of these cheeses.

Approximately 90 % of the citrate in milk is soluble and is lost in the whey; however, the concentration of citrate in the aqueous phase of cheese is ~3 times that in whey, reflecting the concentration of colloidal citrate. Cheddar cheese contains 0.2 to 0.5 % (w/w) citrate which decreases to 0.1 % at 6 months through the metabolic activity of some mesophilic lactobacilli, many of which catabolise citrate to ethanol, acetate and formate (see Chap. 11) late in the ripening when numbers of NSLAB have increased sufficiently.

12.6 Lipolysis and Related Events

Pure lipids elicit an oily sensation in the mouth but are often devoid of flavour *sensu stricto*. However, lipids have a major effect on the flavour and texture of foods, including cheese. The influence of lipids on cheese texture is discussed in Chap. 14.

Lipids contribute to cheese flavour in three ways:

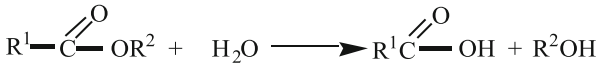
- As a source of fatty acids, especially short-chain fatty acids, which have strong and characteristic flavours. Fatty acids are produced through the action of lipases in a process referred to as **lipolysis**. In some varieties, the fatty acids may be converted to other sapid and aromatic compounds, especially methyl ketones and lactones.
- Fatty acids, especially polyunsaturated fatty acids, undergo oxidation with the formation of various unsaturated aldehydes which are strongly flavoured, causing flavour defects referred to as **oxidative rancidity**. Lipid oxidation is very limited in cheese due to its low redox potential (−250 mV) and the relatively low levels of polyunsaturated fatty acids in milkfat.
- Lipids function as solvents for sapid and aromatic compounds produced not only from lipids but also from proteins and lactose. Lipids may also absorb compounds from the environment, which may cause off-flavours.

Of the various possible contributions of lipids to cheese flavour, lipolysis and modification of the resultant fatty acids are the most significant.

The degree of lipolysis in cheese varies widely between varieties, from ~6 meq/100 g free fatty acids in Gouda to 45 meq/100 g fat in Danish Blue (Gripon 1987, 1993). Lipases in cheese originate from milk, rennet preparation (paste), starter, adjunct starter or non-starter bacteria. Lipolysis in internal bacterially-ripened varieties, such as Gouda, Cheddar and Swiss, is generally low but is extensive in mould-ripened and some Italian varieties, particularly those made using rennet paste. In general, in those varieties in which extensive lipolysis occurs, lipases originate from the coagulant (rennet paste, which contains pregastric esterase), or from the adjunct culture (*Penicillium* spp., which produce a number of lipases (Gripon 1987, 1993) in mould-ripened varieties.

12.6.1 Lipases and Lipolysis

Lipases are hydrolysases which hydrolyse esters of carboxylic acids (EC. group 3.1.1.X):



Lipases have little or no activity on soluble esters and they act at the oil–water interface of emulsified esters. Thus, lipases are distinguished from esterases by the physical state of the substrate rather than by the type of bond hydrolysed.

Lipases exhibit various types of specificity:

- They are usually specific for the outer ester bonds of tri- or diglycerides, i.e., *sn*-1 and *sn*-3 positions; thus, initially they hydrolyse triglycerides to 1,2- and 2,3-diglycerides and later to 2-monoglycerides. The fatty acid at the *sn*-2 position migrates to the vacant *sn*-1 or *sn*-3 position and is then released by lipase (Fig. 12.8). Thus, lipases eventually hydrolyse triglycerides to glycerol and three free fatty acids. In most cheeses, lipolysis probably does not go beyond the first step.
- Lipases usually exhibit specificity for fatty acids of certain chain length.
- Some lipases exhibit specificity for saturated or unsaturated fatty acids.

Although some lipases may be optimally active at neutral or acid pH values, most have an alkaline pH optimum. Lipase activity is inhibited fatty acids and, therefore, lipases are activated by Ca^{2+} which precipitate the fatty acids as insoluble soaps and remove them from the reaction environment. The whey proteins, β-lactoglobulin and blood serum albumin, bind fatty acids and stimulate lipase activity. Lipases are activated by bile salts which emulsify the triglyceride substrates. Some lipases, referred to as lipoprotein lipases, are also stimulated by lipoproteins, which promote the adsorption of the enzyme at the oil–water interface; an example is blood serum lipase, which is the indigenous lipase in milk.

12.6.2 Indigenous Milk Lipase

Milk contains an indigenous lipoprotein lipase (LPL) which is well characterized (Olivecrona and Bengtsson-Olivecrona 1991; Olivecrona et al. 1992; O'Mahony et al. 2013). The enzyme enters milk as a result of leakage through the mammary cell membrane from the blood where it is involved in the metabolism of plasma triglycerides. Bovine milk contains 1–2 mg lipase/L (10–20 nM). Under optimum conditions, it has a turnover of 3000 s^{-1} and could theoretically release sufficient fatty acids in 10 s to cause hydrolytic rancidity. However, most (>90 %) of the lipase

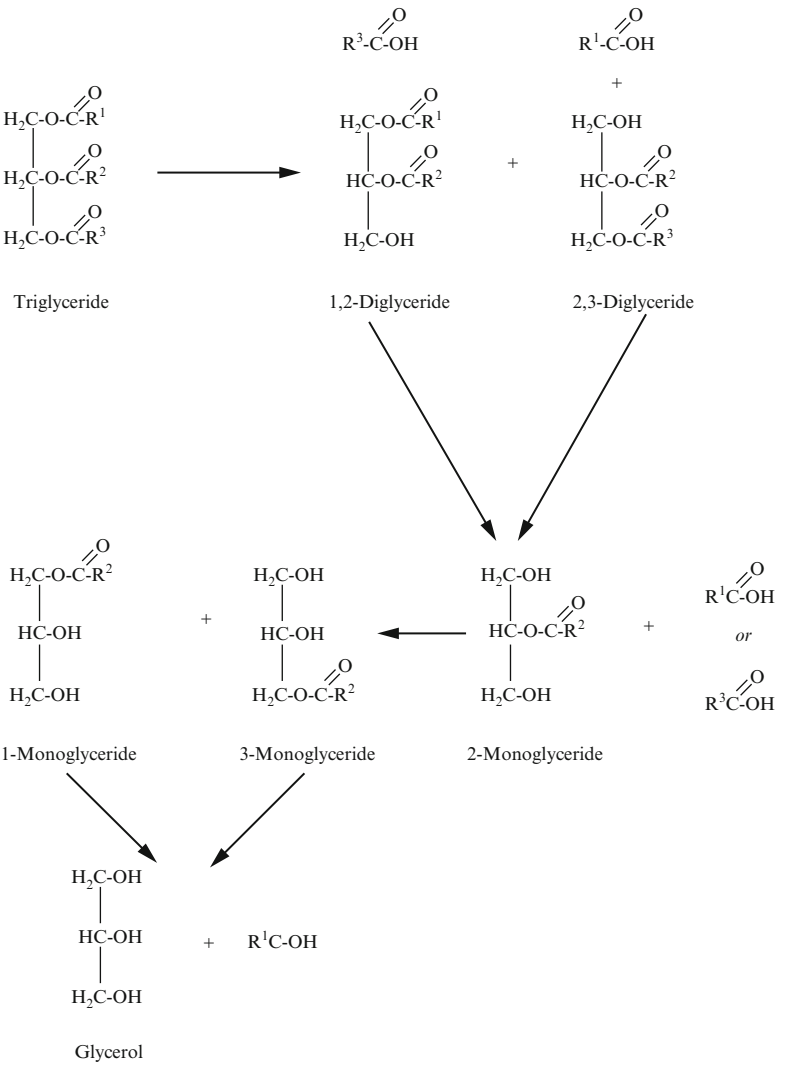


Fig. 12.8 Hydrolysis of a triglyceride by a lipase

is associated with the casein micelles and the fat occurs in globules surrounded by a lipoprotein membrane (the milk fat globule membrane, MFGM). Thus, the substrate and enzyme in milk are compartmentalized and lipolysis does not occur unless the MFGM is damaged by, e.g., agitation, foaming, freezing or homogenization. Inappropriate milking and milk handling techniques at the farm and/or factory may cause sufficient damage to the MFGM to permit significant lipolysis and lead to off-flavours in cheese and other dairy products.

LPL is rather non-specific for the type of fatty acid but is specific for the *sn*-1 and *sn*-3 positions of mono-, di- and tri-glycerides. Therefore, lipolysis in milk leads to preferential release of short and medium chain acids which, in milk triglycerides, are esterified predominantly at the *sn*-3 position. Since more than 90 % of the LPL in bovine milk is associated with the casein micelles, it is incorporated into cheese curd. LPL is relatively heat labile and is extensively inactivated by HTST pasteurization although heating at the equivalent of 78 °C for 10 s is required for complete inactivation. Significantly more lipolysis occurs in raw milk cheese than in pasteurized milk cheese (Fig. 12.9). Milk LPL is probably responsible for difference but the NSLAB microflora of raw and pasteurized milk cheeses also differ markedly.

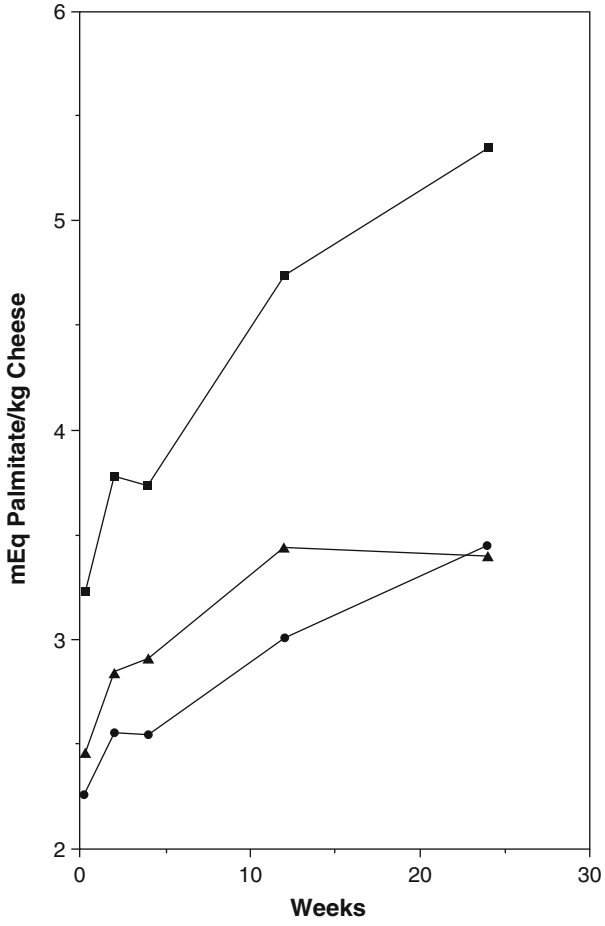


Fig. 12.9 Liberation of free fatty acids in Cheddar cheese made from raw (*filled square*), pasteurized (*filled circle*), or microfiltered milk (*filled triangle*) (from Fox and McSweeney 1998)

12.6.3 Lipases from Rennet

Good quality rennet extract contains no lipolytic activity. However, rennet paste used in the manufacture of certain hard Italian (e.g., Pecorino cheeses, Provolone), Greek and Spanish varieties contain a potent lipase, pregastric esterase (PGE), which is responsible for the extensive lipolysis in, and the characteristic "piccante" flavour of, such cheeses. The literature on PGE was comprehensively reviewed by Nelson et al. (1977) and updated by Fox and Stepaniak (1993).

PGE, also called lingual or oral lipase, is secreted by glands at the base of the tongue. Suckling stimulates the secretion of PGE which is subsequently washed into the abomasum by milk and saliva. Rennet paste is prepared from the abomasa of calves, kids or lambs slaughtered after suckling. The abomasa are partially dried and ground into a paste which is slurried in milk or water before addition to cheesemilk. Rennet pastes are considered unhygienic and their use is not permitted in several countries, e.g., USA; instead, partially purified PGEs or other lipases are used.

Calf, kid and lamb PGEs have been partially purified from commercial preparations and calf PGE from oral tissue. The enzyme is a glycoprotein with an isoelectric pH of 7.0 and a MW of about 49 kDa. The gene for rat lingual lipase has been cloned and sequenced and the primary structure of the enzyme deduced. PGE is highly specific for short-chain acids esterified at the *sn*-3 position and therefore releases high concentrations of highly flavoured short and medium chain acids from milk fat. The specificity of calf, lamb and kid PGEs differ slightly, and, consequently, the flavour characteristics of Italian cheese differ slightly, depending on the source of the PGE used. Most other lipases are unsuitable for the manufacture of Italian cheeses because of incorrect specificity but it has been claimed that certain fungal lipases may be acceptable alternatives (see Fox and Stepaniak 1993).

12.6.4 Microbial Lipases

Lactococcus spp. and *Lactobacillus* spp. have low lipolytic activity compared to other bacteria, e.g., *Pseudomonas*, and moulds. However, in the absence of strongly lipolytic agents and when present at high numbers over a long period, as in ripening cheese, lipases/esterases of lactococci and lactobacilli are probably the principal lipolytic agents in Cheddar- and Dutch-type cheeses made from pasteurized milk; aseptic cheeses acidified with GDL instead of starter have low concentrations of FFA which do not increase during ripening. The lipase/esterase activity of lactic acid bacteria (LAB) appears to be entirely intracellular. Cell-free extracts of various dairy LAB are most active on tributyrin at pH 6–8 and at 37 °C; they have little or no activity on triglycerides of long-chain fatty acids, e.g., $>C_{10}$. There appears to be considerable inter-strain variation in esterase/lipase activity and some strains appear to possess two esterases. Starter bacteria can liberate FFA from mono- and diglycerides in milk produced by other lipases, e.g., milk LPL or lipases from Gram-negative bacteria.

Both mesophilic and thermophilic lactobacilli possess (mainly) intracellular esterolytic/lipolytic activity (Khalid et al. 1990; Gobbetti et al. 1996). The non-starter microflora of cheese may also include *Pediococcus* spp. which are weakly esterolytic and lipolytic (Tzanetakis and Litopoulou-Tzanetaki 1989).

An intracellular lipase of *Propionibacterium freudenreichii* was partially characterized by Oterholm et al. (1970); it probably contributes to lipolysis in Swiss varieties. *Brevibacterium linens,* a component of the surface microflora of smear-ripened cheeses, possesses intracellular lipases and esterases; an intracellular esterase has been purified and characterized (Rattray and Fox 1997).

Extensive lipolysis occurs in mould-ripened cheese, particularly blue varieties. In some cases, up to 25 % of the total FAs may be free (see Gripon 1987, 1993; Cantor et al. 2004). However, the impact of FFAs on the flavour of blue mould-ripened cheeses is less than in hard Italian varieties, possibly due to neutralization as the pH increases during ripening and to the dominant influence of methyl ketones on the flavour of blue cheese. Lipolysis in mould-ripened varieties is due primarily to the lipases of *Penicillium roqueforti* or *P. camemberti*, which secrete potent, well characterized extracellular lipases (see Gripon 1993; Cantor et al. 2004). *P. camemberti* appears to excrete only one lipase which is optimally active at ~ pH 9.0 and at ~35 °C. *P. roqueforti* excretes two lipases, one with a pH optimum at ~8.0, the other at pH ~6.0. The acid and alkaline lipases exhibit different specificities. *Geotrichum candidum* produces two lipases with different substrate specificities (see Sidebottom et al. 1991; Charton et al. 1992).

Psychrotrophs, which usually dominate the microflora of refrigerated milk, are a potentially important source of potent lipases in cheese but are considered not to be very important unless their numbers exceed 10^7 cfu ml^{-1}. Many psychrotroph lipases are heat stable and thus may cause rancidity in cheese over the course of a long ripening period. The subject of psychrotroph enzymes in cheese was discussed by Mottar (1989). Unlike psychrotroph proteinases, which are largely water-soluble and are lost in the whey, psychrotroph lipases adsorb onto the fat globules and are therefore concentrated in cheese.

12.6.5 Pattern and Levels of Lipolysis in Selected Cheeses

Excessive lipolysis is undesirable in most cheese varieties; Cheddar, Gouda and Swiss-type cheeses containing even a moderate level of free fatty acids would be considered rancid; however, certain cheese varieties are characterized by extensive lipolysis (e.g., Italian pecorino cheeses, Parmigiano Reggiano and Blue cheeses). Only small qualitative and quantitative differences in free fatty acids (FFA) ($C_{4:0}$–$C_{18:3}$) occur between Cheddar cheeses differing widely in flavour. The proportions of FFAs ($C_{6:0}$–$C_{18:3}$) in cheese are similar to those in milk fat, indicating that these FFAs are released in a non-specific manner. However, free butyric acid is usually present at a higher concentration than can be explained by its proportion in milk fat, suggesting that it is liberated selectively. Lipolysis in Italian pecorino varieties is

Table 12.2 Typical concentrations of free fatty acids (FFA) in some cheese varieties (compiled from Woo et al. 1984; Woo and Lindsay 1984)

Variety	FFA (mg kg^{-1})	Variety	FFA (mg kg^{-1})
Sapsago	211	Gjetost	1658
Edam	356	Provolone	2118
Mozzarella	363	Brick	2150
Colby	550	Limburger	4187
Camembert	681	Goat's milk cheese	4558
Port Salut	700	Parmesan	4993
Monterey Jack	736	Romano	6754
Cheddar	1028	Blue (US)	32,230
Gruyere	1481	Roquefort	32,453

extensive and due primarily to the action of PGE in the rennet paste used in the manufacture of these cheeses. Lipolysis in blue cheese varieties is extensive due to the action of lipases from *Penicillium* spp. Free fatty acid levels in a number of cheese varieties are listed in Table 12.2.

12.6.6 Catabolism of Fatty Acids

The taste and aroma of blue cheese is dominated by saturated *n*-methyl ketones, a homologous series of which, containing an odd number of carbon atoms from C_3 to C_{17}, is present. Concentrations of methyl ketones in blue cheese fluctuate, presumably due to reduction to secondary alcohols but heptan-2-one, nonan-2-one and undecan-2-one dominate.

The metabolism of fatty acids in cheese by *Penicillium* spp. involves four main steps (Fig. 12.10):

- release of fatty acids by the lipolytic systems in Sects. 12.6.1–12.6.5,
- oxidation of β-ketoacids,
- decarboxylation to a methyl ketone with one less carbon atom, and
- reduction of methyl ketones to the corresponding secondary alcohol; this step is reversible under aerobic conditions.

The concentration of methyl ketones is related to lipolysis. Methyl ketones can also be formed by the action of the mould on the ketoacids naturally present at low concentrations in milk fat (~1 % of total fatty acids). They could also be formed by the oxidation of monounsaturated acids but evidence for such a pathway is equivocal.

A number of factors affect the rate of methyl ketone production, including temperature, pH, physiological state of the mould and the ratio of the concentration of fatty acids to the dry weight of spores; both resting spores and fungal mycelium are capable of producing methyl ketones. The rate of production of methyl ketones does not depend directly on the concentrations of FFA precursors; indeed, high concentrations of FFAs are toxic to *P. roqueforti*.

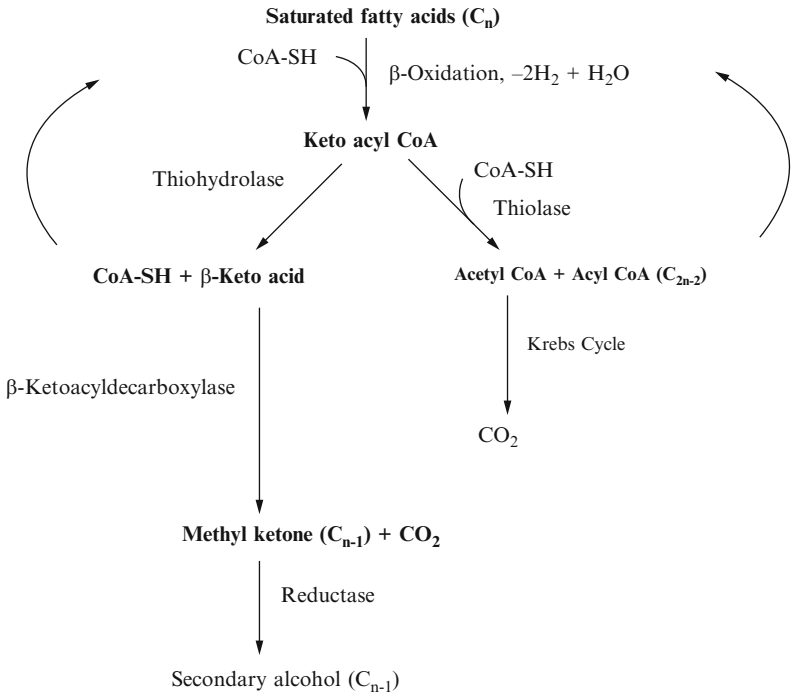
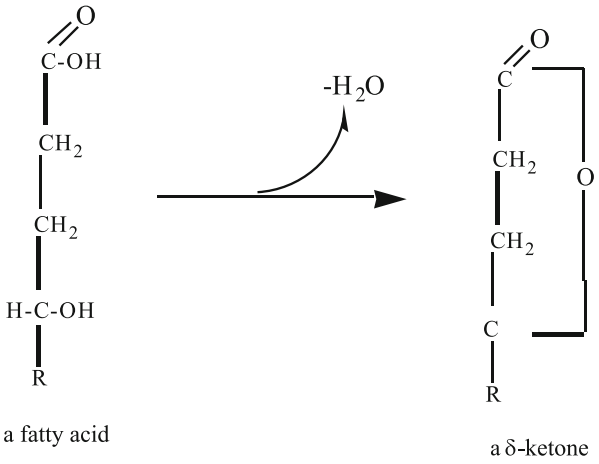


Fig. 12.10 β-Oxidation of fatty acids to methyl ketones by *Penicillium roqueforti* and subsequent reduction to secondary alcohols (from Fox and McSweeney 1998)

Lactones are cyclic esters resulting from the intramolecular esterification of a hydroxyacid through the loss of water to form a ring structure:



a fatty acid

a δ-ketone

α- and β-lactones are highly reactive and are used, or occur, as intermediates in organic synthesis; γ- or δ-lactones are stable and have been found in cheese.

Lactones have a strong aroma, which, although not specifically cheese-like, may be important in the overall flavour of cheese.

γ- and δ-lactones in freshly secreted milk probably originate from the corresponding γ- and δ-hydroxyacids . They may be formed spontaneously following release of the corresponding hydroxyacid. However, there is evidence that precursor hydroxy fatty acids need not be first liberated from triglycerides, but that lactones can be formed by a non-enzymatic intramolecular transesterification, forming the lactone and releasing it from the triglyceride in one step (Alewijn et al. 2007). Lactones could also be produced from keto acids released by lipolysis followed by reduction to hydroxy acids. The mammary gland of ruminants has a δ-oxidation system for fatty acid catabolism and thus oxidation within the mammary gland may be the primary source of lactone precursors. The potential for lactone production depends on such factors as feed, season, stage of lactation and breed.

δ-Lactones have very low flavour thresholds. γ-C_{12}, γ-C_{14}, γ-C_{16}, δ-C_{10}, δ-C_{12}, δ-C_{14}, δ-C_{15}, δ-C_{16} and δ-C_{18} lactones have been identified in Cheddar cheese and their concentration correlates with age and flavour intensity, suggesting that certain lactones are significant in Cheddar cheese flavour, although this has not been confirmed.

The concentration of lactones in Blue cheese is higher than in Cheddar, probably reflecting the extensive lipolysis which occurs in Blue cheese; the principal lactones in Blue cheese are δ-C_{14} and δ-C_{16}.

Fatty acids may also react with methanethiol (produced by catabolism of methionine; see Sect. 12.9) to form *S*-methyl thioesters which have a low flavour threshold and also contribute to cheese flavour. Since it is the most abundant alcohol in cheese, fatty acids can react with ethanol to form a series of ethyl esters. While not possessing specifically cheese-like aromas, ethyl esters have been shown to contribute to cheese flavour (Collins et al. 2003). In addition to the direct reaction of ethanol with a free fatty acid, there is evidence that ethyl esters may be formed by transesterification of fatty acids from glycerol in partial glycerides to ethanol, thus releasing the fatty acid and forming the ester in one step (Holland et al. 2005).

12.7 Proteolysis

12.7.1 Introduction

Of the three primary biochemical events that occur in cheese during ripening, proteolysis is the most complex and, in the view of most investigators, the most important. Together with changes to the calcium equilibrium in cheese, it is primarily responsible for textural changes, e.g., in hardness, elasticity, cohesiveness, fracturability, stretchability, meltability, adhesiveness and emulsifying properties (see Chap. 14), and makes a major contribution to cheese flavour and the perception of flavour (through release of sapid compounds); unfortunately, some small peptides are bitter and, if present at sufficient concentrations, will cause bitterness, a common flavour defect in cheese (see Chap. 13).

Proteolysis during maturation is essential in most cheese varieties. The extent of proteolysis varies from very limited (e.g., Mozzarella) to very extensive (e.g., blue varieties) and the products range in size from large polypeptides only slightly smaller than the intact caseins, through a range of medium and small peptides to amino acids. Clearly, no one proteolytic agent is responsible for such a wide range of products. Small peptides and amino acids contribute directly to cheese flavour and the latter may be catabolised to a range of sapid and aromatic compounds, e.g., amines, acids, carbonyls, sulfur-containing compounds, which are major contributors to cheese flavour. Although the catabolism of amino acids is not proteolysis, it is dependent on the formation of amino acids and thus will be discussed in this section.

12.7.2 Assessment of Proteolysis

Proteolysis is routinely monitored in studies on cheese ripening and is a useful index of cheese maturity and quality. Considering the complexity of proteolysis, a variety of methods may be used, depending on the depth of information required. These methods fall into two general classes: specific and non-specific. The latter include determination of nitrogen soluble in, or extractable by, one of a number of solvents or precipitants (e.g., water, pH 4.6 buffers, NaCl, ethanol, trichloroacetic acid, phosphotungstic or sulphosalicilic acids), or permeable through ultrafiltration membranes and quantified by any of several methods (e.g., Kjeldahl, biuret, Lowry, Hull, absorbance at 280 nm) or by the formation of reactive α-amino groups which may be quantified by reaction with one of several reagents, e.g., trinitrobenzene sulphonic acid (TNBS), *o*-phthaldialdehyde (OPA), fluorescamine, Cd-ninhydrin or Li-ninhydrin. Such methods are valuable for assessing the overall extent of proteolysis and the general contribution of each proteolytic agent. Non-specific techniques are relatively simple and are valuable for the routine assessment of cheese maturity since soluble nitrogen correlates well with cheese age and to a lesser extent with quality.

Specific techniques involve the use of chromatography and/or electrophoresis, which resolve individual peptides. They permit monitoring proteolysis of the individual caseins and identification of the peptides formed. Various forms of chromatography have been used to study peptides in cheese, including paper, thin-layer, ion exchange, gel permeation, metal chelate and, more recently, a variety of high-performance techniques, especially reverse phase HPLC. Electrophoresis is a very effective and popular technique for assessing primary proteolysis in cheese, especially alkaline urea-PAGE, but SDS-PAGE and isoelectric focusing are also used. Gel electrophoretograms are not easy to quantify accurately, which is a major limitation of these techniques. Capillary electrophoresis has also been applied to the analysis of peptides in cheese and has given very satisfactory quantifiable results.

Techniques for assessing proteolysis in cheese during ripening have been the subject of a number of reviews, including Grappin et al. (1985), Rank et al. (1985), Fox (1989), IDF (1991), McSweeney and Fox (1993, 1997) and Fox et al. (1995).

12.7.3 Proteolytic Agents in Cheese and Their Relative Importance

Cheese contains proteolytic enzymes from rennet, milk, starter lactic acid bacteria, adventitious non-starter lactic acid bacteria, and, in many varieties, from secondary cultures.

Several studies using the model cheese systems described in Sect. 12.3, especially on Cheddar and Gouda, have shown that enzymes in rennet (chymosin or rennet substitute) are mainly responsible for initial proteolysis and the production of most of the water- or pH 4.6-soluble N (Fig. 12.11). However, the production of small peptides and amino acids is due primarily to the action of enzymes from starter bacteria. γ-Caseins, formed from β-casein by plasmin, have been found in all cheese varieties that have been studied, indicating plasmin activity and this has been confirmed in cheese supplemented with plasmin or containing a plasmin inhibitor; however, plasmin activity is probably not necessary for satisfactory cheese ripening. The rennet enzymes are extensively denatured in high-cooked cheeses, e.g., Parmesan and Emmental, and in *pasta filata* varieties (e.g., Mozzarella) that undergo high temperature cooking/stretching at the end of acidification (see Chaps. 3 and 8) and therefore the contribution of plasmin to primary proteolysis is considerably higher in these varieties than in Cheddar- and Dutch-type cheeses. The pH optimum for plasmin is ~7.5 and hence cheese (many varieties have pH ~ 5.2) is not a very



Fig. 12.11 Formation of water-soluble nitrogen (WSN) in Cheddar cheese with: (**A**) controlled microflora (free of non-starter bacteria); (**B**) controlled microflora and chemically-acidified (starter-free); (**C**) controlled microflora and rennet-free; (**D**) controlled microflora, rennet-free and starter-free

suitable substrate; however, the pH of many cheeses increases during ripening, e.g., the pH of Camembert increases to ~7, which are therefore more amenable to plasmin action.

Although NSLAB can dominate the microflora of Cheddar-type cheese during much of its ripening, their influence on proteolysis in cheese is relatively limited and mainly at the level of peptidolysis and thus amino acid formation.

Some adjunct/secondary cultures are very proteolytic, e.g., *P. roqueforti, P. camemberti* and organisms from the smear of smear-ripened cheeses (*B. linens* is the best studied). Consequently, these microorganisms make a major contribution to proteolysis in those cheeses in which they are used, especially in blue cheeses, in which extensive mould growth occurs throughout the cheese.

The extent and specificity of proteolysis in representatives of the principal groups of cheese has been characterized and described in Sect. 12.8. The specificity of the principal proteinases and peptidases on the individual caseins in cheese has been established and can be related to proteolysis in cheese. The specificity of the principal proteolytic enzymes found in cheese will be described briefly below and actual proteolysis in some varieties will be discussed in Sect. 12.8.

12.7.4 Specificity of Cheese-Related Proteinases

12.7.4.1 Coagulant

As discussed in Chap. 7, the principal and essential role of the coagulant in cheesemaking is the specific hydrolysis of κ-casein, as a result of which the colloidal stability of the casein micelles is destroyed and coagulation occurs under suitable conditions of temperature and calcium concentration. Most of the rennet added to cheesemilk is lost in the whey or denatured as a result of cooking the curds-whey to a high temperature; typical values for the percentage of added rennet activity retained in the curd range from about 0 for high cook cheese, e.g., Mozzarella, Parmesan, Emmental, through 6 % for Cheddar to ~20 % for high moisture, low-cook cheeses, e.g., Camembert.

Chymosin (EC. 3.4.23.4), the principal proteinase in traditional rennets used for cheesemaking, is an aspartyl proteinase of gastric origin, secreted by young mammals. Its action on the B-chain of insulin indicates that chymosin is specific for hydrophobic and aromatic amino acid residues. Chymosin is weakly proteolytic but highly specific for the Phe_{105}–Met_{106} bond of κ-casein; indeed, limited proteolysis is one of the characteristics to be considered when selecting proteinases for use as rennet substitutes.

The primary chymosin cleavage site in the milk protein system is the Phe_{105}–Met_{106} bond in κ-casein which is many times more susceptible to chymosin than any other bond in milk proteins and its hydrolysis leads to coagulation of the milk (see Chap. 7). Cleavage of κ-casein at Phe_{105}–Met_{106} yields *para*-κ-casein (κ-CN f1-105) and glycomacropeptides (GMP; κ-CN f106-169). Most of the GMP is lost

in the whey but the *para*-κ-casein remains attached to the casein micelles and is incorporated into the cheese. α_{s1}-, α_{s2}- and β-caseins are not hydrolyzed during milk coagulation but may be hydrolysed in cheese during ripening.

In solution, chymosin cleaves the Leu_{192}–Tyr_{193} bond of β-casein very rapidly to form a large peptide, β-CN f1-192 and β-CN f193-209. At very low ionic strength, e.g., distilled water, this bond is the second most susceptible bond to hydrolysis in the caseins, after the Phe_{105}–Met_{106} bond of κ-casein, with a K_M and k_{cat} of 0.075 mM and 1.54 s^{-1}, respectively, for the micellar protein and 0.007 mM and 0.56 s^{-1} for the monomeric protein. However, its hydrolysis is strongly inhibited when the ionic strength is increased, even in 50 mM phosphate buffer; it is strongly inhibited by 5 % NaCl and completely by 10 % NaCl. The bond Ala_{189}-Phe_{190} and bonds in the region of residues Leu_{165} and Leu_{140} of β-casein are hydrolysed less rapidly.

β-Casein undergoes very little proteolysis by chymosin in cheese; undoubtedly, NaCl is an inhibitory factor (Cheddar cheese contains 4–6 % salt-in-moisture) but even in salt-free cheese, proteolysis is limited. Perhaps the concentration of milk salts is sufficient to cause inhibition and protein-protein interactions may also contribute to the low level of proteolysis (the C-terminal region of β-casein is very hydrophobic and intermolecular hydrophobic interactions may cause the chymosin-susceptible bonds to become inaccessible). The small peptide, β-CN f193-209, produced by cleavage of the Leu_{192}–Tyr_{193} bond, and fragments thereof are bitter and hence even limited hydrolysis of β-casein by chymosin may cause bitterness. Because the concentration of NaCl in the interior of most brine-salted cheeses increases slowly due to diffusion from the surface (see Chap. 9), sufficient proteolysis of β-casein by chymosin may occur in Dutch-type and other low-cooked cheese to cause bitterness.

The primary site of chymosin action on α_{s1}-casein is Phe_{23}–Phe_{24} and the small peptide (α_{s1}-CN f1-23) is rapidly hydrolyzed by starter proteinases. The hydrolysis of α_{s1}-casein in solution by chymosin is influenced by pH and ionic strength: in 0.1 M phosphate buffer, pH 6.5, chymosin cleaves α_{s1}-casein at Phe_{23}–Phe_{24}, Phe_{28}–Pro_{29}, Leu_{40}–Ser_{41}, Leu_{149}–Phe_{150}, Phe_{153}–Tyr_{154}, Leu_{156}–Asp_{157}, Tyr_{159}–Pro_{160} and Trp_{164}–Tyr_{165}; these bonds are also hydrolyzed at pH 5.2 in the presence of 5 % NaCl (i.e., conditions similar to those in cheese), and, in addition, Leu_{11}–Pro_{12}, Phe_{32}–Gly_{33}, Leu_{101}–Lys_{102}, Leu_{142}–Ala_{144} and Phe_{179}–Ser_{180} are hydrolyzed (McSweeney et al. 1993b). The rate at which many of these bonds are hydrolyzed depends on the ionic strength and pH, particularly Leu_{101}–Lys_{102}, which is cleaved far faster at the low pH. The k_{cat} and K_M for the hydrolysis of Phe_{23}–Phe_{24} bond of α_{s1}-casein by chymosin is 0.7 s^{-1} and 0.37 mM, respectively.

Primary proteolysis, i.e., the formation of large peptides, in low-cooked cheese, including Cheddar, in which the chymosin is not inactivated during cooking, is due mainly to chymosin and, as discussed in Sect. 12.8, the cleavage sites correspond to those cleaved in α_{s1}-CN in solution at pH 5.2.

α_{s2}-Casein appears to be relatively resistant to proteolysis by chymosin; cleavage sites are restricted to the hydrophobic regions of the molecule, i.e., residues 90–120 and 160–207: the bonds Phe_{88}–Tyr_{89}, Tyr_{95}–Leu_{96}, Gln_{97}–Tyr_{98}, Tyr_{98}–Leu_{99}, Phe_{163}–Leu_{164}, Phe_{174}–Ala_{175} and Tyr_{179}–Leu_{180} were reported by McSweeney et al.

(1994) to be the primary cleavage sites. Although *para*-κ-casein has several potential chymosin cleavage sites, it does not appear to be hydrolyzed either in solution or in cheese.

Good quality calf (veal) rennet contains about 10 % bovine pepsin (EC 3.4.23.1) but values up to 50 % have been reported in "calf" rennet. The principal peptides produced from Na-caseinate by bovine pepsin are similar to those produced by chymosin but the specificity of bovine or porcine pepsins on bovine caseins has not been rigorously determined. The Leu_{109}–Glu_{110} bond of α_{s1}-CN appears to be resistant to chymosin but is relatively susceptible to pepsin—the peptide α_{s1}-CN f110-199 is quite pronounced in electrophoretograms of cheese made with commercial calf rennet but not of cheese made using fermentation chymosin which has the same specificity as purified calf chymosin.

The specificity of the microbial rennet substitutes is quite different from that of chymosin (Fig. 12.12); *C. parasitica* proteinase is much more active on β-casein than chymosin. The principal cleavage sites for *R. miehei* proteinase in α_{s1}-casein are Phe_{23}–Phe_{24}, Phe_{24}–Val_{25}, Met_{123}–Lys_{124} and Tyr_{165}–Tyr_{166}; the principal sites on β-casein are Glu_{31}–Lys_{32}, Val_{58}–Val_{59}, Met_{93}–Gly_{94} and Phe_{190}–Leu_{191}.

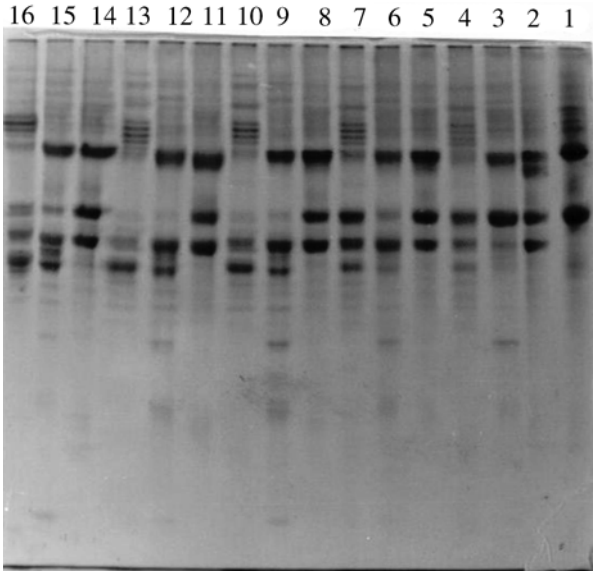


Fig. 12.12 Urea-PAGE of Na-caseinate hydrolyzed by different rennets (0.04 RU/ml) at pH 5.2 for 30 min. *Lane 1*: sodium caseinate. *Lanes 2, 5, 8, 11 and 14*: sodium caseinate containing 0, 1, 2.5, 5 or 10 % NaCl hydrolyzed by chymosin. *Lanes 3, 6, 9, 12 and 15*: sodium caseinate containing 0, 1, 2.5, 5 or 10 % NaCl hydrolyzed by *R. miehei* proteinase. *Lanes 4, 7, 10, 13 and 16*: sodium caseinate containing 0, 1, 2.5, 5 or 10 % NaCl hydrolyzed by *C. parasitica* proteinase (from Rea 1997)

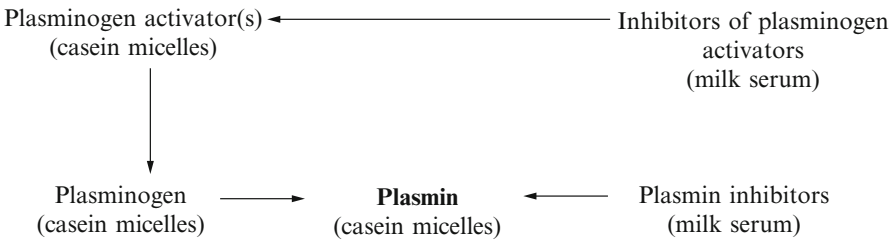


Fig. 12.13 Schematic representation of the plasminogen system in milk (from Fox and McSweeney 1998)

12.7.4.2 Indigenous Milk Proteinases

Milk contains several indigenous proteinases. Plasmin is the principal indigenous proteinase but a low level of cathepsin D is present also; several other proteinases have been reported in milk but are believed to be of little significance in milk and dairy products.

Plasmin Plasmin (fibrinolysin, EC 3.4.21.7) has been the subject of much study (for reviews see Grufferty and Fox 1988; Bastian and Brown 1996; Fox and Kelly 2006a, b; Kelly et al. 2006; O'Mahony et al. 2013). It is a component of blood where its physiological role is solubilization of fibrin clots. Plasmin is a component of a complex system consisting of the active enzyme, its zymogen (plasminogen), activators and inhibitors of the enzyme and of its activators (Fig. 12.13), all of which are present in milk. The components of the plasmin system enter milk via defective mammocyte membranes and are elevated in late lactation and during mastitic infection. Plasmin, plasminogen and plasminogen activators are associated with the casein micelles in milk, and consequently are incorporated into cheese curd, while the inhibitors of both plasmin and plasminogen activators are in the serum phase and are lost in the whey.

Plasmin is a trypsin-like serine proteinase with a pH optimum at about 7.5 and a high specificity for peptide bonds containing lysine at the N-terminal side. It is active on all caseins, but especially on α_{s2}- and β-caseins. Plasmin cleaves β-casein at three primary sites: Lys_{28}–Lys_{29}, Lys_{105}–His_{106} and Lys_{107}–Glu_{108}, with the formation of the polypeptides, β-CN f29-209 (γ_1-CN), f106-209 (γ_2-CN) and f108-209 (γ_3-CN), β-CN f1-105 and f1-107 (proteose peptone 5), β-CN f29-105 and f29-107 (proteose peptone 8-slow) and β-CN f1-28 (proteose peptone 8-fast) (see Chap. 4). Additional cleavage sites include Lys_{29}–Ile_{30}, Lys_{113}–Tyr_{114} and Arg_{183}–Asp_{184}.

Plasmin cleaves α_{s2}-casein in solution at eight sites: Lys_{21}–Gln_{22}, Lys_{24}–Asn_{25}, Arg_{114}–Asn_{115}, Lys_{149}–Lys_{150}, Lys_{150}–Thr_{151}, Lys_{181}–Thr_{182}, Lys_{188}–Ala_{189} and Lys_{197}–Thr_{198}, producing about 14 peptides, three of which are potentially bitter.

Although plasmin is less active on α_{s1}- casein than on α_{s2}- and β-caseins, it hydrolyses α_{s1}-casein in solution at the bonds Arg_{22}–Phe_{23}, Arg_{90}–Tyr_{91}, Lys_{102}–Lys_{103}, Lys_{103}–Tyr_{104}, Lys_{105}–Val_{106}, Lys_{124}–Glu_{125}, and Arg_{151}–Gln_{152} (McSweeney et al. 1993c). The formation of λ-casein, a minor casein component, has been attributed to the action of plasmin on α_{s1}-casein.

Although κ-casein contains several potential sites, it is very resistant to plasmin and the products have not been identified. Even though the pH of cheese is quite far removed from the pH optimum of plasmin, the hydrolysis of β-casein in cheese is due mainly to plasmin. Being quite heat stable, plasmin and plasminogen survive HTST pasteurization and cheese cooking and plays an important role in primary proteolysis in high-cooked cheeses, e.g., Parmesan and Emmental.

Cathepsin D The indigenous acid proteinase in milk, cathepsin D (EC 3.4.23.5), has received little attention. It is relatively heat labile (completely inactivated by 70 °C × 10 min) and has a pH optimum of 4.0. The specificity of cathepsin D on the caseins has not been determined, although electrophoretograms of hydrolyzates indicate that its specificity is similar to that of chymosin; surprisingly, it is unable to coagulate milk (McSweeney et al. 1995).

The contribution of cathepsin to cheese ripening is unclear but is very likely to be less than that of chymosin, which is present at a higher concentration and has a similar specificity. However, cathepsin D may play a minor role in proteolysis during the ripening of cheeses made from unpasteurized mastitic milk.

Other Indigenous Milk Proteinases The presence of other proteolytic enzymes in milk has been reported, including thrombin, a lysine aminopeptidase and proteinases from leucocytes but they are considered not to be significant in cheese (see Grufferty and Fox 1988; Kelly et al. 2006).

12.7.4.3 Proteolytic Enzymes from Starter

Although lactic acid bacteria (LAB) are weakly proteolytic they do possess a proteinase and a wide range of peptidases which are principally responsible for the formation of small peptides and amino acids in cheese. The proteolytic system of *Lactococcus* has been studied thoroughly at the molecular, biochemical and genetic levels; the system of *Lactobacillus* spp. is less well characterized but the systems of both genera are generally similar. *Sc. thermophilus* is less proteolytic than *Lactococcus* or *Lactobacillus* and has been the subject of little research. The extensive literature on the proteolytic systems of LAB has been comprehensively reviewed by Thomas and Pritchard (1987), Monnet et al. (1993), Tan et al. (1993), Visser (1993), Kunji et al. (1996), Law and Haandrikman (1997), Sousa et al. (2001) and Upadhyay et al. (2004).

The proteinase in LAB is anchored to the cell membrane and protrudes through the cell wall, giving it ready access to extracellular proteins; all the peptidases are intracellular. The oligopeptides produced by the proteinase are actively transported

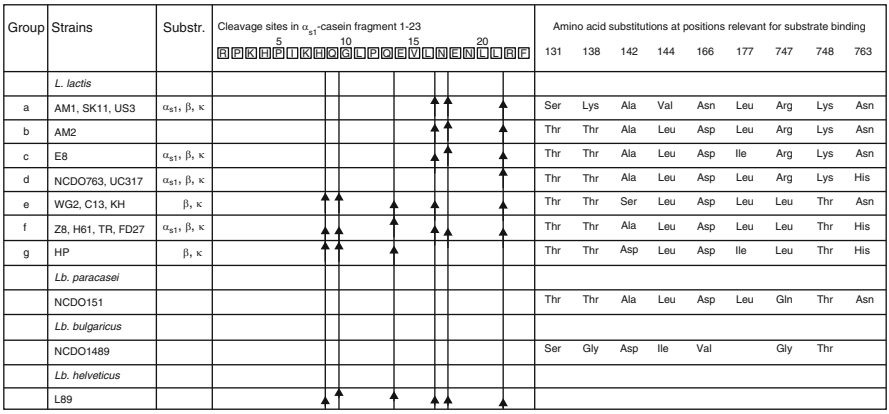


Fig. 12.14 Classification of cell envelope-associated proteinases of lactic acid bacteria according to their specificity toward α_{s1}-casein fragment 1–23 (from Kunji et al. 1996)

into the cell where they are hydrolyzed further by the battery of peptidases (see Chap. 6).

Cell envelope-associated proteinases (CEP) of *Lactococcus* were previously classified into 3 groups, P_I-, P_{III}- and mixed-types; P_I-type proteinases degrade β- but not α_{s1}-casein at a significant rate, while P_{III}-type proteinases rapidly degrade both α_{s1}- and β-caseins; the nucleotide sequences of the genes for both P_I- and P_{III}-type proteinases are very similar. However, more recently and based on their specificity on α_{s1}-CN f1-23 (a peptide produced very rapidly from α_{s1}-CN by chymosin), lactococcal proteinases can be classified into seven groups (Fig. 12.14).

The specificity of the CEP from several *Lactococcus* strains on α_{s1}-, α_{s2}-, β- and κ-caseins and short peptide substrates has been established (see Fox et al. 1994, 1996; Fox and McSweeney 1996; Upadhyay et al. 2004). The lactococcal CEP is involved in the formation of many of the small peptides in cheese while the peptidases are responsible for the release of amino acids.

Lactococcus spp. contain at least the following intracellular proteolytic enzymes:

- Proteinases capable of hydrolyzing caseins with a specificity different from that of the CEP; their activity in cheese has not been demonstrated.
- Endopeptidases ($PepO_1$, $PepO_2$, $PepF_1$, $PepF_2$) which are unable to hydrolyse intact caseins but can hydrolyse internal peptide bonds in large casein-derived peptides (up to 30 amino acid residues); the specificity of these endopeptidases on several casein-derived peptides and on synthetic peptides has been established but their activity in cheese has not been demonstrated.
- Aminopeptidases (PepN, PepA, PepC, PepP); PepN is a broad specificity metalloenzyme; PepC is a broad specificity thiol-enzyme. PepA is a metallo-enzyme with high specificity for N-terminal Glu or Asp residues while PepP is a metalloenzyme which releases the N-terminal residue from peptides with Pro as the penultimate residue.

- An iminopeptidase (PepI) which releases N-terminal Pro.
- A dipeptidyl aminopeptidase (PepX) which has high, but not absolute, specificity for peptides with Pro at the penultimate position, releasing XPro dipeptides, where X may be one of several residues.
- A pyrolidone carboxylyl peptidase (PCP) which releases a cyclicysed Glu (pyroglutamic acid) from the N-terminal.
- A tripeptidase (PepT).
- A number of dipeptidases including a general dipeptidase (PepV), PepL which preferentially hydrolyses dipeptides and some tripeptides containing an N-terminal Leu and proline-specific dipeptidases, prolinase (PepR) and prolidase (PepQ), which hydrolyse ProX and XPro dipeptidases, respectively.

Most of these peptidases have been isolated from more than one strain of *Lactococcus*, and characterized (Table 12.3).

The activity and stability of these peptidases in cheese has not been established in detail but at least some are certainly active, as indicated by the presence of relatively high concentrations of certain peptides and amino acids in cheese. The proteolytic system is capable of hydrolyzing casein completely to amino acids; the sequential action of the peptidase system is shown schematically in Fig. 12.15. This complex proteolytic system is required by LAB for growth to high numbers in milk which contains a low concentration of small peptides and amino acids.

Thermophilic obligately homofermentative *Lactobacillus* spp *(Lb. helveticus, Lb. delbrueckii* spp. *lactis*), alone or paired with *Sc. thermophilus*, are used as starters for high-cooked cheeses. The proteolytic system of the thermophilic lactobacilli is generally similar to that of the *Lactococcus* and includes a CEP. These bacteria die and lyse relatively rapidly in cheese (see Chap. 11), releasing intracellular peptidases which explains the high level of amino acids in cheese made with a thermophilic starter.

Sc. thermophilus is weakly proteolytic (no proteinase has yet been isolated) but it possesses substantial peptidase activity; its contribution to proteolysis in cheese is probably less than that of the thermophilic lactobacilli, but definitive studies are lacking.

12.7.4.4 Proteolytic System of Non-starter Microflora

The starter cells, both *Lactococcus* and thermophilic *Lactobacillus*, reach maximum numbers shortly after curd manufacture and then die off and lyse. In contrast, non-starter lactic acid bacteria grow from very low initial numbers (<50 cfu/g) to about 10^7–10^8 cfu/g within about 3 months and dominate the microflora of long-ripened cheeses for most of their ripening period. As discussed in Chap. 11, the interior of cheese is a hostile environment for bacteria (e.g., a relatively low pH, ~5; a relatively high salt content, 2–4 %; lacks a fermentable carbohydrate; is anaerobic; may contain bacteriocins produced by starter bacteria). Hence, cheese is highly

Table 12.3 Peptidases of lactic acid bacteria (modified from Fox et al. 1996)

Organism	Principal assay substrate	MW, kDa	Opt. Activity		Subunits	Class
			pH	°C		
Oligoendopeptidases (LEP, MEP, NOP, PepO, PepF)						
Lc. lactis ssp. *lactis* CNRZ 267	Peptides	49	–	–	–	–
Lc. lactis ssp. *cremoris* H61	Peptides	98	7–7.5	40	1	M
Lc. lactis ssp. *cremoris* H61	α_{s1}-CN f1-23	80	6	37	2	M
Lc. lactis ssp. *cremoris* Wg2	Metenkephalin	70	6–6.5	30–38	1	M
Lc. lactis ssp. *cremoris* HP	α_{s1}-CN f1-23	180	8–9	42	>2	M
Lc. lactis ssp. *cremoris* C13	α_{s1}-CN f1-23	70	6–7	35	1	N
Lc. lactis ssp. *lactis* MG 1363	α_{s1}-CN f1-23	70	7.5	40	1	M
Lc. lactis ssp. cremoris SK11	Bradykinin	70	6.0	–	1	M
Lc. lactis ssp. *lactis* NCDO763	Bradykinin	70	8.0	40	1	M
Lb. delbr. ssp. *bulgaricus* B14	Metenkephalin	70	7.7	47	1	M
Aminopeptidases						
Aminopeptidase N (general aminopeptidase, AMP, PepN)						
Lc. bv. *diacetylactis* CNRZ 267	Lys-*p*-NA	85	6.5	35	–	M
Lc. lactis ssp. *cremoris* AC1	Lys-*p*-NA	36	7	40	1	M
Lc. lactis ssp. *cremoris* WG_2	Lys-*p*-NA	95	7	40	1	M, -SH
Lb. delbrueckii ssp. *lactis* 1183	Lys-*p*-NA	78–91	6.2–7.2	47.5	1	M
Lb. acidophilus R-26	Lys-*p*-NA	38	–	–	–	M
Lb. delbr. ssp. *bulgaricus* CNRZ 397	Lys-*p*-NA	95	–	–	–	M
Lb. helveticus CNRZ 32	Lys-*p*-NA	97	–	–	–	M
Lb. delbrueckii ssp. *bulgaricus* B14	Lys-*p*-NA	95	7	50	1	M
Lb. helveticus LME-511	Leu-*p*-NA	92	7	37	1	M
Lb. casei ssp. *casei* LLG	Leu-*p*-NA	87	7	39	1	M
Lb. delbr. ssp. *bulgar.* ACA-DC233	Lys-*p*-NA	98	6	40	1	M
Lb. helveticus ITGL1	Lys-*p*-NA	97	6.5	50	1	M

Str. sal. ssp. *thermophilus* CNRZ1199	Lys-*p*-NA	89	6.5	35	1	M
Str. sal. ssp. *thermophilus* CNRZ302	Lys-*p*-NA	97	7.0	36	1	M
Str. sal. ssp. *thermophilus* NCDO573	Lys-*p*-NA	96	6.9–7.0	35	1	M, -SH
Aminopeptidase A (glutamyl aminopeptidase, GAP, PepA)						
Lc. lactis ssp. *cremoris* HP	Glu-/Asp-*p*-NA	130	–	50–55	3	M
Lc. lactis ssp. *lactis* NCDO 712	Glu-*p*-NA	245	8	65	6	M
Lc. lactis ssp. *cremoris* HP	Glu-*p*-NA	520	8	50	~10	M
Lc. lactis ssp. *cremoris* AM2	Asp-*p*-NA	240	–	–	6	M
Aminopeptidase C (thiol aminopeptidase, PepC)						
Lc. lactis ssp. *cremoris* AM2	His-β-NA	300	7	40	6	-SH
Lb. delbrueckii ssp. *bulgaricus* B14	Leu-Gly-Gly	220	6.5–7	50	4	-SH
Pyrrolidonyl Carboxylyl Peptidase (pyroglutamyl aminopeptidase, PCP)						
Lc. lactis ssp. *cremoris* HP	Pyr-*p*-NA	–	–	–	–	–
Lc. lactis ssp. *cremoris* HP	Pyr-*p*-NA	80	8–8.5	37	2	S
X-prolyldipeptidyl aminopeptidase (XPDA, PPDA, XAP, PepX)						
Lc. lactis ssp. *cremoris* P8-2-47	X-Pro-*p*-NA	180	7	45–50	2	S
Lc. lactis ssp. *lactis* NCDO 763	Ala-Pro-*p*-NA	190	8.5	40–45	2	S
Lc. lactis ssp. *cremoris* AM2	Gly-Pro-NH-Mec	117	6–9	–	–	S
Lc. lactis ssp. *lactis* H1	X-Pro-*p*-NA	150	6–9	–	–	S
Lb. delbrueckii ssp. *lactis*	X-Pro-*p*-NA	165	7	50–55	2	S
Lb. helveticus CNRZ 32	X-Pro-*p*-NA	72	7	40	1	S
Lb. delbr. ssp. *bulgaricus* CNRZ 397	X-Pro-*p*-NA	82	7	50	–	S
Lb. delbrueckii ssp. *bulgaricus* B14	Ala-Pro-*p*-NA	170–200	6.5	45	2	S
Lb. acidophilus 357	Ala-Pro-*p*-NA	170–200	6.5	45	2	S
Lb. delbr. ssp. *bulgaricus* LBU-147	Gly-Pro-*p*-NA	270	6.5	50	3	S
Lb. delbr. ssp. *lactis* DSM7290	Ala-Pro-*p*-NA	95	7.0	46–50	1	S
Lb. helveticus LHE-511	Gly-Pro-*p*-NA	90	6.5	50	1	S

(continued)

Table 12.3 (continued)

Organism	Principal assay substrate	MW, kDa	Opt. Activity		Subunits	Class
			pH	°C		
Lb. casei ssp. *casei* LLG	Gly-Pro-*p*-NA	79	7.0	50	1	S
Proline iminopeptidase (PIP)						
Pr. freud. ssp. *shermanii 13673*	–	–	–	–	–	–
Lc. lactis ssp. *cremoris* HP	Pro-Gly-Gly	100	8.5	37	2	M
Lb. delbr. ssp. *bulgar. CNRZ 397*	Pro-*p*-NA	100	6–7	40	3	S
Lb. casei ssp. *casei* LLG	Pro-AMC	46	7.5	40	1	-SH
Dipeptidases (DIP)						
Lactococcus spp.	Dipeptides	25 and 34	7	30	M	
Lc. bv. *diacetylactis* CNRZ 267	Leu-Leu	51	7.5	–	1	M
Lc. lactis ssp. *cremoris* H61	Leu-Gly	100	8	–	–	M
Lc. lactis ssp. *cremoris* Wg2	Dipeptides	49	8	50	1	M
Lb. delbr. ssp. *bulgaricus* B14	Dipeptides	51	7	50	1	M
Prolidase (PRD)						
Lc. lactis ssp. *cremoris* H61	Leu-Pro	43	6.5-7.5	–	–	M
Lc. lactis ssp. *cremoris* AM2	Leu-Pro	42	7.35–9.0	–	–	M
Tripeptidases (TRP)						
Lc. bv. *diacetylactis* CNRZ 267	Tripeptides	75	7	35	–	M
Lc. lactis ssp. *cremoris* Wg2	Leu-Leu-Leu	103–105	7.5	55	2	M
Lc. lactis ssp. *cremoris* AM2	Tripeptides	105	8.6	–	2	M
Lc. lactis ssp. *cremoris* IMN-C12	Leu-Leu-Leu	72	5.8	33	3	-SH
Lb. delbrueckii ssp. *bulgaricus* B14	Leu-Gly-Gly	85	6.0	40	>1	M

M metallo, *S* serine, *-SH* thiol, *AMC* aminomethyl coumarin, *p-NA p*-nitroanalide

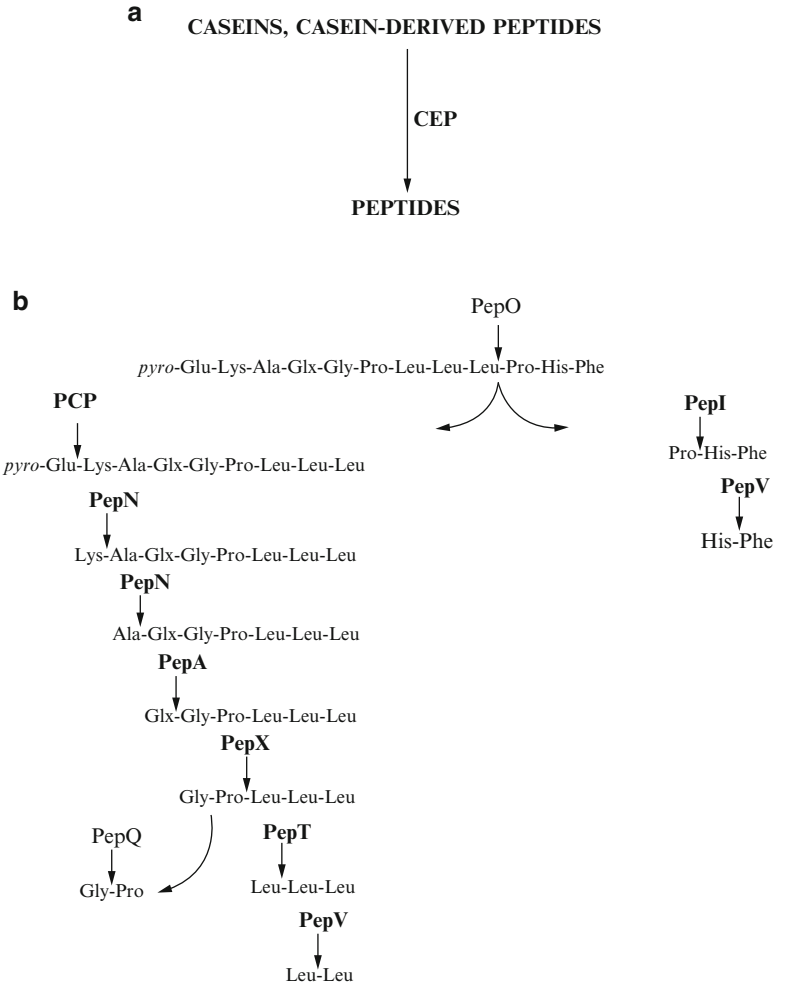


Fig. 12.15 Schematic representation of the hydrolysis of casein (**a**) by lactococcal cell envelope-associated proteinase (CEP), and (**b**) degradation of an hypothetical dodecapeptide by the combined action of lactococcal peptidases; oligopeptidase (PepO), various aminopeptidases (PCP, PepN, PepA, PepX), tripeptidase (PepT), prolidase (PepQ) and dipeptidase (PepV)

selective and the NSLAB microflora is dominated by a few species of mesophilic lactobacilli (principally strains of *Lb. casei* and *Lb. paracasei*.

The proteolytic system of these mesophilic lactobacilli is not as well studied as that of the *Lactococcus* or thermophilic lactobacilli. Since they do not grow well in milk in the absence of an added source of small peptides or amino acids, they may lack a CEP; also, the method used to release CEP from *Lactococcus*, i.e., washing

cells with a calcium-free buffer, fails to do so from mesophilic lactobacilli. They do possess a range of intracellular peptidases but few of these have been studied.

NSLAB appear to contribute little to primary proteolysis in Cheddar cheese but do contribute to the release of amino acids (see Fox et al. 1998). Mesophilic lactobacilli have been used as adjunct starters in Cheddar cheese, in which they are reported to improve and perhaps modify flavour.

12.7.4.5 Proteinases from Secondary Starter

Most cheese varieties have a secondary microflora, the function of which is other than acid production. Originally, this microflora was adventitious and its development occurred as a result of selective conditions, e.g., pH, humidity, temperature and a_w. Today, the secondary microflora may be adventitious but in many cases, the milk or curd is inoculated with selected microorganisms. The principal secondary microorganisms are *Penicillium roqueforti* (blue-mould cheese), *P. camemberti* (surface mould cheese, e.g., Camembert and Brie), *Brevibacterium linens, Arthrobacter* and other corynebacteria (surface smear-ripened cheese), *Propionibacterium freudenfreichii* (Swiss-type cheese) and several species of yeasts (see Chap. 11). Most of these microorganisms are metabolically very active and, consequently, may dominate the ripening of cheeses in which they occur.

P. roqueforti and *P. camemberti* secrete aspartyl and metalloproteinases which have been fairly well characterized, including their specificity on α_{s1}- and β-caseins (see Gripon 1993). Intracellular acid proteinase(s) and exopeptidases (amino and carboxy) are also produced by *P. roqeuforti* and *P. camemberti* but have not been well studied (see Gripon 1993; Cantor et al. 2004). The peptidases of *P. roqueforti* and *P. camemberti* have not been studied in detail.

The proteinase of *Br. linens* ATCC 9174 has been purified and characterized, including its specificity on α_{s1}- and β-caseins (see Rattray and Fox 1998). An extracellular and an intracellular aminopeptidase have also been purified.

Propionibacterium spp. are weakly proteolytic but strongly peptidolytic; they are particularly active on proline-containing peptides and consequently Swiss-type cheeses contain high concentrations of proline, which may contribute to the characteristic flavour of these cheeses. Aminopeptidase, iminopeptidase and X-proline dipeptidolylaminopeptidase have been isolated from *P. freudeneichii*.

12.8 Characterization of Proteolysis in Cheese

The extent of proteolysis in cheese ranges from very limited, e.g., Mozzarella, to very extensive, e.g., blue-mould varieties. PAGE shows that the proteolytic pattern, as well as its extent, exhibit marked inter-varietal differences; the PAGE patterns of both the water-insoluble and water-soluble fractions are, in fact, quite characteristic of the variety, as shown in Figs. 12.16 and 12.17 for a number of Cheddar,

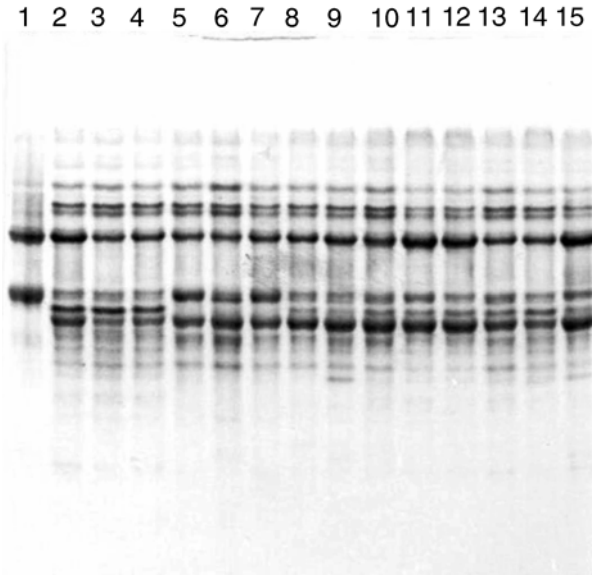


Fig. 12.16 Urea-PAGE of the water-insoluble fraction of a selection of cheese varieties: *1*, Na-caseinate; *2–4*, Cheddar; *5–7*, Emmental; *8*, Maasdamer; *9*, Jarlsberg; *10–12*, Edam; *13–15*, Gouda

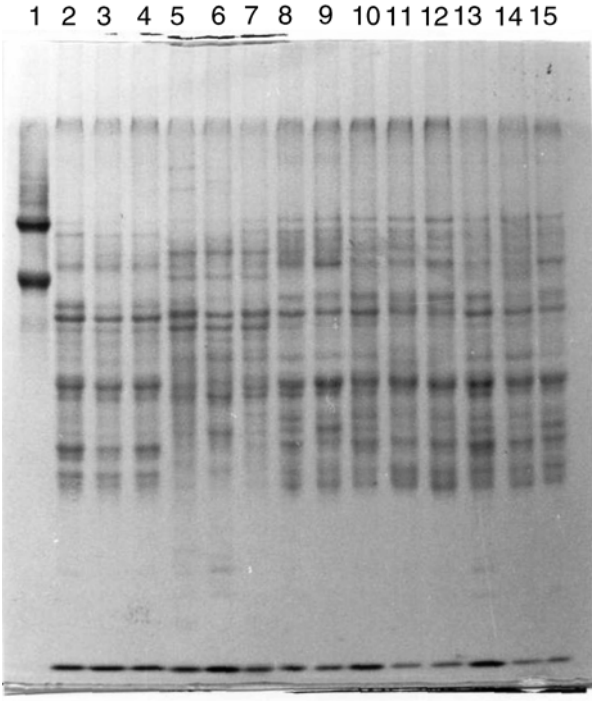


Fig. 12.17 Urea-PAGE of the water-soluble fraction of a selection of cheese varieties: *1*, Na-caseinate; *2–4*, Cheddar; *5–7*, Emmental; *8*, Maasdamer; *9*, Jarlsberg; *10–12*, Edam; *13–15*, Gouda

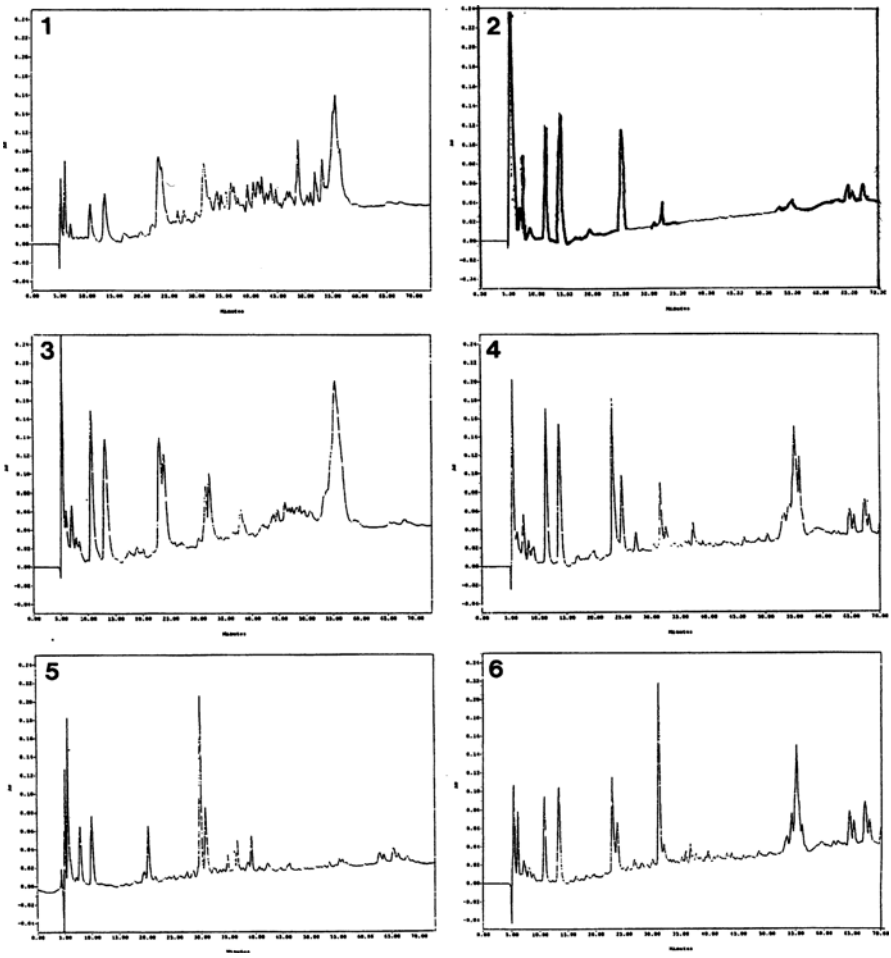


Fig. 12.18 Reverse phase HPLC profiles of the 70 % ethanol-soluble fractions of Cheddar (*1*), Parmesan (*2*), Emmental (*3*), Leerdammer (*4*), Edam (*5*) and Gouda (*6*) cheese

Dutch and Swiss-type cheeses. RP-HPLC of the water-soluble fraction or sub-fractions thereof also show varietal characteristics (Figs. 12.18 and 12.19). Both the PAGE and HPLC patterns vary and become more complex as the cheese matures and are in fact very useful indices of cheese maturity and to lesser extent of its quality. Therefore, they have potential in the objective assessment of cheese quality. The urea-PAGE patterns of Cheddar cheeses at various stages of maturity are shown in Fig. 12.20.

Complete characterization of proteolysis in cheese requires isolation and identification of the individual peptides. A comprehensive fractionation protocol is shown in Fig. 12.21. Many of the water-insoluble and water-soluble peptides in Cheddar cheese have been isolated and identified by amino acid sequencing and mass

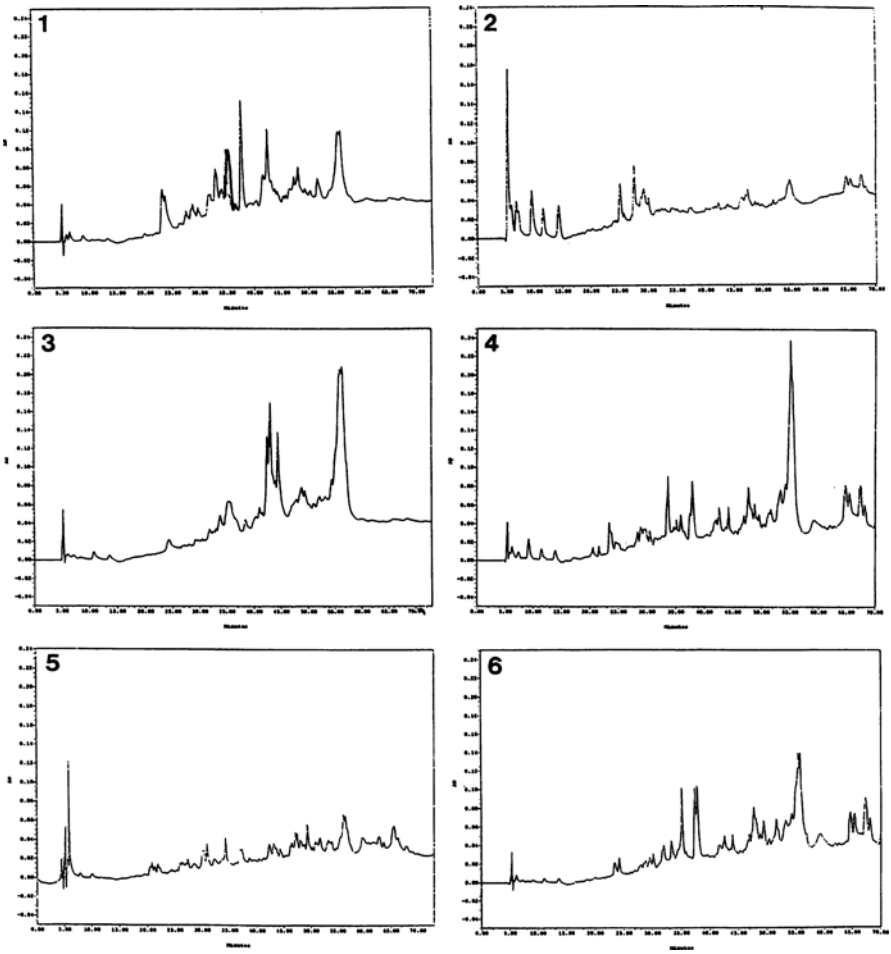


Fig. 12.19 Reverse phase HPLC profiles of the 70 % ethanol-insoluble fractions of Cheddar (*1*), Parmesan (*2*), Emmental (*3*), Leerdammer (*4*), Edam (*5*) and Gouda (*6*) cheese

spectrometry; these are summarized in Figs. 12.22 and 12.23. All the principal water-insoluble peptides are produced either from α_{s1}-casein by chymosin or from β-casein by plasmin and represent the C-terminal fragments of these proteins (Fig. 12.22). In mature Cheddar (>6 months old), all of the α_{s1}-casein is hydrolyzed by chymosin at Phe_{23}–Phe_{24}. The peptide, α_{s1}-CN f1-23, does not accumulate but is hydrolyzed rapidly at Gln_9–Gly_{10}, Gln_{13}–Glu_{14} and/or Leu_{16}–Asn_{17} by the lactococcal cell wall proteinase, depending on its specificity (see Fig. 12.14). A significant amount of the larger peptide, α_{s1}-CN f24-199, is hydrolyzed at Leu_{101}–Lys_{102}. In 6-month-old Cheddar, ~50 % of the β-casein is hydrolyzed, mainly by plasmin, to γ-caseins (β-CN f29-209, f105-209 and f107-209) and proteose peptones (β-CN

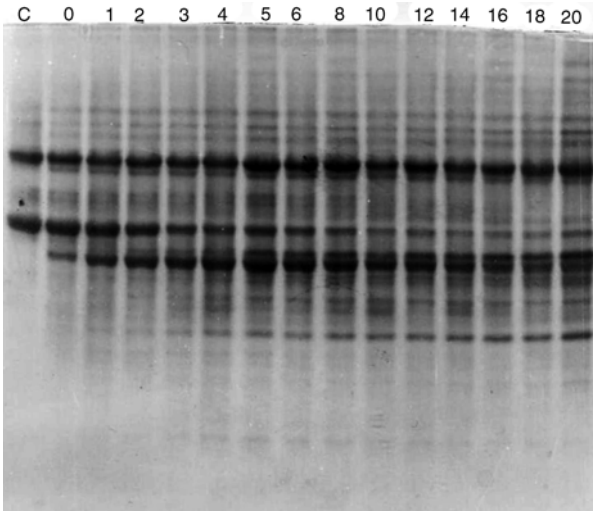


Fig. 12.20 Urea-polyacrylamide gel electrophoretograms of Cheddar cheese after ripening for 0, 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18 or 20 weeks (*lanes 1–14*); C, sodium caseinate (supplied by Mooney et al. 1998)

f1-28, f1-104, f1-106, f29-104, f29-106). These polypeptides do not appear to be hydrolyzed by chymosin or lactococcal proteinase. Although α_{s2}-casein gradually disappears from PAGE patterns of cheese during ripening, few polypeptides produced from it have been identified. *Para*-κ-casein is quite resistant to proteolysis and no peptides produced from it have been identified.

Most of the water-soluble peptides are derived from the N-terminal half of α_{s1}- and β-caseins (Fig. 12.23). The N-terminal of many of these peptides corresponds to a chymosin (α_{s1}-CN) or plasmin (β-CN) cleavage site but some appear to arise from the action of the lactococcal CEP. However, the N-terminal, and especially the C-terminal, of many peptides does not correspond precisely to the known cleavage sites of chymosin, plasmin or lactococcal proteinase, suggesting the action of bacterial aminopeptidases. Carboxypeptidase activity would explain why the C-terminal of many peptides does not correspond to known proteinase cleavage sites but this activity has not been reported in *Lactococcus* spp.. It must be presumed that other proteinase, e.g., from NSLAB, or starter or NSLAB endopeptidases (PepO or PepF types) are involved or perhaps other cleavage sites for lactococcal cell wall proteinase remain to be identified.

The N-terminal sequence of α_{s1}-CN f1-9 and f1-13 is RPKHPIK, which should be susceptible to PepX. The accumulation of these peptides in Cheddar and the

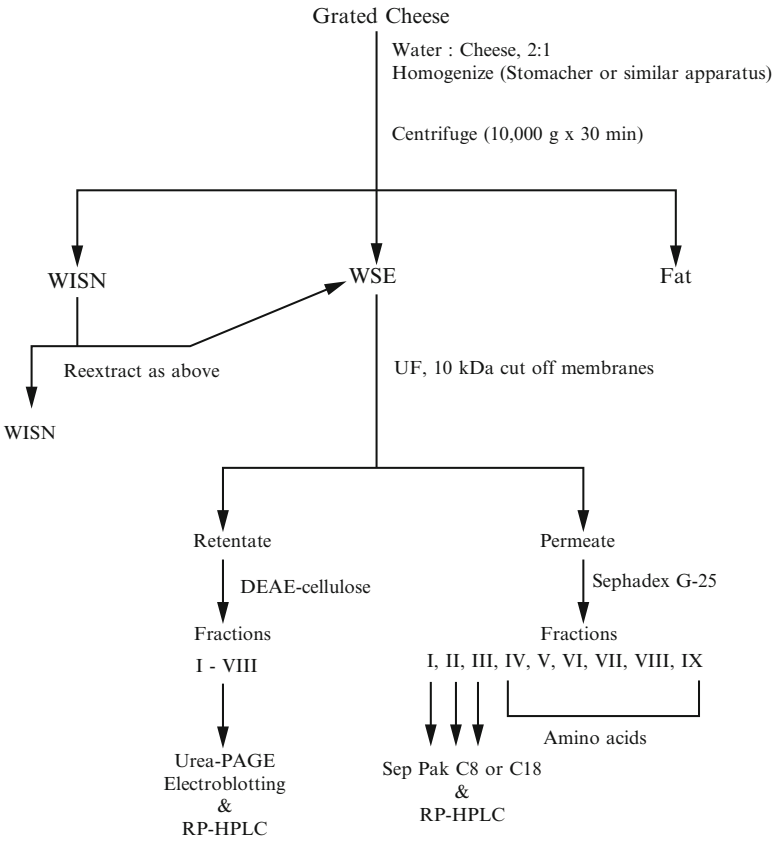


Fig. 12.21 Scheme for the fractionation of cheese nitrogen (Fox et al. 1994)

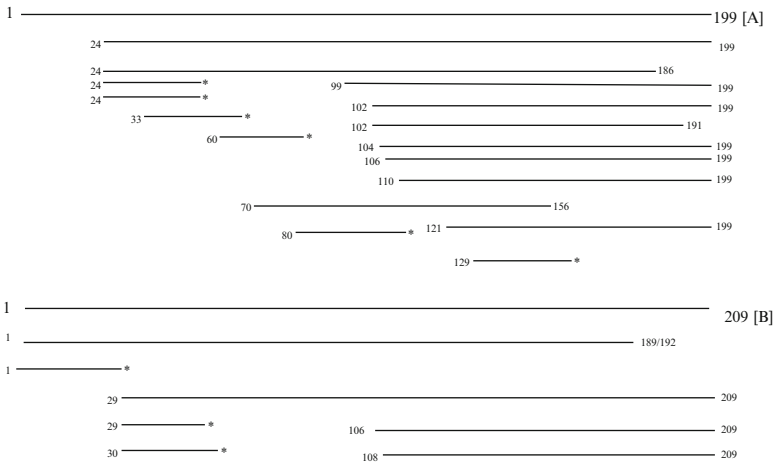


Fig. 12.22 Schematic representation of the principal water-insoluble peptides isolated from Cheddar cheese and identified (Mooney et al. 1998)

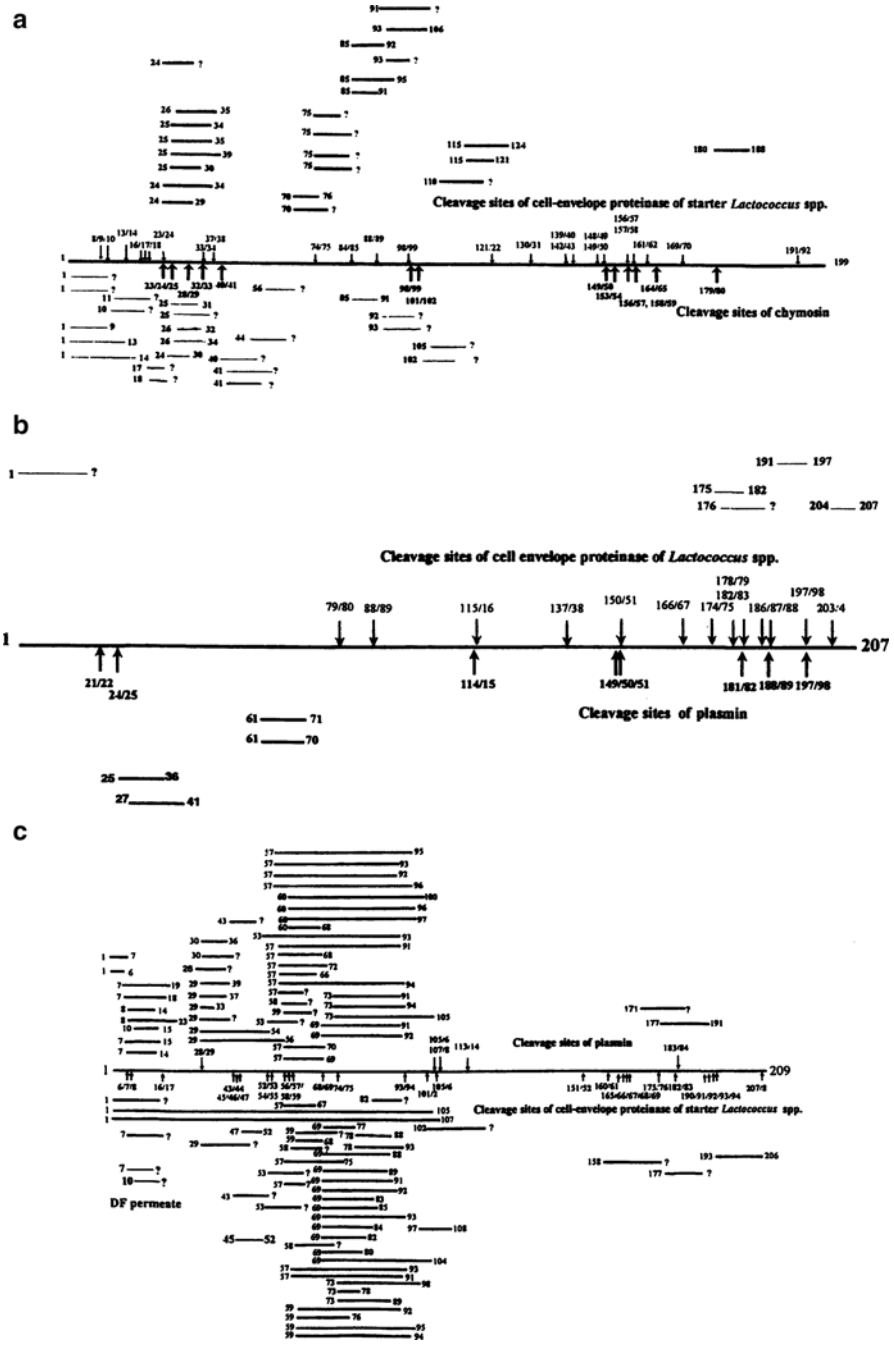


Fig. 12.23 Water-soluble peptides derived from α_{s1}-casein (**a**), α_{s2}-casein (**b**) or β-casein (**c**) isolated from Cheddar cheese and identified. The principal chymosin, plasmin and lactococcal cell envelope proteinase cleavage sites are indicated by *arrows* (from various sources; see Fox and McSweeney 1996)

apparent absence of peptides with a sequence commencing at Lys_3 of α_{s1}-CN suggest that PepX is not active in cheese.

A number of authors have shown that the very small peptides (<500 Da) make a significant contribution to Cheddar flavour but only a few of these peptides have been identified.

A large number of 12 % TCA-soluble and insoluble peptides in the water-soluble extract of Parmesan have been identified by fast atom bombardment mass spectrometry (Addeo et al. 1992, 1994). Although Parmesan undergoes extensive proteolysis and has a very high concentration of amino acids, it contains low concentrations of medium-sized peptides.

Although very extensive proteolysis occurs in blue cheeses and some of the larger peptides detectable by PAGE have been partially identified (see Gripon 1993; Cantor et al. 2004), very little work has been done on the small, pH 4.6-soluble peptides. Some of the peptides resulting from the cleavage of α_{s1}-CN f1-23 (produced by chymosin) by lactococcal CEP have been identified in Gouda. Proteolysis in Swiss-type cheeses has been studied using PAGE and RP-HPLC but small peptides have not been isolated and characterized.

Significant concentrations of amino acids, the final products of proteolysis, occur in all cheeses that have been investigated (see Fox and Wallace 1997; Upadhyay et al. 2004). Relative to the level of water-soluble N, Cheddar contains a low concentration of amino acids. The principal amino acids in Cheddar are Glu, Leu, Arg, Lys, Phe, Ser (Fig. 12.24) (see Fox and Wallace 1997, for a comprehensive compilation of data for amino acids in various cheeses). Parmesan contains a very high concentration of amino acids which appear to make a major contribution to the characteristic flavour of this cheese. The presence of amino acids in cheeses clearly indicates aminopeptidase activity; since these enzymes are intracellular, their action indicates lactococcal cell lysis. On the presumption that amino acids contribute to cheese flavour, interest is now being focussed on a search for fast-lysing lactococcal strains, susceptible to heat, phage or bacteriocin-induced lysis. Amino acids have characteristic flavours (see Chap. 13); although none has a cheese-like flavour, it is believed that they contribute to the savoury taste of mature cheese.

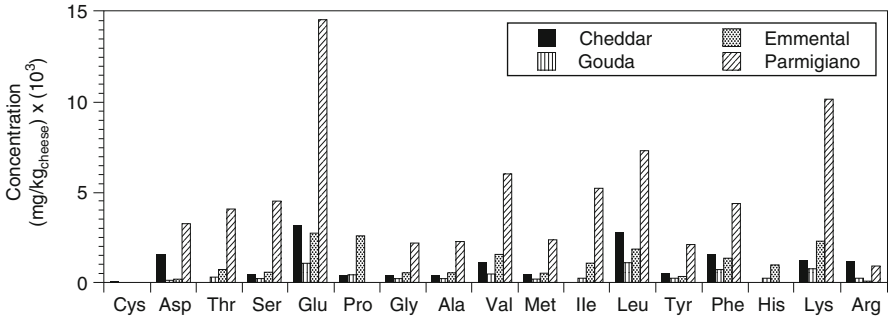


Fig. 12.24 Typical concentraitons of amino acids in Cheddar, Gouda, Emmental and Parmigiano Reggiano (drawn from data in Fox and Wallace 1997)

12.9 Catabolism of Amino Acids and Related Events

Catabolism of amino acids to volatile flavour compounds is one of the key events in the biogenesis of flavour in many cheeses. Catabolism involves decarboxylation, deamination, transamination, desulphuration and hydrolysis of amino acid side chains leading to the production of a wide array of compounds including carboxylic acids, amines, NH_3, CO_2, aldehydes, alcohols, thiols and other sulphur compounds, phenols and hydrocarbons. General pathways of amino acid catabolism are summarized in Fig. 12.25. The catabolism of amino acids has been reviewed by Hemme et al. (1982), Law (1987), Fox and Wallace (1997), Yvon and Rijnen (2001) and Curtin and McSweeney (2004).

The catabolism of most amino acids appears to be initiated by the action of an aminotransferase, which acts on the amino acid to transfer its NH_2 group to an acceptor molecule, which in cheese is principally α-ketoglutarate. This action produces a new α-keto acid corresponding to the original amino acid and glutamic acid. Aminotransferases are intracellular enzymes from the starter and require pyridoxal-5′-phosphate for activity. Aminotransferase activity appears to be the rate-limiting step in amino acid catabolism. The α-keto acids produced in this step are unstable and can be converted to a range of volatile flavour compounds including hydroxy-acids (by the action of 2-hydroxyacid dehydrogenases), aldehydes (which in turn can be oxidized or reduced to the corresponding carboxylic acid or alcohol), or degraded to a range of other compounds. Thus, one amino acid can be converted to a range of volatile compounds (Fig. 12.25), many of which have been shown to be important to cheese flavour.

Decarboxylation involves the conversion of an amino acid to the corresponding amine, with the loss of CO_2. The presence of primary amines in cheese can be explained in terms of simple decarboxylation, although the formation of secondary and tertiary amines is more difficult to explain. The principal amine in cheese is tyramine. A number of amines produced in cheese are biologically active.

Deamination results in the formation of NH_3, which is an important constituent of many cheeses, such as Camembert, Gruyère and Comté. Ammonia can also be formed by oxidative deamination of amines, yielding aldehydes. Transamination results in the formation of other amino acids by the action of transaminases. Aldehydes formed by the above processes can then be oxidized to acids or reduced to the corresponding alcohols.

Amino acid side chains can also be modified in cheese. Hydrolases can release ammonia from Asn and Gln or by the partial hydrolysis of the guanidino group of Arg, forming citrulline or its degradation to ornithine. Phenol and indole can be produced by the action of C–C lyases on Tyr and Trp.

Volatile sulphur compounds, including hydrogen sulphide (H_2S), dimethylsulphide [$(CH_3)_2$-S], dimethyldisulphide (CH_3-S-S-CH_3) and methanethiol (CH_3SH), are found in most cheeses and can be important flavour constituents. Sulphur-containing compounds are produced mainly from methionine by the action of enzymes such as cystathionine-γ-lyase, cystathionine-β-lyase and methionine-γ-

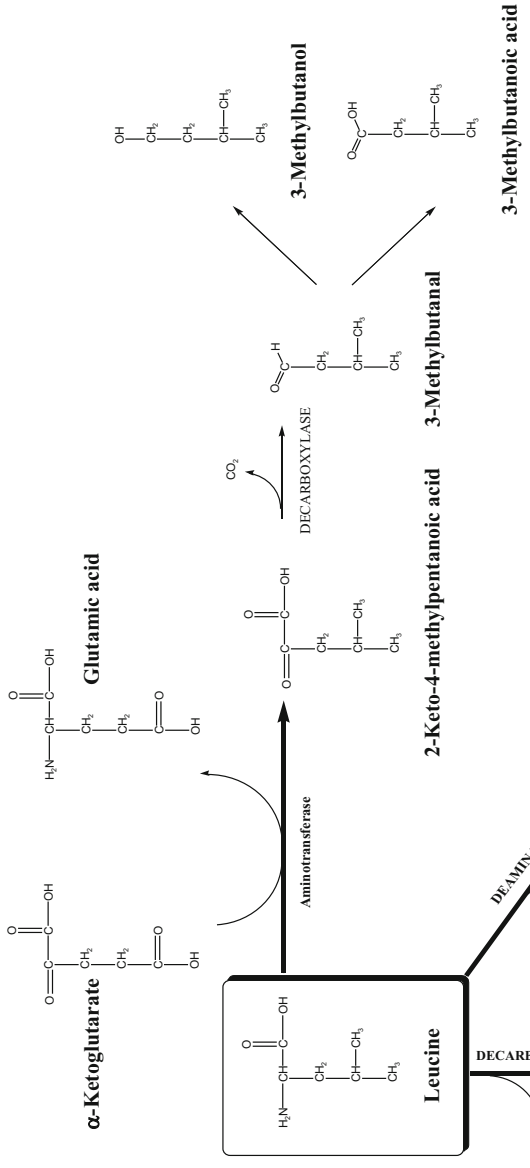


Fig. 12.25 Pathways for the catabolism of leucine in cheese during ripening

lyases. Since Cys is rare in the caseins (it occurs at low levels only in α_{s2}- and para-κ-caseins, which are not extensively hydrolysed in cheese). Methanethiol and related compounds are thought to be particularly important in the flavour of Cheddar cheese.

12.10 Conclusions

Cheese ripening involves a very complex series of biochemical reactions catalyzed by living microorganisms or by enzymes from several sources. The primary events, (1) metabolism of residual lactose and of lactate and citrate, (2) lipolysis and fatty acid metabolism and (3) proteolysis and amino acid catabolism, have been described rather thoroughly. The fermentation of lactose, mainly to lactic acid, is caused by living microorganisms, principally the starter culture or by adventitious bacteria in the case of artisanal cheeses; the fermentation of lactose has been described thoroughly. Lipolysis is quite limited in most cheese varieties and in those varieties in which it is important, lipolysis has been quite well characterized in terms of extent and the enzymes involved. The initial steps in proteolysis and the enzymes responsible have been established for the principal varieties. Secondary proteolysis is characterized to some extent in a few varieties but proteolysis is so complex and variable, both between and within varieties, that it is difficult to characterize it fully.

Many of the enzymes responsible for primary ripening have been isolated and characterized. However, the stability and activity of these enzymes in the cheese environment has received little attention, an area that appears to warrant research. The primary reactions are probably responsible for changes in cheese texture, e.g., increase in pH, due to the catabolism of lactic acid and/or production of NH_3 from deamination of amino acids, or hydrolysis of the protein matrix. With the exception of fatty acids, the products of primary reactions are relatively minor contributors to cheese flavour.

Modifications of the primary products of glycolysis and lipolysis are fairly well characterized. The catabolism of fatty acids to methyl ketones via β-oxidation and decarboxylation is a major contributor to the characteristic flavour of blue-mould cheeses but is not significant in most varieties. The catabolism of lactic acid is of minor significance to the flavour of most cheeses; an exception is Swiss-type cheeses but even in these cheeses, the catabolism of lactic acid is less important for the flavour than for the production of CO_2 for eye formation. The catabolism of amino acids is the least well characterized aspect of cheese ripening. It is very likely that the products of amino acid catabolism are major contributors to the flavour of many cheese varieties. The ammonia produced in many of these reactions contributes to the change in the pH of cheese during ripening and this change in pH affects the texture of the cheese and probably affects the stability and activity of many enzymes which, in turn, probably influences flavour development. It is very likely that future research efforts on cheese ripening will focus on amino acid catabolism.

Since the biochemistry of cheese ripening is responsible for its flavour, texture and appearance, clearly, elucidation of these biochemical reactions is an essential prerequisite for controlling and modifying cheese ripening. Such knowledge is essential for the selection of primary and secondary cultures and for their genetic modification. Without this knowledge, selection of starter cultures will be empirical.

The stability and activity of microorganisms and enzymes in cheese depends on its composition. Although the composition of cheese produced in modern factories using modern technology is controlled within quite narrow limits, the quality of the resultant cheese is at least somewhat variable, even when the cheese is made from pasteurized milk essentially free of indigenous bacteria and using high-quality rennet and starter. This suggests that slight variations in curd composition are important, perhaps owing to their effect on the stability and activity of key enzymes. To date, most studies on these aspects of cheese ripening have been performed on model systems which are well controlled and over-simplified.

Very considerable advances on the biochemistry of cheese ripening have been made during the past 30 years; the general features have been elucidated but the details remain to be established.

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Chapter 13
Cheese Flavour

Kieran N. Kilcawley

Summary Cheese is one of the most diverse product types in the market place, with a wide range of visual, physical and flavour attributes available. As cheese is a dynamic product, its flavour is constantly evolving during ripening and the range of cheese flavours encompasses everything from mild bland dairy notes to complex flavours that are described as intensely putrid, overpowering and nauseous. Cheese flavour is derived from a wide range of compounds resulting from the hydrolysis or metabolism of carbohydrates, proteins and fats, along with compounds added during processing or directly from the milk. This chapter provides an overview of current approaches used to analyse cheese flavour from an industrial and a research perspective. In industrial settings, experienced graders are used to select cheeses for specific markets and in some cases this is carried out in conjunction with key compositional parameters known to be important indicators of cheese quality. However for research purposes a more holistic approach that encompasses a range of different types of sensory, compositional, chemical, biochemical, microbiological, rheological and visual techniques is typically used. Techniques used to assess sensory characteristics of cheese are described in detail, as are chromatographic methodologies used to determine volatile and non-volatile compounds directly implicated in cheese flavour. Our knowledge of taste and odour-active compounds in cheese and the factors that influence their perception continues to grow in tandem with new methodologies and advances in instrumentation and data handling. Although we understand key aspects of the cheese flavour for specific varieties, significantly more research is required to establish the complete flavour profile for specific cheeses/varieties.

Keywords Flavour profile • Cheese volatiles • Gas chromatography • Headspace analysis • Off-flavours • Bitterness

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13.1 Introduction

The range of different natural cheeses commercially available is in excess of 500. Of all the food products available today, cheese is possibly the most diverse with cheeses encompassing a wide range of visual, physical and flavour attributes. Apart from a small number of major varieties, most cheeses are closely associated with a particular geographical region of the world. In many cases, the method of production has evolved with mechanisation and quality control, particularly for large volume production, but the basic principle generally remains unchanged for any given variety. The factors which determine cheese quality in terms of texture and appearance are, in general, well understood and have been explained in more details in Chap. 15. During the production and ripening process, numerous microbiological and biochemical reactions occur, including glycolysis, lipolysis and proteolysis all of which contribute to cheese quality. The extent of these processes in cheeses are generally characteristic of each variety. For example, soft acidic-type cheeses, such as Quarg, have a short shelf-life, a mild acidic flavour and are ripened only for days whereas hard cheeses, such as Parmesan, have a strong piquant flavour and may be ripened in excess of 2 years. In general, the longer the ripening time the more expensive the cheese, due to the costs associated with controlling the cheese ripening environment (temperature and humidity).

Cheese is a dynamic product, with many varieties having up to 100 billion bacteria per gramme, all of which metabolise lipids, carbohydrates and/or proteins to create a myriad of aromatic and sapid compounds that help create cheese flavour. Incorporate all the other factors that influence cheese flavour such as the quality and type of milk, type and concentration of the starter culture, chymosin, the possible inclusion of moulds, yeasts, pregastric lipases, differences in the production parameters and ripening process, it becomes easy to understand the overall complexity of cheese flavour. Problems with any aspect of production can lead to the development of off-flavours or textural or visual faults, all of which reduce the overall appeal of the product. Manufacturers, particularly large-scale producers, focus on quality and consistency as they are very aware of consumer preferences. Brand loyalty is a big factor in the commercial cheese industry. This is especially true for large volume cheeses, such as Cheddar, where manufactures spend a lot of time and money in marketing to ensure that they have excellent brand recognition. Manufactures know that repeat purchase by consumers is driven primarily by flavour and quality. It can take years to develop brand loyalty but a lot less to destroy it and therefore manufactures place huge emphasis on quality and consistency of any cheese they release to the market place.

13.2 Sensory Analysis

Sensory evaluation comprises a set of techniques for the accurate measurement of human responses to foods. Sensory evaluation has been defined as a scientific method used to evoke, measure, analyze and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and hearing (Stone and

Sidel 2004). The dairy industry understands the importance of sensory quality as a crucial aspect of sales and marketing and has been at the forefront of sensory development and used cheese grading and judging for evaluating sensory quality before modern-day sensory analysis techniques were developed (Singh et al. 2003; Drake 2007). Grading and judging of cheeses remain a focal point of the cheese industry to this day. Therefore, sensory analysis in some form has always been a corner-stone of the cheese industry and this is unlikely to change although more scientific approaches are now also used.

The goal of any sensory quality program should be to meet customer requirements. In terms of cheese, sensory evaluation is generally used to assess cheese quality or to characterise cheeses during development or to test consumer acceptance. It is important that a suitable procedure is selected and that test conditions are meticulously controlled to ensure reliability. In general terms, sensory evaluation of cheese can be divided into three separate approaches or techniques: consumer preference studies, cheese grading and descriptive sensory analysis, each of which has different objectives and are typically used independently, but often provide more useful information if used with other product data.

13.2.1 Cheese Grading

Quality is assessed by visual appearance, texture and flavour, typically, by graders or judges very familiar with the variety. A grader's primary purpose is quality control, but they also select cheese for specific markets, for major national or multinational retailers or for individual cheesemongers. During grading, cheeses are scored for overall flavour and texture quality based on an idealized concept of the perfect cheese or graded for a specific market. The unusual aspect of this type of grading is that graders typically look for negative attributes and often have a predetermined list of defects that have been developed for that cheese through experience, which can be either formal or informal (Delahunty and Murray 1997; Delahunty and Drake 2004; Drake 2007). Table 13.1 is an example of defects commonly associated with cheese (Singh et al. 2003; Hersleth et al. 2005; Muir 2009).

A range of different scoring systems are used and have been described for Cheddar cheese. Most scoring systems differentiate between flavour/aroma, body and texture, colour and appearance with different weightings applied to each set of attributes. In most cases, this type of analysis primarily focuses on defects, mainly because more descriptors exist for negative attributes than for positive attributes. Describing or defining defects and their intensities can be subjective, as many of the defects can constitute an "ideal" flavour and become defects only when they are out of balance (Singh et al. 2003; Drake 2007). Obviously, the person or persons analyzing the cheese must be completely knowledgeable of each defect or attribute associated with the cheese; otherwise the analysis is likely to be irrelevant. A critical aspect of this procedure is the control cheese or idealized cheese to which the grader is comparing the test cheese(s). Typically, graders use their own experiences, but are also trying to place the test cheese into specific market categories for what

Table 13.1 An example of common defects associated with cheese

Flavour/aromatic defects	Description
High acid	Excessive acid
Harsh, unclean	Non typical dirty, a taste than lingers
Bitter	Mild to very bitter
Fruity	Aromatic
Rancid	Excessive lipolysis
Yeasty	Earthy aroma
Savoury	Umami—beef stock, soya sauce
Astringent	Acidic like, with a metallic sharpness
Sour	Sourness
Barnyard	Faecal or cowy
Salty	Salty
Sulphur/Eggy	Sulphur or eggy notes
Other	Not described
Textural/consistency defects	
Short	Crumbly
Rubbery	Corky
Weak	Breaks down too easily
Doughy	Sticky
Grainy	Gritty mouthfeel

they believe the cheese is most suited and thus try to achieve the best price. Therefore, they are not physically comparing the test cheese(s) to another cheese, but to a concept they have based on their own experience. The success of this system for quality assessment is dependent upon the quality of the assessor and having standardized frames of reference and a relevant descriptive flavour terminology (Bodyfelt 1981). Grading was never designed to be a specific analytical tool and two cheeses that receive identical scores are still likely to have fundamental sensory differences (Drake 2007). Also what represents a "good" or "bad" cheese to an expert grader or judge may not necessarily represent the views of the consumer (McBride and Hall 1979; McBride and Muir 1999; Lawlor and Delahunty 2000; Singh et al. 2003; Hersleth et al. 2005). The approach used to grade Cheddar cheese in New Zealand was changed from a defect-based system to a more objective system of attribute grading more similar to descriptive profiling (Muir 2009) in order to improve the relationship between consumer perception and factory graders.

Despite the obvious failings of grading, if used correctly it can provide useful information, but has minimal use as a research tool. Grading is used widely in industry because it is cheap, practical and easy to evaluate a large number of samples relatively quickly (Drake 2007; Muir 2009). Some countries have accredited courses for training graders, while others simply assess individuals for their ability to perceive key flavour and aroma attributes in cheese. In the latter case, training typically occurs through "shadowing" an experienced grader or graders. In factories that produce cheeses like Cheddar that can require a long and expensive maturation process, grad-

ing plays a critical role. In such cases, graders will try to identify out-of-specification cheese early in the maturation process to avoid the expense of ripening a cheese that may end up downgraded and sold as less expensive cheese for secondary processing. Therefore, a grader will try to determine whether a cheese is suitable for extended maturation or not. In many cases they will try to identify a specific time at which the cheese should be retailed, or if it should be re-evaluated in the future to ensure that it is following a predicted quality route. In some cases, vintage Cheddar cheese may be ripened for 2 years and evaluated up to six times over this period. In factory settings, graders do not always provide a score but just an overall comment as an assessment of quality. In this situation, a grader can assess in excess of a hundred cheeses in a session. In most cases graders rapidly make visual assessments of large cheese blocks and then make further assessments by taking a sample cheese plug using a cheese trier. Graders assess how cleanly the plug comes out, its appearance, colour and adhesiveness to the trier. A small sample of the plug is repeatedly rolled between the index finger and thumb. The aroma of this warm cheese is inhaled and finally the cheese is rolled within the mouth for aroma and taste. Experienced factory graders can also diagnose problems and provide feedback to those in production to aid improvement and consistency of manufacture (Muir 2009). Some experienced graders also use compositional data to get a better overall assessment of quality, but also potentially gain a better understanding of predicated ripening characteristics. For Cheddar cheese, key compositional parameters have been established that can be associated with overall quality (Lawrence and Gilles 1987); using this knowledge with grading scores can help to provide practical information on predicated quality. However, with cheese grading there is a tendency to be too arbitrary, which gives a false sense of exactness (Bodyfelt 1981). Cheese grading is subjective as no two people have identical perceptions as to the "ideal" cheese. Judging for awards can be even more subjective, especially as judges tend to evaluate cheeses as a group. In this situation, a degree of bias is often encountered as the strongest personality often overly influences the group rather than obtaining a true representative assessment Typical grading systems used in major Cheddar cheese-producing countries were reviewed by Muir (2009).

13.2.2 Consumer Studies

Focus groups are used to provide an insight into consumer perceptions, needs and desires. Understanding consumer perception of cheese flavour is crucial for effective marketing and product development (Drake 2007). Typically, 8–12 participants are guided through a process by an experienced moderator and asked to describe the cheese. Such sessions are undertaken to obtain information on preferences, motivations and product attributes. As the number of participants is low, results need to be interpreted with caution (Drake 2007). A big problem with this approach is clarification of the language used. Consumers may often use terms that have multiple meanings, are ambiguous and infer either positive or negative attributes, or use

combinations of various terms (Delahunty and Drake 2004). It is often difficult or impossible to discern why a consumer likes or dislikes a cheese in such studies.

In preference or discrimination testing, consumers are presented with two or more cheeses and asked, for example, to indicate which they prefer. If more than two samples are used, consumers can rank their preference; this is termed "ranked preference testing" (Drake 2007). A range of different discrimination tests exist. However, a major limitation of this type of study is that no information on liking or disliking is determined. Consumers can dislike all the cheeses, but still rank them (Delahunty and Drake 2004). Effective studies measure consumer responses and can provide information on consumer likes and dislikes (hedonic responses). Consumers are presented with cheeses and asked specifically to indicate liking on a nine point hedonic scale (Drake 2007). The use of rating scales that measure relative likes and dislikes is based on preference rather than difference. These tests should be carried out with subjective assessors or with untrained consumers. The assessors should be regular consumers of cheese or, even better, regular consumers of the type of cheese to be studied. At least 50 personal are required, the more assessors the greater the reliability of the results or statistical relevance (Delahunty and Murray 1997; Delahunty and Drake 2004).

13.2.3 Descriptive Sensory Analysis

Descriptive analysis is the most powerful sensory tool in cheese flavour research. It can be used to differentiate cheeses based on a full complement of sensory characteristics/attributes and determine a quantitative description of all the sensory aspects that can be identified (Singh et al. 2003). Each descriptive test has three stages: (a) selecting a panel, (b) establishing the language or vocabulary to describe the cheese, and (c) quantifying the sensory results (Delahunty and Drake 2004). A panel of, typically, 12 individuals is used. Upwards of 50 or more individuals may need to be screened before a trained group is achieved; a trained group consists of individuals who are able to discern each required attribute. Assessors are trained to identify and quantify a wide range of specific sensory attributes. Such attributes relate to flavour, aroma and texture and, in some cases, appearance. Training typically involves exposure to a wide range of attributes using foods, beverages or chemical standards (Drake 2007). Precise definitions and references (food or chemical) for each attribute or lexicon are necessary to be able to "calibrate" panels over time or to compare different panels. Assessors quantify the perceived intensity of each attribute by giving a score on a line scale. In order to use the scale effectively, both its lower and upper extremes must be defined for each assessor. Panellists are constantly re-trained and evaluated to ensure that they are calibrated with one another. It is relatively easy to determine if any individual panellists are failing for any given attribute as all results are evaluated statistically. The power of descriptive analysis is that the panel and its training can be adjusted to meet specific objectives (Drake 2007).

Table 13.2 Descriptive attributes used to characterise Cheddar cheese

Attribute	Definition
Cheddary	Flavour associated with typical Cheddar cheese
Creamy	Creamy notes
Buttery	Butter-like
Pungent	Strong irritant
Mouldy	Earthy, dirty, stale, musty notes
Caramel	Burnt sugar or syrup or toffee
Burnt-milk	Sweet with burnt aroma
Soapy	Soapy
Smoky	Charred wood, tainted
Fruity	Fruity
Mushroom	Raw fresh mushrooms
Rancid	Oxidised fat, sour milk
Nutty	Nut-like, roasted nuts
Sweaty	Stale, cheesy, smelly feet
Balanced	Mellow, in equilibrium
Processed	Packaging, artificial
Salty	Salty
Sweet	Sucrose-like
Acidic	Sharp, lactic acid
Bitter	Tonic water
Strength	Intense or harsh
Astringent	Mouth-drying, puckering
Whey	Cheddar whey
Brothy	Boiled meat or vegetable stock
Catty	Cat urine
Diacetyl	Buttery aroma
Sulphur	Sulphurous
Cowy/Barnyard	Cow shed/barn

Evaluation of the cheeses is vcarried out in a controlled environment to eliminate distractions and bias (subconscious or conscious). Specific sized booths are used with controlled lighting and atmosphere, where distractions, including sound, are minimised or eliminated. Samples are analyzed in a controlled manner to eliminate any order of preference that may influence the overall results. Assessors also have access to fresh water and slices of fresh green apple to cleanse their palate between samples. Assessors are asked to avoid eating or drinking foods prior to the test that may interfere with their ability to perceive subtle aromatic and flavour differences. Non-smokers are always preferred. Panels act as a highly trained instrument and require regular "calibration" or training to ensure accuracy. These panels are costly to operate, mainly because panels operate best when regularly active. Table 13.2 gives examples of typical attributes used to characterise Cheddar cheese. A more detailed description of attributes for cheese in general is given in Delahunty and Drake (2004).

Muir (2002) described the protocols required to establish a relevant sensory language. Different panels across the world use different sensory languages to describe cheese; this is confusing when the same cheese type is discussed. Differences between panels across countries have been highlighted by Drake et al. (2005). Development of flavour attributes/lexicons are discussed in detail by Drake and Civille (2003) and details on how to set up panels and assess their performance were described by Muir (2009). When a test is complete, the results are analysed statistically to determine which attributes best discriminate the cheeses. Because descriptive attributes are not independent of each other, a true representation of the data is obtained only by using multivariate statistical analysis. The most common way to graphically represent the descriptive attributes by multivariate analysis is spider diagrams or principal component analysis (PCA). Spider diagrams consist of a sequence of equi-angular radii, with each representing a descriptor. The point on the radius, represents the magnitude of the variable of an individual cheese sample relative to the other sample scores for the same variable. The final plot is an easy way to quickly view sensory differences. Figure 13.1 is an example where the cheeses are plotted in separate colours. Figure 13.2 highlights the differences in specific flavour characteristics of a range of different cheese types. PCA allows a multi-dimensional matrix to be simplified and describes graphically the maximum discrimination between cheeses. Results are typically displayed in a two-dimensional plot, where discriminating attribute scores show the relationship between the cheeses studied. Figure 13.3 shows discrimination on specific flavour and odour attributes between three individual cheeses in triplicate; each colour corresponds to a separate cheese.

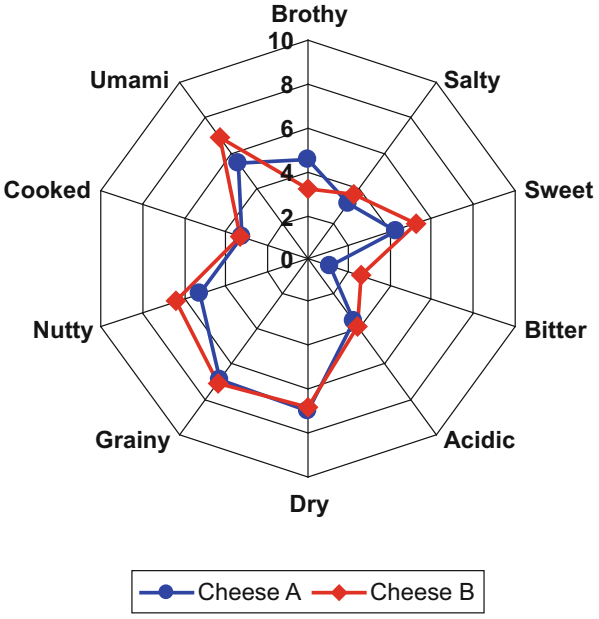


Fig. 13.1 Spider diagram highlighting differences in the perception of defined attributes/lexicons between two cheeses

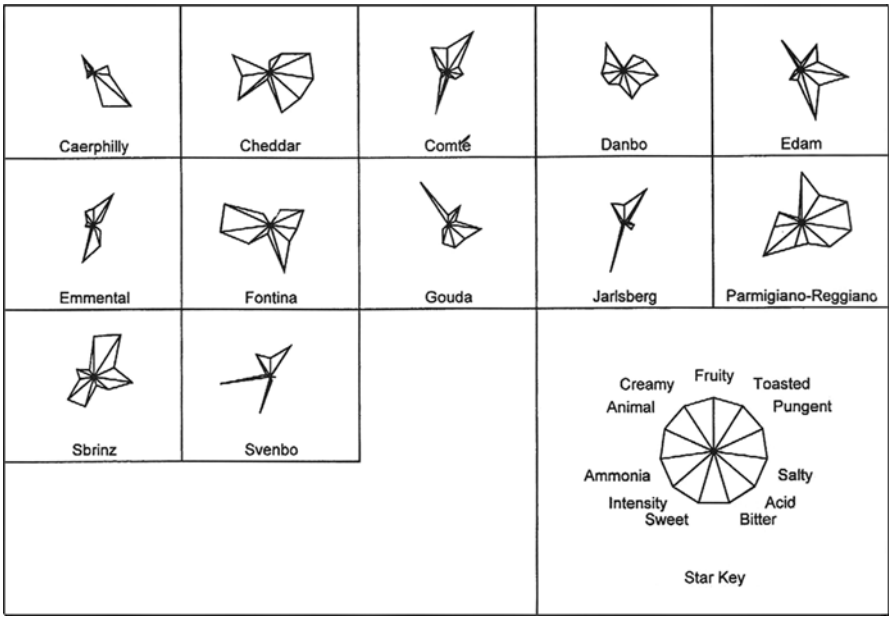


Fig. 13.2 Spider diagrams representing the flavour profiles of 12 individual hard cheeses

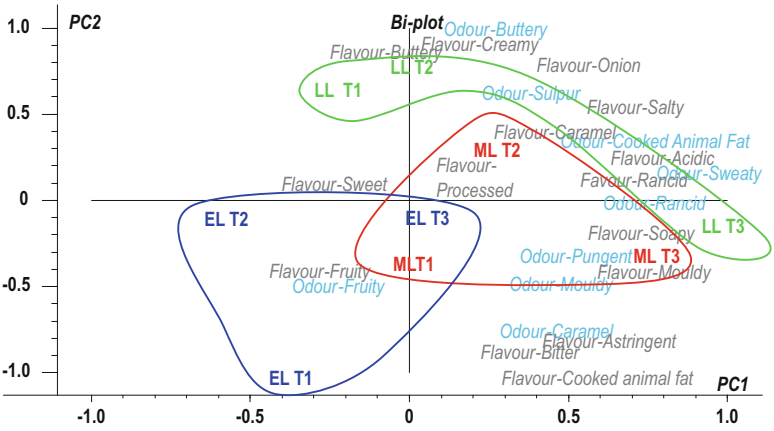


Fig. 13.3 Principal component analysis highlighting the association of cheeses (coded EL, ML & LL) with different flavour attributes

13.3 Characteristics of Cheese Flavour

Flavour actually comprises taste and aroma. It is estimated that taste contributes only approximately 25 % of flavour, the remaining 75 % being from aromatic compounds which are perceived orthonasally and retronasally. It has been postulated that cheese flavour is the result of the correct balance and concentration of a wide variety of volatile flavour compounds, called the "component balance theory" (Mulder 1952); however it is also obvious that non-volatile taste components have a significant role.

13.4 Analysis of Cheese Flavour

Research on cheese flavour can be divided into analysis of volatile (aromatic) and non-volatile (taste) components. Initial research concentrated on taste as it was easier to comprehend and analyse with the equipment available. Advances in high performance liquid chromatography (HPLC) and gas chromatography (GC) coupled to specialised detection systems have vastly improved the ability to identify and quantify the complexity of cheese flavour.

13.4.1 Analysis of Non-volatile Compounds

There are only five basic taste sensations: sour, sweet, salt, bitter and umami, however, a host of other sensations and interactions exist that increase the complexity of taste. Most taste compounds are water-soluble and contribute to a number of sensations in the mouth. These include salty, sweet, bitter and acid, together with umami, 'hot' and 'cooling', astringency and mouth-coating. It is well established that only low molecular weight water-soluble compounds are responsible for cheese taste (Aston and Creamer 1986; Warmke et al. 1996; Rychlik et al. 1997; Kubickova and Grosch 1998) and that the contribution of amino acids, organic acids, biogenic amines, peptides and mineral salts varies between different cheese types. Direct associations between proteolysis and the development of mouth-feel, texture, taste and aroma of maturing cheese have been made (Pripp et al. 2006). Cheese flavour intensity has also been correlated with small peptides and free amino acids and with off-flavours and bitterness (Pripp et al. 2006). Another critical aspect is flavour release which is strongly affected by cheese composition (Delahunty and Piggott 1995; Urbach 1993; Subramanian et al. 2009; Overington et al. 2010).

The concentration of amino acids in cheese increases during ripening due to proteolysis, arising mainly from the action of intracellular peptidases of lactic acid bacteria (LAB). The properties of the various amino acids are given in Table 13.3. Most bitter amino acids are those with non-polar or hydrophobic side chains, such

Table 13.3 Properties of L-amino acids in cheese

L-Amino Acid	Properties	Sensory character
Glutamic acid (Glu)	Acidic, charged	Umami, sour, slightly sweet
Aspartic acid (Asp)	Acidic, charged	Sour, slightly bitter, slightly acidic
Histidine (His)	Basic, negatively charged, polar	Sour, medium bitter
Arginine (Arg)	Basic, negatively charged, polar	Bitter
Lysine (Lys)	Basic, negatively charged, polar	Medium bitter and sweet
Isoleucine (Ile)	Aliphatic, hydrophobic, neutral, non-polar	Bitter
Leucine (Leu)	Aliphatic, hydrophobic, neutral, non-polar	Bitter, medium sweet
Valine (Val)	Aliphatic, hydrophobic, neutral, non-polar	Bitter, slightly sweet
Alanine (Ala)	Aliphatic, hydrophobic, neutral, non-polar	Sweet
Glycine (Gly)	Aliphatic, hydrophobic, neutral, non-polar	Sweet
Proline (Pro)	Imino acid, neutral, non-polar	Sweet, medium bitter
Phenylalanine (Phe)	Aromatic, non-polar, neutral, hydrophobic	Bitter
Tryptophan (Trp)	Aromatic, slightly polar, neutral, hydrophobic	Bitter
Tyrosine (Tyr)	Aromatic, polar, neutral, hydrophobic	Bitter
Methionine (Met)	Sulphur, hydrophobic, non-polar, neutral	Bitter, slightly sweet, slightly sulphurous,
Cysteine (Cys)	Sulphur, slightly hydrophobic, slightly polar, neutral	Sulphurous, unpleasant
Glutamine (Gln)	Amide, polar, neutral, hydrophilic	Bland, slightly sweet, slightly umami
Asparagine (Asn)	Amide, polar, neutral, hydrophilic	Sour, slightly umami, possibly bitter
Threonine (Thr)	OH, hydrophilic, neutral, polar	Sweet, slightly bitter, possibly sour
Serine (Ser)	OH, hydrophilic, neutral, polar	Sweet, slight umami, sour

as isoleucine, phenylalanine, leucine, methionine, proline and valine, although lysine (basic), and tryptophan and tyrosine (polar, but normally uncharged), histidine and arginine (polar, negatively charged) are also considered bitter. Bitterness in cheese is due to the accumulation of small peptides, 300–400 Da that have hydrophobic end sequences due to the presence of certain amino acids at the carboxy or amino terminal. The intensity of bitterness is related directly to the number of hydrophobic amino acids, the sequence of amino acids and the size of the peptide. However, some authors have proposed that bitterness is not related solely to hydrophobicity but appears to be influenced by $CaCl_2$ and $MgCl_2$ and bitter amino acids

(Salles et al. 2000; Toelstede and Hofmann 2008a, b, 2009). The widespread use of adjunct cultures with high concentrations of intracellular aminopeptidase and proline-specific peptidase activities that lyse rapidly during ripening has greatly reduced bitterness in commercial cheese. Bitterness is also thought to be masked by other cheese components such as free fatty acids (Homma et al. 2012), glutamic acid, NaCl and sodium lactate (Toelstede and Hofmann 2009).

Proline and lysine are sweet and bitter, alanine, glycine, serine and threonine are also sweet, while glutamic acid, histidine and asparagine are sour (McSweeney 1997). Asparagine and glutamic acid also have umami properties and have the lowest taste thresholds; however the concentrations of several amino acids in a water-soluble extract of mature Cheddar cheese are typically above their flavour thresholds. Glutamic acid, leucine and tyrosine have been identified as major taste compounds in Camembert and Cheddar cheese and are associated with umami taste (Kubickova and Grosch 1998; Drake et al. 2007; Andersen et al. 2008). Glutamate and lactate have also been associated with umami taste in Gouda cheese (Toelstede and Hofmann 2008a, b) and in Cheddar (Drake et al. 2007). An additional taste sensation was identified in Gouda cheese, which was described as mouthfulness and thickness termed "kokumi", which was linked to a range of γ-glutamyl (γ-G) dipeptides, and also associated with umami flavour (Toelstede and Hofmann 2009). γ-G dipeptides contain a peptide bond between the γ-carboxylic acid group of L-glutamic acid and another amino acid (McSweeney 1997). The intensity of kokumi flavour depends on NaCl, amino acid concentration and pH (Toelstede and Hofmann 2009). γ-G peptides have been associated with salty, sour, brothy, metallic and sour flavours in Comte cheese. Organic acids and mineral salts have been shown to be important compounds in Cheddar cheese and were correlated with umami flavour (Drake et al. 2007; Andersen et al. 2010).

NaCl is an important contributor to the taste of the non-volatile fraction of cheese. The direct impact of NaCl on taste is important and is particularly important to the flavour and quality of cheese. NaCl was added to cheese originally as a preservative, acting to reduce water activity (a_w). Salty taste is stimulated by small inorganic ions; the taste of chlorides varies from acid (HCl) through salty (e.g., NaCl) to bitter (e.g., KCl and CsCl). Salt level also impacts on proteolysis and thus subsequently on bitterness and texture (McSweeney 1997). Salt has been identified as a major contributor to the flavour of Camembert cheese, and the impact of salt in cheese depends upon its concentration and the composition and age of the cheese. Saltiness in Gouda cheese was affected by salt, sodium phosphate and arginine. Salt perception is usually greater in young cheese as there are less flavour-active compounds present to reduce its perception. In relation to the major commodity cheeses, such as Cheddar, a decrease in salt level has been apparent in recent years brought about by health-related concerns due to excessive levels of sodium in the diet. Some studies have involved partial replacement of NaCl by KCl but this can lead to excessive bitterness as the taste of most high molecular weight salts is bitter, rather than salty. Also, the inclusion of non-sodium salts must be listed on the ingredient list of the cheese, which may have an adverse impact on sales, something which cheese producers are likely to avoid if possible.

Acid taste is caused by H^+, the taste threshold of which is about 2 mM. The principal acid in cheese is lactic acid, the concentration of which, and consequently the pH of cheese, varies considerably with variety. The concentration of lactic acid in cheese is influenced by its initial production by the starter, the extent of loss in the whey and its metabolism by the secondary microflora. Total lactate concentration may not be a good index of cheese acidity since in certain varieties (e.g., mould-ripened cheeses) the pH increases during ripening, caused by ammonia released by deamination of amino acids and/or by the metabolism of lactate. Acids produced from carbohydrate and protein metabolism and fat hydrolysis are also likely to contribute to acidity (McSweeney 1997). Sourness in cheese is linked directly to organic acids, and in particular the level of lactic acid, but also to mineral salts and some free amino acids (as previously mentioned). Lactic acid and NaCl have been associated with sourness perception in Gouda cheese (Toelstede and Hofmann 2008b).

Sweetness in cheese may not be linked directly to sugars (lactose, glucose and galactose), but as in the case of Swiss cheese it is linked to products of proteolysis, such as proline. It is also thought that the sweet flavour of Swiss cheese results from the interaction of calcium and magnesium ions with amino acids and small peptides and from organic acids (acetate, propionate, lactate, glutamate, succinate and phosphoric acids) and their sodium, potassium, calcium, magnesium and ammonium salts (Preininger et al. 1996; Drake et al. 2007).

Biogenic amines also contribute to the flavour of Camembert cheese (Kubickova and Grosch 1998), although high levels are toxic and are becoming an issue of concern in dairy products, including cheeses (Linares et al. 2012).

13.4.2 Methods of Analysis for Non-volatile Compounds in Cheese

Analysis of the non-volatile compounds in cheese is dependent upon the compound of interest. Peptides are well separated by chromatographic procedures such as Reverse Phase HPLC and depending upon the information required (identification and/or quantification) a range of different detection methods can be used, such as UV/Vis absorption spectroscopy or different types of mass spectrometry (MS). Electrophoresis has also been applied widely, using techniques such polyacrylamide gel electrophoresis (PAGE) or urea-PAGE, capillary electrophoresis or spectroscopic techniques, such as infrared spectroscopy, Raman spectroscopy or fluorescence spectroscopy. A detailed review of methods used to separate, identify and quantify peptides is given by Recio and Lopez-Fandino (2010). Amino acids are typically analysed using specific amino acid analysers or HPLC or post-derivatization by GC. Biogenic amines are also typically analysed by HPLC, using UV/Vis absorption spectroscopy or MS. Organic acids are most commonly analysed by HPLC using selective columns with detection by refractive index, evaporative light scattering or charged aerosol detection. Some organic acids and fatty acids are analysed by GC flame ionization detection or MS and mineral salts are typically analysed by atomic

adsorption, inductively-coupled plasma MS or photometrically. Carbohydrates are typically analysed using enzymatic methods or HPLC using specific columns with evaporative light scattering or pulsed amperometric detection or MS.

13.5 Analysis of Volatile Compounds

13.5.1 Analysis of Volatile Compounds

The aroma of cheese is complex. Many different volatile compound classes are present, most of which are liposoluble. These compounds are formed via a series of enzymatic and chemical reactions from free fatty acids, peptides, amino acids and carbohydrates. Some volatile aromatic compounds are further reduced or converted to other volatile compounds and some interact to form more stable complexes. However, not all volatile compounds are aromatic and the contribution of those that are varies with their concentration, aroma threshold (odour activity) and with cheese composition (matrix effect).

The sensitivity of the human nose remains greater than any GC detection system, and therefore, it is necessary to concentrate volatiles prior to identification by GC. It is best to extract aromatic compounds in cheese from non-volatile proteins, lipids, carbohydrates and water, as these can interfere with analysis. Isolation methods based on volatility are complicated by the fact that the concentration of water in cheese is high. Water not only dilutes aromatic compounds but also causes partitioning of the chemical classes based on polarity and causes problems with GC-MS detection. The presence of proteins and carbohydrates can also complicate the extraction process, due to their emulsification, viscosity and foaming properties. Most aromatic compounds in cheese are lipophilic which is why polar solvents are widely used to extract aroma compounds from cheese. However, the presence of non-volatile lipids can interfere with extraction and analysis. As aroma compounds must be volatile to be sensed, it is therefore logical that volatility is commonly used as a basis for extraction.

The study of volatile compounds in cheese is not new, but it is only in the last couple of decades that significant progress has been made due to advances in volatile extraction/concentration techniques, chromatographic separation and utilization of MS detection (improved sensitivity, single ion monitoring, library searching and deconvolution software). The study of cheese volatiles is difficult due to the complexity of the compounds involved, the array of concentrations present, their odour activity, the dynamism of their production, difficulty of their isolation, concentration and identification. It is also important to note that since only individual compounds can be identified by GC techniques, the synergist impact of combined volatiles in foods cannot be identified as their volatile profiles together may be very different from their individual volatile profile. A wide range of extraction and concentration techniques have been applied to aid the understanding of cheese flavour development. However, each technique has an inherent degree of bias and the application of each depends

upon its effectiveness, cost, degree of automation and practicality. Some techniques extract all the volatiles in a cheese sample, but this is unlikely to be a true reflection of the actual volatiles released during mastication in the mouth. Thus, at present no single technique will provide the true aromatic profile of cheese, the choice depends upon a compromise between what is affordable/available and what works best. Some useful reviews and manuscripts on different extraction and concentration techniques are: Sides et al. (2000), Harmon (2002), Werkhoff et al. (2002), Qian et al. (2007), Lancas et al. (2009), Spietelun et al. (2010) and Croissant et al. (2011).

13.5.2 Distillation/Solvent Extraction Techniques

The most basic distillation methods simply involve placing an aqueous cheese/water homogeneous slurry in a rotary flask and heating; the distillate is collected. However, as the distillate contains a high level of water and as the volatiles are significantly diluted, they must be concentrated subsequently and the water removed. This is usually achieved by solvent extraction prior to GC analysis. This method can be problematic for complex samples, such as cheese, as the elevated temperature applied during distillation may lead to artefact formation and the analytes extracted are dependent upon the pH of the cheese slurry and the solvent used. It is also common practice to separate the distillate into acidic, basic and neutral fractions. This is done to aid separation of chemical classes in order to make the volatile profiles generated by GC techniques less complicated and thus easier to interpret. Each phase is concentrated separately prior to injection onto a GC.

As most aromatic compounds are lipophilic, solvent extraction is used to separate specific aromatic classes of compounds. However, as all lipids are extracted further purification techniques are required. In addition, analytes are separated depending upon polarity and thus a degree of discrimination exists. Solvent purity is also very important as any impurities can interfere with analytes of interest and may be identified incorrectly as part of the volatile profile of the cheese sample. The efficiency of extraction is aided by grating the cheese and agitating during the extraction process. The most common solvent used is diethyl ether, possibly because it is used widely in the extraction of fat from dairy products, although it is not as effective for extracting a wide range of volatile compounds as it is non-polar. Other solvents have been shown to be more effective for certain chemical classes, but considerations such as toxicity, flammability and cost need to be considered (Alewijn 2006). Figure 13.4 is a schematic of a basic solvent extraction apparatus. In addition, high concentrations of solvents in a sample introduced to a GC can mask very volatile aromatics that elute with the solvent. This can be alleviated to some degree by using a cryogenic cooling trap which helps separate very volatile components. With solvent extraction, it is often necessary to concentrate solvent extracts prior to injection into the GC. This is achieved typically by vacuum distillation. Techniques such as high vacuum transfer have been used, which use a temperature gradient to volatilize the sample, which is subsequently transferred to a

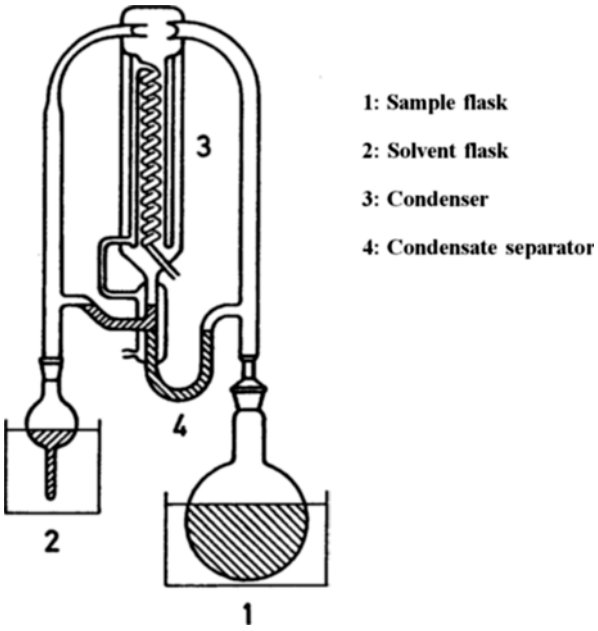


Fig. 13.4 1: Sample flask. 2: Solvent flask. 3: Condenser. 4: Condensate separator. Solvent extraction apparatus. Reproduced with permission, from Chap. 5 Aroma Compounds in Food Chemistry, eds, Belitz, HD., Grosh, W & Schieberle, P, 4th Edition, Published by Springer-Verlag, Berlin, 2009

collecting flask. Sometimes, it is necessary to fractionate the extract further to separate chemical classes in order to ease subsequent separation and identification by GC. Another solvent extraction technique is simultaneous distillation-extraction (SDE), which simultaneously distils and extracts volatiles in the solvent (Fig. 13.5). This technique has the advantage that a distillation step separates the volatiles from the non-volatiles in a continuous process. The condensing water vapour is extracted by the condensing vapour of a low boiling point solvent, which results in a high extraction rate. The condensed solvent vapours are separated by density. It is relatively quick and easy to perform and results in an aroma extract that can be injected directly into a GC. However, it is possible to induce artefacts during heating, cause decomposition of thermo-labile compounds or lose very volatile components during the process. Distilling under vacuum enables a lower temperature to be used which can reduce risks outlined above, but as only steam-distillable volatiles are extracted, polar compounds are recovered poorly.

Solvent Assisted Flavour Extraction (SAFE) is a separate technique that allows the analysis of samples without extensive preparation, with or without the use of solvents (Fig. 13.6). It is a robust method that uses a vacuum to isolate flavour compounds from the cheese. This technique is especially suitable for GC—olfactometry (GC-O) as it removes volatiles at a low temperature under

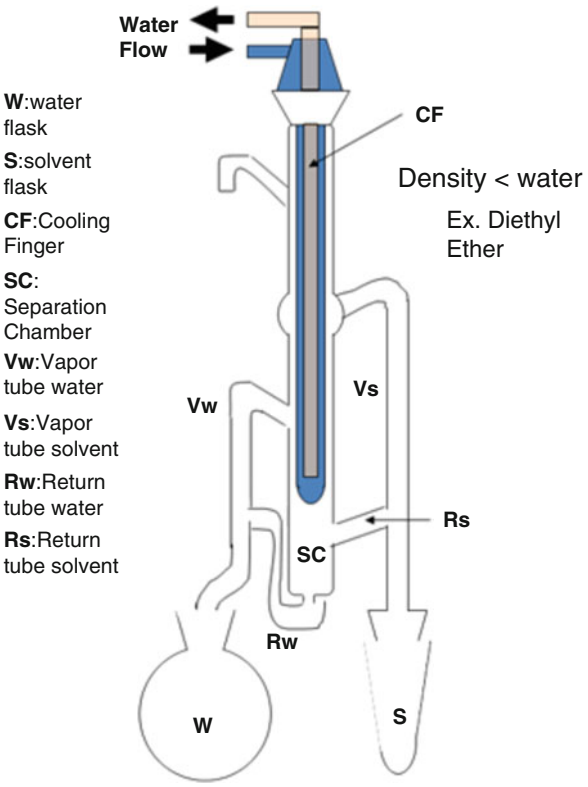


Fig. 13.5 Simultaneous distillation-extraction. Reproduced from the MSc Thesis of Drew Watson "Optimization of recovery of key flavour compounds from full-fat and reduced-fat Cheddar cheeses" 2009 from North Carolina State Theses and Dissertations at the North Carolina State University Institutional Repository—http://www.lib.ncsu.edu/resolver/1890.16/6943

vacuum. The equipment consists of a distillation unit in combination with a high vacuum pump and two cooling traps using liquid nitrogen. A vacuum is pulled and the pressure reduced. As solvent extract from the cheese sample is fed into a heated round-bottom flask the volatiles are pulled through a cooling arm and into an initial trap and cooled by liquid nitrogen which condenses the solvent. A second trap in series after the first trap collects impurities, such as water. The condensed solvent containing the volatiles from the first trap is concentrated, typically by purging with nitrogen prior to injection onto a GC. An advantage of using diethyl ether to extract cheese volatiles in this apparatus is that the non-polar nature of the diethyl ether also solvates any lipid present. The technique distils the diethyl ether extract phase from the lipid phase yielding the volatile extract free of non-volatile material that can be concentrated further and analysed. A disadvantage of solvent extraction techniques is the removal of any associated matrix effect (due to the composition of the cheese), and thus the technique favours the extraction of heavier volatiles, rather than volatiles released during mastication of the cheese in the mouth.

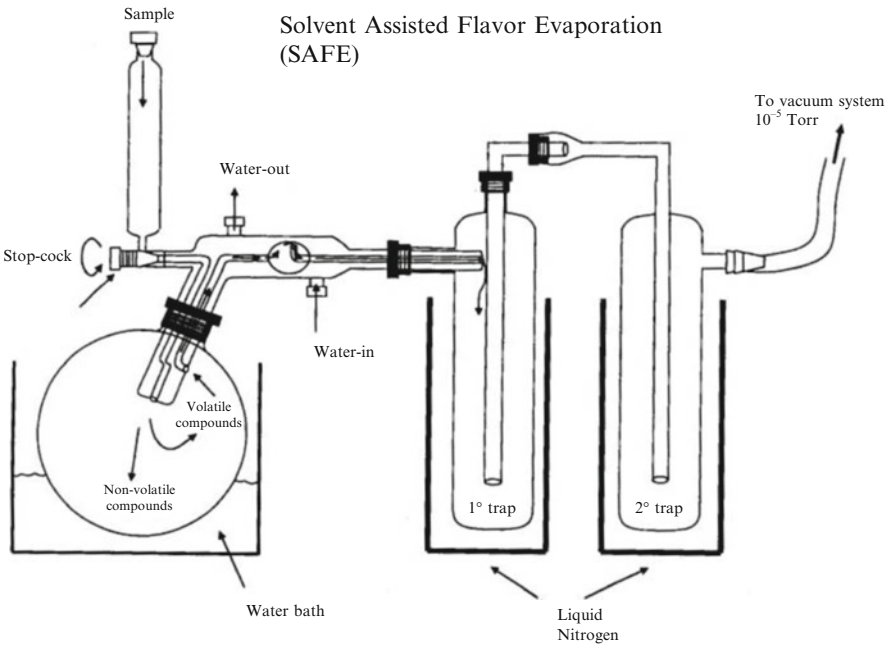


Fig. 13.6 Solvent assisted flavour evaporation. Reproduced from the MSc Thesis of Drew Watson "Optimization of recovery of key flavour compounds from full-fat and reduced-fat Cheddar cheeses" 2009 from North Carolina State Theses and Dissertations at the North Carolina State University Institutional Repository—http://www.lib.ncsu.edu/resolver/1890.16/6943

13.5.3 Passive and Dynamic Extraction Techniques

13.5.3.1 Passive Techniques

Passive static techniques involve exposing the sample or headspace to a sorbent in one step. Equilibrium is established and the volatiles are subsequently desorbed and then analysed by GC. The technique is simple and automatable but the sorbents are biased for certain volatiles based mainly on polarity. The technique works best if the compounds of interest are at a relatively high concentration in relation all other volatiles present. The most routinely used static techniques in cheese research are solid phase micro-extraction (SPME) and stir bar sorptive extraction (SBSE). Both techniques are automatable and do not require the use of solvents.

SPME involves exposure of a fibre-coated needle directly into a liquid or to the headspace of a sample as shown in Fig. 13.7. Headspace SPME is best used with a robotic autosampler to minimize operator error/deviation which can be significant with manual injection. Automation also has the advantage of increasing the longevity of the fibres which are fragile and expensive. The method uses a silica fibre coated with different adsorbent or absorbent materials that are exposed to the headspace of cheese (grated or as a slurry) in a specialised headspace vial under controlled

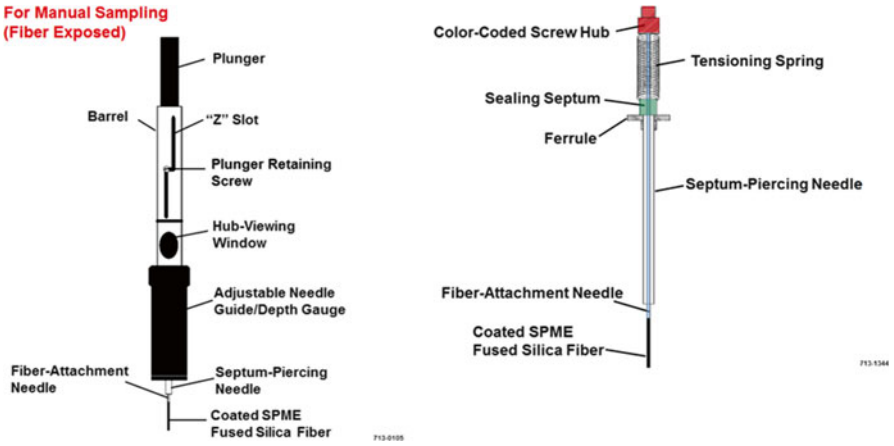


Fig. 13.7 Solid phase micro-extraction (manual assembly). Reproduced with permission of Sigma-Aldrich Corporation, St Louis, USA

conditions (temperature, agitation and time). Once equilibrium is reached, the fibre is desorbed in the GC inlet at a high temperature and introduced to the column for separation and subsequently detected, typically by either MS or flame ionization detection. A small number of different fibres are available commercially which are either single polymer coatings or mixed film coatings with different absorption and/or adsorption properties. Optimal conditions in terms of fibre selection, pre-conditioning, exposure and desorption times/temperatures must be determined for each sample type. The desorption temperature typically exceeds 250 °C and thus may introduce thermal artefacts and be problematic for sulphur compounds due to their inherent instability and reactive nature. A major limitation of this technique is the relatively low volume of sorbent available on the fibre. A direct SPME method can be used for liquid samples, where the fibre is immersed in the liquid sample and subsequently desorbed in a GC inlet. Efficient agitation is required to counteract the low diffusion coefficients of analytes in liquid to improve extraction. The fibres require cleaning between injections after either headspace or direct SPME and this is achieved by baking out the fibre at a high temperature, which varies according to the fibre type. This can be achieved within the GC injector or in a specific bake-out station as part of the robotic handling system on the GC instrument.

SBSE is a similar technique to SPME in principle, but different in application. The technique is based on sorption instead of adsorption. The principle involves a magnetic stirring bar in a glass jacket coated with a sorbent layer, Fig. 13.8. The magnetic stirring bar is usually in direct contact with an aqueous cheese slurry. Once the volatiles are absorbed, they are subsequently thermally desorbed. It is also possible to do headspace SBSE. Like SPME, optimal conditions (time and temperature) must be determined for each sample type. The fact that sorption is a weaker interaction than adsorption, a lower desorption temperature can be used, reducing the risk of the formation of thermal artefacts. At present, only polydimethylsiloxane

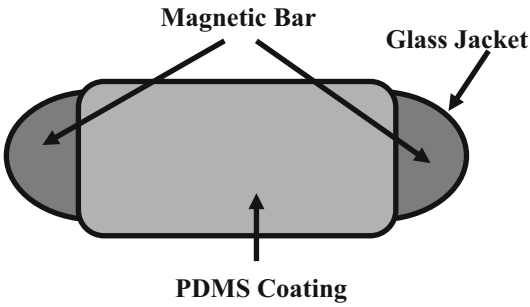


Fig. 13.8 Stir bar sorptive extraction magnetic bar

(PDMS) coatings are available, but the capacity available is significantly greater than on a SPME fibre which increases the sensitivity of the technique. Complete robotic automated extraction and injection systems are also available for GC instruments for this technique.

13.5.3.2 Dynamic Techniques

Dynamic methods continually absorb or cold trap volatiles and therefore a true equilibrium is never reached. These methods can increase volatile concentration and thus the overall sensitivity, however they can be time-consuming and potentially less reproducible.

Purge and Trap (P&T) is a dynamic technique widely used mainly because of its effectiveness in concentrating a relatively large sample (Fig. 13.9). This technique is best suited to the isolation of analytes with the greatest vapour pressure. The cheese sample is typically dispersed in water or NaCl solution or finely grated. The sample is placed in a clean glass vessel, and heated and purged with an inert gas, such as nitrogen or helium, in order to generate a gas stream containing the volatiles. These volatiles are subsequently trapped on an adsorbent. Tenax is used widely, as it retains little water (low affinity for polar compounds), although Tenax and graphitized carbon mixes are useful as they have increased adsorption capacity over Tenax alone. Conditions (sample volume/time/temperature/gas flow rate) need to be optimised for each sample type. The fact that the technique is dynamic ensures that more volatiles are flushed from the sample and thus concentrated onto the sorbent. However, the technique has some disadvantages, mainly the potential for water to be retained in the trap and subsequently injected onto the GC; the system includes a lot of connections and therefore can be prone to leaks, sample foaming can be an issue if the sample is dispersed as a solution. Water can be removed by venting using a dry purge gas as a pre-step but it also increases the risk of losing volatiles. Cryogenics are often required to aid trapping but this increases costs and can increase problems with water retention, blockages, column damage and MS damage.

Solid phase dynamic extraction (SPDE) is a relatively new extraction procedure that is effectively a dynamic form of SBSE and has some similarities to SPME. The procedure uses a specially designed needle which is coated on the inside with a

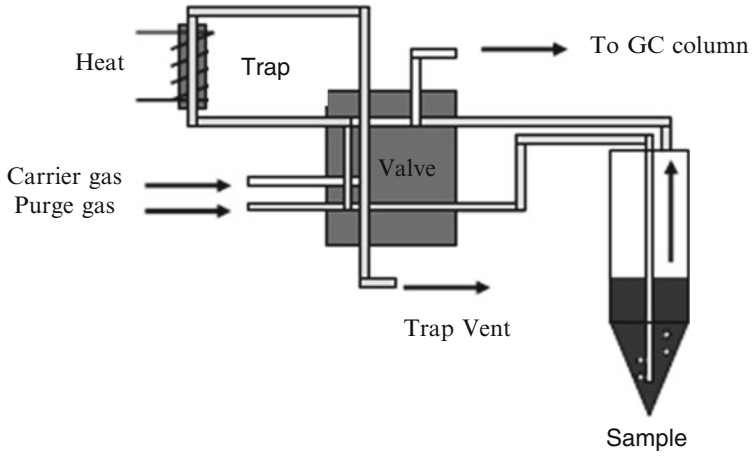


Fig. 13.9 Schematic of purge and trap system

polymer/sorbent. The needle, assembled in a gas-tight syringe, is exposed to the headspace of a cheese sample in a similar manner to SPME and the efficiency of adsorption is improved by repeated plunging of the gas-tight syringe which forces the headspace on the sorbent on the needle. Adsorption conditions need to be optimised for each sample, after which the needle is desorbed in the GC inlet and cleaned either in the inlet or in a specialised bake-out station on the autosampler. This is an automatic technique best performed using a robotic autosampler system. The system works best with cryogenic cooling to focus volatile peaks on the column front. The advantage of this system is the greater capacity of the adsorbent material, the dynamic nature and the greater range of polymer coatings available.

In Tube Extraction (ITEX) is another dynamic automatic technique that uses a robotic autosampler and is similar to P&T (Fig. 13.10). This technique uses a microtrap filled with an adsorbent material which is placed between a heated headspace syringe and a syringe needle. The cheese sample is placed in a headspace vial in a similar manner to either SPME or SPDE and heated for a fixed period under controlled conditions. The headspace volume is pumped by repeated syringe strokes and concentrated on the microtrap. A range of microtrap adsorbents is available, such as Tenax TA and graphitized carbon. Optimal extraction conditions need to be established for each sample type. The microtrap is flash-heated and the volatiles passed into the GC inlet. The microtrap is cleaned by flushing with inert gas and heated between samples. The main difference between this technique and other automatable dynamic techniques is that desorption is independent of the GC inlet and is thus temperature gradient free, nor is the needle used as a support for the adsorbent. The advantages are its automation, relatively large volume capacity, range of adsorbents available and the independent desorption factor.

Another widely used dynamic technique is direct thermal desorption (TD). A range of manufacturers offer specialised automatable equipment for TD which is directly connected to the column or via the GC injection system. Sample size varies consider-

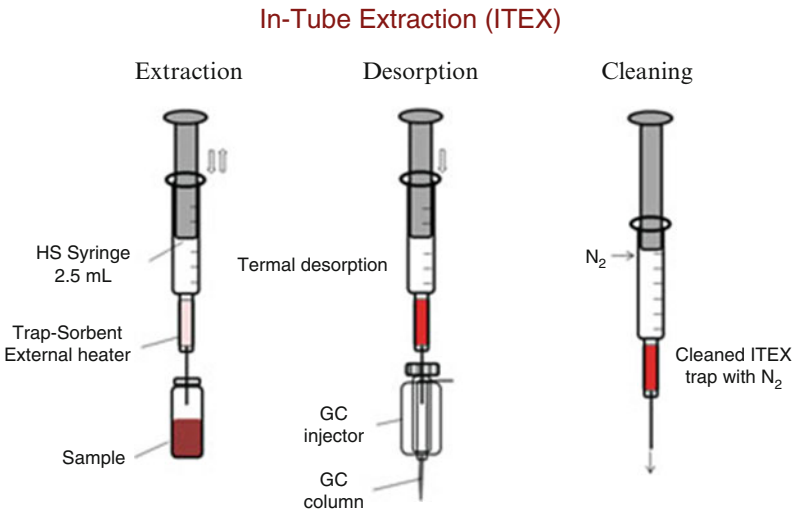


Fig. 13.10 In tube extraction (ITEX). Reproduced with permission from CTC Analytics AG, Zwigen, Switzerland

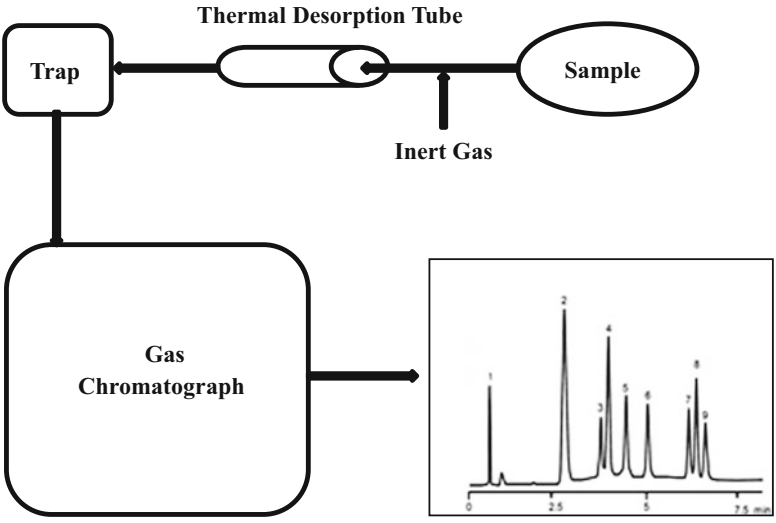


Fig. 13.11 Thermal desorption

ably depending on the choice of TD equipment, but the principles of operation are similar. Volatiles are purged from a heated cheese sample under controlled conditions and swept into an adsorbent trapping material using an inert gas such as helium or nitrogen (Fig. 13.11). A wide range of trapping materials is available which can be used to target specific chemical classes. Carrier gas is passed through the desorption tube for an initial purge. The traps are desorbed but often the volatiles are cryogenically focussed

(liquid nitrogen, liquid carbon dioxide or thermoelectrically/peltier) in a cooled trap prior to injection onto the GC in order to improve resolution. Parameters can be altered automatically in TD to optimise sensitivity and improve extraction and isolation of key volatiles which is increasing the popularity of the technique.

13.5.4 Gas Chromatography Techniques for Aroma Analysis

GC is the most useful method for the analysis of volatile compounds. Initially, a sample is injected into the inlet and volatilised in the injector and swept onto a column by a carrier gas. Individual volatile compounds are separated by their vapour pressure, interaction (or lack of) with the column stationary phase and usually by a temperature gradient applied to the column. Compounds are then identified using a specific detector. The key elements of the GC system that can be altered to optimise separation are inlet liners, type of injection (split, splitless, on-column), type of column (phase, length, diameter, phase thickness), carrier gas (flow rate, pressure) and oven temperature (use of cyro traps, ramping rates). Choice of column is very influential. The most common phases for volatile separation are high polarity polyethylene glycol (Wax) or nitroterephthalic acid modified polyethylene glycol columns which are good for the separation of polar volatiles, such as acids and some alcohols, or low polarity acid-modified 5 %-phenyl-95 %-dimethylpholsiloxane which are good for the separation of less-polar volatile compounds, such as aldehydes, lactones, ketones and esters.

A range of detectors is commercially available but the following are the most widely used for volatile analysis. MS detectors are the most commonly used for cheese volatile research. MS detectors operate by using ionization energy (electron or chemical) to fragment molecules exiting a GC column in a gas stream. The fragments are pre-selected based on their mass to charge ratio (m/z) and detected. The ion profiles generated are compared to an on-line reference mass spectral library which in conjunction with retention indices of standards are used to positively identify the compound. Initially, ion-trap mass spectrometers were used but have in general been replaced by quadrupole mass spectrometers. This is partially due to more robust instruments, reduced costs, greater sensitivity and scan rates and the fact that spectral data generated by ion-traps do not match spectral data available in on-line libraries. These instruments work in both full scan (SCAN) mode or in selective ion monitoring (SIM) mode. SCAN is used to identify unknowns based on their fragmentation pattern and SIM is used to selectively target specific ions of interest. Triple quadrupole mass spectrometers are now also widely available, which are effectively two single quadrupole analysers on either side of a collision cell, which enable further mass selection and fragmentation, which can be really useful for confirmatory analysis. However, their use in the analysis of cheese volatiles is limited by the low mass of most volatiles of interest, where further fragmentation often results in the formation of less unique base fragmentation patterns. Recently, Time of Flight (TOF) detectors have been used in cheese research as manufactures create more competitively priced bench-top units.

These mass analyzers have very high mass acquisition rates and generate more data. These instruments are very useful for two-dimensional (2D) GC×GC analysis due to their higher scan rates. As many food matrices, including cheese, are very complex, it is difficult, if not impossible, to fully separate all volatile components in a single chromatographic elution. Therefore, 2D GC×GC was developed where two separate columns of different polarity and length are used to increase analyte resolution. The principle is quite simple in that everything going through the first column can be transferred to the second column (comprehensive 2D GC) and detected, or individual peaks or groups of peaks can be selectively transferred to the second column (heart-cutting). The flows between and from the columns can be split into different detectors to maximise the amount of information generated from a single sample run. The advent of electronic flow controllers and pressure regulation in modern GC equipment has made this possible. With comprehensive 2D GC, components can be trapped cryogenically between the columns to improve peak focussing. The acquisition rate from the second column is critical in order to gain sufficient data from two very similar components, which is why TOF detectors are very useful for this type of analysis (GCxGC-TOF-MS). Data are actually plotted three-dimensionally (3D) showing the retention time on the first column versus the retention time of second column and the third dimension is the height or the total ion current (in the case of TOF). This technique has great potential in volatile research on cheese as it is possible to resolve complicated matrices simultaneously in a single chromatographic run. This technique generates a huge amount of data and requires suitable software in order to easily manage the data and generate useful information. A key aspect of flavour chemistry research using MS is data interpretation and processing. It is essential to utilise deconvolution algorithms in order to improve and speed up data processing. A number of deconvulation software packages are available for this purpose.

A flame ionization detector is a non-selective detector which is excellent for organic compounds and has a wide liner response. It is by far the most widely used detector with GC. Its response is proportional to the number of carbon-hydrogen bonds present in a molecule. It is particularly useful for detection of acids, aldehydes, ketones, alcohols and terepenes and easy to obtain quantifiable information.

Pulsed flame photometric detectors are highly selective detectors used mainly for the quantification of sulphur or phosphorus compounds. It is a highly sensitive, robust, relatively economical detector. Sulphur compounds are very important in cheese aroma as they are positively associated with cheese quality at low concentrations, but negatively associated with quality at high concentrations. They are notoriously difficult to detect due to the fact that they degrade easily and are highly reactive. The sulphur compounds of most interest are hydrogen sulphide, methanethiol, dimethyl sulphide, dimethyl disulphide, dimethyl trisulphide, methyl thiols, dimethylsulfoxide, dimethylsulfone, carbonylsulphide and carbon disulphide.

In order to interpret volatile analysis in a user-friendly format, it is also necessary to use multivariate statistical methods. Principal component analysis is widely used as it graphically highlights discrimination between samples based on their volatile profiles and can also be used to associate volatile data with descriptive sensory analysis data (Fig. 13.3).

13.5.5 Gas Chromatography-Olfactometry

The human nose as a detector in chromatography has become a basic tool for sensory-oriented flavour analysis. In this technique, an experienced assessor inhales analytes from the effluent of the column using a specialised transfer line. This is usually achieved after the outflow from the column has been split, where a portion passes into a detector (usually MS detector or a flame ionization detector) at the same time as the other gas stream passes through a specially designed olfactory port. A key aspect is ensuring that outflows after the split are synchronised exactly. This has become easier with the advent of electronic flow controllers, especially with MS detection as the vacuum can influence gas flow (Fig. 13.12). GC-O can be used to help to identify an off-odour or a character-impact compound and is often used in conjunction with time intensity techniques or dilution techniques to determine the perceived odour intensity of an analyte. Delahunty et al. (2006) gave a very comprehensive overview of GC-O techniques, equipment, environment and factors impacting on odour perception and on the assessor. A simple approach is "detection frequency" which can use a relatively small untrained panel who evaluate the same extract. Those compounds which are detected most frequently are assumed to be the most important. The inclusion of a measure of the duration of the aroma can then be used to compare the relative importance of each detected peak to each other, as those detected most frequently for the longest duration are assumed to have the greatest relative importance. Another approach is "dilution to threshold" which is used to quantify odour potency. Two common dilution methods are aroma extract dilution analysis (AEDA) and combined hedonic aroma response methodology (CHARM) (Grosch 1993; D'Acampora Zellner et al. 2010). Both methods generally involve a trained panel. The AEDA technique calculates the dilution factor of each compound as the maximum dilution in which the compound can still been perceived by the assessor (Mariaca and Bosset 1997). The assessor usually also describes the aroma/odour, a significant number of injections of diluted samples may be required before

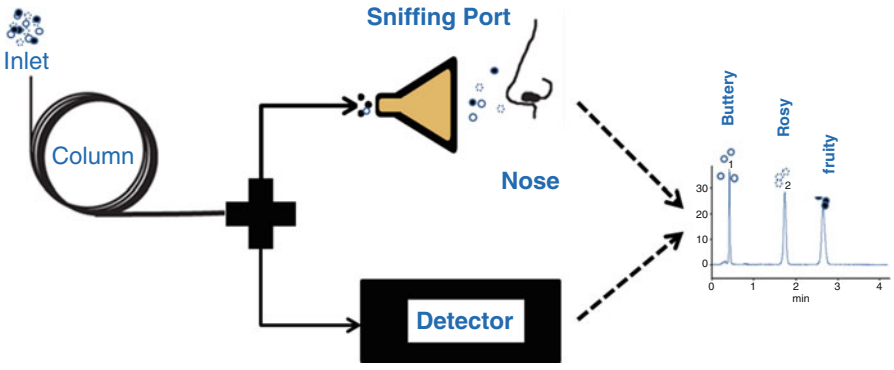


Fig. 13.12 Gas chromatography-olfactometry

the assessor can no longer detect the odour. With the CHARM method, serial dilutions of the sample are injected into the GC, duration and intensity, and numbers of panellists detecting the odour are then used to generate aromagrams that correspond to the intensity of perception of each odour detected. These techniques can also be carried out with descriptive sensory analysis in order to determine relationships between odour-active compounds and individual sensory descriptors. Another approach is "direct intensity" where trained assessors use a scale to quantify the perceived intensity. The most common method is called OSME (Greek for odour) where the intensity and duration of a perceived aroma are noted by the assessor.

13.6 Aroma Compounds in Specific Cheeses

The volatile compounds that predominate are mainly responsible for a specific cheese aroma, but this cannot be taken for granted as the odour activity of a volatile is also an important factor. Therefore, an analyte present at a very high concentration may have less influence on aroma perception than a very odour-active analyte at a very low concentration. This is also one of the reasons why in GC-O analysis an aroma can be perceived by the assessor but no corresponding peak is evident on the chromatogram. Other issues relate to the extraction/concentration techniques, polarity of the column and sensitivity of the detector used.

In general, a number of key aromatic compounds are derived from the metabolism of carbohydrates (lactose and citrate) by LAB resulting in acetate, diacetyl (2,3-butanedione), acetaldehyde, acetoin (3-hydroxy 2-butanone), ethanol, 2,3-butanediol, propionate and lactate. Citrate-positive heterofermentative cultures are used only in certain cheese varieties but contribute to a much greater diversity of aroma-active compounds than homofermentative cultures that only use lactose.

Short-chain free fatty acids (butyrate, caproate, caprylate and caprate) are the products of lipolysis and are both volatile and odour-active. These acids are present in the milk but significant portions are lost during whey removal in the production of most cheese varieties as they are water-soluble. However, esterases from starter cultures (Hickey et al. 2006) produce significant levels of these short-chain fatty acids during ripening as well as in cheeses containing moulds, yeasts and pregastric esterases. Both acetate and propionate, as mentioned, are also products of lactose metabolism. Some other short-chain acids, such as isovalerate (3-methyl butanoate), isobutyrate (2-methyl proponate), n-butyrate, proponate, n-valerate are also products of amino acid metabolism. Short-chain fatty acids with less than 12 carbons can be oxidised to β-ketoacids and decarboxylated to methyl ketones with one less carbon. Methyl ketones are volatile odour-active and predominate in some cheese varieties. These methyl ketones can be reduced subsequently to their corresponding secondary alcohol, which are also volatile and odour-active. Free fatty acids can also react with alcohol groups to form esters which are, again, volatile and odour-active and are important compounds influencing the final flavour of many cheeses. Esters can also be produced by alcoholysis via a reaction of acylglycerols with alcohols or from the transfer of fatty acid groups of fatty acyl CoAs (derived from the metabolism of fatty

acids, amino acids or carbohydrates) to alcohols (Liu et al. 2004). Water activity and the availability of alcohols are the rate-limiting factors in this reaction. Free fatty acids can also react with methanethiol to produce S-thioesters (S-methyl thioacetate, S-methyl thiobutyrate and S-methyl thiopropionate), which are present in some cheese varieties at low concentrations, but are very odour-active. Another group of volatiles that are odour-active and are present in many cheese varieties are lactones. Lactones are cyclic compounds, the precursors of which are hydroxylated fatty acids. These hydroxylated fatty acids are esterified in milk glycerides and are released as free hydroxylated fatty acids (not odour-active) by heating (pasteurisation) or by the action of lipases. These hydroxylated free fatty acids are then converted to lactones by a one-step intermolecular transesterification reaction (Alewijn 2006). Hydroxyl fatty acids are also produced by lipooxygenases or hydratases by catabolism of unsaturated fatty acids by microorganisms. The most common lactones present in cheese are δ-decalactone, δ-dodecalactone, γ-decalactone, γ-dodecalactone. δ-octalactone and δ-octadecalactone.

Many important cheese aroma compounds are formed from the metabolism of amino acids. The metabolism of branched chain amino acids (leucine, isoleucine and valine) can produce volatile acids (acetate, propionate, n-caproate, isobutyrate and isovalerate), but also aldehydes (2-methyl butanal, 2-methyl propanal and 3-methyl butanal), and secondary alcohols (2-methyl butanol, 2-methyl propanol, 3-methyl butanol and 3-methyl propanol) that can be esterified with fatty acids to produce esters (ethyl-3-methylbutanoate, ethyl isobutanoate). In the metabolism of aromatic amino acids (phenylalanine, tyrosine, tryptophan and histidine), which have an aromatic ring structure, the amino carbon is transaminated to pyruvate which is subse quently reduced to aldehydes (benzylaldehyde, phenylacetaldehyde, indole-3-acetaldehyde), aromatic acids (benzoic acid, phenylacetic acid, indole-3-acetic acid, ρ-hydroxy phenyl acetic acid, ρ-hydroxy phenyl lactic acid) and alcohols phenylmethanol, phenylethanol). These alcohols can be esterified subsequently to esters (phenylethyl acetate, ethyl benzoate). Some of the aromatic amino acids can be metabolised further or catabolised to odour-active compounds such as ρ-cresol, indole and skatole that have a negative aroma perception. Tryptophan, tyrosine, phenylalanine and hisidine can also be decarboxylated to their corresponding amines (tryptamine, tyramine, 2-phenylethylamine and histamine) which are also odour-active. Metabolism of the sulphur amino acids, methionine and cysteine, results in the formation of propionate, methanethiol and methional *via* aminotransferase, decarboxylase and lyase activity. Methanethiol can be further oxidised to di-methyl sulphide, di-methyl di-sulphide and di-methyl tri-sulphide. These sulphur compounds react with acids, aldehydes and ketones to produce thio-esters and thio-carbonyls. Metabolism of methionine produces, methanethiol, phenol and indole, all of which are highly odour-active. Catabolism of cysteine is mainly responsible for hydrogen sulphide which can be an important aroma compound in some cheese varieties. It also appears that serine can act as a precursor for methionine formation by LAB in cheese. Threonine can be deaminated to produce α-ketobutyrate and propionate and can be metabolised to acetate, n-butyrate and n-caproate. Threonine is important for cellular energy and pH regulation and may also serve as a precursor for some branched chain free fatty acid formation in cheese. Table 13.4

Table 13.4 Major volatile odour-active compounds in some cheese varieties

Volatile compounds	Odour descriptor	Cheese types
Acids		
Acetic	Vinegar, peppers, green, fruity floral, sour	1, 3, 4
Butyric (n-butyric acid)	Sweaty, buffer, cheese, strong, acid, facel, rancid, dirty sock	1, 2, 3, 6
Propionic	Pungent, sour milk, cheese, gas, burnt, cloves, fruity	5
Capric	Stale, butter, sour, fruit, grassy, fatty, cheese, aged Cheddar	1, 2
Caproic	Sweaty, cheesey, sharp, goaty, bad breath	1, 2, 3
Caprylic	Cheesey, rancid, pungent, sweat	2, 3
Isovaleric	Cheesey, sweaty, socks, rancid, rotten fruit	2
Palmitic	Waxy, lard, tallow	2
Esters		
Ethyl acetate	Solvent, fruity, pineapple	3, 5
Ethyl butyrate	Fruity, green, apple, banana	1, 2, 3, 5
Ethyl caproate	Pineapple, sweet, fruity, banana	1, 3, 6
Ethyl octanoate	Pear, apricot, sweet, fruity, banana, pineapple	3
Ethyl phenyl acetate	Floral, rose, lily-jasmine, honey	4
Ketones		
Acetone	Sweet, fruity, ethereal, wood pulp, hay	5
Acetoin	Buttery, sour milk	1, 2
Butanone	Buttery, sour milk	2,
2-Butanone	Sweet, ethereal, slighty nauseating	5
1-Octan-3-one	Mushroom-like	1, 4
2-Undecanone	Floral, fruity, green, musty, tallow	2, 4, 6
2-Nonanone	Malty, rotten fruit, hot milk, green, earthy	2, 3, 4, 5, 6
2-Tridecanone	Goaty, citrus	2, 5
2-Pentanone	Orange peel, sweet, fruity	2, 3, 4, 5
2-Heptanone	Blue cheese, spicy, Roquefort, fruity	1, 4, 5
Diacetyl	Strong buttery, caramel, toffee	
Aldehydes		
Phenylacetaldehyde	Honey-like, rosey, violet-like, styrene	1
3-Methyl butanal	Malty, powerful, cheese, green, dark chocolate	1, 2, 3, 4, 5, 6
Alcohols		
Ethanol	Dry, dust, alcohol	5
1-Butanol	Banana like, wine-like, fusel oil	3
1-Propanol	Sweet, wine-like	4, 5
2-Propanol	Fruity, ethereal, wine-like	5
2-Butanol	Sweet, fruity, fusel oil, wine-like	5
2-Pentanol	Green, alcoholic, fruity, fresh	3
2-Heptanol	Fruity, earthy, green, sweetish, dry, dusty carpet	4
2-Honanol	Fatty green	4
1-Octen-3-ol	Mushroom-like, mouldy, earthy	4

(continued)

Table 13.4 (continued)

Volatile compounds	Odour descriptor	Cheese types
Phenylethanol	Unclean, rose, violet-like, honey, floral	4, 6
2-Methylbutanol	Malty, wine, onion	5
3-Methylbutanol	Fresh cheese, breathtaking, alcoholic, fruity, grainy, solvent-like	2
Sulphurs		
Methional	Baked potato	1, 3, 4
Methanethiol	Rotting cabbage, cheese, vegetative, sulphur	4
Di-methyl sulphide	Sulfur, cabbage-like, pomegranate	4
Di-methyl-disulphide	Green, sour, onion	4
Di-methyl-trisulphide	Vegetable-like, sulfurous, garlic, putrid, cabbage-like	1, 2, 3, 4
Lactones		
δ-Decalactone	Coconut-like, peachy, creamy, milk fat	1, 2, 4
δ-Dodecalactone	Cheesey, coconut, sweet, soapy, buttery, peach, milk fat	2
γ-Dodecalactone	Peach, almonds, herbs, lilacs, fruit, toffee	2
Pyrzaines		
Tetra-methyl pyrazine	Baked, beans, raw potato	3

1 Cheddar, *2* Gouda, *3* Parmesan, *4* Cambembert, *5* Swiss, *6* Blue

lists the key volatile impact compounds associated some of the major cheese varieties based on a wide range of international studies. The odour descriptive of each volatile is included. The following reviews of volatile cheese flavour production are recommended (Urbach 1997; McSweeney and Sousa 2000; Yvon and Rijnen 2001; Singh et al. 2003; Marilley and Casey 2004; Smit et al. 2005). It is interesting to note the importance of acids in Cheddar, Gouda and Parmesan cheese, with propionate being important in Swiss cheese and butyrate being important in Blue cheese. Alcohols are very prominent in Swiss and Camembert, as are sulphur compounds in Camembert cheese. 3-Methyl butanal is a key odour compound in all of the above cheeses. Ketones are widespread in Gouda and Swiss cheeses, as are lactones in Gouda and esters in Parmesan cheese. Identification of the key volatile compounds associated with any cheese variety is important and many studies have been carried out on different cheese varieties. However, it is also worth highlighting that all extraction/concentration, separation and identification techniques have inherent bias and it is quite likely that not all the key aroma compounds have been identified to date mainly due to limitations in detector sensitivity.

13.7 Conclusion

Significant advances are been made about the volatile and non-volatile compounds that are involved in cheese flavour perception and the many external factors that influence their production. This has been made possible by advances in analytical

capability and in the use of statistical chemometric approaches that can manage diverse data streams in order to elucidate relevant aspects associated with cheese flavour formation. Information on the biochemical reactions that create cheese flavour and the impact of the cheese environment on these enzymatic and metabolic reactions plus the inherent capabilities of lactic acid bacteria, yeasts and moulds continues to expand, enabling cheese manufacturers to create more consistent high-quality cheese for targeted markets.

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Chapter 14
Cheese: Structure, Rheology and Texture

Summary The rheology of cheese characterizes its deformation behaviour when subjected to stress or strain. Based on stress/strain behaviour, materials may be generally classified as ideal elastic solids, ideal viscous (Newtonian) liquids, or viscoelastic. Cheeses, like most other solid- and semi-solid foods that contain moisture and solids such as protein, fat and/or carbohydrate, exhibit the characteristics of both an elastic solid and a viscous fluid, and are thus termed viscoelastic. The rheological behaviour of cheese can be measured by an array of tests. Some tests, for example creep and low strain oscillation rheometry, involve application of a low strain (e.g., <0.05) within the linear viscoelastic range, the region where stress in the sample is directly proportional to the applied strain and the sample, like an elastic solid, recovers fully from the deformation on removal of the strain (at least after short time scales). Low strain rheology tests give information on fundamental intrinsic rheological quantities such as storage modulus, G′, and elastic creep compliance. These tests are useful also for characterizing the viscoelasticity of cheese, in terms of how close it behaves to a solid or a liquid. In other tests such as large strain compression, large strain torsion and cutting tests, the cheese is subjected to a large strain (e.g. ≫0.5) outside the viscoelastic range. The strains applied in these tests simulate more closely those applied during consumption and commercial size-reduction operations, which include portioning/cutting, shredding or grinding of cheese. Large strain deformation provides information on the fracture properties of the cheese, including the stress required to fracture, the strain at fracture, and the stress required to achieve a given degree of deformation, such as compression.

Cheese texture is a composite sensory attribute resulting from a combination of physical properties that are perceived by the senses of touch (tactile texture), sight (visual texture) and hearing (oral texture) during consumption. The tactile texture component comprises mechanical properties of the cheese that characterize the response of the cheese to the stresses applied during consumption (e.g., cutting with the incisors, compression between the molars, shearing between tongue and cheeks). Texture profile analysis (TPA) measures the response of cheese to large strain compression (e.g. to ~70 % of original height of cheese sample) in two consecutive compressions, referred to as bites. TPA, therefore, simulates the repeated compression of a piece of cheese during mastication and chewing, which involves several compressions between the molar teeth. The stress/time profile generated during the

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double compression enables the determination of an array of mechanical properties such as hardness, cohesiveness, adhesiveness, chewiness and gumminess which correspond to the sensory parameters of the same name.

The rheology and texture of cheese are controlled by its macrostructure, microstructure, composition and internal environment (e.g., pH, temperature). The primary structural component controlling the deformation of cheese for a given stress is the protein network, with the concentration of protein and degree of protein hydrolysis and hydration being critical.

Keywords Rheology • Structure • Texture • Measurement • Factors affecting structure • Rheology and texture

14.1 Introduction to Cheese Rheology

Rheology involves the deformation and flow of materials when subjected to stress or strain. The rheological properties of cheese are those that determine its response to stresses or strains (e.g., compression, shearing or cutting) that are applied during processing (e.g., portioning, slicing, shredding, and grating) and consumption (slicing, spreading, masticating and chewing). These properties include intrinsic characteristics such as elasticity, viscosity and viscoelasticity that are related primarily to the composition, structure and the strength of attractions between the structural elements of the cheese. The rheological characteristics of cheese are measured instrumentally by the application of stress or strain under defined conditions and measuring the response of the cheese. The output variables from these tests (e.g., creep, stress relaxation or compression tests), may include changes in dimensions over time, the ratio of stress-to-strain for certain strain levels and stress or strain required to induce fracture. These parameters enable the determination of quantities such as shear modulus, fracture stress, fracture strain and firmness. In general terms the behaviour of the cheese when subjected to these stresses and strains can be described by terms such as hardness, firmness, springiness, crumbliness or adhesiveness.

The rheological properties of cheese are of considerable importance as they effect its:

(a) handling, portioning and packing characteristics.
(b) texture and eating quality, which are determined by the effort required to masticate the cheese, or alternatively the level of mastication achieved for a given level of chewing (the latter may, in turn, influence its flavour/aroma properties and the suitability of the cheese for different consumer groups such as children or aged people);
(c) physical behaviour (e.g., tendency to fracture, crumble, bend or be flexible) when subjected to different size-reduction methods (grating) or blending/shearing operations such as the preparation of pasteurized processed cheese products and sauces;

(d) use of cheese as an ingredient, as they influence its physical behaviour (e.g., tendency to fracture, crumble, flow) when subjected to different size-reduction methods (such as shredding, grating or shearing) and how the cheese interacts with other ingredients in foods in which cheese is used an ingredient, for example in the preparation of cheese-based sauces and desserts;
(e) ability to retain a given shape when stacked
(f) ability to retain gas and, hence, to form eyes, cracks or to swell.

In short, the rheological properties of cheese are quality attributes that are important to the manufacturer, packager, distributor, retailer, industrial user and consumer.

The rheological properties of cheese are a function of various cheese characteristics (cf. Chap. 18):

- composition (e.g., levels of moisture, fat and protein),
- microstructure, which represents the spatial distribution of the compositional components (casein, minerals, fat, moisture and dissolved solutes such as lactose, lactic acid, soluble salts and peptides) at the micro-sale level (e.g. >25× magnification) and the level of intra- and intermolecular attractions between the components;
- macrostructure which represents the arrangement of, and attractions between, the different macro-components (e.g., curd particles, curd chips, gas pockets, veins and/or rind) and determines the presence of heterogeneities such as curd granule junctions, cracks and fissures;
- the physico-chemical state of its components (e.g., ratio of solid-to-liquid fat as affected by temperature, and degree of protein aggregation or hydration, and extent of protein hydrolysis).

Cheese rheology and the factors that affect it have been reviewed extensively (van Vliet 1991; van Vliet and Peleg 1991; Visser 1991; Prentice et al. 1993; O'Callaghan and Guinee 2004; Guinee 2011, 2016).

14.2 Relationship Between Cheese Rheology and Texture

Cheese texture is a composite sensory attribute resulting from a combination of physical properties that are perceived by the senses of touch (tactile texture), sight (visual texture) and hearing (oral texture) during consumption (Brennan 1988; Delahunty and Drake 2004). In contrast to rheology, which measures the response of the cheese to stress or stain using an instrument, texture is a sensory characterisation of the cheese that is measured by a sensory panel using agreed textural terms or descriptors, with the objective being to acquire an impression of how the cheese is perceived during consumption.

A classification of the sensory textural attributes relating to food in general was first proposed by Szczesniak (1963a) and Brandt et al. (1963). The classification was based on the integration of sensory data from trained consumer panels with

rheological data acquired using an instrument known as the General Foods Texturometer (Rao and Quintero 2005; see Sect. 14.8.2). The Texturometer, designed to imitate the compressing action of molar teeth while masticating food in the mouth, subjected the food sample to two successive compressions, referred to as bites (Szczesniak 1963a; Peleg 1976; Bourne 1978). This system proposed that the textural characteristics of foods, including cheese, may be categorised as mechanical, geometrical and other (Table 14.1). Mechanical properties are manifested by the reaction of food to stresses applied during consumption (e.g., cutting with the incisors, compression between the molars, shearing between tongue and cheeks) and by hearing in the case of fracture. They are in turn divided into five primary parameters and three secondary parameters. Geometrical characteristics are mostly sensed visually but are also partly sensed by touch (Table 14.1). The other characteristics are "mouthfeel" qualities, described subjectively by terms such as hard, soft, firm, springy, crumbly, adhesive, moist or dry. The secondary parameters were considered to be composed of various intensities of hardness and cohesiveness. The geometrical parameters were divided into two classes, i.e., those related to particle size and hardness, and those related to particle shape and orientation.

Subsequently, an alternative method of classification in which the properties of a food contributing to its texture may be divided into two broad groups based on perceptions of the material before and during consumption (Fig. 14.1; Brandt et al. 1963; Sherman 1969). Characteristics contributing to the initial perception of cheese texture, before eating, include its:

Table 14.1 Classification of textural characteristics and their relationship to popular sensory terms

Characteristic	Primary parameters	Secondary parameter	Popular terms
Mechanical	Hardness	–	Soft, firm, hard
	Cohesiveness	Brittleness	Crumbly, crunchy, brittle
		Chewiness	Tender, chewy, tough
		Rheology:classification of textural characteristics Gumminess	Short, mealy, pasty, gummy
	Viscosity	–	Thick, thin
	Springiness	–	Plastic, elastic
	Adhesiveness	–	Sticky, tacky, gooey
Geometrical	Particle size and shape	–	Gritty, grainy, coarse
	Particle shape and orientation	–	Fibrous, cellular, crystalline
Other	Moisture content	–	Dry, moist, wet, juicy
	Fat content	Oiliness	Oily
		Greasiness	Greasy

Adapted from Szczesniak (1963a)

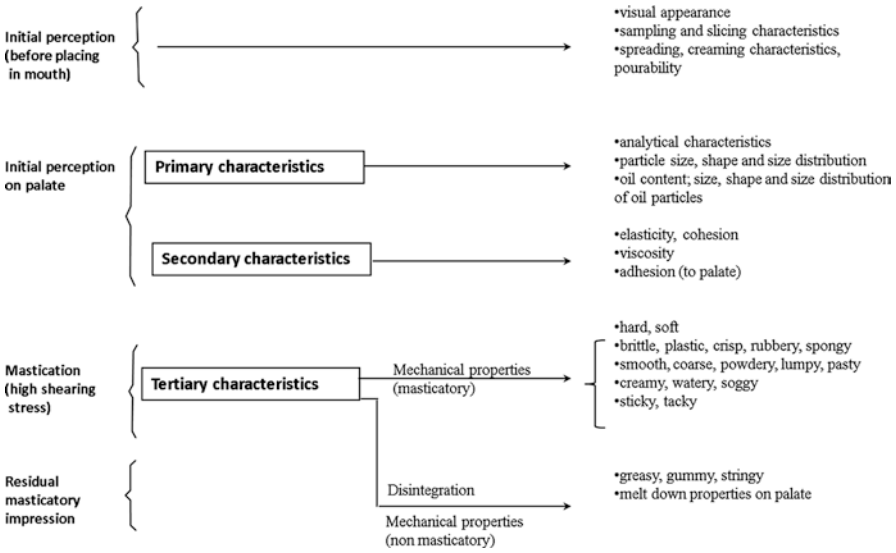


Fig. 14.1 Classification of the textural characteristics of foods. Redrawn from Sherman (1969)

(a) visual appearance (e.g., presence of holes, eyes, granules, surface roughness or smoothness, surface matt or gloss),
(b) sampling (e.g., crumbliness, springiness, stickiness of cheeses) and slicing characteristics,
(c) spreading characteristics (e.g., important with pasteurized processed cheese spreads)

The characteristics contributing to the texture of cheese during eating were classified as primary, secondary (e.g., adhesiveness) or tertiary (e.g., firmness). The primary characteristics, from which all others are derived, include composition, micro- and macro structure, and molecular properties. The secondary and tertiary categories of textural properties include many characteristics which are related directly to the rheological properties as it is subjected to various stresses and strains during eating e.g., hardness, brittleness, adhesiveness (Fig. 14.1). According to this classification, the secondary characteristics are associated with initial perception in the mouth, i.e., upon contact with tongue, palate and teeth prior to mastication. The attributes relating to the secondary characteristics include elasticity (E), viscosity (η) and adhesion to palate, where elasticity is understood as the tendency to recover shape after removal of stress (Sherman 1969).

More detailed considerations of cheese texture include those by Szczesniak (1963a, b, 1998); Brennan (1988); Rosenthal (1999) and Delahunty and Drake (2004).

14.3 Cheese Structure

The structure of cheese may be considered at two levels, namely the microstructure and the macrostructure. The former represents spatial distribution of the compositional components (casein, minerals, fat, moisture and dissolved solutes such as lactose, lactic acid, soluble salts and peptides) at the micro-sale level (e.g. >25× magnification) and the type of intra- and intermolecular attractions between the components. The macrostructure, representing the gross aspects of structure within the moulded cheese, such as curd granule junctions, eyes, and slits/cracks, is observed visually by the unaided eye or at very low levels of magnification (<10× magnification) with the aid of light microscopy. From a practical viewpoint, the microstructure may be defined arbitrarily as the structure within the smallest piece of the cheese (e.g., curd particles), and the macrostructure as an assembly of curd particles (in the case of brine-salted cheeses), or curd chips or pieces (in the case of dry-salted cheeses such as Cheddar and Stilton), that cohere and fuse into a moulded cheese during pressing and ripening (Figs. 14.2, 14.3, and 14.4).

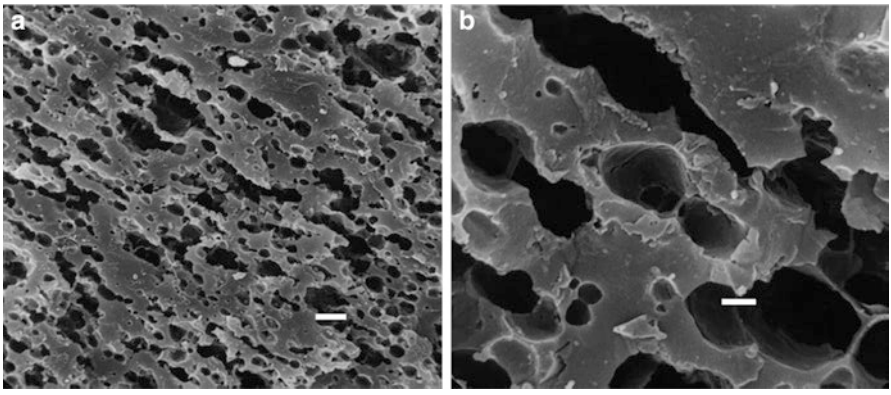


Fig. 14.2 Scanning electron micrographs of Cheddar cheese, showing a continuous *para*-casein network (*grey area*) permeated by holes and fissures corresponding to discreet fat globules and/or pools of clumped/coalesced fat globules (*black area*). Moisture in the cheese is not visible, being entrapped within the *para*-casein network. Bar indicates 5 μm (**a**) or 1 μm (**b**). Modified from Guinee et al. (1998)

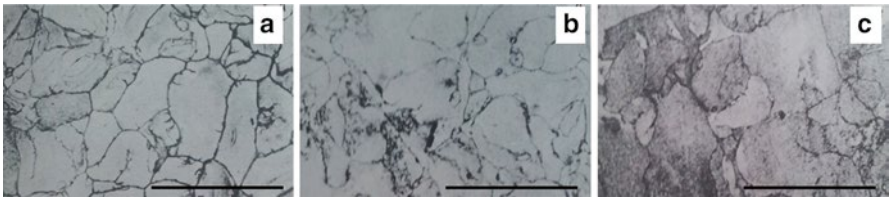


Fig. 14.3 Light micrographs of Mozzarella (**a**), Gouda (**b**) and Edam (**c**) cheeses showing curd granule junctions which appear as dark lines on the outside of the curd particles. Bar indicates 5 mm. From Kaláb (1977)

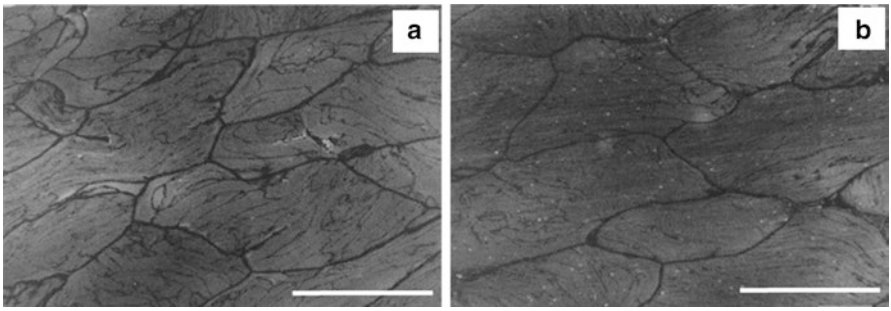


Fig. 14.4 Light micrographs of Cheddar cheeses subjected to pressing in a Wincanton block former (**a**) or in a mould as in traditional Cheddar manufacture (**b**). Curd chip junctions appear as heavy *dark lines* while the junctions of curd particles within the curd chips are also discernible as a tracery of fine *black lines*. Bar indicates 10 mm. From Lowrie et al. (1982)

14.3.1 Microstructure

14.3.1.1 Rennet-Curd Cheeses

The microstructure of rennet-curd cheese may be defined as a highly concentrated matrix (Fig. 14.2), consisting of:

- a calcium phosphate *para*-casein network of dehydrated extensively, fused *para*-casein micelles;
- a fat phase (in the form of globules, coalesced globules and/or pools) that is encased within the casein network;
- a solvent phase comprised of water and dissolved solutes (e.g., lactose, lactic acid, soluble salts, water-soluble peptides, enzymes) entrapped within the *para*-casein micelles, and that corresponds largely to residual solvent not removed from the micelles during manufacture;
- microorganisms, mainly starter and non-starter lactic acid bacteria, located mainly at the fat-protein interface

The fusion of *para*-casein molecules within the network is mediated by various interactions, including calcium bridges formed by divalent calcium ions which bind to dissociated acidic amino acid residues (glutamate and aspartate) and to phosphate (serine phosphate groups), and hydrophobic interactions between uncharged amino acid residues of individual casein molecules. Overall, the microstructure of the *para*-casein network may be envisaged as a polymer network, comprised of polymer chains of fused *para*-casein micelles that are interconnected by numerous calcium- and hydrophobic cross-links to form a single macroscopic entity. The volume fraction of the *para*-casein network and degree of fusion (aggregation) of the *para*-casein particles making up that network increase as the protein concentration increases. Hence, the volume fraction is higher in high-protein cheeses (e.g., Emmental, Edam, Cheddar) than low-protein cheeses (e.g., Feta, Blue cheese), and increases as the fat content of a particular cheese variety is reduced, for example

from 33 % in full-fat Cheddar to <4 % (w/w) in low-fat Cheddar-type cheese. Enzymes such as residual chymosin and plasmin associated with the *para*-casein micelles hydrolyze the *para*-casein molecules to large peptides slowly over time and contribute to the softening of the cheese during maturation.

The fat in cheese occurs as globules in varying degrees of coalescence (Fig. 14.2; Mistry and Anderson 1993; Fenelon et al. 1999; Rowney et al. 2003; Lopez et al. 2006). Clumping and coalescence are the result of shear stresses imposed on the fat globule membrane by various manufacturing steps (cutting, stirring, pressing, plasticization) and the shrinkage of the surrounding *para*-casein matrix, which forces the occluded globules into close contact.

The cheese matrix also contains starter and non-starter bacteria, which are concentrated at the fat-casein interfaces (Laloy et al. 1996). Bacterial proteinases and intracellular peptidases, released on autolysis, further degrade large casein peptides (produced by chymosin or plasmin) to free amino acids and, thereby, contribute to cheese flavour (cf. Chaps. 11–13)

14.3.1.2 Acid-Curd Cheeses

The microstructure of acid-curd cheeses is similar generically to rennet-curd cheese, in that it consists of a matrix comprised of a continuous protein network, which occludes fat globules and moisture. However, it differs from the microstructure of rennet-curd cheeses in the following respects:

- the polymer network is comprised of casein instead calcium phosphate *para*-casein;
- the degree of calcium bridging contributing to casein interconnectivity and casein network formation is significantly lower, owing to the low pH (~4.5–4.8) at whey drainage which results in solubilisation of all the colloidal calcium phosphate and its removal in the cheese whey (see Chap. 16);
- the volume fraction of the casein network is much lower and the degree of fusion between the casein particles (acidified casein micelles) making up the network is generally much lower (Kaláb 1979) because of the lower casein concentration and higher moisture content.
- the fat globules are generally less coalesced (Heertje et al. 1985), owing to the lower degree of concentration, and to homogenization of milk prior to gelation in the case of some products, such as Cream cheese.

14.3.2 Macrostructure

14.3.2.1 Rennet-Curd Cheeses

The macrostructure of rennet-curd cheeses consists of an assembly of curd particles that are fused with neighbouring particles to varying degrees (Fig. 14.3; Kaláb 1977). Discontinuities at the macrostructural level exist in the form of curd granule

junctions or curd chip junctions in Cheddar and related dry-salted varieties (Fig. 14.4; Lowrie et al. 1982). Unlike the interior of the curd particles, the junctions are comprised mainly of casein, being almost devoid of fat. Factors which contribute to the formation of curd granule junctions include leaching of the fat from the surface of the individual curd particles and dehydration of surface protein during the cutting, acidification, cooking and pressing stages of curd manufacture. The difference in cheese composition at junctions, compared to the interior of the curd particles, probably leads to differences in the molecular attractions between the *para*-casein layers in the interior and exterior of curd particles. However, on going fusion of particles during maturation leads to a more coherent structural continuum as reflected by the disappearance of inter-particle boundaries and the formation of a more homogeneous mass (Kimber et al. 1974; de Jong 1978a).

Curd chip junctions occur in Cheddar and related dry-salted varieties owing to the milling of the acidified curd mass (at pH ~5.1–5.3) into chips (typically 1 cm × 1 cm × 7 cm) that are mixed with dry salt prior to moulding and pressing (see Chap. 9). The boundaries between the salted chips remain clearly discernible on examination of the pressed cheese by light microscopy and, like curd granule junctions, have a higher casein-to-fat ratio than the interior of the chips. This is due to a high degree of protein dehydration and a low protein-to-fat ratio at the surfaces of curd pieces compared to the interior, owing to contact with dry salt and ensuing losses of moisture and fat.

The knitting of individual curd particles or chips/pieces into a macrostructure (intact moulded cheese) is influenced by many factors (Guinee 2016), including:

- the microstructural-related properties of the particles or chips, which determine their potential to deform and flow into, and fuse with, other curd particles when subjected to moulding and pressing; critical factors include, *inter alia*, the degree of *para*-casein hydration (g water/g protein), level of calcium bound by *para*-casein, protein-to-fat ratio, salt content and pH.
- the surface properties of the curd particles such as composition (protein-to-fat ratio, salt content, moisture content), degree of protein hydration (as affected by the temperature of curd particle/whey mixture during scalding, moulding and pressing), and dimensions (e.g., shape, size, aspect ratio, uniformity);
- the size distribution and surface area of curd particles or pieces, which affects the packing arrangement, i.e., the neatness of fit into a continuum;
- temperature of curd particles, which affects the liquid–solid fat ratio and the ratio of protein-entrapped moisture to expressed (free) moisture, and, hence, their ability to flow;
- pressing conditions including temperature and pressure which affect the extent of protein solubilisation, fat crystallisation and the ability of curd particles to flow and knit into a seamless whole, without notable inter-particle microstructural discontinuities and junctions;
- the presence of air pockets between curd particles;
- storage conditions (temperature, time) which affect the extent of age-related changes in pH, equilibrium between soluble and *para*-casein bound calcium, proteolysis, fat coalescence, and, hence, ability of curd particles to fuse further.

14.3.2.2 Acid-Curd Cheeses

Apart from Cottage cheese and Ricotta, acid-curd (e.g., Cream cheese, Quark, *Fromage frais*), and acid-heat coagulated (e.g., Mascarpone) cheeses tend to be devoid of a macrostructure, being homogeneous throughout. This is because of several factors, including the concentration of the gel pieces (particles) shortly after cutting or breaking the gel (without stirring and cooking of individual curd particles in whey) and the relatively high level of moisture (compared to rennet-curd cheeses) which causes the pieces to lose their identity and fuse into a seamless structural continuum. In contrast, Cottage cheese has a very distinctive macrostructure consisting individual curd granules (dry-curd Cottage cheese), or individual curd granules covered with a cream layer (Cottage cheese).

14.4 Rheological Concepts

The general terminology used to describe the rheology of materials has been reviewed extensively (Rao and Steffe 1992; Collyer and Clegg 1998). Rheologically, cheese is a viscoelastic material, exhibiting the characteristics of both solid and liquid materials. Hence, defining cheese rheology requires an examination of the two forms of ideal rheological behaviour, i.e., elastic behaviour and viscous behaviour (Visser 1991; Gunasekaran and Ak 2003, O'Callaghan and Guinee 2004). The terms most commonly applied to the rheology of cheese are described in Table 14.2.

14.4.1 Stress and Strain

Stress is defined as the distribution of force over an area of a material, i.e., stress = force/area. The 'area' over which a force is distributed may be a surface, (e.g., the surface of a cylindrical sample exposed to a compression plate) or an imaginary section within a material (e.g., an internal fracture plane). The force applied at a surface is distributed throughout the material and is borne by the structural elements, e.g., in the case of cheese, the casein/*para*-casein strands of the network and the occluded fat globules.

The applied stress results in displacement, known as deformation. The deformation is manifested as a change in dimensions (for example a reduction in height) and shape (i.e., form) which may be temporary, permanent or partly recoverable. The fractional displacement (ratio of change in dimension, such as height, to the original dimension) that occurs under an applied stress is referred to as strain. The dynamic measurement of instantaneous force and displacement, for example during compression, results in a stress/strain curve which describes the rheological characteristics of a material under the assay conditions. The conditions which affect the force-displacement response include temperature, type of deformation (compression,

Table 14.2 Rheological properties of raw cheese and their definitions, showing the relationship to rheological quantities as measured instrumentally[a]

Rheological property	Definition	Cheese type displaying property
Elasticity (rubberiness)	Tendency of cheese to recover its original shape and dimensions upon removal of an applied stress	Swiss-type cheese, low-moisture Mozzarella
Springiness	Bouncing property of cheese. Tendency to recover from large deformation (strain) after removal of deforming stress	Swiss-type cheese, low-moisture Mozzarella
Elastic fracturability	Tendency of hard cheese to crack, with very limited flow (near the crack); after fracture, the broken surfaces can be fitted closely to each other	Parmesan, Romano, Gruyere cheese
Brittleness	Tendency of (hard) cheese to fracture at a relatively low deformation	Romano, Parmesan,
Firmness (hardness)	High resistance to deformation by applied stress	Cheddar, Swiss-type cheese, Romano, Parmesan, Gouda
Longness	The failure of cheese to fracture until a relatively large deformation is attained	Mozzarella, Swiss
Toughness (chewiness)	Length of time required to masticate cheese to make it suitable for swallowing. A high resistance to breakdown upon mastication	Mozzarella, String cheese, Halloumi
Softness	Low resistance to deformation by applied force.	Blue cheese, Brie, Cream cheese
Plastic fracturability	The tendency of cheese to fracture, accompanied by flow (the broken parts do not easily fit together)	Mature Cheddar, Blue cheese, Chaumes, Raclette
Shortness	The tendency to plastic fracture at a small deformation; low resistance to breakdown upon mastication	Camembert, Brie
Adhesiveness (stickiness)	Force required to remove cheese from palate during eating. The tendency to resist separation from another material it contacts (e.g., another ingredient or a surface such as a knife blade or palate)	Mature Camembert
Cohesive	Tendency of cheese to resist breakage into pieces by application of force.	Mozzarella, string cheese
Crumbliness	The tendency to break down easily into small, irregular shaped particles (e.g., by rubbing)	Cheshire, Wensyledale, Blue cheese, Stilton, Feta
Shear thickening	The tendency to increase in apparent viscosity when subjected to an increasing shear rate (especially upon heating)	Cream cheese (when heated), 'creaming' of processed cheese products
Shear thinning	The tendency to exhibit a decrease in apparent viscosity when subjected to an increasing shear rate	Quarg (especially at a low temperature, i.e., <4 °C)
Grainy	Inhomogeneities in the cheese evaluated in the mouth. Ranging from smooth to grainy (gritty, mealy)	Defect in some fresh acid-curd cheeses; Parmesan, mature Cheddar cheese with crystals of calcium lactate or calcium tyrosine.

[a]Compiled from data of van Vliet (1991) and Fox et al. (2000)

extension or shear), level of deformation in relation to the elastic limit, rate of deformation and deformation history).

The rheological behaviour of the material is affected by the response of the structural elements to the applied stress. The initial response of a cheese to stress is determined mainly by the casein/*para*-casein network. At larger deformations the moisture and fat phases contribute to the rheological response.

14.4.2 Relationship Between Stress and Strain

The application of a force to a material generally results in deformation, which is manifested as a change in sample shape and dimensions. Stress, denoted σ or τ, is the force applied per unit surface area of the material. It may be applied to the surface in a normal direction (σ), resulting in compression (e.g., reduction in the sample height) or extension (e.g., increase in sample height) (Fig. 14.5a). Alternatively, the stress may be applied tangentially to the surface (τ), causing adjoining layers of the material to slide relative to each other in a direction parallel to their plane of contact (Fig. 14.5b). The deformation is the displacement relative to a fixed plane in the material as stress is applied, and strain is the displacement as a fraction of a particular dimension (e.g., height).

Normal stress (σ), which occurs during extension or compression of a sample, is defined as

$$\sigma = F / A$$

where A is the cross-sectional area of the sample over which force (F) is applied (Fig. 14.5a). The applied stress results in normal strain (also referred to as Cauchy strain, apparent strain or engineering strain), defined as the deformation relative to the original sample dimension, i.e.

$$\varepsilon = \Delta H / H_o$$

where H_o corresponds to the original height of the sample and ΔH to the change in height (displacement) under the applied stress, σ (Fig. 14.5a). An alternative expression is Hencky strain, also referred to as true strain, which is defined as the natural logarithm of the ratio of the sample height under pressure to the original length, i.e.

$$\varepsilon_t = \ln(H / H_o),$$

where H is height of the sample under stress and Ho is the original sample height.

Shear stress (τ), which occurs when a force is applied parallel to the plane of a surface, is defined as

$$\tau = F / A$$

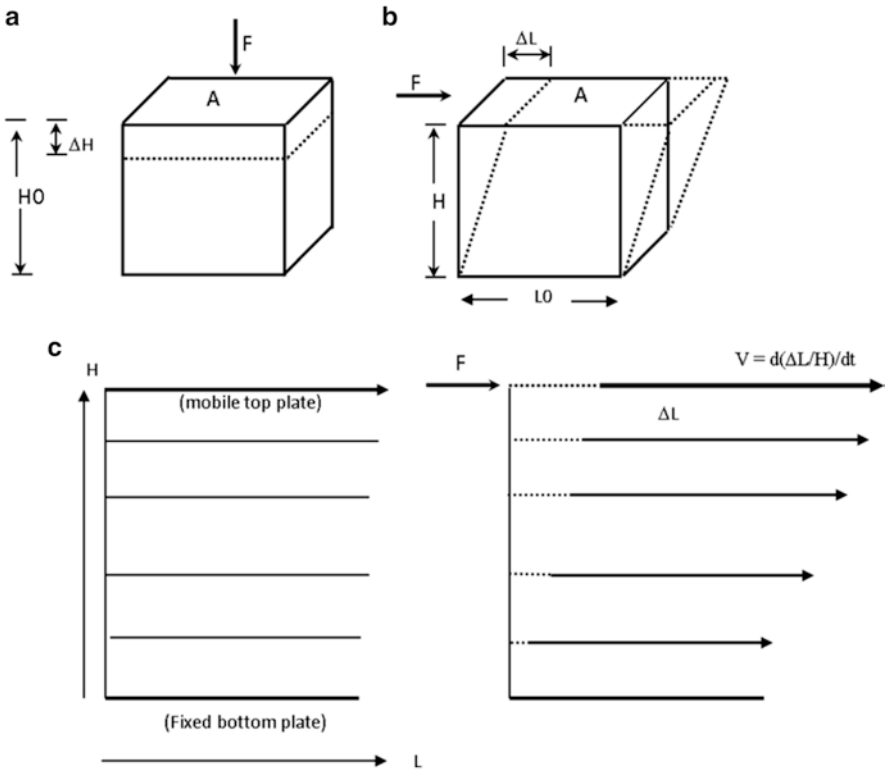


Fig. 14.5 Application of stress (i.e., force, F, per unit surface area, A) to a solid (**a**, **b**) or liquid (**c**) material. For the solid material, the stress may be applied (**a**) in a direction normal to the surface resulting in compression (ΔH) or (**b**) tangential to the surface resulting in shear deformation (ΔL). For the liquid material, confined between two parallel plates (a fixed bottom plate and mobile top plate) separated by a distance H, application of a shear force to the top plate results in movement in the direction x at a velocity V

where A is the cross-sectional area over which the shear force (F) is distributed (Fig. 14.5b). The resulting shear strain is defined as

$$\gamma = \Delta L / H$$

where ΔL is the shear (tangential) displacement on the application of shear stress, τ.

Compression testing is generally used in the rheological evaluation of cheese because of the relatively low tensile strength of most cheeses. Exceptions include members of the *pasta filata* family of cheeses, such as String cheese and Mozzarella, which when heated are able to undergo a high degree of stretching when pulled. This characteristic is associated with the presence of *para*-casein fibres which are formed during the exposure of the curds to high temperatures (e.g., 58–60 °C) at low pH (e.g., 5.1–5.4) during manufacture (see Chap. 18).

In practice, normal and shear stresses occur simultaneously during testing, size reduction at commercial level (e.g. comminution, shredding, grating), and consumption (mastication).

14.4.3 Definitions of Different Types of Rheological Behaviour Based on Creep and Recovery Experiments

14.4.3.1 Ideal Elastic Solid

A material is described as an ideal elastic, or Hookean, solid if the relationship between σ and ε, or τ and γ, is linear, with the σ versus ε curve passing through the origin (Fig. 14.6a). Two types of moduli (i.e., proportionality constant between σ and ε) are obtained for an ideal solid based on the method of stress application (Fig. 14.5):

(a) modulus of elasticity, or Young's modulus (E), where the stress is normal to the stress-bearing area, $\sigma = E.\ \varepsilon$, and $\varepsilon = \Delta H/H$;
(b) elastic shear modulus, where the stress is tangential to a fixed plane, $\tau = G.\ \gamma$, and $\gamma = \Delta L/H$.

The moduli E and G for an ideal solid are independent of the time, and the rate at which the stress is applied; hence, the stress/strain curve is always linear.

A plot, known as a creep/recovery curve, shows the variation of ε with time on the instantaneous application of a constant σ to a solid and its removal some time later (Fig. 14.7). An elastic solid deforms instantly on the application of σ and recovers instantly to its original shape and dimensions on the removal of σ (Fig. 14.7a). During the holding of a force (F) and hence, σ, the stress energy is absorbed and stored by the structural elements of the material and no breakage of the bonds between the structural elements occurs. On removal of σ, the stored

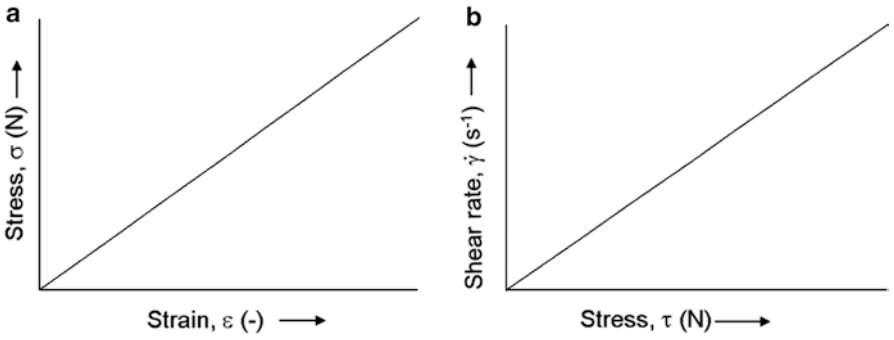


Fig. 14.6 Relationships between stress, δ, and strain, ε, for an elastic solid (**a**) and between shear stress, τ, and strain rate, $\dot{\gamma}$, for an ideal liquid (**b**)

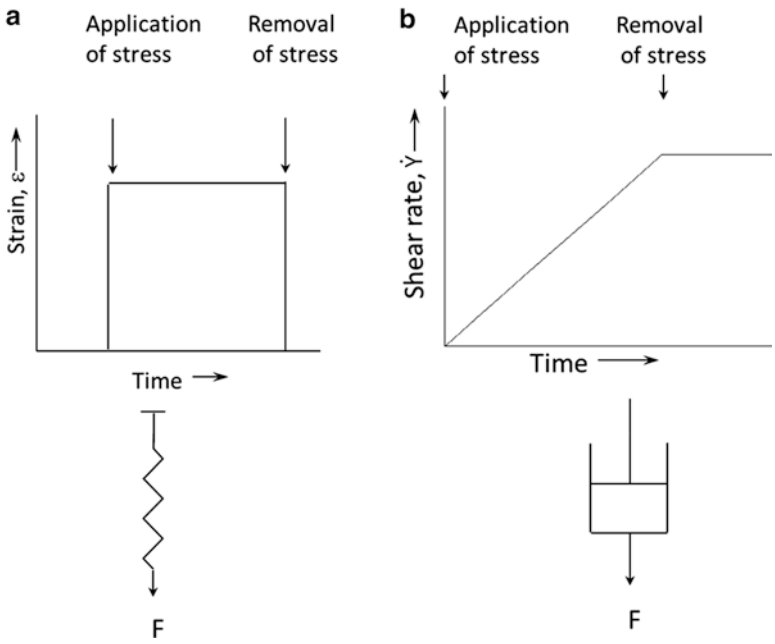


Fig. 14.7 Creep/recovery curves showing time-related changes in the strain of an ideal elastic solid (**a**) and in the shear (strain) rate of an ideal liquid (**b**) on the application, holding and removal of a constant stress. The rheological behaviour of an ideal elastic solid is mechanically represented by a single spring, and that of an ideal liquid by a dashpot

energy is released immediately and enables the material to counteract the deformation and regain its original dimensions instantly. This rheological behaviour may be represented mechanically by the simple spring, which deforms instantly and where the degree of extension (i.e., ε) is directly proportional to the mass (and hence force per unit area, σ) pulling/hanging from the spring.

14.4.3.2 Ideal Viscous Liquid

In contrast to an ideal solid, a liquid does not support a permanent stress; the strain changes constantly as long as the stress is maintained. On the application of τ to a liquid confined between two parallel plates (upper floating plate and lower stationary plate) separated by a distance y (Fig. 14.5c), the top plate moves a distance (Δx) in the x-direction at a velocity (v), given by $v = \Delta x/y$. Assuming that the liquid remains in contact with each plate by surface tension, the rate of change of strain (denoted shear rate and abbreviated $\dot{\gamma}$) is thus given by the differential change in v with position, since $\dot{\gamma} = d(\Delta x / y) / dt = dv / dt$. A material is defined as an ideal, or Newtonian, liquid if $\dot{\gamma}$ is directly proportional to τ, with the τ versus $\dot{\gamma}$ curve passing through the

origin (Fig. 14.6b). The proportionality constant between τ and $\dot{\gamma}$ is known as the coefficient of viscosity or kinematic viscosity, η, where $\eta = \tau/\dot{\gamma}$.

A creep curve for a Newtonian fluid shows that a fluid starts to flow instantly at a constant $\dot{\gamma}$ on the application of a constant τ, and ceases flow (but is permanently deformed) immediately on its removal (Fig. 14.7b). Unlike a solid material, the energy due to stress is not stored but is dissipated (due to breakage of the interactions between the structural elements of the liquid) in the form of flow, with the strain being proportional to the time over which the stress is applied. This rheological behaviour may be represented mechanically by a dashpot (i.e., a piston enclosed in a cylinder filled with a viscous liquid) (Fig. 14.7b), where the rate of movement of the piston ($\dot{\gamma}$) is directly proportional to the applied τ.

14.4.3.3 Viscoelastic Materials

Most solid- and semi-solid-like materials, including cheese, exhibit characteristics of both an elastic solid and a Newtonian fluid and are thus termed viscoelastic. The relationship between stress and strain for these materials is not linear except at very low strains, as discussed below. The stress increases less than proportionally with strain, resulting in a curve which has a concave downwards shape (Fig. 14.8). The rheological properties (i.e., E, G, η) of viscoelastic materials differ from those of perfectly elastic and viscous materials in that they are dependent on: (1) time, being a function of the time over which a fixed stress or strain is applied and (2) magnitude of the stress. However, on the application of a strain that is sufficiently small so as not to cause permanent damage or fracturing of the microstructure, viscoelastic materials behave as elastic. The strain at which linearity between stress and strain is lost is referred to as the critical strain (i.e., linear viscoelastic range), and for most solid-like foods, including cheese, is relatively small, e.g., of the 0.02–0.05 (Walstra and van Vliet 1982; Subramanian and Gunasekaran 1997a). At short time scales most hard cheeses are essentially elastic at a strain less than the critical strain.

14.5 Model of Cheese Rheology Based on Creep and Recovery Experiments

14.5.1 Creep and Stress Relation Behaviour of Cheese

Cheese is a viscoelastic material, exhibiting the characteristics of both an ideal solid and an ideal liquid, but also differing from these materials in that the relationship between stress and strain depends on the magnitude of the applied stress/stain and the duration of the application. On the application of a low stress, that is sufficiently small so as not to induce permanent damage or fracturing (breaking of bonds

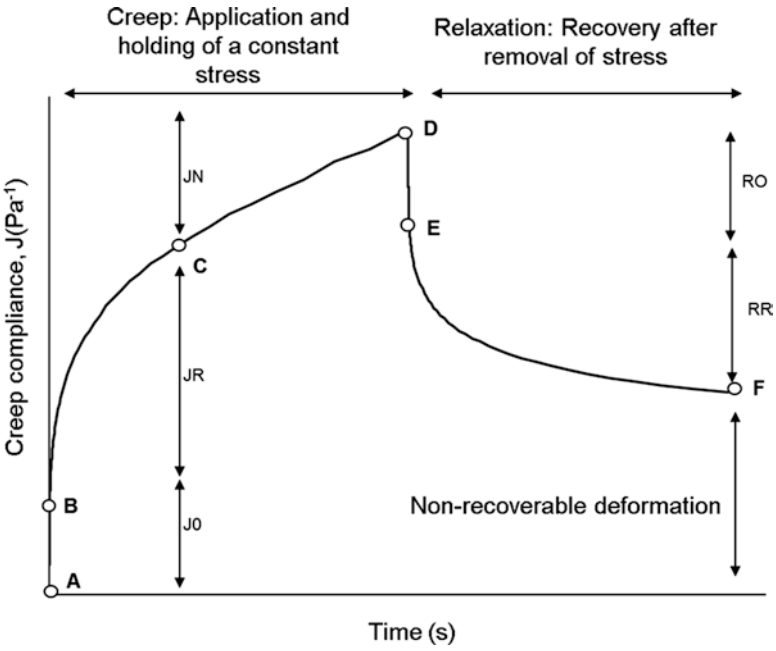


Fig. 14.8 Creep compliance and recovery curve of a 3-month-old Cheddar cheese showing the following regions: elastic creep compliance (J_0, AB), retarded elastic compliance (J_R, BC), Newtonian compliance (J_N, CD), elastic recovery (RO, DE), and delayed elastic recovery (RR, EF); the creep compliance, J, ranged from 0 to 187×10^{-6} Pa^{-1} and time from 0 to 1000 s. The various terms are discussed in text. Guinee (unpublished results)

between the structural elements) of the microstructure, for a short time, cheese behaves as an ideal solid. However, a low stress applied over a relatively long time results in an increasing strain, a gradual failure of the structure and eventual flow. Hence, the relationship between τ (or σ) and γ (or ε) is linear only at very low τ and short time scales. The γ at which linearity between τ and γ is lost is referred to as the critical strain (i.e., linear viscoelastic range), which for most solid-like foods, including cheese, is relatively small, e.g., 0.02–0.05. The time-dependent rheological behaviour of cheese is evaluated using creep or stress relation tests (Visser 1991; Ma et al. 1996; Pereira et al. 2001; Venugopal and Muthukumarappan 2001). Creep is the time-related change in strain on application of a constant stress to a material such as cheese.

Practical examples of creep occur when curd or cheese is gradually compressed under its own weight, pressed or stacked, e.g. during retailing. A creep test generally involves the instantaneous application of a low (e.g., 10–50 Pa) constant stress (τ) and the measurement of the resultant strain (γ) or creep compliance as a function of time (e.g., Fig. 14.8). Typically, testing involves placing a disc-shaped test sample

between the two parallel plates of a rheometer cell, i.e., a stationary bottom plate and a top plate which applies the shear. The results are expressed as strain, or creep compliance (J) at a particular time (t). Creep compliance is the ratio of strain to applied stress:

$$J(_t) = \gamma(\tau) / \tau, \text{or } J(_t) = 1 / G_t \text{ where } G_t \text{ is the ratio of } \tau / \gamma \text{ at time, t.}$$

The typical change in shear strain (γ) with time on the application of a low (constant) shear stress (τ) to Cheddar cheese is shown in a creep curve (Fig. 14.8), where creep compliance (J) is the ratio of γ to τ. Three distinct regions are evident:

- elastic deformation (region A-B), where the γ is instantaneous and fully reversible; the creep compliance is referred to as being elastic (J_0)
- viscoelastic deformation (region B-C), where γ is partly elastic and partly viscous; the creep compliance is described as being retarded elastic (J_R) and the recovery of the elastic component of γ, on the removal of τ, as delayed.
- viscous deformation (region beyond C-D), where γ increases linearly with time and is permanent; the creep compliance is referred to as being Newtonian (J_N) and γ is non-recoverable.

On removal of the stress at point D, the strain recovery curve shows three identifiable regions: an instantaneous elastic recovery (D-E), a delayed elastic recovery (EF), and an eventual flattening. The vertical distance from the flat portion of the recovery curve to the time axis is the non-recoverable strain per unit stress, which is related to the amount of structural damage (lasting deformation) to the sample during the test. In the elastic region of the creep curve, the strands of the cheese matrix absorb and store the stress energy, which is instantly released on removal of τ, enabling the cheese to regain its original dimensions. The extent and duration of the elastic region depends on the magnitude of τ and the structural and compositional characteristics of the cheese. At γ > critical strain, the structure of the cheese is altered via the breaking of bonds between structural elements, which are stressed beyond their elastic limit. Eventually, when the stress-bearing structural casein network has fractured, the cheese flows.

Generally, a stress relaxation test entails the instantaneous application of a low constant deformation or strain, ε, (typically 0.10–0.20) by compression of the cheese sample between two parallel plates of a texture analyser. On the application of ε, σ increases instantaneously to σ_0 (i.e., zero-time value) but decays exponentially with time (t) (Shama and Sherman 1973). The resultant σ-time curve is used to determine the stress relaxation time, t, which may be defined as the time required for σ to decrease to a fraction of σ_o, e.g., t at which $\sigma = \sigma_o/e$, where e = inverse natural logarithm of 1 ($\ln^{-1}$ 1) = 2.718. In a variation on such a test, Emmons et al. (1980) compressed full-fat (35 %) and reduced-fat (17 %) Cheddar cheeses, having a common level of moisture-in-non-fat substance, at a constant speed to a strain of 0.2 and held the strain for 1 min. They showed that the initial compression slope (or modulus of deformability), the relaxation slope and the residual force (after 1 min) were much higher for reduced-fat cheese, with or without homogenisation, than for full-fat cheese.

14.5.2 Mechanical Models of Cheese Rheology

The modelling of the rheology of viscoelastic solid materials, such as cheeses, begins with simple relationships such as Hooke's Law for small displacements in the elastic region. In the region beyond the elastic limit, sometimes referred to as the elastoplastic region (i.e., where recovery following deformation is partial on removal of the stress), the modelling of rheology in cheese requires more complex models. Various mechanical models have been used to simulate creep and relaxation effects of viscoelastic materials (Rao and Steffe 1992; Gunasekaran and Ak 2003; Abd El-Maksoud et al. 2009). These models contain different arrangements of dashpots and springs in series or in parallel (Fig. 14.9), with springs representing the elastic element, and dashpots (piston/cylinder), the fluid element. Models include Maxwell (two-element model consisting of spring and dashpot in series), Kelvin (two-element model with a spring and dashpot in parallel), Burger (four-element model comprising a combination of Maxwell and Kelvin model bodies), or other arrangements. The Maxwell model gives an initial instant increase in strain (elastic deformation due to spring element) followed by a linear increase in strain (viscous deformation due the dashpot element) on application of a small stress (τ). Removal of the stress is characterized by an instant decrease in strain (elastic recovery), but there remains a non-recovered strain associated with the viscous deformation. The Kelvin model, also known as the Kelvin (Voigt) model shows an exponential increase in strain, followed by an exponential decay in strain to full recovery, on application of stress and its removal. A creep curve for the Burger model indicates that it affords a much closer approximation of the stress relation behaviour of cheese (Fig. 14.8) than either the Maxwell or Kelvin models, on the application and removal of stress.

Subramanian and Gunasekaran (1997b) showed that the viscoelastic behaviour of Mozzarella cheese within the linear viscoelastic strain range (0.05) over the frequency range 0.05–20 Hz could be simulated by a model consisting of eight Maxwell elements. Ma et al. (1996) showed that a six-element Kelvin model could simulate creep compliance in full-fat and reduced-fat Cheddar cheese.

The viscoelastic nature of cheese implies that the ratio of elastic to viscous properties depends on the time scale over which the deformation is applied. At short time scales, cheese is essentially elastic, whereas after a long deformation time, cheese flows, albeit very slowly for hard cheeses (Fig. 14.8). However, even hard cheese flows eventually when stressed and will not recover completely on removal of the stress. Failure to appreciate this characteristic can often lead to loss of shape (e.g., manifested by bulging, inclined surface) during distribution and retailing, where cheeses of different consistencies are often laid upon each other haphazardly.

The flow of rigid materials is not always readily apparent (because of the relatively long time required to produce a notable deformation) and the notion may even appear somewhat abstract. However, there are many natural examples that reveal the slow flow of rigid materials over very long time periods (equivalent to creep experiments where the time approaches infinity) e.g., the flow of glass in window panes,

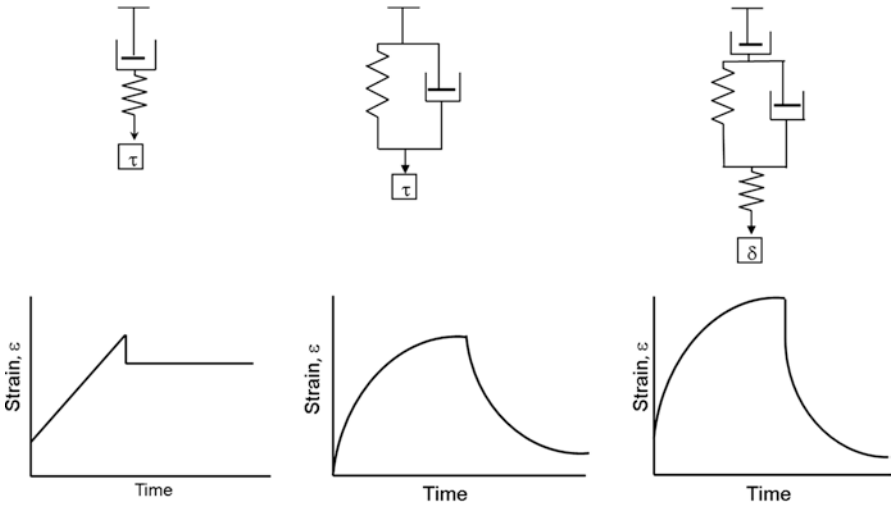


Fig. 14.9 Mechanical models (bodies/elements) representing viscoelastic behaviour and their corresponding creep and recovery curves: (**a**) Maxwell, (**b**) Kelvin and (**c**) Burger models

due to the force of gravity, as reflected by the increase in the thickness of individual window panes of old buildings (e.g., churches) with increasing distance from the top of the pane.

14.6 Measurement of the Rheological Behaviour of Cheese

The methods used to assess the rheological characteristics of cheese may be classified broadly as sensoric or instrumental. Instrumental methods may be classified arbitrarily as empirical or fundamental. In general, the nature of the stresses and strains in empirical methods are less well-defined than in fundamental methods. Moreover, unlike fundamental methods, the measurements obtained with some empirical methods are on an arbitrary scale (e.g., the ball compressor).

14.6.1 Sensoric Methods

The aim of sensoric methods, which are routinely performed by cheese graders, is to acquire an impression of how the texture of the cheese is perceived during consumption. As discussed in Sect. 14.2, cheese texture may be defined as a composite sensory attribute resulting from a combination of physical properties that are perceived by the senses of touch (including kinaesthesis and mouthfeel), sight and hearing. The test conditions are arbitrary and frequently involve subjecting the

cheese to a deformation which results in visual fracture, e.g., as when rubbing cheese between the fingers until it becomes pliable, bending of a cylindrical cheese plug between the fingers and gauging mentally the force required to bend or break it. Alternatively, the cheese may be assessed by the application of forces or deformations which cause no visible fracture, e.g., pressing the ball of the thumb onto the surface of a whole cheese and mentally gauging the degree of indentation or the force exerted on the fingers. In all cases, a mental impression is formed and the grader assigns a score, based on one or more criteria, such as test conditions and response.

14.6.2 Instrumental Empirical Methods

These tests involve subjecting a cheese sample to a stress or strain using various instrumental devices (Gunasekaran and Ak 2003; O'Callaghan and Guinee 2004). The different test types include:

- penetration tests, where the force required to insert a probe a given distance into the cheese, or alternatively the depth of penetration of a probe under a constant load for a given time, is measured (penetrometer test);
- compression tests, where the extent of compression under a constant load for a specified time is measured (e.g., the ball-compressor test)
- cutting tests, where the resistance to the passage of a wire through a cheese is measured (e.g., Cherry-Burnell Curd Tension Meter)

The aim is to measure a parameter which experience indicates or suggests is related to the textural characteristics of the cheese. Hence, while the test conditions are arbitrary and the stresses and strains involved are not defined, a value is obtained which gives some indication of the textural characteristics of the cheese and differentiates one sample from another. However, they provide only single values that are an overall measure of the many different facets of rheological behaviour.

14.6.2.1 Penetrometer Tests

Penetrometer tests measure the depth to which a penetrometer (e.g., needle, cone or probe) can be forced into a cheese under a constant stress for a given time. As the needle or cone penetrates the cheese, the cheese in its path is fractured and forced apart. The progress of the penetrometer is retarded to an extent depending on the hardness of the cheese in its path, the adhesion of the cheese to its surface (which increases with the depth of penetration into the cheese) and its surface area of contact with the cheese (regulated by the thickness of the needle or angle of the cone used). The test, which is used to provide an index of hardness (i.e., resistance of a surface to penetration), is suitable for closed-textured cheeses (such as Gouda and Mozzarella) which are macroscopically homogeneous. Conversely, it is unsuitable

for open-textured cheeses (e.g., with small mechanical openings or small eyes, e.g., Tilsiter or Gruyere) or cheeses which are macroscopically non-uniform (e.g., Cheddar due to the presence of chip boundaries). Hennequin and Hardy (1993) used a cylindrical probe (5 mm diameter at a speed of 10 mm/min to a depth of 10 mm) to penetrate soft cheeses (e.g. Camembert, Coulommier, Munster) and found that the force at 10 mm penetration was highly correlad with sensory firmness ($r=0.94$, $n=19$). They concluded that the technique is suitable as a rapid method for texture measurement in soft cheese. Breuil and Meullenet (2001) found a significant correlation between measurements obtained using a cone penetrometer (30°) or a 2 mm needle, and textural characteristics of a wide range of commercial cheeses (e.g., Colby, Cream cheese, Edam, Gouda, Cheddar, Mozzarella, Fontina, Muenster) as measured by a sensory panel.

14.6.2.2 Compression Tests

Compression tests involve determination of the force required to deform cheese as an index of the hardness perceived by the consumer. Hence, these tests are imitative of some aspect of consumption. The General Foods Texturometer, which measures the force to compress a cheese sample placed on a fixed plate to 25 % of its original height by a tooth-shaped plunger (see Sect. 14.2), was designed to simulate the biting of food by the jaws and the teeth (Friedman et al. 1963; Bourne 1978). The ball compressor which measured the deformation after applying a fixed force for a specified time simulated that of a thumb pressing against cheese when making a sensory evaluation of the product (Szczesniak 1963b).

The General Foods Texturometer, based on a modification of a previous instrument known as the MIT texturometer (denture tenderometer), was designed to simulate the compression of food between the molars (Friedman et al. 1963; Szczesniak 1975; Bourne 1978; Rao and Quintero 2005). Essentially, a cheese sample is loaded onto a fixed plate and subjected to a deforming force by a tooth-shaped plunger which is mounted on a hinge and driven by an eccentric wheel to simulate the vertical action of a human jaw. The sample is compressed to 75 % of its original height in each of two successive deformations (referred to as bites). Mimicking jaw movement, the plunger decelerates as it reaches the end of the first bite, then accelerates upward as it withdraws, and the second bite is repeated. When the plunger deforms the sample, strain gauges attached to the sample-holding beam detect the movement of the beam *per se* and a force-time trace (Fig. 4.24) is recorded (Brennan 1988; Rao and Quintero 2005). The force-time profile, which could be converted into a time-deformation (since deformation rate was known) is denoted a texture profile, and the measurement as Texture Profile Analysis. The relationships between the trace and textural descriptors are outlined in Table 14.5 (Peleg 1976; Brennan 1988; van Vliet 1991).

The Ball-Compressor test measures the depth of indentation after a given time made by a small ball or hemisphere when placed under a given load (stress) on the cheese surface. Hence, the test simulates the action of a grader who in the course of

examination presses the ball of the thumb into the cheese. The depth of penetration has been used directly as an index of firmness, or, alternatively, making a number of simplifying assumptions, may be used to calculate a modulus, analogous to an elastic modulus, i.e., G, given by the equation (Prentice et al. 1993): $G = 3\,M/[16(RD^3)^{1/2}]$, where M is the applied force, and R and D correspond the radius and depth of the indentation, respectively. The test is generally non-destructive and can be performed on the whole cheese at several locations. However, it provides only single datum values that are an overall measure of the many different facets of rheological behaviour. Nevertheless, the test results may be of relevance if a correlation has been established between measurements made using the instrument and some characteristic of the cheese that is important during consumption (e.g., hardness) or when using the cheese (e.g., suitability for cutting and portioning). Cox and Baron (1955) found that the total deformation measured using the ball compressor was capable of discriminating between Cheddar cheeses from successive cheese vats, and deformation, measured in different layers, increased from the surface to the centre of the cheese. In contrast, the elasticity index did not discriminate between the cheeses. Green et al. (1985) reported a significant correlation between the elasticity of Cheddar cheese, as measured using the ball compressor, and graininess as evaluated by a sensory panel.

14.6.2.3 Cutting Tests

Cutting tests measure the force required to push a wire, of a given diameter, at constant velocity through a cheese mass (Green et al. 1986; Marshall 1990). Luyten et al. (1991b) investigated the fracture properties of Gouda cheese using wire-cutting. A typical force-time curve showed an initial rise in force, which reaches a maximum as the wire penetrates the sample surface. Once the surface is broken, the force drops rapidly to a constant level, F_c, as the wire 'ploughs' through the sample (van Vliet et al. 1991). F_c increases somewhat with cutting speed (~ doubling for a 20-fold increase in speed) and with wire diameter.

Since fracture develops around a crack, a specific fracture energy (in units, J/m^2), R_f, can be defined as the energy needed per unit area (of crack) to cause a fracture to spread. While it is not possible to determine specific fracture energy precisely, because of the inherent heterogeneity in cheese structure (Sect. 14.3.1), its order of magnitude can be determined by measuring cutting force using wires with a series of diameters and extrapolating to a diameter of zero. The specific fracture energy is calculated as,

$$R_f = \frac{F_c}{d}$$

where F_c is the cutting force, extrapolated to cutting with a wire of zero diameter, and d is the sample width, i.e., the length of wire in contact with the cheese (Luyten et al. 1991b). The fracture energy obtained with the wire-cutting method

may give a more accurate prediction of the behaviour of cheese during cutting (e.g., portioning, slicing) than that obtained using large strain shear or compression deformation tests.

14.6.3 Instrumental Fundamental Methods

There are a number of fundamental instrumental methods, which can be used to measure the rheological characteristics of cheese. These may be classified into three main groups (Tunick 2000; O'Callaghan and Guinee 2004):

- Creep tests and stress relaxation tests, used to measure the time-dependent viscoelastic behaviour of cheese on the application of a relatively small stress (cf. Sect. 14.5);
- Low-amplitude strain (e.g., <0.05) oscillation rheometry (LASOR) for linear viscoelastic measurements, and which involve the respective application of a constant stress or strain of low magnitude;
- Large strain deformation for measurement of fracture properties, involving the application of a large strain that may result in structural alteration and fracture.

The tests most commonly used are discussed below.

14.6.3.1 Creep and Stress Relation Tests

Cheese is subjected to a very small stress or strain so as to minimize structural alteration of the cheese sample. In a creep test, as described in Sect. 14.5.1, the sample is instantaneously subjected to a low constant stress (e.g., 10–50 Pa) and the ensuing increase in deformation or strain is measured over time (e.g., Fig. 14.8). The constant stress may be applied by placing a cheese sample between two parallel plates of a rheometer (e.g., from Anton Paar, Malvern Kinexus, TA Instruments) and applying a load to the top plate. Alternatively, a stress relaxation test entails the instantaneous application of a low constant deformation or strain (e.g., <0.05) to a cheese sample and measuring the reduction in stress over time. The constant strain may be applied by placing the sample between two parallel plates of a texture analyser and allowing the top plate to compress the sample to a particular deformation and then stopping it. The rheological outputs from creep and stress relation tests (stress or strain as a function of time) provide information on the time-dependent viscoelastic behaviour of cheeses, and indirectly gives information on structure. Hence, for a given stress, an increase in the elastic deformation (region AB, Fig. 14.8) and elastic recovery is indicative of a higher degree of casein cross-linked structure, and a more rigid, solid-like structure for example in cheeses such as Gouda, Cheddar, Mozzarella and Emmental. Conversely, increasing levels of viscous and non-recoverable deformation are suggestive of a lower degree of casein-casein interaction, and

a more viscous-like character, as for example in cheeses such as mature Camembert and fresh acid-curd cheeses, such as Quark and Labneh.

Creep and stress relaxation tests are seldom used in practice as measures of the rheological properties of cheese, because the stresses and strains involved are low and do not result in fracture, and the time scales are relatively long (e.g., at least a few minutes). Hence, the tests are not suitable for predicting the force required to induce fracture, as for example during consumption and size reduction (e.g., cutting, shredding and mincing). Nevertheless, creep tests may be useful for predicting how the behaviour (change in dimensions and shape) of cheese when subject to stresses over relatively long time scales, for example should the cheeses be stacked during transport or retail.

14.6.3.2 Low-Amplitude Strain Oscillation Rheometry (LASOR)

As discussed in Sect. 14.5.1, creep tests show that cheese is viscoelastic, displaying the characteristics of both ideal elastic (Hookean) and viscous (Newtonian) materials. At low strains, normally <0.10, cheese recovers fully, although not instantaneously, when the stress is removed. In the elastic region, where there is a linear relationship between stress and strain (typically ≤ 0.05), the behaviour is described as linear viscoelastic. Linear viscoelastic measurements are typically carried out by applying low oscillating strain to the cheese (which could be semi-solid, like Quark, or solid/rigid like hard rennet-curd cheeses) using a controlled strain rheometer (e.g., Bohlin VOR, Bohlin Rheologi, Sweden; Rotovisco RV 100/CV 100, Haake Buchler Instruments, USA), and the measurement of the resultant stresses within the sample. Alternatively, a small stress is applied to the sample using a control stress rheometer (e.g., Bohlin CS, Bohlin Rheologi, Sweeden; Cari-med CSL^2, TA Instruments, USA; Rheometric Scientific SR5, Rheometric Scientific Inc, USA) and the resultant strain is measured. The rheometer applies a dynamic oscillating shear deformation (γ) to the sample and measures the resultant stress (τ). Typically, measurement involves placing a disc-shaped sample of cheese (typically 20–40 mm diameter, 2 mm height) between two parallel plates with serrated surfaces (parallel plate geometry), one of which is fixed, while the other applies a low-amplitude torsional harmonic motion. The use of serrated plates ensures that the sample is gripped firmly between the plates, and thereby minimizes the risk of slippage (development of a free fat layer between plate and cheese surface), associated with fat liquefaction and leakage (Guinee et al. 1999), especially when rheological measurements are performed at high temperatures of 20–90 °C. It is noteworthy that some of the fat in hard rennet-curd cheeses, such as Cheddar, is free or partially free (occurring as globules in varying degrees of coalescence) (Fig. 14.2; Bryant et al. 1995; Mistry and Anderson 1993; Guinee et al. 1998; Fenelon et al. 1999; Rowney et al. 2003; Lopez et al. 2006; Richoux et al. 2008). Furthermore, most of the fat in *pasta filata* (stretched-curd) cheeses, such as Mozzarella, is free, occurring in the form of elongated pools trapped between, and having the same orientation as, the *para*-casein fibres (Guinee 2016). As milk fat is almost fully liquid at 30–40 °C (Norris et al. 1973; Lopez et al. 2006),

free fat moves through the cheese matrix to the surface of the cheese sample, thereby creating a layer of free oil between cheese surface and the top plate of the parallel plate geometry where smooth, non-serrated plates are used.

While the top plate remains stationary, a motor rotates (oscillates) the bottom plate, thereby imposing a time-dependent shear strain on the sample, where the strain (γ) at any time (t), γ(t) is: γ(t) = γ sin (ωt). Simultaneously, the time-dependent shear stress σ (t) is quantified by measuring the torque that the oscillated sample imposes on the top plate (O'Callaghan and Guinee 2004; Wyss et al. 2007; Mezger 2011). For an ideal elastic material, the stress is in phase with, and proportional to, the applied strain; the proportionality constant of stress-to-strain is the elastic shear modulus, or storage modulus (G′), of the cheese. For a Newtonian fluid, the stress is proportional to the rate of strain deformation, and is out-of-phase with the strain, with a phase angle δ = π/2. In the case of viscoelastic materials, such as cheese, the stress response contains both in-phase and out-of-phase components, reflecting the respective contributions of the elastic and viscous structural elements of the sample. Consequently, the total stress response shows a phase shift, δ, with respect to the applied strain deformation that lies between that of an elastic solid (0) and a Newtonian liquid (π/2). The viscoelastic behaviour of the system at a strain oscillation frequency, ω, is characterised by the storage modulus, G′, and the loss modulus, G′, which characterize the solid-like and fluid-like contributions to the measured stress response.

The derivation of these parameters has been described by O'Callaghan and Guinee (2004). At any time *t*, the angle of rotation, θ, of the oscillating plate is defined by: $\theta = a \sin \omega t$, where *a* is the maximum angle of rotation and ω is the angular velocity. The shear applied by the plate results in a strain $\gamma(t)$ at any radius *r*: $\gamma(t) = \gamma_o \sin \omega t$, where γ_o is the amplitude of $\gamma(t)$. For viscoelastic materials, such as cheese, the resultant oscillating stress is out-of-phase with the applied shear by a phase angle, δ. The total stress (τ) is given by: $\tau = \tau_o^{'} \sin \omega t + \tau_o^{''} \cos \omega t$, where $\tau_o^{'}$ and $\tau_o^{''}$ indicate the stress components which are in-phase and out-of-phase with the strain γ, and are related by the phase angle, δ. The tangent of the phase angle, $\tan \delta, = \tau_o^{''} / \tau_o^{'}$. The storage modulus (or elastic shear modulus) G′, and loss modulus (or viscous modulus), G′, may be defined from the relationship between τ and γ, where, $G^{'} = \tau_0^{'} / \gamma_0$ and $G^{''} = \tau_0^{''} / \gamma_0$. Therefore, $\tan \delta = G''/G'$

Oscillatory rheometry has been used to characterize the viscoelasticity of cheeses as a function of frequency or stress at specific temperatures. Ma et al. (1997) found that G′ and G′ for full-fat and reduced-fat cheeses at 20 °C decreased significantly on increasing stress in the range 1000–10, 000 Pa, and increased significantly on increasing the frequency in the range 0.01–10 Hz. In both scenarios, G′ and G′ were higher for full-fat cheese over the frequency and stress range than those for reduced-fat cheese. Similarly, Guinee and McSweeney (2006) found that G′ of full-fat (32 % fat) and reduced-fat (17 % fat) Cheddar cheeses increased with frequency of oscillation 0.1–10 Hz. G′ for half-fat Cheddar was lower than that of full-fat Cheddar at 4 °C, when the fat is mainly solid, but similar at 40 °C when fat is fully liquid, even though the dry matter content of the full-fat cheese was higher than that of the

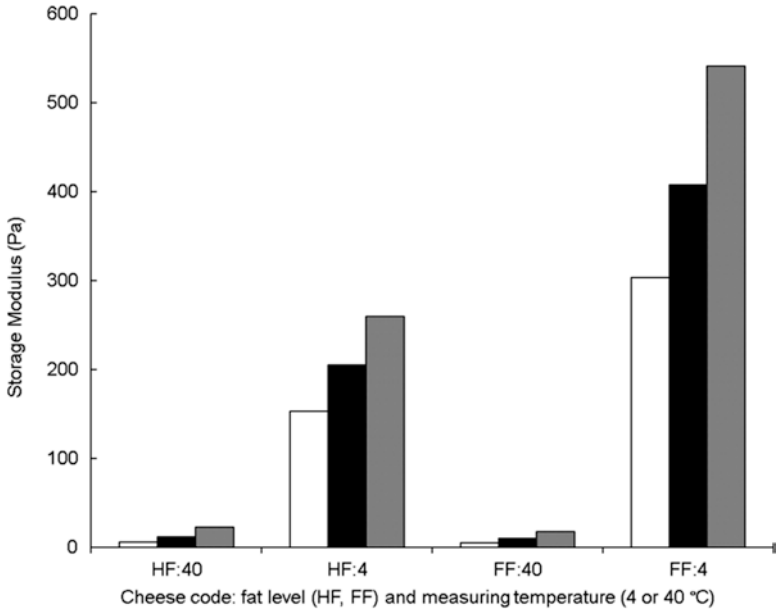


Fig. 14.10 Effect of fat level (half-fat, HF, 17 %, w/w; full-fat, FF, 32 %, w/w) and assay temperature (4 or 40 °C) on the elastic shear modulus of Cheddar cheeses measured using low strain amplitude oscillation at frequencies of ~0.1 (*white bar*), 1 (*black bar*) or 10 (*grey bar*), Hz. Redrawn from Guinee and McSweeney (2006)

reduced-fat cheese (Fig. 14.10). The higher G' of the full-fat cheese, compared to the reduced-fat cheese, at low temperature (4 °C) was attributed to the fat globules encased within the *para*-casein network being largely crystallised (solid) and augmenting the elastic contribution of the casein network and thereby increasing the stress required to achieve a given deformation. Conversely, the contribution of fat to G′ decreases as the ratio of solid-to-liquid fat decreases with increasing temperature, and is very low at 40 °C. At the higher temperatures, the fat behaves more as a fluid and the fat globules confer viscosity rather than elasticity or rigidity to the cheese mass. This effect of temperature is readily apparent from the sharp decrease in elastic shear modulus, G', and increase in phase angle, δ, when cheese is heated from 20 to 40 °C (Fig. 14.11).

Oscillatory rheometry has been used widely to follow the changes in viscoelasticity (under defined conditions of oscillation frequency and strain) as a function of temperature in the range 20–90 °C (Horne et al. 1994; Guinee et al. 1999, 2000b; Lucey et al. 2003; Shirashoji et al. 2006; Guinee and O'Callaghan 2013; Schenkel et al. 2013). Characteristic changes in G′, G′ and phase angle, δ, as a function of temperature on heating cheese are shown in Fig. 14.11 for full- and half-fat Cheddar cheeses. Typically, G′ and G′ indicate a softening of the cheese, an effect which is due to:

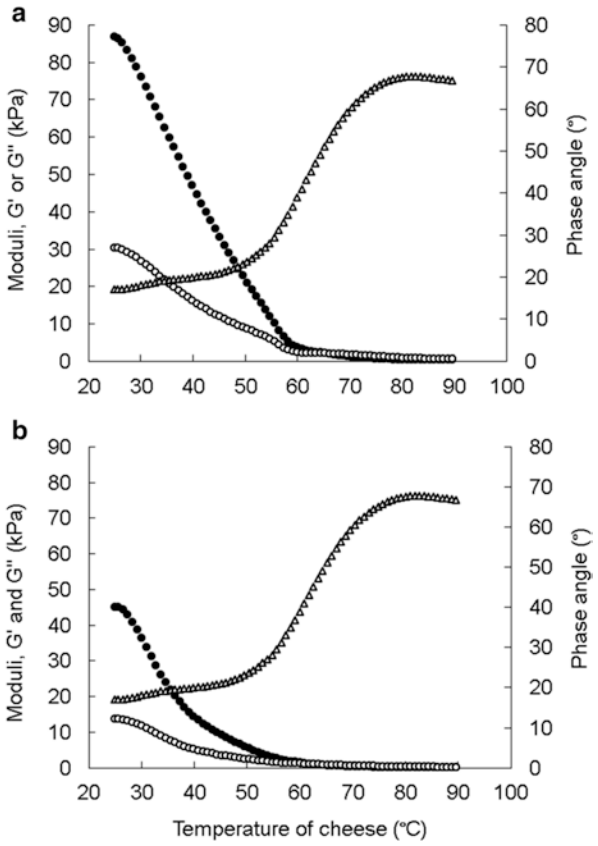


Fig. 14.11 Changes in storage modulus, G′ (*filled circle*), loss modulus, G″ (*open circle*) and phase angle (*open triangle*) of 270 day half-fat (**a**, 16.5 %, w/w, fat) and full-fat (**b**, 33 %, w/w, fat) Cheddar cheeses on heating from 25 to 90 °C. These changes were measured using low strain oscillation on a Physica MCR 501 Rheometer (Anton Paar GmbH) under the following conditions: strain amplitude, 0.006; frequency, 1 Hz. McCarthy and Guinee (unpublished data)

- liquefaction of the fat phase, which is fully liquid at ~40 °C (Norris et al. 1973), and to protein aggregation
- contraction and shrinkage of the *para*-casein network (owing to a temperature-induced increase in the extent of hydrophobic interactions between the casein molecules) and a simultaneous expulsion of moisture from the casein network (Guinee 2016).

Simultaneously, as the melting cheese mass becomes more liquid, the phase angle increases from ~20 to 70°, reflecting a transformation from a viscoelastic solid ($\delta < 45°$) to a viscoelastic liquid ($\delta > 45°$). These measurements give an insight into the effects of different treatments in manufacture (e.g., milk heat treatment, milk homogenization, make-procedure alterations to affect calcium content) and composition (e.g., levels of fat, protein, or calcium) on the cooking (melting) properties of

cheese which are important in applications where cheese is heated to a high temperature during baking, grilling or melting, e.g. toasted sandwiches, pizza, lasagna and sauces. Several parameters are of relevance: the maximum phase angle (δ_{max}) or loss tangent (LT_{max}) is indicative of the fluidity of the melted cheese and the degree to which it flows; the cross-over temperature, where G′ equals G″, is indicative of the temperature required for the cheese to transform from a viscoelastic solid to a viscoelastic liquid and becomes more fluid-like; the temperature of LT_{max} is indicative of the temperature which gives the maximum fluidity. In relation to the latter, it is frequently observed that the LT decreases at elevated temperatures (>70 °C), probably as an increase in hydrophobic-induced protein interactions (Bryant and McClements 1998). Oscillatory rheometry, may be used also to investigate how rapidly, and the temperatures at which, the melted cheese begins to congeal ($\delta < 45$ °C) on cooling, as reflected by the increases in G′ and G″ and decrease in δ (Guinee et al. 2015). This is important in designing ingredient cheese products for inclusion in various heated cheese dishes such as pizza, gratins and sauces (see Chaps. 17 and 18).

14.6.3.3 Large Strain Uniaxial Compression

In practice, cheese is subjected to large stresses and strains (i.e., $\gg 0.05$) which usually result in visible fracture, i.e., when all the interactions and or bonds between the structural elements (e.g., strands in the *para*-casein matrix) in a given macroscopic plane fail. Fracture is very evident as a result of compressive and shear forces applied during typical size-reduction operations undertaken at commercial level, e.g., pre-cutting of large blocks and/or comminution (e.g., by a conveying auger crushing and forcing pre-cut cheese through die plates with narrow apertures during processed cheese manufacture), shredding, dicing/or cubing, and portioning and slicing of table cheese. Cheese is also fractured during mastication, which reduces it to a pulp capable of being swallowed. Thus, a prediction of how cheese behaves under large σ and τ is desirable in many instances. The most common types of rheological measurement for large strain deformation of cheese involve linear (uniaxial) displacement (O'Callaghan and Guinee 2004). Other tests which are used less frequently include for large strain deformation testing of cheese include:

- Large strain shear (torsion) tests using rheometers with parallel plate geometry (Bowland and Foegeding 1999; Tunick 2000), or using vane rheometry (Truong and Daubert 2001)
- Bending tests, which involve subjecting a finger of cheese loaded on two fixed support beams to compression by a mobile beam, and measurement of the resultant compression and tension forces (Rosenthal 1999; Everard et al. 2007).

Large strain shear and bending of cheese have been reviewed by O'Callaghan and Guinee (2004), and will not be discussed further.

Large strain uniaxial compression measurement involves compression of a rectangular or cylindrical sample between parallel plates, a bottom (base) plate that is fixed, and a top plate (cross-head) which is programmed to descend and compress

the sample at a fixed rate (e.g., 60 mm/min) to a predetermined height (e.g., 30 % of original) (Fig. 14.12). The test may involve one or two cycles or bites, where a compression cycle denotes a compression of the sample and withdrawal of the top plate to its pre-compression position. During a compression cycle, the force (F) developed during compression is recorded as a function of time, enabling a force/displacement (deformation) curve to be obtained, where displacement (distance) is cross-head velocity multiplied by the time that the cross-head travels. A typical force-displacement curve, obtained on compression of a sample of Cheddar cheese at constant velocity to a strain of 0.8 (i.e., final height of sample is 20 % of original height), is shown in Fig. 14.13. Alternatively, the force-displacement curve may be converted into a stress (σ)-strain (ε) curve, where σ = force per unit area of the sample face in contact with the cross-head ($\sigma = F/A$), and ε = fractional displacement at a given force, calculated as the change in height of the sample on compression ($[h_0\text{-}h]$) as a fraction of the original sample height before compression ($\varepsilon = [ho\text{-}h]/h_0$); h is the height of the sample at the end of compression. Conversion of a force-displacement curve to a stress–strain curve removes the effect of sample dimensions, and facilitates inter-laboratory comparison of results on the same cheese type. Nevertheless, comparison of the stress–strain curves for different cheese types may be complicated by sample dimensions, especially at large strains ($\varepsilon > \varepsilon f$, where εf denotes stress at fracture) because of differences in composition and structure which affect the distribution of stress and strain within the sample and how they fracture (whether they undergo elastic fracture or plastic fracture; Table 14.2).

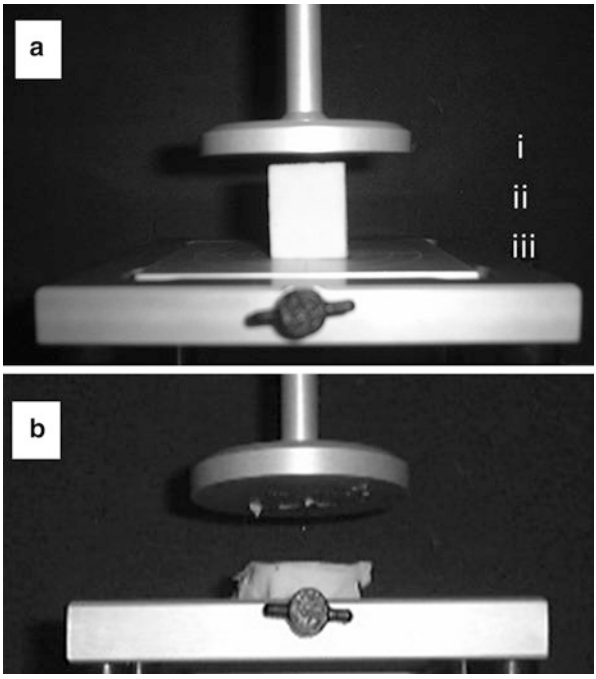


Fig. 14.12 Sample of mature Cheddar cheese before (**a**) and after (**b**) compression at a rate of 60 mm/min to 80 % of original height on a TA HDI texture analyser. The sample (ii) was paced on a fixed base plate (iii) and compressed with the mobile top plate (cross-head, i). As seen in (**b**), the sample fractured during compression and some adhered to the cross-head on retraction. Adapted from O'Callaghan and Guinee (2004)

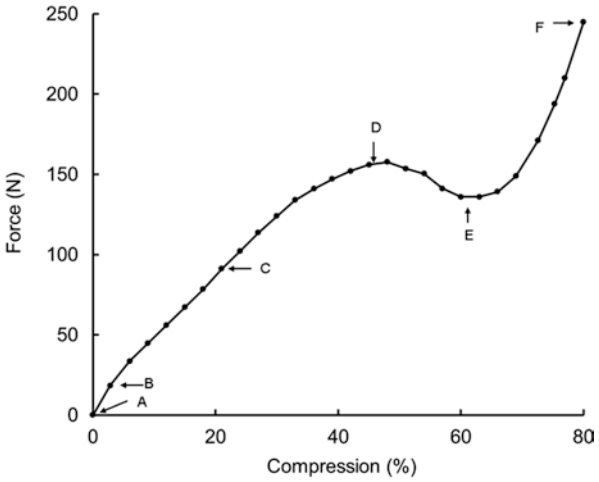


Fig. 14.13 Force-compression curve of a 6-month-old mature Cheddar cheese sample, compressed to 75 % of original height at a rate of 5 cm/min. Several regions are identifiable: AB, elastic region, BC, CD, DE, EF (see text, Sect. 14.6.3, for details). Redrawn from Guinee (2011)

The force-displacement curve for mature Cheddar cheese (Fig. 14.13) shows a number of distinct regions and enables the determination of a number of rheological parameters:

- Region A-B, where, at very low displacement, the force (and hence, σ) increases proportionally with displacement (and hence, ε). The slope of this linear region defines the compression modulus, E (i.e., $E=\sigma/\varepsilon$), which is an indication of the textural parameters, springiness or elasticity. This region may or may not be evident depending upon the cheese type, composition and degree of maturity. From a practical viewpoint, E may be an indication of springiness (e.g., where a grader sensorically monitors the resistance to small deformation, as in pressing the thumb into the outside of the cheese block; the force applied during this hand deformation is typically 18 N and σ is ~40 kPa.
- Region B-C, where the force increases less than proportionally with displacement. The slightly lower slope (dF/dh, where h is sample height) of the curve in this region compared to that in A-B is probably due to the formation of micro-cracks that do not spread throughout the sample but which allow some stress to be dissipated;
- C-D, where the increase in force with time is less than that in Region BC, and the slope force decreases markedly. The cheese begins to fracture at C, as cracks grow and spread throughout the entire sample at an increasing rate. Eventually, at D the rate of collapse of the cheese matrix overtakes the build-up of force within the cheese sample through further compression and a peak force is reached. The force and displacement at D may be used to calculate the fracture stress, σ_f, and fracture strain, ε_f, which are respective measures of the stress and strain required to bring about complete fracture of the sample.

- D-E, where the force decreases with further compression due to the fracture of the sample into pieces, an increase in surface area of the cheese, and the loss of contact of the sample with the cross-head which results in dissipation of stress energy stored within the individual pieces.
- E-F, where the force increases again as the cross-head begins to compress the fragmented pieces of cheese. The force at the end of the compression (point F) is a measure of firmness, as judged in the first bite of mastication.

The various quantities obtained from the stress/strain curve and their interpretations are given in Table 14.3.

Hence, large strain uniaxial compression is a dynamic method for which the calculated parameters (e.g. σ_f, ε_f) depend on a range of stress–strain data accumulated during the test. It is a widely used method in both research and commercial practice for the measurement of fracture properties. It is a relatively simple and rapid method that is applicable to most cheese products (natural cheese, processed cheese products and analogue cheeses), apart from cheeses that are soft or have a sticky consistency (e.g., mature Camembert with a runny-consistency, Labneh, reduced-fat Cream cheese) or cheeses that are very brittle (low εf, e.g., Club cheese, some Cream cheeses and Feta cheeses). With the latter cheese types, preparation of samples (cylindrical, rectangular) with uniform, defined dimensions may not be possible even with specialised cutting equipment such as wires (e.g., Cheese Blocker, Box Kaasgereedschap, Bodengraven, The Netherlands) or borers, owing to fracturing or sticking of the sample to the sampling equipment. Methods more

Table 14.3 Rheological parameters derived from force-compression (stress/strain) curve obtained using one-bite uniaxial compression test[a]

Parameterb[b]	Abbreviation	Interpretation	Units
Compression modulus	E	Measure of true elasticity or springiness Portion of curve where force (or stress) is proportional to strain Slope of curve in region AB, Fig. 14.13	Pa
Fracture stress	δ_f	Stress (or force) required to cause fracture of the cheese Calculated from the inflexion point (slope = 0) of the force/compression curve Stress (force per unit surface of sample) at point D, Fig. 14.13	Pa or kPa
Fracture strain	ε_f	Strain (% compression) at which the cheese fractures Strain at fracture force, point D, Fig. 14.13	Unit-less (cm/cm)
Firmness	δ_{max}	Force required to reach a given compression e.g., force at maximum compression	N

[a]*Sources*: Bourne (1978), Szczesniak (1963a), Yang and Taranto (1982), van Vliet (1991b)
[b]Fracturability was originally known as brittleness (Bourne 1978), and firmness as hardness (Szczesniak 1963a).

suited to these cheese types include those that do not require sampling with measurements being performed on the whole cheese, for example vane rheometry for soft sticky products or penetration tests using probes of different diameters for soft cheeses or brittle cheeses.

As for all large strain deformation methods, a serious limitation of uniaxial compression is the difficulty in obtaining results for cheeses with eyes.

14.7 Factors That Influence the Rheological Characteristics of Cheese as Measured Using Large Strain Uniaxial Compression

The exact shape of the force-displacement, or stress–strain, curve and the magnitude of the various rheological parameters obtained from force-deformation curves depend on test conditions, cheese variety, composition and degree of maturity (Shama and Sherman 1973; Culioli and Sherman 1976; Dickinson and Goulding 1980; Sherman 1988; Visser 1991; Prentice et al. 1993).

14.7.1 Test Conditions

14.7.1.1 Sample Dimensions

Increasing the height of cylindrical samples, in the range 0.75–3.5 cm, reduces the stress at fracture stress (σ_f) and full compression (σ_{max}), and increases the fracture strain, ε_f (Culioli and Sherman 1976; Fig. 14.14), with the effects being more pronounced as the compression rate is increased in the range 5–50 cm min^{-1}. Analogously, the stress required to obtain a given deformation increases as the diameter:height ratio of cylindrical samples is increased, a trend which is more accentuated with the degree of compression (Sherman 1988; Fig. 14.15). The influence of sample dimensions is related to surface friction (between the contact surfaces of cheese sample and instrument plates) that is associated with squeeze-flow behaviour of the sample into a barrel shape as the cheese is increasingly compressed. Friction shear forces impede the lateral movement of the cheese surfaces along the texture analyser plates during compression and contribute to the development of a transverse compressive stress, in addition to the axial compressive stress. Hence, there is a progressive increase in diameter (cylindrical samples) or width (rectangular or cube samples) of the cheese sample from the flat surfaces to a maximum in the central region of the cheese (Fig. 14.16). Experiments with cylindrical samples of Gouda cheese (h = 2.5 cm, diameter 2.5 cm) showed that barrelling, which was first observed at 20 % compression, increased with degree of compression in the range 20–80 %, especially at high compression rates (i.e., >50 cm min^{-1}) (Sherman 1988). Barrelling affects the axial force per unit area (and hence stress) at

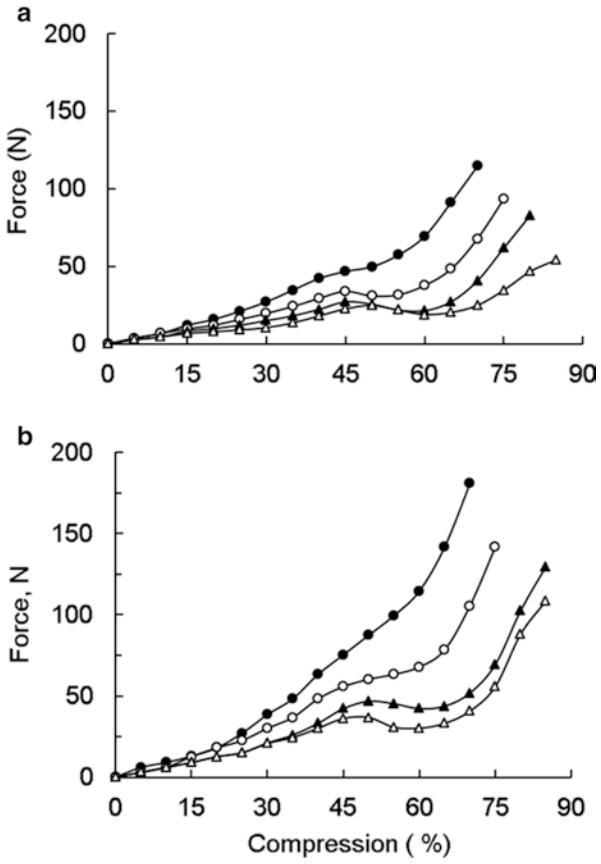


Fig. 14.14 Effect of sample height on the force-compression behaviour of cylindrical samples (2.5 cm diameter) of Gouda cheese, compressed at 5 (**a**) or 50 (**b**) cm/min. Sample height: 0.75 (*filled circle*), 1.5 (*open circle*), 2.5 (*filled triangle*) or 3.5 (*open triangle*) cm. Redrawn from Culioli and Sherman (1976)

different layers within the sample, with the axial stress decreasing with distance from the plates as the sample area over which the axial force is exerted increases. Nevertheless, the stress is calculated based on the area of contact between the cheese and plates; hence, the apparent stress is higher than the true stress.

Friction can be reduced by lubrication of the contact surfaces with mineral oil or grease. In the absence of surface friction, there is greater lateral movement of the sample surfaces along the plates during compression, resulting in an increase in the actual surface area over which the force is applied; consequently, with lubrication, the actual stress does not increase as rapidly as in the absence of lubrication (Sherman 1988). Lubrication can reduce the stresses by as much as 50 % and increase the observed fracture strain (ε_f) from ~0.45 to 0.55, in the case of Gouda

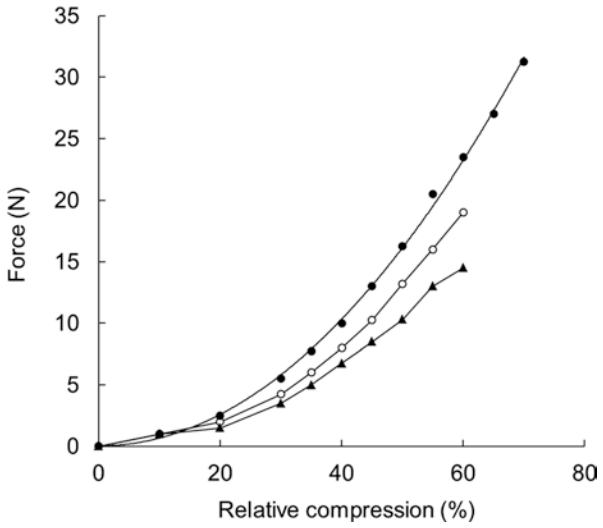


Fig. 14.15 Effect of diameter/height ratio on the force-compression behaviour of cylindrical samples (1.64 cm diameter) of German Loaf Cheese. Sample height (cm) and diameter/height ratio:1.65 and 1.0 (*filled triangle*), 1.3 and 1.25 (*open circle*), 1.1 and 1.5 (*filled circle*). Redrawn from Sherman (1988)

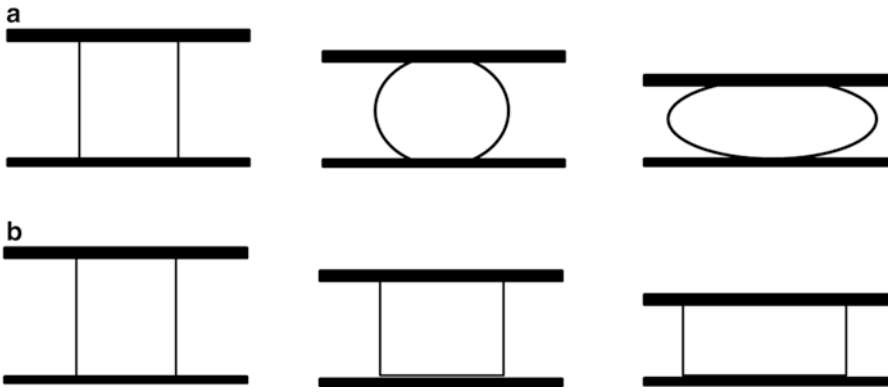


Fig. 14.16 Schematic of barrelling of: (**a**) A cylindrical cheese sample during compression. Barreling leads to a progressive increase in the length of the central region of the cheese sample relative to the sample surface where friction between the cheese and the compression plate (crosshead) restricts the cheese surface from spreading uniformly. This results in larger surface area in the central region and a lower stress (force per unit surface area) in the central region than at the surface. (**b**) Ideal compression where the sample spreads uniformly during compression, maintaining a constant force per unit area across the sample. Compiled using data from various sources: Culioli and Sherman (1976), Vernon Carter and Sherman (1978) and Prentice et al. (1993)

cheese at 20 °C with an aspect ratio (i.e., height/width) of unity and a cross-head speed of 500 mm/min (Sherman 1988). However, the frictional effect increases with cross-head speed. At a low cross-head speed (5 mm/min), lubrication reduced σ_f by ~20 % for Cheddar cheese where the aspect ratio was 0.35, with the effect becoming more pronounced (of the order of 20–30 %) at $\varepsilon > \varepsilon_f$ (Casiraghi et al. 1985). In contrast to lubrication, bonding of the cheese surfaces to the compression plates (e.g. using cyanoacrylate ester adhesive) had relatively little effect on σ_f, ε_f and σ_{max}. A similar trend was found for Mozzarella and processed cheese spread (Casiraghi et al. 1985).

14.7.1.2 Sample Shape

Sample shape (i.e., cubical or cylindrical) affects both the form of the stress–strain curve, and the changes in magnitude of stress on increasing strain. For both shapes, only slight differences have been reported in the stress–strain behaviour of Gouda cheese up to the fracture point (~40 % compression). However, on further increasing strain (to $\varepsilon > \varepsilon f$), compression of cubic samples resulted in significantly higher stress (and σ) than in cylindrical samples (Culioli and Sherman 1976). Moreover, cylindrical samples (1 cm high, 2 cm diameter) showed a clearly defined fracture stress peak at 40 % compression, after which stress decreased moderately (by ~16 %) to a minimum at 60 % compression, and thereafter increased more steeply to 80 % compression (Culioli and Sherman 1976). Cube samples (1 cm sides) exhibited neither a well-defined fracture stress peak nor a minimum stress, and stress increased very steeply following fracture.

For both sample shapes, increasing the sample dimensions resulted in higher stress being required to achieve a given deformation. However, for comparable dimensions (a cylinder with a height, diameter, and volume of 1 cm, 1 cm and 0.78 cm^3, respectively, and a cube with a volume 1 cm^3), cubic samples were found to require a higher stress to achieve a given percentage compression than cylinders at compression levels >40 % (Culioli and Sherman 1976). This effect may related to differences in sample volume, area of sample contact with texture analyser plates and surface friction.

14.7.1.3 Deformation Rate (Compression Velocity)

Generally, increasing the rate of deformation (also referred to as strain rate, rate of deformation, or compression speed) has been found to increase both σf and σ at strains $< \varepsilon f$, but has little effect on the ε_f for various cheese types including Cheddar, Cheshire, Double Gloucester, Gouda, Leicester, and Stilton (Figs. 14.14 and 14.17; Shama and Sherman 1973; Culioli and Sherman 1976; Dickinson and Goulding 1980; Sherman 1989; Creamer and Olson 1982; Luyten et al. 1991a; Ak and Gunasekaran 1992; Xiong et al. 2002). Comparison of instrumental force-compression data obtained at different compression rates with

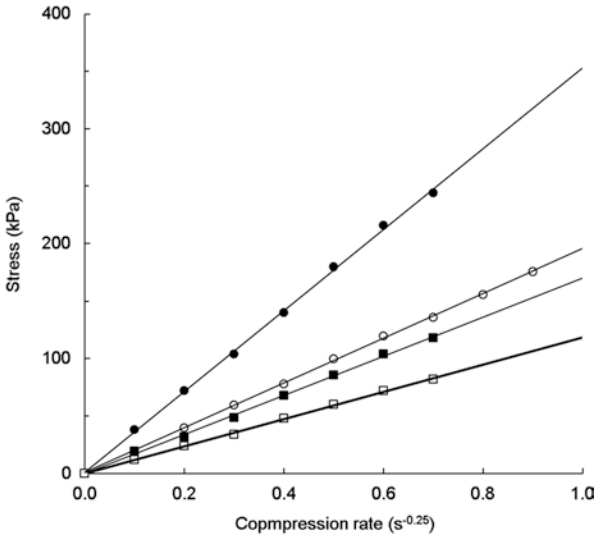


Fig. 14.17 Effect of rate of compression on stress at fracture for different cheese types: Double Gloucester (*filled circle*), Cheddar (*open circle*), Leicester (*filled square*) and Cheshire (*open square*). Redrawn from Prentice et al. (1993)

consumer evaluations (both orally and using the fingers) of a wide range of solid foods suggests that low compression rates (≤50 %) correspond to measurement of firmness or hardness by squeezing the cheese between the fingers, whereas higher compression rates (~≥70 %) correspond to firmness as judged on chewing the cheese (Sherman 1989). Evaluation of food by compressing between the fingers depends more on the cheese elasticity, whereas in the mouth it depends on the cumulative contributions of elasticity, fracture and flow.

Various studies (Boyd and Sherman 1975; Sherman 1989; Xiong et al. 2002) showed that high compression rates (e.g., >50 cm/min, ideally up to 100 cm/min) for cheese are essential to obtain close correlation between cheese hardness (as measured by a consumer panel) and firmness measured by uniaxial compression. Xiong et al. (2002) investigated the effects of altering the % deformation (10–90 % compression) and deformation rate (1–60 cm/min) on the relationship between scores for the hardness of a range of cheese types (including Cheddar, Havarti, Muenster, Monteray Jack, Gouda) obtained using a sensory panel with the firmness scores (maximum compression force, δ_{max}) for the same cheeses measured using a texture analyser. The optimal % deformation and deformation rate for maximizing the correlation between sensory and instrumental scores 70–90 % and 60 cm/min, respectively. Such a trend is expected based on chewing studies which have found that people chew typically at a rate of 40–80 masticatory strokes per minute, oral mastication involves compression to ≥70 %, and hardness is evaluated during the first down stroke.

14.7.1.4 Assay Temperature

Raising the sample assay temperature (e.g., in the range 0–32 °C) during compression results in a marked reduction the elastic modulus, fracture stress and firmness. This effect is attributed to liquefaction of the fat fraction, which reduces the elastic contribution of fat globules enclosed in the calcium phosphate *para*-casein network, contributes to lubrication of fracture surfaces, and a probable reduction in the surface friction between the sample and the instrument plates as liquid fat is exuded at the flat surfaces (Culioli and Sherman 1976; Dickinson and Goulding 1980; Casiraghi et al. 1989; Ak and Gunasekaran 1992)

14.7.2 Cheese Structure, Composition and Maturity

The viscoelasticity of cheese results from the interactive rheological contributions of its individual constituents. Cheese rheology (and texture) is complex, with the final outcome influenced by the interacting effects of many parameters including:

- volume fraction of the calcium phosphate-protein network,
- levels of fat and moisture (solvent) and the ratio of solid-to-liquid fat
- solvent quality (pH, salt level),
- degree of protein mineralisation and calcium-induced cross-linking of the casein comprising the network,
- degree of casein (network) hydrolysis as affected by maturation/storage conditions (time, temperature, moisture loss)
- microstructure
- macrostructure

Moreover, it is important to stress that it is difficult to elucidate the direct effects of altering the concentration of any one compositional component, including protein, on the rheology of cheese since the levels of the major compositional components (fat, protein, moisture) tend to vary simultaneously, e.g., fat reduction is accompanied by increases in the levels of protein and moisture and decreases in the levels of moisture-in-non-fat substance and fat-in-dry matter. Hence, caution is required in categorically assigning particular rheological attributes to any one parameter. Nevertheless, analysis of the rheological results from various studies on cheeses differing in composition does provide insight on the effects of different compositional components.

14.7.2.1 Protein Concentration and Protein Hydrolysis

On the application of a stress to a cheese product, the *para*-casein network will at first control the deformation. As the concentration of casein increases, the intra- and inter-strand linkages become more numerous and the network displays greater

elasticity and is more difficult to deform (Fig. 14.18). Hence, reduced-fat Cheddar which contains a higher concentration of structural network per unit volume than full-fat Cheddar is firmer and has a higher σ_f than the latter (Fig. 14.19; Emmons et al. 1980; Mackey and Desai 1995; Fenelon and Guinee 2000). This is confirmed by the positive correlations between the content of protein and fracture stress and/or firmness of various cheese products, including Cheddar cheese, Meshanger cheese and processed cheese (de Jong 1978b; Guinee et al. 2000a; Guinee and O'Callaghan 2013)

Factors that promote weakening of the casein matrix reduce the level of stress required to achieve a given deformation. Hence, the σ_f and firmness at maximum compression (δ_{max}) of cheese usually decrease with ripening time due to hydrolysis (proteolysis) and hydration of the casein strands, both of which reduce the rigidity of the casein network (Fig. 14.20); this is analogous to the ease with which spaghetti can be twisted with a fork upon chopping it with a scissors. These changes are inversely correlated with the extent of primary proteolysis, as measured by the increase pH 4.6-soluble N, or by reduction in level of intact casein (Guinee et al. 2001; Rynne et al. 2004; Hou et al. 2014). The structure of cheese is weakened significantly by the early hydrolysis of αs_1-CN by residual chymosin, at the Phe_{23}-Phe_{24} peptide bond. αs_1-CN f1-24 is strongly hydrophobic and interacts with the hydrophobic regions of other αs_1- and β-CN molecules, and thus contributes to the overall continuity and integrity of the *para*-casein network (Creamer and Olson 1982; Lawrence et al. 1987). Nevertheless, O'Mahony et al. (2005) concluded that the softening of Cheddar cheese during the early stages of ripening was essentially independent of the hydrolysis of αs_1-CN at Phe_{23}-Phe_{24} and was instead more closely correlated to solubilisation of colloidal calcium phosphate associated with the *para*-

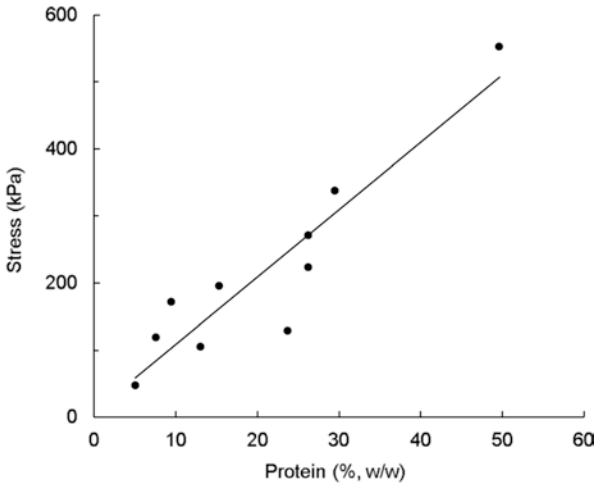


Fig. 14.18 Effect of protein content on cheese firmness; data refer to ten different types of hard cheese and a pasteurized processed Cheddar cheese. Drawn from data of Chen et al. (1979)

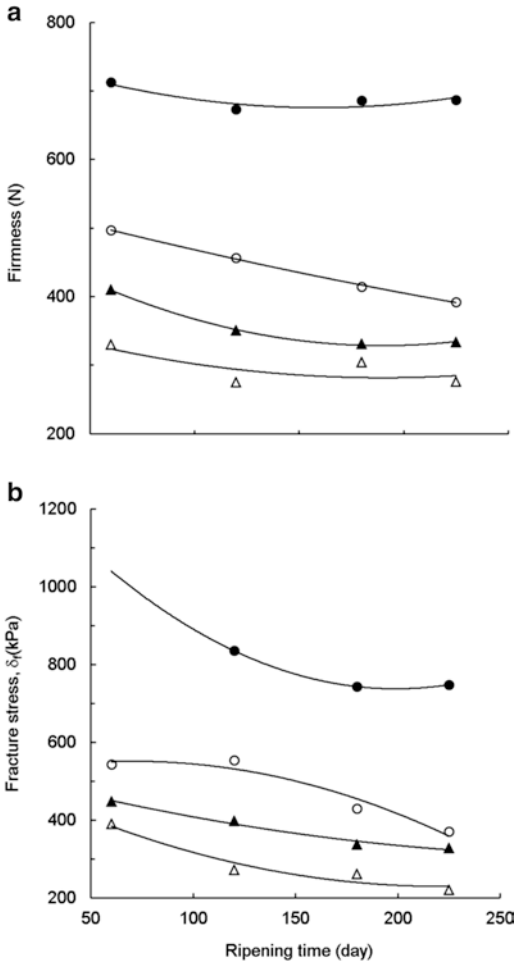


Fig. 14.19 Effect of fat level (6 %, *filled circle*; 17 %, *open circle*; 22 %, *filled triangle*; 33 %, *open triangle*) on the firmness (**a**) and fracture stress (**b**) of Cheddar cheese, compressed at 5 cm/min. The protein contents of the cheeses were 39.3, 33.4, 31.2 and 26.0 % (w/w), respectively. Redrawn from Fenelon and Guinee (2000)

casein network of the curd. These authors reported that the addition of pepstatin (a potent competitive inhibitor of chymosin) at a level of μmol/L to the curds/whey mixture at the start of cooking led to a notable reduction in the hydrolysis of α_{s1}−casein, inhibited the rate of formation of αs_1-CN (f24-199) completely, but had little effect on the hydrolysis of β-casein during the first 21 days of ripening. Yet, the hardness of all cheeses decreased significantly during this period, albeit at a much lower rate in the pepstatin-containing cheeses. The degradation of β-casein probably contributes also to the softening of cheeses during maturation, especially in cheese scalded at a high temperature (e.g., 50–55 °C) as in Emmental, low-moisture Mozzarella and Parmesan. This suggestion is supported by the findings of Bogenrief and Olson (1995) and Yun et al. (1993a, b) which showed that an increase in the degradation of β-casein in Cheddar and Mozzarella cheeses, respectively, coincided with lower fractures stress or lower firmness during maturation.

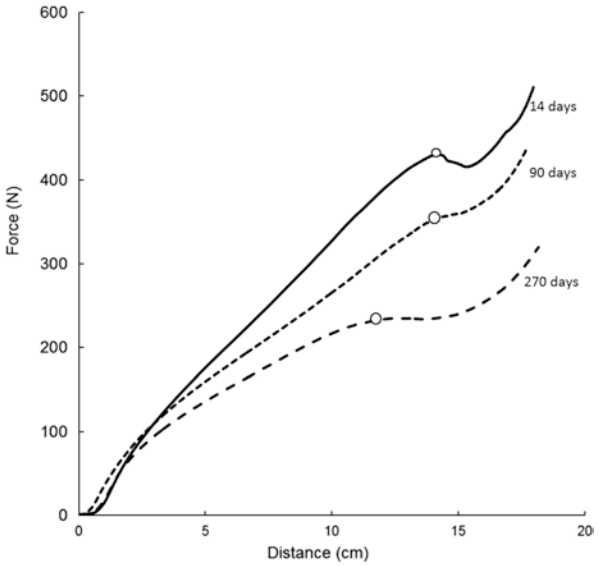


Fig. 14.20 Force-compression curves for experimental Cheddar cheeses (2.5 cm cubes) compressed to 70 % at a rate of 60 mm/min. The cheeses were ripened for 14 (*solid line*), 90 (*dotted line*) or 270 (*broken line*) days at 8 °C; the corresponding levels of proteolysis, as measured by % pH 4.6-soluble N (as % of total N) were 3.7, 13.9 and 21.3, respectively. The fracture point, determined from the inflection point of the curve prior to the slope becoming negative, is indicated by the open circle (*open circle*). McCarthy and Guinee (unpublished data)

In contrast to the above, the firmness of some cheeses (e.g., brine-salted and/or surface dry-salted varieties that are not packaged for part of their ripening period) may increase initially even though proteolysis occurs during this period. The increase in firmness is a consequence of the loss of moisture and the concomitant increase in the protein concentration. Other factors such as changes in pH and the increase in the salt content in the inner regions as a result of inward diffusion from the surface rind zone, may also contribute to the initial increase in firmness. However, when the composition has stabilised, the softening associated with proteolysis becomes dominant, and the firmness and fracture stress decrease with ripening time (de Jong 1977; Visser 1991).

14.7.2.2 Fat Content

The contribution of fat, at a given volume fraction, to the rheological properties of cheese depends on its physical state, and therefore on the temperature, which controls the ratio of solid-to-liquid fat. At a low temperature (i.e., <5 °C), where the milk fat is predominantly solid, the fat adds to the elasticity of the casein network.

The solid fat globules limit the deformation of the casein matrix, as the deformation of the latter would also require deformation of the fat globules enmeshed within its pores. As the proportion of liquid fat increases (e.g., from ~20 % at 5 °C to ~60 % at 20 °C), the fat behaves more as a fluid and confers viscosity rather than elasticity or rigidity to the cheese. Moreover, liquid fat acts as a lubricant on fracture surfaces of the casein matrix, and thereby reduces the stress required to fracture the matrix. Hence, for a given fat level, raising the assay temperature (e.g., in the range 0–32 °C) during compression results in a marked reduction the elastic modulus, fracture stress and firmness (Dickinson and Goulding 1980; Visser 1991). Increasing the fat-in-dry matter level of cheese, while retaining the other compositional parameters constant, is paralleled by a decrease in σ_f, with the effect becoming more pronounced as the temperature is increased. Generally, an increase in fat content of cheese is accompanied by a reduction in the levels of protein and moisture and reductions in the σ_f and firmness (Fig. 14.19).

14.7.2.3 Moisture

The third major component of cheese is moisture, which acts as a plasticizer in the protein network, thereby making it less elastic and more susceptible to fracture on compression. Thus, increasing the moisture content of cheese, while maintaining the compositional parameters relatively constant, results in reductions in E, σ_f and firmness (Creamer and Olson 1982; Luyten 1988; Visser 1991). The ε_f increases slightly with moisture content to an extent dependent on cheese pH, maturity and moisture range (Visser 1991).

14.7.2.4 Calcium Content

Generally, it is considered that increasing the calcium content of cheese, and more specifically the insoluble, casein-bound calcium, increases the firmness and force required to fracture cheese. For a given calcium content, the ratio of insoluble-to-soluble calcium increases as the pH is increased (Guinee et al. 2000c; Kindstedt et al. 2001; Ge et al. 2002).

Pastorino et al. (2003) varied the calcium content of Mozzarella cheese from ~300 mg to 1600 mg/100 g by injection of the final cheese with $CaCl_2$ solution. The increase in calcium content resulted in increased *para*-casein aggregation, a linear reduction in moisture content (from ~50 to 38 %) and pH, and a three fold (linear) increase in harness. An analogous approach by O'Mahony et al. (2006) increased the calcium content of Cheddar cheese from 420 to 890 mg/100 g by immersing cheese slices in $CaCl_2$ solutions of different concentrations. In corroboration with the findings of Pastorino et al. (2003), increasing calcium content reduced moisture content and pH but increased firmness and fracture stress.

14.7.2.5 pH

In milk and cheese, pH affects casein charge (due to its effect on the extent of dissociation of amino acid side chain groups) and the level of calcium bound to the casein; bound calcium occurs as colloidal calcium phosphate, CCP (Ca_3PO_4 nanoclusters) bound electrostatically to serine phosphate groups, and as caseinate calcium complexed electrostatically with dissociated carboxyl groups of aspartate and glutamate. CCP dissolves as the pH is reduced and is fully soluble at pH 5.3, while caseinate calcium is fully soluble at pH 4.6. For a dilute casein dispersion with ~ ≤5 % casein, pH affects the degree of casein hydration, which as a function of pH in the region 4.6–6.6, is maximal at ~5.3 and minimal at 4.6 (Creamer 1985); the pH value for maximum hydration may differ somewhat for more concentrated casein/*para*-casein systems such as hard/semi-hard cheeses. The changes in casein hydration with pH reflect concomitant alterations in the balance of forces favouring interaction of molecules (e.g., reduction in negative charge on casein) or dissociation (solubilization of CCP and casein-bound calcium).

The interrelationship between pH and casein-bound calcium and its influence on the texture is widely recognized in practice. Hence, hard rennet-curd cheeses with a relatively high pH (e.g., 5.3–5.6, e.g., low-moisture Mozzarella, Gouda, Maasdam, Emmental) are generally considered to be longer and more bendable (higher εf) than cheeses with a lower pH (e.g., 4.9–5.3, e.g., Cheshire, Cheddar, Parmesan, Stilton) which are comparatively shorter and more crumbly (lower εf). Consequently, Lawrence et al. (1987) illustrated a spectrum of texture attributes across different cheese varieties as a function of pH: the pH region 5.5–5.3 with springy; 5.35–5.25 with plastic, 5.2–5.0 with cheddary, 5.05–4.9 with mealy, 4.9–4.8 with short and 4.8 – 4.5 with non-cohesive. These observations are consistent with rheological trends, showing a reduction in ε_f of Cheddar and Gouda (Luyten et al. 1991a; Visser 1991a) cheeses, as the pH was reduced from 5.25 to 5.0 and 5.25 to 4.9, respectively. Furthermore, reducing the pH of Gouda cheese, while retaining the moisture content relatively constant (~44.5 % moisture) resulted in a decrease in the elastic modulus (E) and σ_f (Visser 1991; Fig. 14.21), with the level of the effect depending on age (Luyten 1988).

In contrast, increasing the pH in the range 5.2–5.6 results in a marked increase in σ_f (to values much higher than those at pH < 5.2) and a slight increase in E (Fig. 14.21). The ε_f of young (1-week-old) Gouda cheese, was maximal at pH 5.2 and decreased on lowering or raising the pH to 4.8 and 5.6, respectively. However, the pH at which ε_f is maximal increases with ripening time, e.g., from ~5.2 in a 1-week old Gouda ~ to 5.4 in 3-month-old Gouda cheese. Differences in pH may help to explain the different rheological characteristics exhibited by some common cheeses on compression. Low pH cheeses (e.g., Cheshire, Feta) tend to have low values of σ_f and ε_f and crumble into many pieces on fracturing, whereas relatively high pH cheeses (e.g., i.e., pH 5.35–5.50), such as Emmental and Gouda exhibit higher values of σ_f and ε_f and tend to fracture into larger pieces (Prentice et al. 1993). The effect of pH is probably a consequence of its influences on (1) the ratio of soluble-to-colloidal Ca, (2) the degree of *para*-casein hydration (which

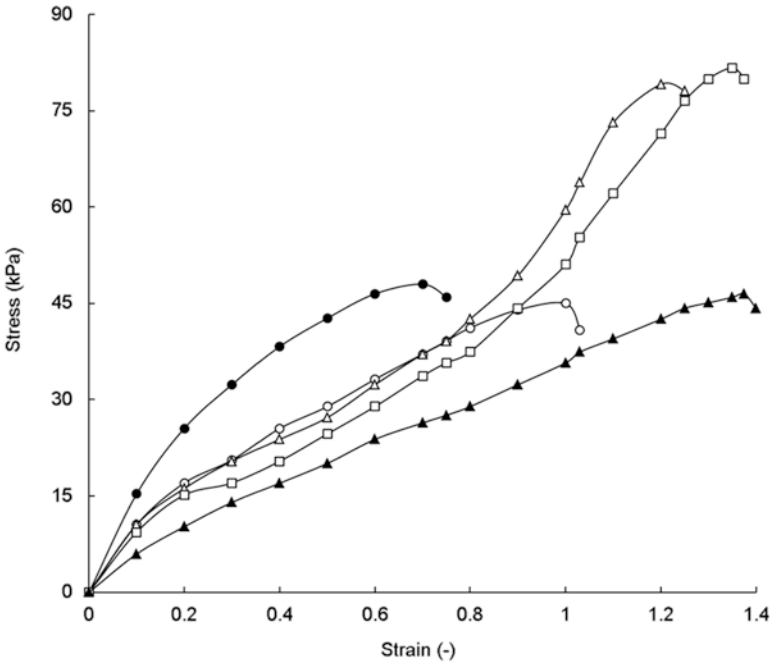


Fig. 14.21 Effect of pH on the force-compression behaviour of 1-week-old Gouda cheeses with similar gross composition. Cheese pH: 5.02 (*filled circle*), 5.1 (*open circle*), 5.2 (*filled triangle*), 5.43 (*open square*), 5.58 (*open triangle*). Redrawn from Visser (1991)

is maximal at ~ pH 5.2) and (3) the type/extent of protein interactions. Moreover, the effect of pH appears to be related to other factors such as the levels of moisture, NaCl and Ca, and degree of casein hydrolysis (Walstra and van Vliet 1982; Strange et al. 1994; Carr et al. 2002).

14.7.2.6 Salt Content

The salt content of rennet-curd cheeses varies from about 0.79 % g in Emmental to about 3–4 % in Feta (see Chap. 9); however, because salt is dissolved in the moisture phase, the effective concentration is much higher (~2 and 6 g/100 g, respectively). Increasing the salt content of a given cheese variety reduces its moisture content (Kelly et al. 1996; Rulikowska et al. 2013), by ~1.5–2 % for every 1 % increase in NaCl (Guinee and Fox 1986). Simultaneously, the firmness and fracture stress increase (Pagana and Hardy 1986; Rulikowska et al. 2013) probably as a result of the reduction in moisture content, but probably also as a consequence of an increase in casein hydration and swelling of the *para*-casein network as the salt level in the cheese moisture increases to ~6 %; this would have the effect of creating a more voluminous, continuous network which becomes increasingly more

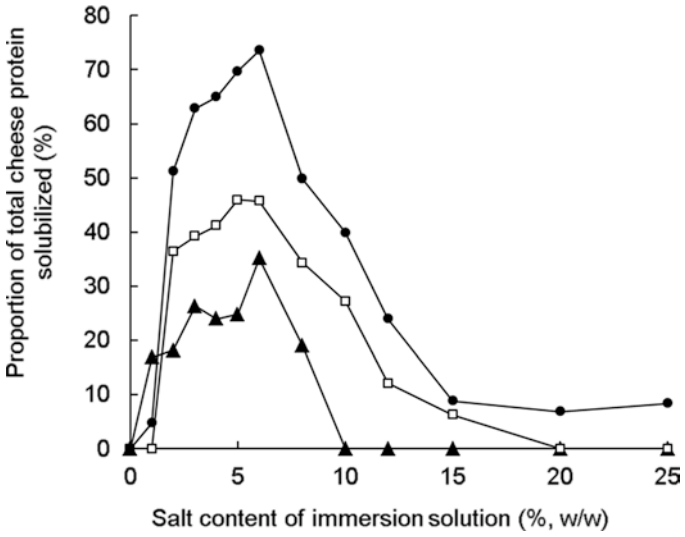


Fig. 14.22 Effect of NaCl concentration and time (1, *filled triangle*; 2, *open square*; or 3, *filled circle* days) on the solubilization of protein in cheese discs placed in salt solutions. In the model experiment, designed to simulate the effect of salt content in the moisture phase of cheese on protein solubilization, cylinders of unsalted Cheddar cheese curd (7.3 g) were placed in NaCl solutions (400 mL) of different concentrations (0–25 %, w/w, NaCl with 0.02 % NaN_3) in capped containers and incubated at 20 °C. The brine solutions containing the cheese sample were gently agitated every 8 h, and a portion was analyzed daily for protein content, which was expressed as a percentage of the total protein in the unsalted cheese. Guinee (unpublished results)

constrained and difficult to deform. The effect of salt content on casein hydration has been demonstrated in model experiments in which discs of unsalted Cheddar cheese (~7.2 g) were placed in NaCl solutions (400 mL) with a salt content ranging from 0 to 25 %(w/w) (with ~0.03 % NaN_3 added as preservative) for up to 72 h to simulate the potential effects of varying salt-in-moisture content, as found in the moisture phase of different cheese varieties (e.g., ~ from ~1.2 % in Emmental to ~9 % in Parmesans and Roquefort). The % of total cheese protein solubilized in the salt solution following salting was used as an index of casein hydration. These experiments showed that casein hydration increased progressively with time from ~35 % of total cheese protein after 1 day to ~75 % after 3 days in 6 % NaCl (Fig. 14.22). Casein hydration after 72 h increased from ~0 at 0 % NaCl to a maximum of ~75 % at 6 % NaCl and decreased sharply at higher salt levels (>6 %) to ~7 % of total protein at ≥ 15 % NaCl. These trends suggest salting-in of the casein (hydration and network swelling) at ≤ 6 % NaCl in moisture and salting-out (casein dehydration and network contraction) at > 6 % NaCl. Thus, increasing the mean level of NaCl in brine-salted low-moisture Mozzarella from 0.13 % (w/w) (unsalted) to 1.4 % (w/w) increased protein hydration significantly (Guo et al. 1997).

Visser (1991) reported on the effect of increasing the level of salt-in-moisture (S/M) in the range 0–11 % on the rheology of Gouda cheeses in which the moisture

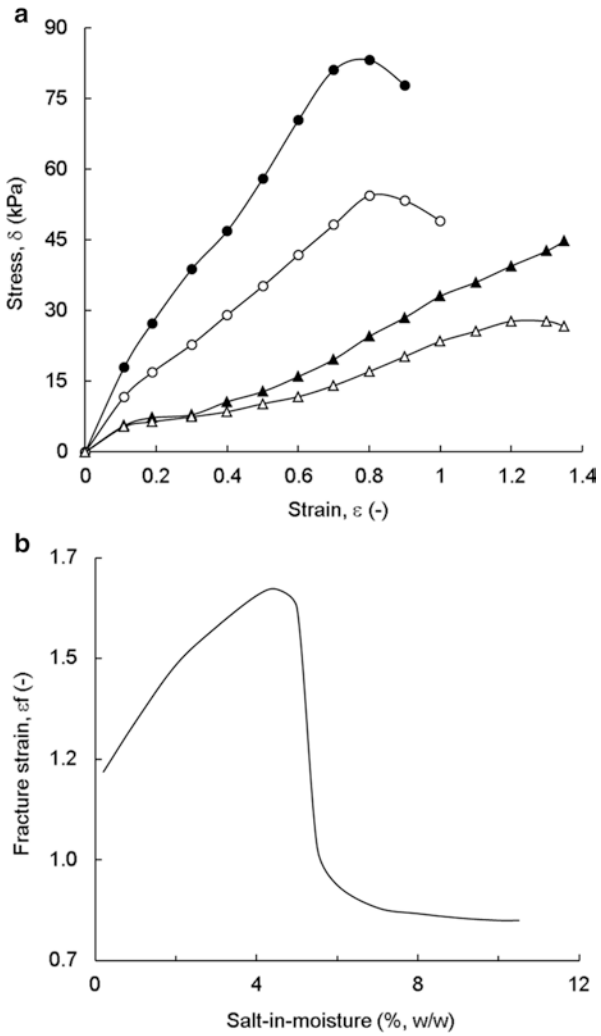


Fig. 14.23 Effect of salt-in-moisture (S/M) level on the force-compression behaviour (**a**) and fracture strain (**b**) of Gouda cheeses, where the composition was otherwise similar. S/M levels: 0.4 (*open triangle*), 3.3 (*filled triangle*), 7.3 (*open circle*), 11.3, (*filled circle*), g/100 g. Redrawn from Visser (1991)

content was held constant through alteration of the manufacturing process. Increasing S/M concentration coincided with increases in E and σ_f (Fig. 14.23a). The fracture strain, ε_f, increased slightly to a maximum at 6 % S/M, then decreased sharply (to a value of approximately half the maximum) at 7 % S/M and thereafter decreased slightly (Fig. 14.23b). The changes in εf are consistent with the increase in casein hydration/swelling in the range 0–6.0 % S/M and the subsequent dehydration/contraction at higher S/M levels (Fig. 14.22).

14.7.2.7 Macrostructure

The macrostructure, representing the gross aspects of structure within the moulded cheese, such as curd granule junctions, eyes, and slits/cracks, is observed visually by the unaided eye or at a very low (<10) level of magnification by light microscopy. The knitting of individual curd particles or chips/pieces into a macrostructure is influenced by many factors including their microstructural-related properties (which affects their potential to deform and flow into, and fuse with, other curd particles when subjected to moulding and pressing), level of calcium bound by *para*-casein, protein-to-fat ratio, salt content, pH, curd particle dimensions and surface properties, degree of protein hydration, pressing conditions (temperature and pressure) and storage conditions (Guinee 2016; see Chap. 18). After manufacture, discontinuities at the macrostructural level exist in the form of curd granule junctions or curd chip junctions (Kaláb 1977, 1979; Lowrie et al. 1982) to a degree dependent on factors which influence their ability to knit and fuse together (see Sect. 14.3.2). Dry-salted cheeses are macroscopically more heterogeneous than brine-salted cheeses owing to the method of manufacture, which involves milling or breaking fused curd mass into chips (Leicester, Cheddar, Cheshire) or pieces (Stilton) close to the end of manufacture when the desired pH (~5.0 5.3) has been reached, dry salting the chips/pieces, pressing/moulding. As discussed in Chap. 9, the dry salt dissolves at the surface of the chips forming a concentrated brine which results in a high degree of protein dehydration and a low protein-to-fat ratio at the chip surfaces compared to the interior. In effect, milling and dry salting result in the development of a dehydrated surface 'skin' which impairs the ability of the curd chips to knit closely during subsequent moulding and pressing. Conversely, the generic manufacturing method of brine-salted cheeses (moulding of curd particles immediately after whey drainage while the curd particles are still warm and relatively moist, pre-pressing and pressing of moulded curds while inverting) is more amenable to the formation of uniform packing and knitting of the curd particles and the development of a more uniform macrostructure. However, dynamic changes during ageing of all cheeses (reduction in the intra- and inter-particle differences in composition, and increases in protein hydrolysis and fat coalescence) facilitate graduation to a more coherent structural continuum as reflected by the disappearance of inter-particle boundaries and the formation of a more homogeneous mass (Guinee 2016).

While little direct evidence is available on the contribution of cheese macrostructure to rheology, it is likely to have a major effect, especially in terms of fraction strain (εf), and hence the shortness/crumbliness (low εf) or longness/pliability/rubberiness (high εf). Hence, the % deformation at fracture of 3 month-old Leicester (a cheese formed by pressing dry-salted curd chips in a similar manner to Cheddar) was found to be markedly lower than that of Gouda or Emmental cheese of comparable age (~25, 45 and 65 % respectively) (Walstra and van Vliet 1982).

14.8 Cheese Texture

As discussed in Sect. 14.2, cheese texture is a sensory characteristic which can be measured directly only by sensory analysis. Texture perceptions arise from a complex array of sensory inputs that occur both prior to and during food consumption (Brennan 1988; Delahunty and Drake 2004). Consumption of cheese involves the following series of events:

- Visual assessment (e.g., eye distribution in a Swiss-type cheese, granularity of Cottage cheese, whiteness of Feta cheese). The visual perception may create an important first impression about the anticipated taste and texture of the cheese. For instance, the granular, dry (non-glossy) appearance of Parmesan may create the perception that it will likely have a dry, grainy mouthfeel; the surface sheen of a freshly sliced Camembert, or its absence, may be taken as evidence of its state of maturity, flavour and palatability.
- Assessment by touch (e.g., the resistance of a piece of string cheese to touch or that of Cheddar or Camembert when it is cut with a knife or punctured with a fork).
- Eating, which occurs in four phases:
  - Placement in mouth, including contact with nerve endings in the tongue and cheeks that contribute to sensations referred collectively to as somaesthesis (e.g., sensations of touch, pain, warmth and cold). The ingested cheese is compressed by various parts of the oral cavity (palate and inside of lips and cheeks), and concomitantly the counter stress exerted by the cheese is detected by nerve endings.
  - An initial bite by the teeth (resistance to cutting by the incisors may be involved)
  - Chewing and mastication. The cheese is compressed repeatedly, mainly between the molars, moved towards the teeth by the tongue, and mixed with saliva. This results in diminution of the cheese to a pasty bolus ready for swallowing. Evidence suggests that the tooth socket is elastic, permitting vertical and horizontal movement of the teeth (Brennan 1988). The degree of deformation of the periodontal membrane (the structure which surrounds the tooth in the socket and attaches it to the jaw bone) is the stimulus which detects the mechanical properties of the cheese being eaten.
  - Swallowing of the cheese, during which the bolus is pressed out of the oral cavity

In the process of eating, the cheese is subjected to cutting, shearing and compression forces which fracture it and reduce it to a state ready for swallowing. The objective of sensory texture evaluation is to translate the cumulative sensations perceived into descriptors or scores.

14.8.1 Sensory Measurement of Cheese Texture

The sensory methods used to measure food texture are of three general types (Brennan 1988; Powers 1988; Jack et al. 1995; Delahunty and Drake 2004):

- attribute (or profiling) methods,
- difference methods
- preference methods.

Attribute methods, which are used most widely, include:

- the texture profile analysis (TPA) method,
- descriptive analysis,
- free choice profiling.

In the texture profile method, three categories of texture characteristics were proposed by Szczesniak (1963a): (1) mechanical characteristics, which are related to the reaction of food to stress; (2) geometrical characteristics, which are related to size, shape and orientation of particles within the food, and (3) other characteristics, which are related to the perception of moisture and fat contents of a food. The mechanical characteristics were in turn divided into primary characteristics such as hardness, cohesiveness, viscosity, elasticity and adhesiveness, and secondary characteristics such as brittleness, chewiness and gumminess. The definitions of the mechanical characteristics are summarized in Table 14.4. Each of the above attributes is scored on a nine-point equidistant scale relative to a standard reference cheese.

Table 14.4 Textural profile parameters derived from two-bite compression[a]

Primary parameters	Secondary parameters
1. Hardness—measure of force required to achieve a given deformation 2. Cohesiveness, a measure of: • strength of internal bonds making up the body of the product, • tendency of cheese to remain together, and resist breaking into several pieces, during compression. 3. Springiness or elasticity—a measure of: • the rate at which a deformed material returns to its original dimensions after the deforming force is removed, • the distance (height) the cheese recovers during the time that elapses between the end of the first compression and the start of the second compression 4. Adhesiveness—the work necessary to overcome the attractive forces between the surface of a food and surface of other materials with which it comes in contact, e.g., the teeth, palate and tongue.	1. Fracturability (brittleness)—measure of the force at which the material fractures, with a brittle material being one that is characterized by a high degree of hardness, and low degree of cohesiveness. 2. Chewiness—the energy required to masticate a solid food material to a state ready for swallowing 3. Guminess—the energy required to disintegrate a semi-solid food to a state ready for swallowing

[a]Compiled using information from different sources. Based on Szczesniak (1963a), Bourne (1978) and van Vliet (1991)

Descriptive textural analysis involves the scoring of samples in terms of intensity, using a fixed consensus vocabulary of textural descriptors that are agreed upon jointly by the sensory panel and the trainers during training sessions. Standard products (of the same generic type as the product being evaluated) considered to exhibit one or more descriptors may be used to define characteristics, with the panel members being repeatedly exposed to the standard products during training.

The free choice profiling (FCP) method of texture evaluation is similar to that of descriptive analysis, except that the sensory descriptors are derived by the individual assessor, who quantifies the intensity of a particular attribute by assigning a score on a line scale.

14.8.2 Evaluation of Cheese Texture by Instrument-Based Texture Profile Analysis

Cheese texture, being a sensory property, is ultimately expressed in sensory terms or descriptors. However, trained texture panels may be difficult and costly to establish and maintain. Instrumental methods are easier to perform, standardize and reproduce and require the use of fewer trained people. Hence, much research effort has focussed on the measurement of textural properties using instrumental methods.

Texture evaluation by instrumental methods is generally based on forced-compression tests, designed to simulate the compression of cheese between the molars during chewing. The first apparatus of this kind was the forced-compression test using the General Foods Texturometer (Friedman et al. 1963; Bourne 1978), as discussed in Sect. 14.6.2. The texturometer has been superseded by uniaxial compression instruments, such as Instron Universal Testing Instrument and TA HDi Texture Analyser, for the purpose of texture profile analysis. The latter instruments compress the sample between parallel plates at a fixed velocity, and therefore do not simulate the human jaw as precisely as the texturometer which decelerates as it reaches the end of the compression stroke, and then accelerates upward as it withdraws. Effective simulation of sensory texture evaluation by instrumental compression tests necessitates test conditions (e.g., compression rate) which resemble those during consumption and that are carefully standardized between samples (e.g., temperature, sample size and shape, size and shape of the compression plates). Research has shown that most people compress food to >70 %, and chew at a rate of 40–80 masticatory strokes per minute–indicating that the duration of the downstroke in a bite is ~ 0.5 s, depending on the food (Sherman 1988). The importance of a high deformation rate (e.g., >40–100 cm/min) during compression was demonstrated by Sherman (1989) and Xiong et al. (2002). In contrast to consumer evaluation scores, force-compression measurement at a low compression rate of 5 cm/min found Stilton cheese to be firmer than Gouda cheese, whereas compression at a higher rate (50–100 cm/min) found, in agreement with consumers, that Gouda was firmer than Stilton.

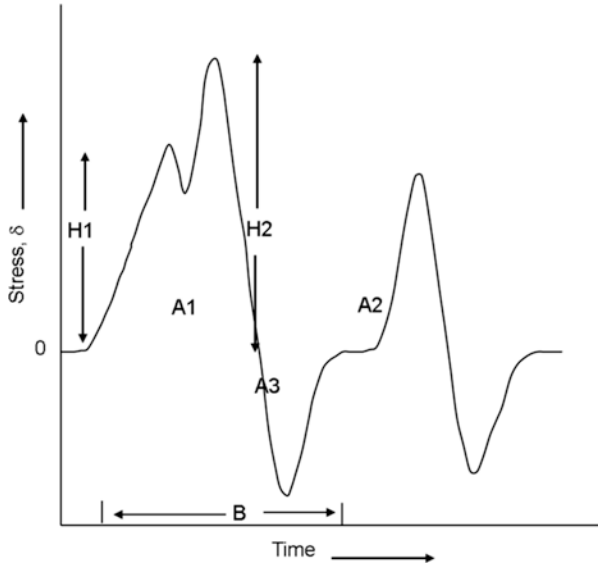


Fig. 14.24 Typical stress/time curve obtained during a two-bite compression test on cheese, which forms the basis of texture profile analysis. The corresponding stress/deformation or stress/strain curve is obtained from the stress/time curve by converting time into distance (deformation = time x velocity of cross-head). The curve is used to obtain various texture parameters, as explained in Table 14.5

From the forgoing it is clear that large strain deformation testing and TPA of cheese are very similar. Nevertheless, they differ subtlety. Typically, large strain deformation rheological evaluation involves only one deformation cycle (bite) which allows the determination of firmness, and, where the cheese fractures, fracture stress and strain. TPA measures the response of cheese to two-bite deformation (Fig. 14.24), and in addition to firmness, fracture stress and strain, also allows a prediction of parameters (e.g., cohesiveness, adhesiveness, chewiness and gumminess, Table 14.5) that are important during consumer mastication.

14.8.3 Correlation Between Texture TPA and Sensory Evaluation of Texture

Several studies have demonstrated relationships between the textural properties of cheese as measured by sensory panel and rheological parameters measured using texture profile analysis, using single- or double-bite compression (Lee et al. 1978; Vernon Carter and Sherman 1978; Green et al. 1985; Breuil and Meullenet 2001; Xiong et al. 2002). Some of these are discussed below.

Table 14.5 Textural profile parameters derived from a two-bite uniaxial compression test[a,b]

Parameter	Interpretation	Units
Fracturability	Height of first peak in first bite. Height H1, Fig. 14.24.	N (force/displacement curve) Pa (stress/displacement curve)
Firmness (hardness)	Height of second peak in first bite. Height H2, Fig. 14.24	N (force/displacement) Pa (stress/displacement curve)
Springiness	Height that the cheese recovers during the time that elapses between the end of the first compression and the start of the second compression Difference between distance B (measured from the initial point of contact with the sample in bite 1 to the point of contact with the sample in bite 2) and distance C (the same measurement made on a completely inelastic standard material such as clay). Distance B–C, Fig. 14.24	cm
Adhesiveness	Area of the negative peak formed when the cross-head (plunger) is withdrawn from the sample after the first bite, due to cheese adhering to cross-head and retarding its retraction. Area A3, Fig. 14.24	N × cm = J
Cohesiveness	Ratio of area in bite 2 to area in bite 1. A2/A1, Fig. 14.24	Unit-less
Guminess	Product of hardness × cohesiveness. H1 × (A2/A1), Fig. 14.24	N (force/displacement) Pa (Stress/displacement curve)
Chewiness	Product of hardness × cohesiveness × springiness. H1 × (A2/A1) × (B-C), Fig. 14.2	Nm (force/displacement) Pam(Stress/displacement curve)

[a]*Sources*: Friedman et al. (1963), Peleg (1976), Bourne (1978); Szcesniak (1993a); Brennan (1988), van Vliet (1991), O'Callaghan and Guinee (2004) and Rao and Quintero (2005)
[b]Fracturability was originally known as brittleness (Bourne 1978), and firmness as hardness (Szczesniak 1963a)

Chen et al. (1979) found significant positive correlations between hardness, cohesiveness, chewiness and adhesiveness of 11 different hard and semi-hard cheese types as measured by a sensory panel and the corresponding parameters measured by TPA (compression to ~25 % original height at 2.5 cm/min) using linear regression. Green et al. (1985) reported significant correlations between the fracture stress (δ_f) of Cheddar and Cheshire cheeses measured using large strain compression to 80 % at 50 mm/min and sensory firmness and springiness, and between fracture strain (ε_f) and sensory crumbliness. Hennequin and Hardy (1993) reported that TPA-hardness, i.e., force at 70 % compression, also had a high correlation with sensory hardness ($r = 0.78$, $n = 19$) for four soft cheeses. Noël et al. (1996) found that five sensory attributes (elasticity, firmness, deformability, grainy, crystals) of Parmigiano

Reggiano chesses could be estimated from four large strain deformation parameters (elastic modulus, δ_f, ε_f, work to fracture) through prediction equations determined using partial least squares. Antoniou et al. (2000) carried out sensory and TPA analyses on 15 French cheeses, which were classified into 3 groups based on the range of dry matter (DM) content: 49–61 % DM (Mûnster, Valencay, Tomme de Savoie, Fourme de Salers, Roquefort, Bleu d'Auvergne), 41–53 % DM (Camembert, Pont l'Eveque, Reblochon, Saint Nectaire, Brie de Meaux), and 65–66 % DM (Emmental, Beaufort, Pyrenees Brebis, Comte Vieux). Instrumental fracture force (δ_f) and firmness at 80 % compression ($\delta_{max\ 80}$) and 10 % compression ($\delta_{max\ 10}$) were both significantly correlated with sensory hardness, cohesiveness and adhesiveness.

Despite the significant correlations between some sensory textural parameters and rheological measurements, instrumental analysis of texture, e.g., using texture analysers, is not considered a complete substitute for sensory evaluation, because of several factors: complexity of mastication, differences between individuals in the perception of texture, effect of time of day upon the perception of texture, and others. Hence, while instrumental methods alone cannot be relied upon to determine consumer acceptance precisely, they are, nevertheless, useful for evaluating the influence of alterations in cheesemaking recipe or manufacturing procedure on changes on the physical characteristics of the cheese, that are likely to affect texture.

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Chapter 15
Factors that Affect Cheese Quality

Summary Cheese quality relates to, and depends on, many attributes, including appearance, texture, functionality, flavour, safety and nutritional value, the relative importance of which depend on the application of the cheese. Since cheese is the end-product of a long and complex process, which may last for two or more years, its quality depends on many factors, some of which are generally pertinent but some are variety-specific. The most important are:

1. The milk supply, including its composition, microbial quality, preparation (standardization and heat treatment) and consistency.
2. The bacterial culture(s) used for acidification and which play(s) major roles during ripening.
3. The rennet used to coagulate the milk and which is the principal proteolytic agent during ripening.
4. The non-starter bacteria which are either indigenous to the milk or gain entry to the milk or cheese from the environment during manufacture.
5. Composition of the cheese.
6. Ripening of the cheese curd, including temperature and duration.

These aspects are described in several chapters throughout the text; in this chapter, an attempt is made to integrate the numerous factors that affect cheese quality.

Keywords Cheese quality • Milk composition • Cheese microflora • Cheese composition • Ripening temperature

15.1 Introduction

As discussed in Chap. 12, the ripening of cheese, and hence its quality, is due to the activity of microorganisms and enzymes from 4 or 5 sources. Therefore, it might reasonably be expected that it should be possible to produce premium quality cheese consistently by controlling these agents; however, in spite of considerable research and quality control efforts, it is not yet possible to do so.

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A very wide and diverse range of factors interact to affect the composition of cheese curd and hence the quality of the final cheese; an attempt to summarize these is shown in Fig. 15.1. Some of these factors/agents can be manipulated easily and precisely while others are more difficult, or perhaps impossible, to control. It should be possible to apply the principles of Hazard Analysis Critical Control Point (HACCP) analysis to cheese production. However, the influence of many of the factors included in Fig. 15.1 on cheese ripening and quality is not known precisely and many of the factors are interactive. The interactions between the principal factors that affect cheese quality were also reviewed by Lawrence and Gilles (1980). Figure 15.1, which is more comprehensive than the scheme presented by Lawrence and Gilles (1980), is not considered to be definitive but it is hoped that it may stimulate others to apply HACCP principles to cheesemaking.

15.2 Milk Supply

It is well recognised that the quality of the milk supply has a major impact on the quality of the resultant cheese. Three aspects of quality must be considered: microbiological, enzymatic and chemical.

15.2.1 Microbiological

In countries with a developed dairy industry, the quality of the milk supply has improved markedly during recent years—total bacterial counts (TBC) are now usually <20,000 cfu/ml ex-farm. The TBC probably increases during transport and storage at the factory, but growth can be minimized by keeping the temperature below 4 °C. Thermization (65 °C × 15 s) of the milk supply on receipt at the factory, which is standard practice in some countries (see Chap. 5), also prolongs the storage stability of milk.

Although many cheeses are made from raw milk, in quantitative terms, most cheese is made from milk pasteurized at or close to 72 °C × 15 s. If produced from good quality raw milk and if subsequently handled under hygienic conditions, pasteurized milk should have a very low TBC and therefore represents a very uniform raw material from a microbiological viewpoint. With increasing storage time of the raw milk at the factory, greater attention should be given to its microbiological quality just prior to pasteurization.

15.2.2 Indigenous Enzymes

Milk contains about 60 indigenous enzymes (see Andrews et al. 1992; O'Mahony et al. 2013), several of which have the potential to affect cheese quality, especially lipase, proteinase(s), acid phosphatase and perhaps xanthine oxidase,

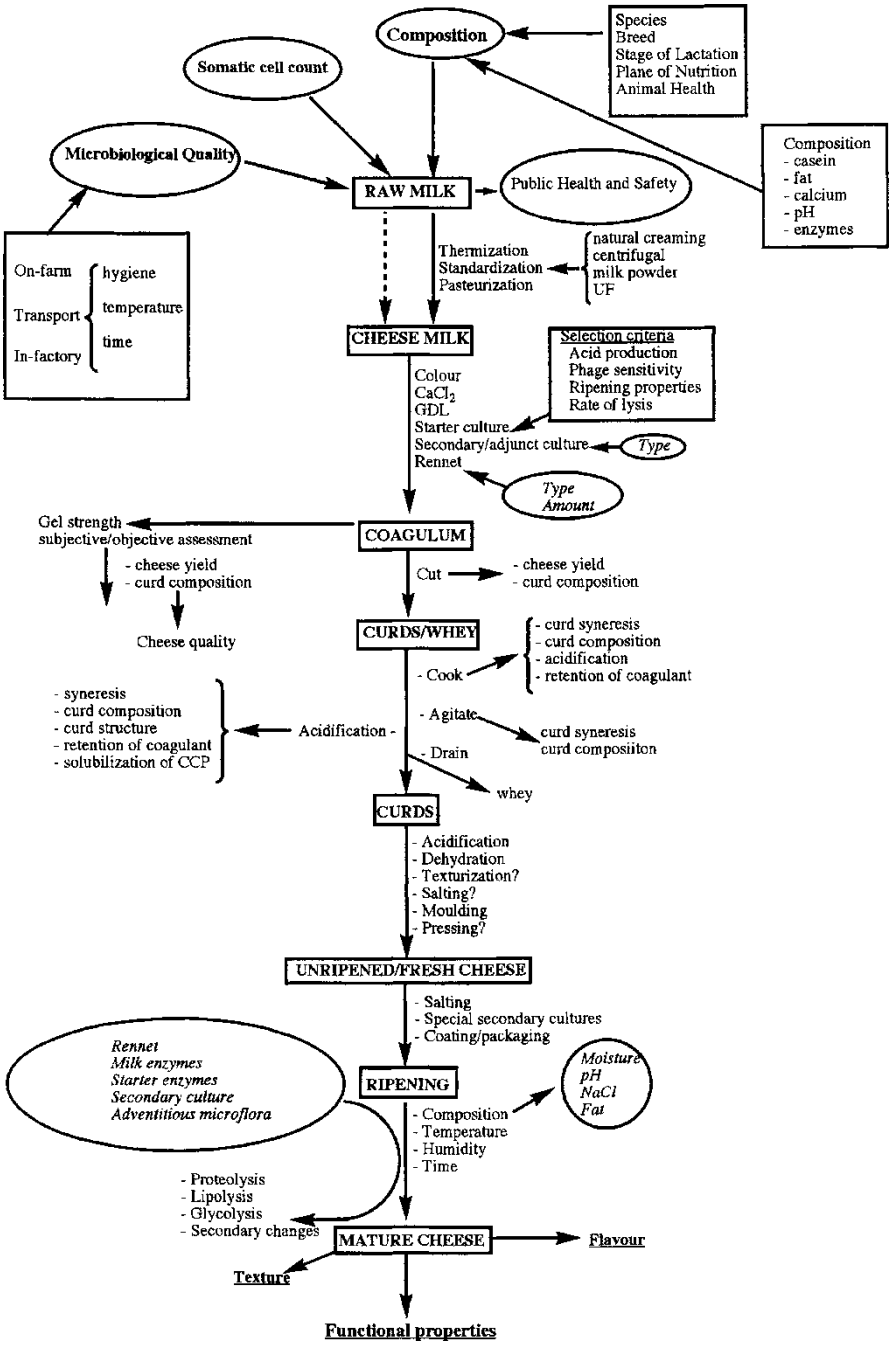


Fig. 15.1 Factors that affect the quality of cheese. The figure is intended to show the multiplicity of factors that impact, directly or indirectly, on the quality of rennet-coagulated cheeses. It is possible to standardize and control many of the factors involved. Knowledge of the factors that affect cheese quality should enable a HACCP approach to be applied to cheese production

sulphydryl oxidase, lactoperoxidase and γ-glutamyl transpeptidase. Many of these survive high temperature-short time (HTST; 72 °C × 15 s) pasteurization at least partially and at least some, e.g., plasmin, acid phosphatase and xanthine oxidase are active during cheese ripening (see Chap. 12).

Somatic cells are an important source of enzymes, particularly proteinases, in milk. Somatic cell count (SCC) is negatively correlated with cheese yield (see Chap. 10) and quality. A SCC of < 300,000/ml is recommended.

Although precise information is lacking, it is unlikely that indigenous milk enzymes are a major cause of variability in cheese quality; some of these enzymes contribute to cheese ripening and may contribute to the superior quality of raw milk cheese, a possibility that warrants investigation.

15.2.3 Chemical Composition

The chemical composition of milk, especially the concentrations of casein, fat, calcium and pH, has a major influence on several aspects of cheese manufacture, especially rennet coagulability, gel strength, curd syneresis, and hence cheese composition and cheese yield. When seasonal milk production is practiced, as in New Zealand, Ireland and Australia, milk composition varies widely and there is some variability even with random calving patterns due to nutritional factors. It is possible to reduce, but not eliminate, the variability in the principal milk constituents by standardizing the concentrations, not just the ratio, of fat and casein (protein content can be standardized by adding UF retentate), the pH (using gluconic acid-δ-lactone) and calcium content (by adding $CaCl_2$), as discussed in Chap. 2.

15.3 Coagulant (Rennet)

It is generally accepted that calf chymosin produces the best quality cheese. An adequate supply of chymosin from genetically engineered microorganisms is now available (although its use is not permitted in all countries) and therefore rennet quality need not be a cause of variability in cheese quality.

As discussed in Chap. 12, the proportion of added rennet retained in cheese curd varies with rennet type, cook temperature and pH at draining; these variables should be standardized if cheese of consistent quality is to be produced. Increased retention of the coagulant in the curd results in greater initial hydrolysis of α_{s1}-casein although this does not appear to be reflected in sensory assessment of cheese texture and flavour. It has been suggested that the activity of chymosin in cheese curd is the limiting factor in cheese ripening; however, excessive rennet activity leads to bitterness. There have been relatively few studies on the significance of chymosin activity in cheese quality, an aspect which appears to warrant further research.

15.4 Starter

Since the starter plays a key role in cheese manufacture and ripening, it might reasonably be expected that differences between the enzyme profile of starter strains affect cheese quality. Modern single-strain starters produce acid very reproducibly and if properly managed, show good phage resistance. *Lactococcus* strains have been selected mainly on the basis of acid-producing ability, phage resistance and compatibility. Based on pilot-scale studies and commercial experience, strains that produce unsatisfactory, especially bitter, cheese have been identified and excluded from commercial usage. However, systematic studies on strains with positive cheesemaking attributes are lacking. This probably reflects the lack of information on precisely what attributes of a starter are desirable from a flavour-generating viewpoint. Studies on genetically engineered strains that super-produce proteinase and/or the general aminopeptidase (PepN) showed that cheese quality was not improved, although proteolysis was accelerated. Since all lactococcal enzymes, except the cell wall-associated proteinase, are intracellular, the cells must lyse before these enzymes can participate in ripening; therefore, the rate of lysis of *Lactococcus* strains is being studied with the objective of selecting strains with improved cheesemaking properties.

Sulphur compounds have long been considered as major contributors to the flavour of many cheese varieties. Some strains of *Lc. lactis* subsp. *cremoris*, but not *Lc. lactis* subsp. *lactis*, can absorb glutathione (γ-Glu.Cys.Gly; GSH) from the growth medium. Release of GSH into the cheese on cell lysis may affect the redox potential of cheese, and hence the concentration of thiol compounds. Comparative cheesemaking studies using starter strains that accumulate glutathione and those that do not are warranted. The variations between strains in their ability to degrade methionine need to be studied systematically.

Although considerable information is available on the individual enzymes of *Lactococcus* and to lesser extent of *Lactobacillus*, especially on the glycolytic and proteolytic systems, there have been few studies on the proportions of different enzyme activities in starter strains. There have been even fewer studies on the relationship between different starter enzyme profiles and cheese quality. It would appear to be highly desirable that studies should be undertaken to relate cheese quality to the natural enzyme profile of starter strains or genetically engineered starters. The availability of starter strains deficient in or over-producing one or more enzymes will facilitate such studies.

It is very likely that the desirable cheesemaking properties of starters are due to a balance between certain, perhaps secondary, enzymatic activities, which have not yet been identified.

15.5 Non-starter Lactic Acid Bacteria (NSLAB)

The significance of lactobacilli for Cheddar and Dutch cheese quality is controversial (see Chap. 12). Many researchers consider their contribution to be negative (in the Netherlands, a maximum of 2×10^6 NSLAB/g is specified for Gouda).

Although there are several studies on controlled microflora cheeses, we are not aware of studies in which cheese free of NSLAB was compared with "control" cheeses containing "wild" NSLAB. Several comparative studies on cheese made under aseptic or non-aseptic conditions using *Lactococcus* starter alone or with selected *Lactobacillus* adjuncts, indicate that inoculation of cheesemilk with selected strains of *Lactobacillus* improves cheese flavour and possibly accelerates ripening. Thermophilic *Lactobacillus* spp. are more effective as adjuncts than mesophilic lactobacilli, probably because they die rapidly in cheese, lyse and release intracellular enzymes. Both mesophilic and thermophilic lactobacilli, and *Sc. thermophilus,* are being used commercially as adjunct cultures for Cheddar cheese, and possibly for other varieties.

Since the numbers and strains of NSLAB in cheese are uncontrolled, it is likely that they contribute to variability in cheese quality. It is impossible to eliminate NSLAB completely, even under experimental conditions; therefore, it appears worthwhile to determine what factors affect their growth. The number of NSLAB in Cheddar is strongly influenced by the rate at which the curd is cooled and subsequently ripened. Rapid cooling of the curd after moulding is the most effective way of retarding the growth of NSLAB, although they will grow eventually to ~10^7 cfu/g. The growth of NSLAB can be prevented by ripeing at ~1 °C but all ripening reactions are retarded at this temperature. The growth of NSLAB does not appear to be influenced by the composition of cheese (moisture, salt or pH) within the ranges normally found in commercial cheese.

NSLAB grow mainly after the lactose has been metabolized by residual starter activity. Although the growth substrates in cheese for *Lactobacillus* are not known, it is likely that they are limited (NSLAB normally plateau at ~10^7 cfu/g) and hence it might be possible to out-compete wild NSLAB by adding selected strains of *Lactobacillus* to cheese milk, thereby offering better control of the ripening process. NSLAB may also be controlled by including a broad spectrum bacteriocin-producing strain in the starter culture.

15.6 Cheese Composition

The quality of cheese is influenced by its composition, especially moisture content, NaCl concentration [preferably expressed as salt-in-moisture (% S/M)], pH, moisture in non-fat substances (MNFS; essentially the ratio of protein to moisture) and % fat-in-dry matter (FDM). At least five studies (O'Connor 1971; Gilles and Lawrence 1973; Fox 1975; Pearce and Gilles 1979; Lelievre and Gilles 1982) have attempted to relate the quality of Cheddar cheese to its composition. While these authors agree that moisture content, % S/M and pH are the key determinants of cheese quality, they disagree as to the relative importance of these parameters (see Fig. 15.2).

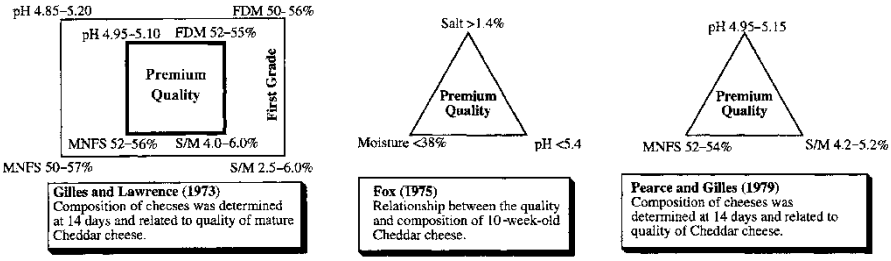


Fig. 15.2 Relationships between composition (determined at various stages during ripening) and the quality of mature Cheddar cheese (moisture-in-non-fat substances [MNFS]; fat-in-dry matter [FDM], and salt-in-moisture [S/M]

O'Connor (1971) found that flavour, texture and total score were not correlated with moisture content but were significantly correlated with % NaCl and particularly with pH. Salt content and pH were themselves strongly correlated with each other, as were salt and moisture.

Based on the results of a study on experimental and commercial cheeses in New Zealand, Gilles and Lawrence (1973) proposed a grading (selection) scheme which is used commercially in New Zealand for young (14 day) Cheddar cheese. The standards prescribed for Premium grade were: pH: 4.95–5.10; % S/M: 4.0–6.02 %; MNFS: 52–56 %; FDM: 52–55 %. The corresponding values for First Grade cheeses were: 4.85–5.20 %, 2.5–6 %, 50–56 % and 50–56 %; young cheese with a composition outside these ranges was considered unlikely to yield good quality mature cheese. Quite wide ranges of FDM are acceptable; Lawrence and Gilles (1980) suggested that since relatively little lipolysis occurs in Cheddar cheese, fat content plays a minor role in determining cheese quality but if FDM falls below about 48 %, the cheese is noticeably more firm and has a less attractive flavour. Pearce and Gilles (1979) found that the grade of young (14-day-old) cheeses produced at the New Zealand Dairy Research Institute was most highly correlated with moisture content; the optimum compositional ranges were: MNFS: 52–54 %; S/M: 4.2–5.2 %; pH: 4.95–5.15.

Fox (1975) reported a weak correlation between grade and moisture, salt and pH for Irish Cheddar cheeses but a high percentage of cheeses with compositional extremes were downgraded, especially those with low salt (<1.4 %), high moisture (>38 %) or high pH (>pH 5.4). Salt concentration seemed to exercise the strongest influence on cheese quality and the lowest percentage of downgraded cheeses can be expected in the salt range 1.6–1.8 % (S/M: 4.0–4.9 %); apart from the upper extremes, pH and moisture appeared to exercise little influence on quality. High salt levels tend to cause curdy textures, probably due to insufficient proteolysis; a pasty body, often accompanied by off-flavours, was associated with low salt and high moisture levels. In the same study, the composition of extra-mature cheeses was found to vary less and the mean moisture content was 1 % lower than that of regular cheeses.

A very extensive study of the relationship between the composition and quality of nearly 10,000 Cheddar cheeses produced at five commercial New Zealand factories was reported by Lelievre and Gilles (1982). As in previous studies, considerable compositional variation was evident but was less for some factories than others. While the precise relationship between quality and composition varied between plants, certain generalizations emerged:

- within the compositional range suggested by Gilles and Lawrence (1973) for "premium" quality cheese, composition does not have a decisive influence on grade, which decreases outside this range;
- composition alone does not provide a basis for grading as currently acceptable in New Zealand;
- MNFS was again found to be the principal factor affecting quality;
- within the recommended compositional bands, grades declined marginally as MNFS increased from 51 % to 55 % and increased slightly as S/M decreased from 6 to 4; pH had no consistent effect within the range 4.9–5.2 and FDM had no influence in the range 50–57 %.

The authors stress that since specific inter-plant relationships exist between grade and composition, each plant should determine the optimum compositional parameters pertinent to that plant.

The results of the foregoing investigations indicate that high values for moisture and pH and a low salt level lead to flavour and textural defects. The desired ranges suggested by Gilles and Lawrence (1973) appear to be reasonable, at least for New Zealand conditions but within the prescribed zones, composition is not a good predictor of Cheddar cheese quality. Presumably, several other factors, e.g., microflora, activity of indigenous milk enzymes, relatively small variations in cheese composition and probably other unknown factors, influence cheese quality but become dominant only under conditions where the principal determinants, moisture, salt and pH, are within appropriate limits.

Although the role of calcium concentration in cheese quality has received occasional mention, its significance was largely overlooked until the work of Lawrence and Gilles (1980) who pointed out that the concentration of calcium in cheese curd determines the cheese matrix and, together with pH, indicates whether proper procedures were used to manufacture a specific cheese variety. As the pH decreases during cheese manufacture, colloidal calcium phosphate dissolves and is removed in the whey. The whey removed after cooking comprises 90–95 % of the total whey lost during cheesemaking and this whey contains, under normal conditions, ~85 % of the calcium and ~90 % of the phosphate lost from the cheese curd. Thus, the calcium content of cheese reflects the pH of the curd at whey drainage; there are strong correlations between the calcium content of cheese and the pH at 1 day, pH at 14 days and the amount of starter used (see Lawrence et al. 1984). Since the pH of cheese increases during ripening, the pH of mature cheese may be a poor index of the pH of the young cheese. Therefore, calcium concentration is probably a better record of the history of a cheese with respect to the rate of acidification than the final pH. Reduction in calcium phosphate concentration by excessively rapid acid

development also reduces the buffering capacity of cheese and hence the pH of the cheese will fall to a lower value for any particular level of acid development. Unfortunately, no recent work on the level and significance of calcium in Cheddar cheese appears to be available.

15.7 Ripening Temperature

The rate of ripening and cheese quality are strongly influenced by ripening temperature. Ripening at an elevated temperature is normally considered with the objective of accelerating ripening but it also affects cheese quality. Cheddar cheese of good composition can be ripened at a 16 °C without adverse effects but fat exudation occurs at a higher temperature (Folkertsma et al. 1996). The literature on the accelerated ripening of cheese is discussed in Chap. 12.

15.8 Conclusions

Through increased knowledge of the chemistry, biochemistry and microbiology of cheese, it is now possible to consistently produce cheese of an acceptable quality although this is not always achieved owing to failure to control one or more of the key parameters that affect cheese composition and ripening. Milk is a variable raw material and although it is possible to eliminate major variations in the principal milk constituents, some variation persists. Variability in milk composition can also be compensated by manipulating some process parameters during cheesemaking. Most large factories operate on a strict time schedule and hence subtle process manipulation on an individual vat basis may not be possible. Therefore, strict control of milk composition and starter activity are critical.

From a microbiological viewpoint, the milk supply to modern cheese factories is of very high quality and after pasteurization is essentially free of bacteria. In modern factories where enclosed vats and other equipment are used, the level of contamination from the environment is very low; cheese curd containing $<10^2$ NSLAB/g at 1 day is normal. However, these adventitious NSLAB grow to ca. 10^7 cfu/g and dominate the microflora of long-ripened cheese. Since the adventitious NSLAB grow slowly in cheese, they are most significant in long-ripened cheese. Although the significance of the adventitious NSLAB in long-ripened cheese is unclear, it would appear to be desirable to control them, either by eliminating them or standardizing them. It is not possible to eliminate NSLAB, even in cheese made on a pilot scale under aseptic conditions. Their growth can be prevented by ripening at ~1 °C but the overall ripening process is reduced to an unacceptable rate. Outcompeting indigenous NSLAB by an adjunct culture of *Lactobacillus* isolated from good quality raw milk Cheddar, has become widespread; this also gives cheese a characteristic factory-specific flavour.

Although it is now possible to avoid major defects in cheese produced using modern technology, further research on the biochemistry of cheese ripening is required to enable the process of cheese manufacture and ripening to be refined to an extent that will allow the consistent production of premium quality cheese.

The key to successful cheesemaking is a good reliable starter, both from the viewpoint of reproducible acid production and subsequent ripening. If properly managed, modern starters are generally satisfactory and their performance is being improved progressively.

The use of starter adjuncts, usually mesophilic lactobacilli, for some varieties, especially Cheddar, is increasing, with the objective of intensifying and modifying cheese flavour, accelerating ripening and perhaps controlling adventitious NSLAB and thus standardizing quality.

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Chapter 16
Fresh Cheese Products: Principals of Manufacture and Overview of Different Varieties

Summary Fresh cheese represents a diverse group of varieties produced by the coagulation of milk, cream or whey via acidification, acidification with a small quantity of rennet, or a combination of acid and heat and which are ready for consumption once manufacturing is complete. The essential steps in the manufacture of acid-coagulated (or acid-curd) and acid/rennet coagulated verities involves slow quiescent acidification of the standardized, pasteurized cheese milk to pH values (4.6–4.8) close to the isoelectric point of casein, cutting or gently braking the gel, and concentration of the gel to a curd using various means including pouring the broken gel onto cheese cloth or perforated moulds, mechanical separation or membrane filtration. The resultant cheese is then packaged cold (<30 °C) in the case of cold-pack varieties such as Quark, Cottage cheese, Fromage frais and Baker's cheese. For other varieties such as Cream cheese and Neufchâtel, the curds after whey separation is subjected to further treatments including heating to 75–85 °C, shearing, addition of salt (and hydrocolloids for some varieties), and/or homogenization. The treated curd is then hot-packed typically at 70–85 °C; hence, the term hot pack. The manufacture of acid/heat coagulated varieties such as Mascarpone, Ricotta, Paneer and some Queso blanco cheese types typically involves acidification of the cheese milk to a pH value in the range 5.4–6.0, heating to 80–90 °C and recovery of the curds by various means, as for acid- and acid/rennet-coagulated varieties. The quality of fresh cheeses is influenced by many parameters including gel structure, conditions of whey separation, and treatments of the curd.

Keywords Acid-curd • Acid-heat curd • Different varieties • Principles of manufacture

16.1 Introduction

Fresh cheeses refer to those varieties produced by the coagulation of milk, cream or whey via acidification, acidification together with a small quantity of rennet, or a combination of acid and heat and which are ready for consumption once the

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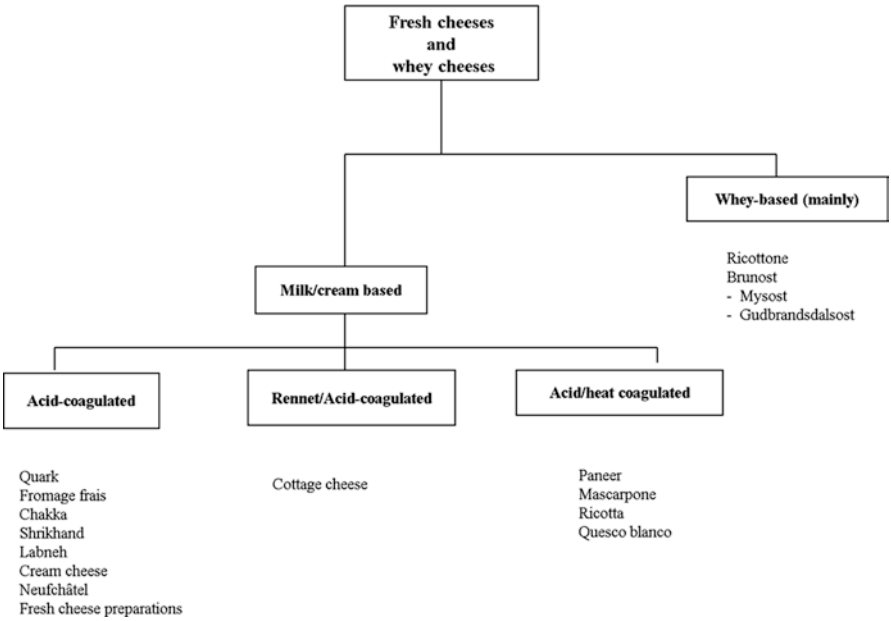


Fig. 16.1 Fresh acid-curd cheese and whey cheese varieties. Brunost is a fat-protein-enriched concentrated heated whey, and hence is not cheese in the classical sense

manufacturing operations are complete (Fig. 16.1). They differ from rennet-curd cheeses, for which coagulation is induced by the action of rennet at pH 6.4–6.6, in that coagulation occurs close to the isoelectric pH of casein, i.e., pH 4.6, or at a higher value when a higher temperature is used, e.g., Ricotta (~ pH 5.8–6.0 at 80 °C). While a very small amount of rennet may be used in the production of Quark, Cottage cheese and Fromage frais to give firmer coagula and to minimize casein losses during subsequent whey separation, its addition is not essential. They also differ from rennet-curd cheeses in the following respects (Table 16.1):

- they generally have higher moisture (e.g., 55–84 % versus 32–60 %), lower content of protein (e.g., 10–15 % versus 16–34 %) and calcium (<0.15 % versus >0.4 %) and lower pH (e.g., 4.4–5.0 versus 4.9–6.0);
- they are softer and less chewy
- they do not require a ripening period, and are consumed fresh
- they generally have a shorter shelf-life (e.g., <4 weeks for cold-packed fresh cheeses to <6 months for some hot-packed products, compared to ~1 month up to 2 years for rennet-curd cheeses).

Global cheese manufacture is ~21.3×10^6 tonnes per annum (OECD/FAO 2014). It is estimated that fresh cheese accounts for ~30 % of total cheese

Table 16.1 Approximate composition of various fresh cheeses

Cheese group/variety	Dry matter	Fat	Protein	Salt	Ca	pH
	% (w/w)				mg/100 g	
Acid-curd						
Skim Quark	>18	<1.8	>12	0.15	100	4.6
Labneh	25.5	10.2	10.5	na	na	4.4
Double cream cheese	45.0	33	10	0.75	70	4.7
Single cream cheese	30.0	14	20	0.75	84	4.6
American Neufchâtel	≥35.0	20–33	10	0.75	na	4.6
Petit suisse	45.0	35.0	7.3	1.0		4.6
Acid/Rennet curd						
Cottage cheese	21.0	4.2	12.5	1.0	60	<5.2
Low-fat Cottage cheese (1 %)	17.5	1.0	12.4	1.0	61	4.8
Dry-curd Cottage cheese	>20	0.4	17.3	0.03	40	4.8
Baker's cheese	26	0.2	19.0	<0.1	<60	4.4
Acid/heat coagulated						
Mascarpone	39.0	54.0	2.6	0.2	na	5.8
Whole milk Ricotta	28.0	13.0	11.0	<0.5	207	5.7
Part-skim Ricotta	25.5	8.0	11.5	<0.5	272	5.7
Ricottone	23.0	2.5	16	<0.5	na	na
Buffalo milk Paneer	45.0	19.0	21.0	na	na	na
Cow milk Paneer	43.0	18.1	18.4	na	208	na
Full-fat Paneer	46	23.4	18.3	na	na	na
Low-fat Paneer	38.3	8.6	21.6	na	na	na
Skim milk Paneer	35.4	0.2	25.8	na	na	na
Minas						
Queso Blanco (Queso de Pais)	49.0	15.0	22.9	3.9	na	na

Compiled from data from various sources: USDA (1976), Kosikowski and Mistry (1997), Guinee et al. (1993), Schulz-Collins and Senge (2004), Farkye (2004a, b), Guinee and Hickey (2009), Umer Khan and Ahraf Pal (2011) and Kumar et al. (2014)
na no published data available

(Schulz-Collins and Senge 2004). Factors contributing to the popularity of fresh cheese include:

- their soft, ingestible consistency which makes them safe for and attractive to very young children.
- the healthy perception of these products by diet-conscious consumers
- the large variety they offer in terms of consistency and flavour, made possible by changes in cheesemaking protocols, the addition of sugar, fruit purees, spices or condiments,
- their retail presentation in an array of attractive and convenient packages.

16.2 Overview of the Manufacturing Process for Fresh Acid-Curd Cheese Products

Production of acid-curd cheeses generally involves pre-treatment of milk (standardization, pasteurization and perhaps homogenization), inoculation of the milk with a lactic acid starter culture, incubation of the inoculated milk at 20–35 °C depending on type of cheese, slow quiescent acidification, gel formation, dehydration of the gel (whey separation) and, in some cases, further treatments of the curd (pasteurization, shearing, addition of salt, condiments and stabilizers and homogenization) (Fig. 16.2; Guinee et al. 1993; Lucey 2011; Schulz-Collins and Senge 2004; Farkye 2004a, b). Acidification is generally slow, 12–16 h at 21–23 °C (long set) or 4–6 h at 30 °C (short set) and is usually brought about by the *in situ* conversion of lactose to lactic acid, by an added starter culture, and/or by the addition of food-grade acid (e.g., lactic, citric) or acidogen, such as glucono-δ-lactone (GDL, which hydrolyses to gluconic acid). The structure of the gel has a major influence on the texture (e.g., spreadability, firmness) and sensory attributes (smoothness) of the final product and its physico-chemical stability (i.e., stability to wheying-off and/or to the development of a chalky/grainy mouthfeel) during storage. The structure of the gel is influenced by many processing factors such as the protein level, milk pasteurization treatment, homogenization, temperature during acidification, and the pH at which the gel is broken and subjected to dehydration (Kim and Kinsella 1989; Gastaldi et al. 1996; Lucey et al. 1997a, b; Lucey et al. 1998a, b; Schkoda et al. 1999; Horne 1999). The effect of gel structure on product quality is most pronounced in products, such as Quark and Fromage frais, for which the curd, following whey separation and concentration, is not treated further (Guinee et al. 1993). In hot-pack products, such as Cream cheese and other fresh cheese products and preparations, the curds are subjected to various treatments (i.e., heat treatment, shear, homogenization, hydrocolloid addition) which vary according to product type. These treatments provide major opportunity for differentiation of product texture characteristics (e.g., smoothness, granularity, firmness/softness, spreadability, mouthfeel) and have a major impact on the quality of the final product (Guinee et al. 1993; Sects. 16.5–16.7).

16.3 Principles and Mechanism of Acid-Induced Gelation of Milk

The various physico-chemical and microstructural changes that occur during acidification and acid-induced gelation of milk have been studied and reviewed extensively (Harwalkar and Kaláb 1980; Snoeren et al. 1984; Creamer 1985; Roefs et al. 1985; Schmidt and Poll 1986; Dalgleish and Law 1988; Kim and Kinsella 1989; Walstra 1990; Guinee et al. 1993; Gastaldi et al. 1996; Brulé et al. 2000; Vasbinder et al. 2001; Lucey and Singh 2003; Lucey 2004; Schulz-Collins and Senge 2004; Karlsson et al. 2005; Phadungath 2005a, b).

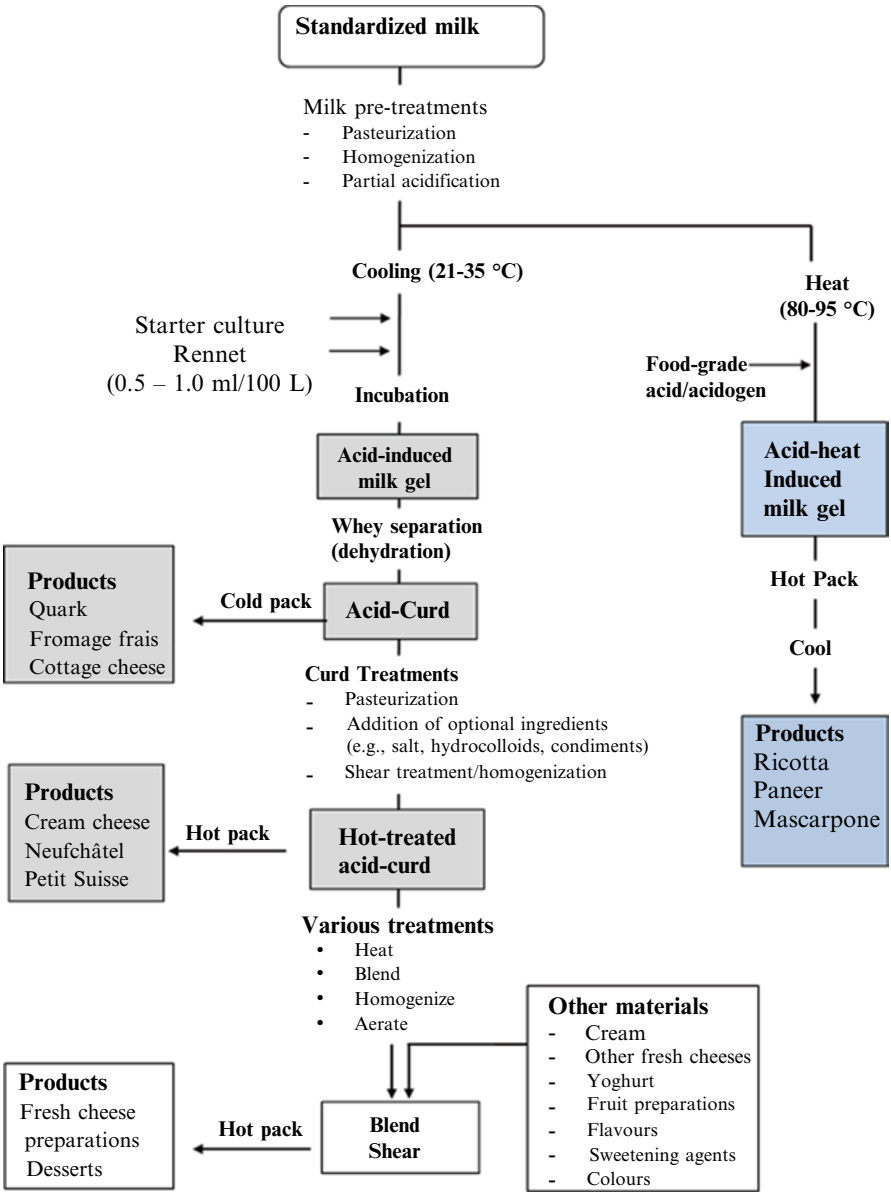


Fig. 16.2 Generalized production protocol for fresh cheeses and related products

16.3.1 *Physico-Chemical Changes During Acidification and Gelation*

During the manufacture of acid-curd cheeses, the milk is inoculated with a starter culture and incubated, which ferments lactose to lactic acid. Slow acidification of milk under quiescent conditions is accompanied by two opposing sets of physico-chemical changes:

- a tendency towards disaggregation of the casein micelles into a more disordered system as a result of:
  - solubilization of the internal casein micelle cementing agents, colloidal calcium phosphate (CCP), which is fully soluble at pH ~ 5.2–5.3 at 20–30 °C (Fig. 16.3, van Hooydonk et al. 1986), and casein calcium to a degree dependent on the final pH of the product (see Chap. 18).
- a pH- and temperature-dependent dissociation of individual caseins, especially β, from the micelles with a concomitant increase in the level of serum casein. Casein dissociation decreases with decreasing pH to ~pH 6.2, then increases to a

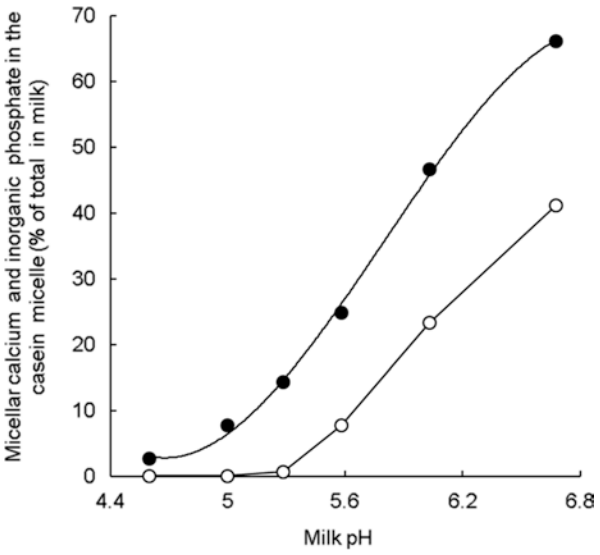


Fig. 16.3 Micellar calcium (*filled circles*) and inorganic phosphate (*open circles*) remaining in casein micelles as a function of pH. Milks were adjusted to different pH values, equilibrated at 30 °C for 30 min and ultracentrifuged at 88, 000 g to give a pellet of sedimented casein micelles (containing micellar calcium and inorganic phosphate) and a supernatant comprising the serum and soluble calcium and inorganic phosphate. The concentrations of calcium and inorganic phosphate in serum at each pH were measured and expressed as a % of the corresponding values in the native milk (pH 6.6). Using these data, the calcium and inorganic phosphorus in the micelle at each pH was derived and expressed as a % of that at pH of the native milk (pH 6.7). Redrawn from van Hooydonk et al. (1986)

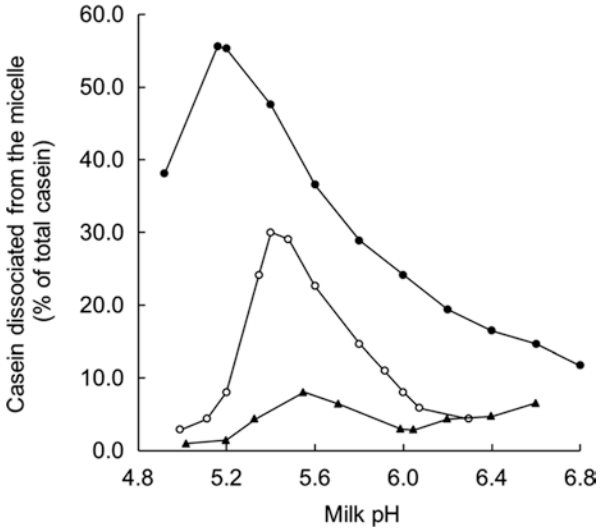


Fig. 16.4 Serum (non-sedimentable) casein in skim milk as a function of pH at 4 °C (*filled circles*), 20 °C (*open circles*) or 30 °C (*filled triangles*). Milks were adjusted to different pH values, equilibrated at 30 °C for 30 min and ultracentrifuged at 70, 000 g to give a pellet of sedimented casein micelles and a supernatant (serum). The casein in the serum was expressed as a % of total casein in the milk. Redrawn from Dalgleish and Law (1988)

maximum at pH 5.3–5.6 (depending on the temperature) and thereafter decreases to a minimum at the isoelectric pH (Fig. 16.4).
– an increase in casein hydration with pH reduction in the range 6.7–5.3 (Fig 16.5).

- a tendency for the casein micelles to aggregate into a more ordered system due to:
  - the reduction of the negative surface charge on the casein micelles, and hence inter-micellar repulsive forces, due to the production of lactic acid;
  - a decrease in casein hydration in the pH range 5.3–4.6 (Fig. 16.5);

16.3.2 Microstructural Changes During Gel Formation

The changes in the casein micelle at the various stages of acidification, from pH 6.6 to 4.6, have been monitored using freeze-fracture electron microscopy (Heertje et al. 1985), which presents a two-dimensional view. At pH values ≥5.9, no major changes are observed, with the casein micelles retaining their shape, integrity and dimensions. At lower pH, ~5.5, the casein micelles become more porous owing to the solvation of some casein (mainly β- and κ-caseins) and its permeation into the surrounding serum phase as the CCP (which contributes to electrostatic cross-linking of the caseins into a network within the micelle) solubilizes. The residual micelle is considered as a framework enriched in α_s-casein. Simultaneously,

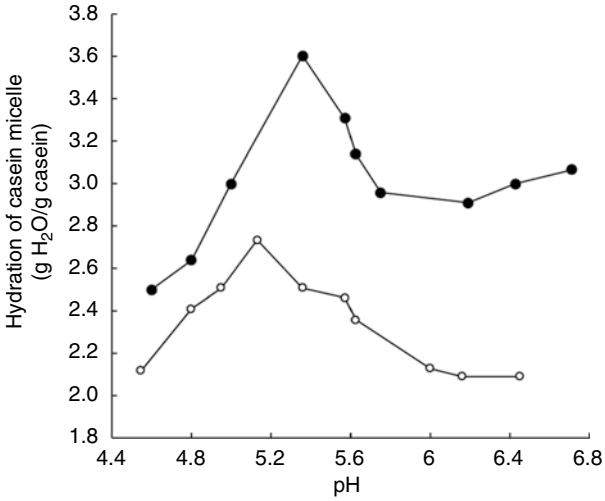


Fig. 16.5 Hydration of casein micelles as a function of pH at 20 °C for skim milk (*filled circles*) and *para*-casein micelles following rennet-treated of the skim milk (*open circles*). Redrawn from Creamer (1985)

dissociated caseins begin to self-associate in the serum into particles that are smaller than the original micelles, giving rise to a larger particle size distribution of casein particles in the milk than that observed at higher pH (i.e., ≥pH 5.9). As the pH falls further towards 5.2, where all the CCP is solubilized, the number of smaller casein particles increase, reflecting the greater degree of casein dissociation from the micelle. At lower pH values (≤5.1) as the onset of gelation approaches, the casein particles begin to rearrange, shrink and aggregate as the *zeta* potential decreases to <~4 mV. As the pH decreases further towards the isoelectric pH of the casein (~4.6), where the *zeta* potential is ~0 mv, the casein aggregates fuse into a larger particles, separated by large void spaces. These changes in the casein micelle structure lead to the formation of an acid-induced casein network (gel), the development of which can be easily monitored using scanning electron microscopy (Gastaldi et al. 1996; Figs. 16.6 and 16.7) which presents a three-dimensional view of the forming gel.

Three distinct stages of structure development are discernible based on pH:

(a) Stage 1 (pH 6.7–5.8): individual casein micelles begin to lose their identity, and clusters of casein particles begin to develop
(b) Stage 2 (pH 5.8–5.3–5.2): the casein particles from into a loose open particulate network comprised of strands of aggregated casein particles, in which the considerable extension and swelling of casein particles is evident
(c) Stage 3 (pH 5.3–4.6): shrinkage and contraction of the casein particles, and hence strands and network as a whole. The re-arrangement and shrinkage of the casein particles leads to a tighter casein network which has more numerous linkages than that observed in Stage 2.

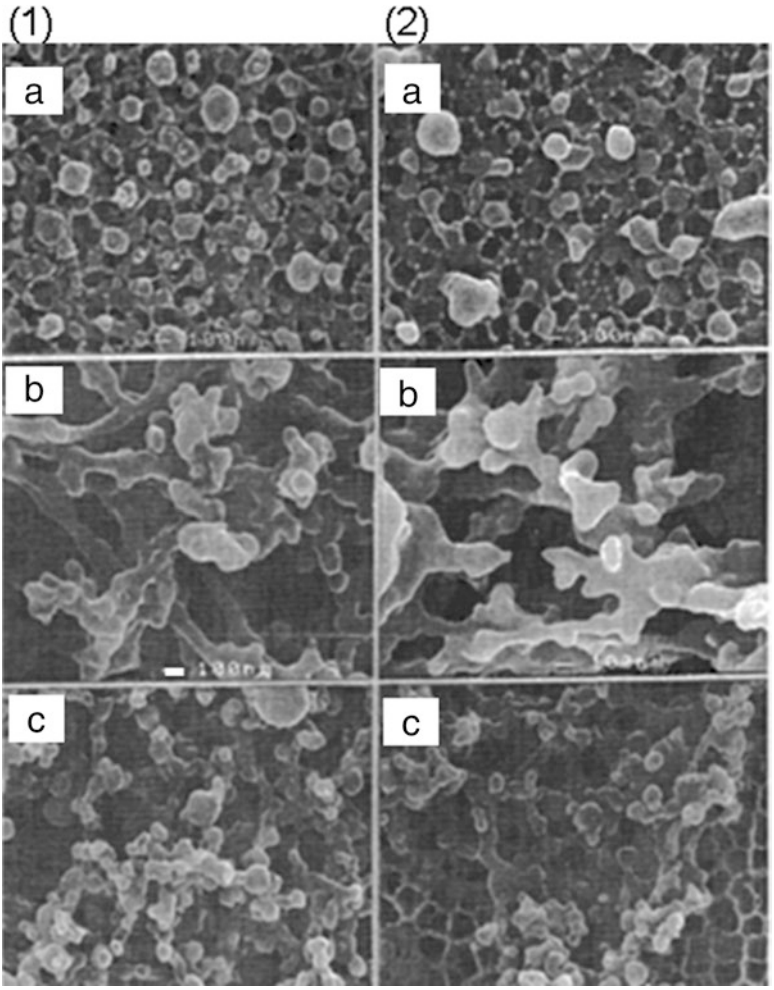


Fig. 16.6 Scanning electron micrograph of acidified milk samples obtained using two different techniques of sample preparation Critical-point drying (1**a**, **b**, **c**) or freeze-drying samples (2**a**, **b**, **c**). The pH of the milk samples was pH 6.7 (**a**), 5.3 (**b**) or 4.6 (**c**). Scale = 1 μm. From Gastaldi et al. (1996)

The gel is described as particulate, because when viewed by scanning electron microscopy, the individual gel strands are found to be composed of particles (casein aggregates) which undergo limited touching (over part of their surfaces) and are linked together, rather like the beads in a necklace. Compared to rennet-curd cheese, the degree of calcium bridging contributing to casein interconnectivity and casein network formation is significantly lower; owing to low pH (~4.5–4.8), all the CCP has been solubilised during gel formation and removed in the whey during subsequent concentration.

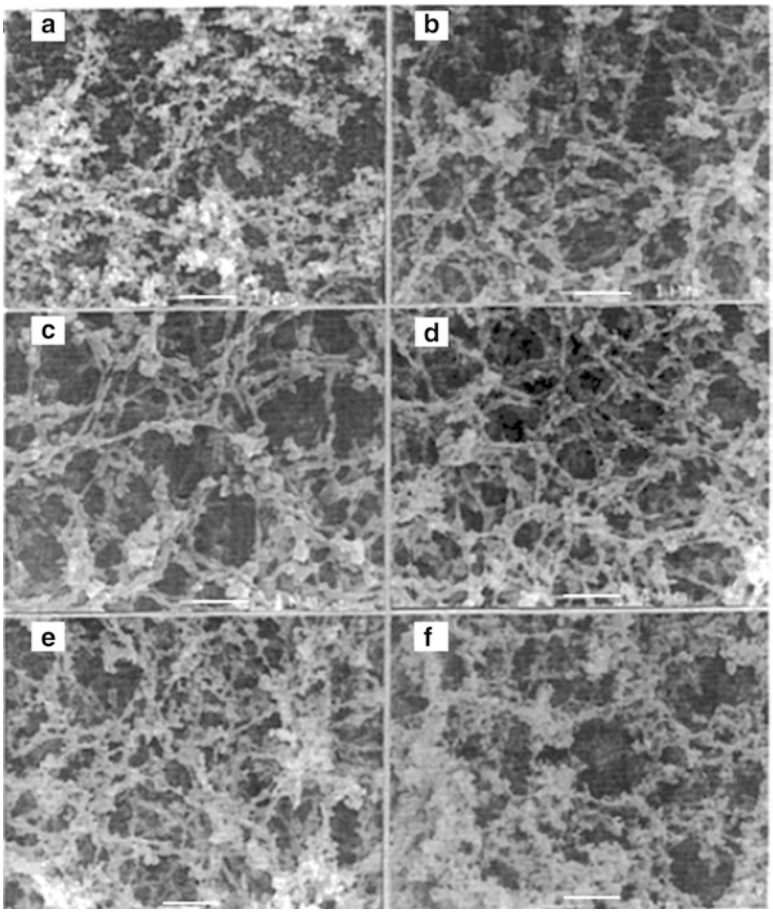


Fig. 16.7 Scanning electron micrograph of critical-point dried samples of acidified milk. The pH of the milk samples was 5.8 (**a**), 5.5 (**b**), 5.3 (**c**), 5.0 (**d**), 4.8 (**e**) or 4.7 (**f**). Bar = 1 μm. From Gastaldi et al. (1996)

The structure of the acid-induced milk gel has a major influence on its rheological and syneretic properties (Harwalkar and Kaláb 1981; Gastaldi et al. 1996; Lucey et al. 1997a, b, 1998a, b, 2001; Renault et al. 2000; Niki et al. 2003; Liu et al. 2013), as discussed in Sects. 16.5 and 16.6. The impact of gel structure on the quality characteristics of the final product depend on the extent to which the gel is subjected to further treatments, including shearing, heating, cooling and the addition of other materials, as discussed in Sect. 16.6.

16.3.3 Rheological Changes During Gel Formation

Gel formation is accompanied by a marked increase in the elastic shear modulus (index of rigidity), which increases sigmoidally as the pH of the gelling milk continues to decrease towards 4.6 during incubation. The principal interactions between the casein molecules are electrostatic and hydrophobic, with the strength or contribution of each type governed by the residual charge on the casein molecule (which is influenced directly by pH, ionic strength and Ca binding) and the temperature of the gel. However, covalent (disulphide) bonding between κ-CN and β-lactoglobulin are considered to contribute significantly to gel formation and elasticity of acid-induced milk gels formed from high heat-treated milk and to acid-heat induced milk gels (Fig. 16.8; Lucey et al. 1999; Lucey and Singh 2003; Vasbinder and de Kruif 2003).

The overall physico-chemical and microstructural changes accompanying the conversion of milk to an acid-induced gel are summarized in Fig. 16.9.

16.3.4 Prerequisites for Gel Formation

Acidification of milk may result in the formation of a gel or a precipitate, depending on the rate and extent of casein aggregation. Gelation of milk occurs when forces that promote aggregation of the casein particles slowly overcome those that

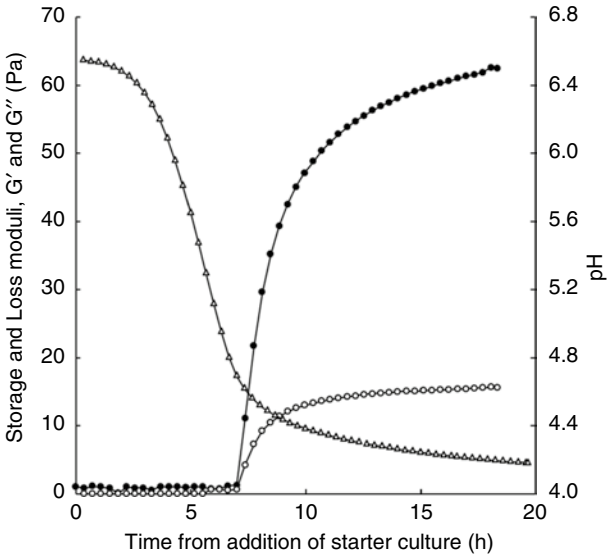


Fig. 16.8 Dynamic changes in pH (*open triangles*), storage modulus, G′ (*filled circles*) and loss modulus, G″ (*open circles*) during acid-induced gelation of skim milk. The skim milk (3.1 % protein) was pasteurized at 72 °C, cooled to 30 °C, inoculated with 2 % (w/w) *Lactococcus lactis* subsp. *cremoris* and incubated for 18 h. Modified from Guinee and Hickey (2009)

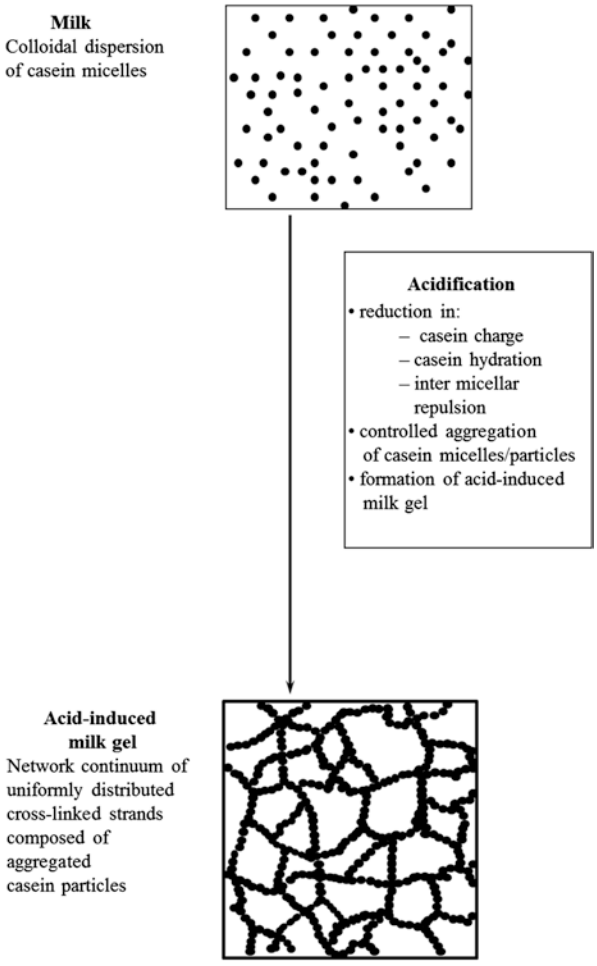


Fig. 16.9 Schematic representation of the conversion of milk to an acid-induced gel by slow quiescent acidification using a starter culture or on addition of glucono-δ-lactone

promote repulsion of the micelles. These conditions result in the formation of relatively loose, porous, hydrated aggregates of casein micelles, which are only slightly more dense than the serum phase in which they are dispersed. Owing to the relatively small density gradient between the aggregates and the serum phase, the aggregates have sufficient time to link together, via strand formation, to form a continuous casein network, which physically entraps the serum (whey) phase and fat globules.

In contrast, rapid acidification while agitating at a high temperature, as in the manufacture of isoelectric casein, leads to precipitation, rather than gelation, of the casein (Fig. 16.10). These conditions are conducive to rapid aggregation, a very

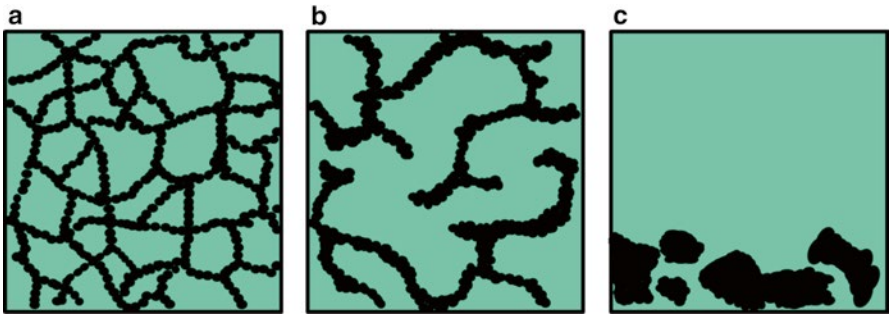


Fig. 16.10 Schematic representation of a fine-structured gel (**a**), a coarse-structured gel (**b**) and a precipitate (**c**). The gels differ in relation to the degree of casein aggregation and network structure: the fine gel is characterized by a limited degree of interaction between the casein particles and has a highly branched, continuous casein network (*black area*) that encloses the serum phase (*green area*); a coarse gel has a higher degree of casein interaction, thicker strands (branches) with a lower degree of interconnectivity and overall less continuity with large serum-filled spaces (pores); a precipitate has a very high degree of casein interaction, is non-continuous and does not imbibe the serum, which unlike a gel, forms the continuous phase. Gels are inherently unstable and network re-arrangement progressively leads to increasing shrinkage of the gel and accompanying expression of the enclosed serum (syneresis) and protein (casein) aggregation (which leads to sensory defects such as chalkiness and graininess) in fresh acid-curd cheese products. However, optimization of gel structure and the use of stabilizers such as hydrocolloids, where allowed, slow this progression

high degree of fusion between the surfaces of neighbouring casein particles and shrinkage. This leads to the formation of smaller, less porous and less hydrated aggregates, which owing to their relatively high density, sediment as a precipitate. Compared to a gel, the casein in a precipitate is highly aggregated, has a very low water-holding capacity and occupies a much lower specific volume, i.e., has a low voluminosity (Fig. 16.10). Thus, although the production of both acid casein and fresh acid-curd cheeses, such as Quark, involves the acidification of skim milk to ~pH 4.6, the conditions of acidification differ markedly (Fig. 16.11), resulting in the production of two very different types of casein structure, i.e., a network continuum (gel) structure which occludes the serum (Quark) or a precipitate from which the moisture is expelled rapidly (acid casein curd). Thereby, varying acidification conditions enables the recovery of casein as a food ingredient (as in acid casein) or a cheese (Quark).

The gel formed during acidification of milk may be described as coarse- or fine-structured. The structures of fine and coarse gels and of a precipitate, in which the concentration of gel-forming protein is equal, are depicted schematically in Fig. 16.10. Compared to a coarse gel, a fine gel is more highly branched and has thinner network strands, a more uniform distribution of, and greater interconnectivity between, the strands of the casein network, a higher network voluminosity (specific volume) and a lower pore size, i.e., a lower permeability coefficient. A finer-structured gel has a lower permeability coefficient, a higher water-holding capacity

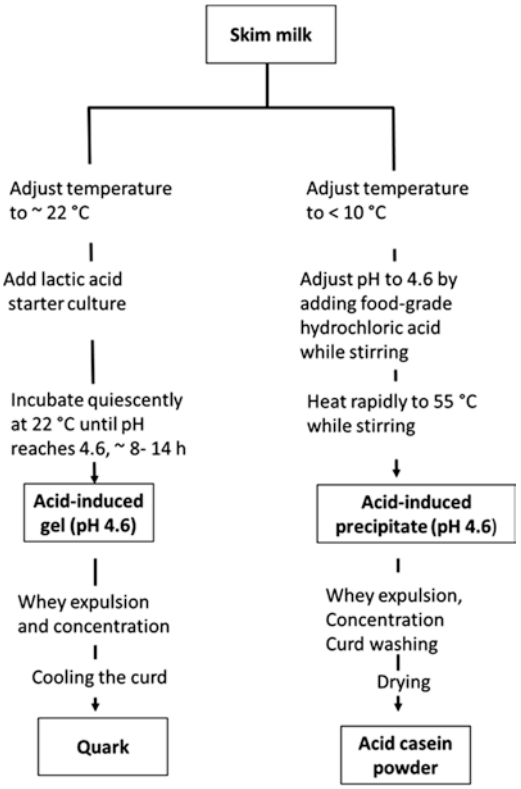


Fig. 16.11 Effect of acidification rate of skim milk to pH 4.6 on the casein structure formed. Slow quiescent acidification results in the formation of a casein gel that is subsequently concentrated to a curd and cooled to give Quark. In contrast, rapid acidification, agitation and heating to a high temperature results in a casein precipitate that is concentrated, washed and dried to give acid casein powder

and is less prone to syneresis; a finer gel also tends to be more rigid, but this depends on the types of interactions between the proteins. Conditions that favour the formation of a coarse gel over a fine gel are those that lead to a more rapid and higher degree of aggregation of the casein particles such as the use of unheated milk rather than pasteurized or high heat-treated (HHT) milk, a higher concentration of milk protein, a more rapid rate of acidification and a higher gelation temperature in the range 20–40 °C.

In extreme situations (e.g., if the rate of acidification is very slow, i.e., ≫16 h for the pH to decrease from ~6.6 to 4.6), when the number of inter-particle attraction sites is lower than optimum, slowly-forming casein aggregates may have sufficient time to precipitate before fusing and linking with neighboring aggregates to form a network. An example of the latter is the defect in Cottage cheese production known as 'major sludge formation' whereby phage infection of the starter, after acid development has progressed to an advanced stage (~pH 5.2–5.3), leads to casein precipitation rather than gelation (Brooker 1986; Grandison et al. 1986).

16.4 Gel Syneresis

Syneresis of acid-gelled based products may be categorized as induced, where it is affected by the application of forces to the acid milk to increase its dry matter content to that of the final cheese, or as spontaneous, where it occurs as an endogenous expression of whey (wheying-off) from the final product that is generally undesirable.

16.4.1 Induced Syneresis

Induced syneresis of, or whey expulsion from, the acid milk gel is necessary to concentrate the casein and fat, and to attain the desired dry matter content of the target cheese, e.g., ~14 % for Fromage frais to ~45 % for Cream cheese. This is achieved by subjecting the gel, following incubation, to concentration (i.e., whey removal) by cutting, stirring, heating, whey drainage by mechanical centrifugation or gravitational force (e.g., by pouring the broken gel onto muslin bags which are suspended on a frame). The application of stress by these actions results in breakage of the gel structure into particles, re-arrangement and shrinkage of the gel network within the individual particles, and whey expulsion.

Breaking of the gel enhances syneresis by:

- increasing multifold the surface area available for whey escape
- enabling re-arrangement and shrinkage of the protein network, as the broken gel strands come into closer proximity and to re-knit into a more compact arrangement
- the attendant development of a pressure on the serum entrapped within the network, which forces it through the pores of the network to the surfaces of the curd particles where it is released.

The prevailing conditions of low pH and relatively high temperature (e.g., 30 °C during the manufacture of Quark and ~80 °C during the manufacture of Cream cheese) enhance casein dehydration and aggregation.

Once syneresis has started, the outward flow of whey through the gel matrix becomes increasingly impeded over time due to the reduction in the porosity of the gel as it is concentrated. As the gel contracts and the cross-sectional area between the pores (spaces or channels between the strands of the casein network) decreases and exerts an increasing frictional effect on moisture passing through. For one-dimensional flow through a porous medium, such as an acid milk gel, the rate of syneresis, may be derived according to Darcy's law as follows (Walstra and van Vliet 1986):

$$v = \frac{B}{\eta} \times \frac{P}{l}$$

where, v is the average linear velocity of the whey (serum) flowing through the network in the direction of the pressure gradient $\Delta P/l$, ΔP is the pressure gradient arising from syneretic pressure exerted on the entrapped whey by the matrix, l is the distance over which the whey flows (from the high pressure region inside the curd matrix to the low pressure outside the matrix), η is the viscosity of the whey, and B is the permeability coefficient of the gel matrix, which corresponds to the average cross-sectional area of the gel pores. The permeability coefficient, B, depends on the volume fraction of the protein matrix and the spatial distribution of the matrix strands, i.e., gel fineness/coarseness. Compared to a coarse gel, a fine gel has a lower permeability coefficient (Fig. 16.10). For a given syneretic pressure, the resistance to the passage of serum through the gel decreases as the permeability coefficient increases. Hence, a fine gel structure, with a relatively low porosity, has a lower permeability to out-flowing whey than its coarse-structured counterpart. Consequently, while it is more difficult to remove whey from fine-structured gels during manufacture, they are less prone to spontaneous wheying-off during storage of the final product.

The influence of gel structure on its susceptibility to wheying-off may be easily explained by reference to Fig. 16.10, which depicts the structural differences between fine and coarse gels in which the concentration of gel-forming protein is equal. In the fine gel, A, the casein particles have formed into thin strands (chains), giving a highly branched and continuous gel network. Conversely, in the coarse gel, B, the casein particles have fused to a much greater degree to give thicker gel strands and a less continuous, more porous network structure, which is more susceptible to serum (whey) leakage on storage; whey drains easily through the large open channels between the network strands. In contrast, the gel-forming protein in 'A' is more uniformly distributed, resulting in a finer gel with smaller interstitial spaces or pores. The relatively low porosity of gel A retards the outflow of whey and so endows the gel with superior water-holding capacity compared to gel B, and a low tendency towards syneresis. Several factors enhance induced syneresis of the milk gels (Harwalkar and Kaláb 1983; Snoeren et al. 1984; Roefs et al. 1985; Lucey et al. 1997a, b; Schkoda et al. 1999; Schorsch et al. 2002; Lucey 2001; Lucey 2004; Castillo et al. 2006; Lee and Lucey 2010; Liu et al. 2013), including *inter alia*:

- reducing the heat treatment of the cheese milk prior to fermentation;
- addition of a small quantity of rennet to the milk (e.g., ~0.5–1.0 ml of single strength rennet/100 L) shortly (~2 h) after the beginning of fermentation when the pH is ~6.3;
- reducing the gel firmness (lower storage modulus, G′) at breakage and whey separation;
- increasing the rate of acidification during gelation by optimizing the temperature for growth of the starter culture;
- reducing the pH of the gel closer to the isoelectric pH before whey separation;
- increasing the temperature of the broken gel prior to whey separation;
- increasing the temperature and centrifugation force at whey separation.

The above factors exert their effects on syneresis principally by increasing protein aggregation and gel permeability (pore size). Conversely, syneresis is generally impeded by reducing the protein-to-fat ratio of the milk, high heat treatment of the milk (e.g., 95 °C for 2–5 min), high gel firmness at cutting, increasing gel pH, lower separation temperature, and the presence of materials which enhance the viscosity of the aqueous phase (e.g., addition of hydrocolloids prior to whey separation, production of exopolysaccharide by ropy starter cultures). High heat treatment of milk prior to fermentation depresses syneresis by giving a finer gel structure with a reduced permeability to whey, as affected by the denaturation of whey proteins and their complexation with κ-casein at the surface of casein micelles. The latter interaction leads to the onset of gelation at a higher pH (e.g., pH 5.5 for milk heated at 90 °C for 15 min *versus* pH 5.1 for unheated milk at an incubation temperature of 43 °C; Heertje et al. 1985; Lucey et al. 1998a), creates steric impedance by a higher degree of fusion of casein particles during acidification and gives a finer gel network with lower porosity.

16.4.2 Spontaneous Syneresis

Acid milk gels formed in situ in the package, such as set natural yoghurt, or fresh acid-curd cheese products made from milk concentrated to the final dry matter content prior to acidification and gelation (e.g., by ultrafiltration) generally show little tendency to syneresis if left undisturbed. However, spontaneous syneresis sometimes occurs to a greater or lesser degree during storage and is generally considered undesirable or a defect. Nevertheless, a small quantity of whey on the surface of some high-moisture acid-curd cheese products (e.g., fromage frais products) may be acceptable or even considered a desirable attribute that conveys an impression of naturalness or additive-free to the consumer.

Fresh acid-curd cheeses have an inherent susceptibility to wheying-off owing to their relatively high moisture-to-protein ratio and low pH compared to rennet-curd cheeses (e.g., ~6.5 g H_2O/g protein in Quark *versus* ~1.44 g H_2O/g protein in Cheddar cheese). Consequently, the protein network of fresh acid-curd products tends to be less constrained than that of rennet-curd cheeses. This is conducive to uncontrolled post-manufacture protein aggregation and dehydration because of rearrangement of the casein network, especially when subject to stresses, for example during transport/distribution and retailing. The increase in the degree of casein aggregation results in internal stresses within the gel and re-arrangement of the casein matrix, which, in turn, leads to syneresis. This is the basis of major sensory defects, including excessive wheying-off and the development of a sandy/grainy texture during storage. The tendency to protein aggregation, and the occurrence of these defects, is accentuated by a reduction in product pH, an increase in product temperature, and fluctuations in storage temperature. It has been suggested that variations in casein hydrolysis, due to starter culture properties (activity and acidifying potential, autolysis, proteolytic activity), may also be a contributory factor for

the widely different practical experience regarding syneresis in set fermented milk products (day-to-day in-factory and inter-factory inconsistencies) (Walstra et al. 1985). Other factors may include:

- an increase in the lactose content of milk, which is likely to cause differences in the level of lactic acid, lactate-to-protein ratio, and pH of the final product;
- variation in product composition (pH, salt content);
- manufacturing protocol and process, e.g., non-standardization of gel pH at whey separation and gel concentration and cooling conditions, including the time of cooling, and the temperature to which the concentrated gel (curd) is cooled.

Avoidance of such defects requires optimization of the degree of casein aggregation at the different stages of manufacture through the use of appropriate means such as temperature, pH, ionic strength, whey protein denaturation and their complexation with the casein micelles prior to fermentation. These influence the extent of casein aggregation during gel formation, whey separation and curd treatment (post-separation), and the structure and porosity of the casein network in the final acid-curd cheese.

16.5 Factors that Influence the Rheology and Syneresis of Acid-Induced Milk Gels

The structure of acid-induced milk gels is important since the structure of the gel affects syneresis of the gel during subsequent whey separation. To date, most published information on acid-curd cheeses relates to the factors affecting the structure and syneretic properties of acid milk gels prior to concentration and curd treatments. Nevertheless, from experience, gel structure is also likely to influence the quality of the final cheese (concentrated gel following whey separation). Some of the main factors that affect gel structure are discussed below.

16.5.1 Gel Structure

For networks of similar composition, gel strength is primarily dependent on the homogeneity of the gel, which determines the number of stress-bearing strands per unit volume of the network. Considering a gel to which a relatively small, one-dimensional stress (i.e., much less than yield stress) is applied in a direction x, the storage modulus (G′, i.e., ratio of shear stress to strain, σ/γ), which is an index of rigidity, elasticity and strength of the gel, can be related to the number of strands per unit area according to the following equation (Walstra and van Vliet 1986):

$$G' = cN \cdot d_2 A / dx^2,$$

where N is the number of strands per unit area of the gel in a cross section perpendicular to x, bearing the stress; c is a coefficient related to the characteristic length determining the geometry of the network; dA is the change in free energy when the elements of the network between which the bonds act, are moved apart over a distance dx on the application of stress (per unit cross-sectional area). Hence, the strength of the network is determined to a large extent by the number of strands, which in turn is controlled by:

- the total protein content,
- the level of gel-forming protein, which depends on the level of total protein and the degree to which whey proteins present are denatured and interact with the casein,
- the integration of emulsified (homogenized) milk fat globules into the network,
- the fineness or coarseness of the network

For a given level of gel-forming protein, a fine gel network has a greater number of stress-bearing strands than a coarse gel. The thickness, and hence the strength, of the stress-bearing strands is, on average, greater in the coarser gel because of the greater number of attractions between the aggregates. However, a fine gel generally has greater gel firmness than a coarser gel with a similar composition and concentration of gel-forming protein owing to the greater number of finer strands.

Compared to a gel, a precipitate (and its accompanying expressed whey) with the same level of gel-forming protein has a much lower G′ value, as the rheological contribution ensues mainly from the continuous whey phase (Walstra and van Vliet, 1986).

16.5.2 Level of Gel-Forming Protein

Higher concentrations of gel-forming protein generally result in denser gel matrices that have a higher number of strands per unit volume, and are more highly branched and less porous. The resultant gels are generally less prone to syneresis and are firmer and more elastic (i.e., higher G′) (Kaláb et al. 1976; Harwalkar and Kaláb 1980, 1983; Guirguis et al. 1984). The lower susceptibility to syneresis is particularly desirable in products where the gel is essentially the final product, e.g., set and stirred-curd yoghurts. Thus, it is widespread practice in the commercial manufacture of yoghurt and cold-pack fresh cheese products to increase the level of milk protein prior to acidification, e.g., by ultrafiltration of the milk or the addition of dairy ingredients, e.g., milk protein concentrate, skim milk powder, whey protein concentrate or blends of dairy ingredients.

The effective concentration of milk protein may also be increased, while maintaining the actual protein level constant, by:

- Homogenization of the milk (as practiced in the production of yoghurt and Cream cheese) which disrupts the native fat globule membrane, disperses the free fat, and coat (emulsifies) the newly-formed fat globules with a protein layer

consisting of casein micelles and whey protein, referred to as recombined fat globule membrane (RFGM). The emulsified fat globules behave like protein particles that become an integral part of the protein network. Consequently, homogenization of milk in the manufacture of Cream cheese leads to a more rigid gel (higher G′) at the end of fermentation (van Vliet and Dentener-Kikkert 1982; Ortega et al. 2000) and a firmer end-product (Phadungath 2005a).

- high heat treatment of the milk (e.g., 95 °C × 5 min) which causes denaturation and binding of whey protein and their complexation with κ-casein at the surface of the micelles or with κ-casein that has dissociated from the micelles into the serum phase (Vasbinder and de Kruif 2003; Mėnard et al. 2005); the denatured whey protein becomes part of the resultant acid-induced gel. In contrast, undenatured whey proteins are soluble at their isoelectric pH (i.e., ~4.6) and do not participate in gel formation.

16.5.2.1 Heat Treatment of Milk

High heat treatment (HHT) of milk (e.g., 90 °C × 5 min) prior to fermentation and gelation is widely practiced in the manufacture of fresh acid-curd cheeses, such thermoQuark, Fromage frais and Cream cheeses, and fresh cheese-based desserts (e.g., Shrikhand-type products). Compared to unheated milk or milk pasteurised at 72–73 °C for 15–30 s, HHT treatment of milk leads to the onset of acid-induced gelation at a higher pH (pH ~5.4 for milk heated at 90 °C for 30 min versus 5.2 for milk heated at 72 °C for 30 min) and formation of gels that are finer (more branched with a lower pore size) and more rigid (higher G′) and that generally have a lower tendency to wheying-off than the corresponding products made from unheated milk or milk pasteurized under standard conditions, e.g., 72 °C for 15 s (Figs. 16.12, 16.13 and 16.14; Table 16.2; Kaláb et al. 1976; Harwalkar and Kaláb 1981, 1983; Parnell-Clunies et al. 1986; Dannenberg and Kessler 1988a, b; Lucey et al. 1998a, b; Hinrichs 2001; Lucey 2004). These effects are attributable to extensive denaturation of the whey proteins (e.g., >70 % of total whey protein in HHT milk compared <5 % in pasteurized milk) and their binding (especially of β-lactoglobulin) to κ-casein via thiol-disulphide interaction. The denatured whey protein co-aggregates with the casein and becomes integrated into the acid-induced gel network. Microscopic analysis of high heat-treated milk shows that these denatured whey proteins form filamentous appendages that protrude from the surface of the micelle and reduce the extent of aggregation of casein on subsequent acidification. Compared to HHT milk, the casein in unheated milk or pasteurised milk undergoes a higher degree of aggregation during acidification and forms a coarser, less-continuous gel network that has a lower water-binding capacity (Fig. 16.13) and is more prone to syneresis during subsequent separation and concentration of the gel; the undenatured whey proteins in milk are soluble at the pH of acid gels (i.e., 4.5–4.8) and do not contribute to gel formation. Following gel concentration and whey separation, acid-curd cheese from unheated milk or pasteurized milk generally has a higher dry matter content, is heavier and more granular/curdy, and is more prone to

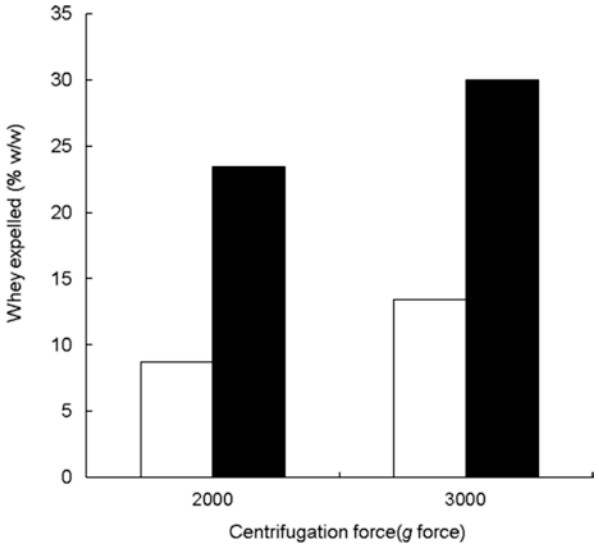


Fig. 16.12 Level of wheying-off (syneresis) from acid-coagulated gels formed from skim milk pasteurized at 72 °C × 15 s (*open bars*) or at 90 °C × 300 s (*filled bars*), as described in Table 16.2. After fermentation of the skim milk with a starter culture at 22 °C, the gels (pH 4.6) were stirred gently and samples were weighed into centrifuge tubes and held at 8 °C for 36–48 h. The samples were then centrifuged at 2000 or 3000 *g*; the weight of whey expelled was expressed as a percentage of the original sample weight. Guinee et al. (unpublished results)

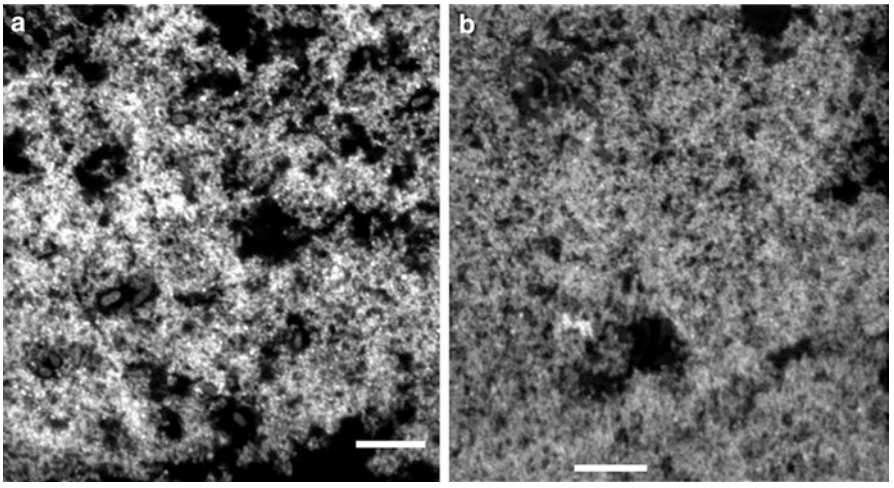


Fig. 16.13 Confocal laser scanning micrograph of an acid-induced gel from skim milk pasteurized at 72 °C × 15 s (**a**) or at 90 °C × 300 s (**b**), as described in Table 16.2. Bar = 10 μm. Guinee et al. (unpublished results)

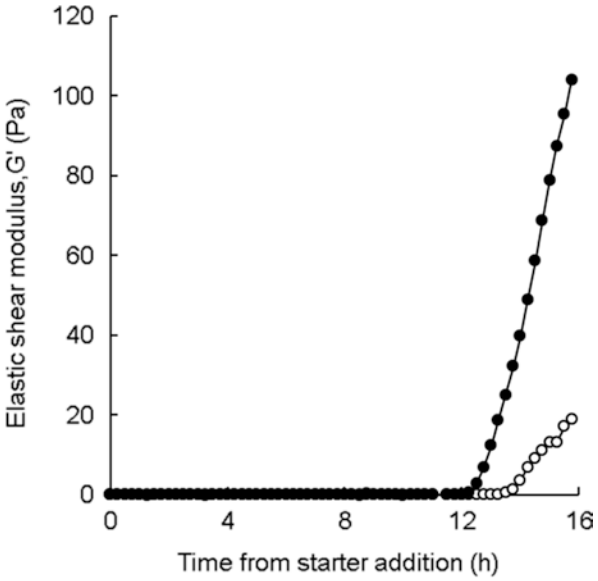


Fig. 16.14 Development of storage modulus, G′, index of rigidity, during the fermentation of skim milk pasteurized at 72 °C × 15 s (*open circles*) or at 90 °C × 300 s (*filled circles*), as described in Table 16.2. Guinee et al. (unpublished results)

Table 16.2 Effect of heat treatment on the level of whey protein denaturation in skim milk and the permeability coefficient of the resultant skim milk gels

	Heat treatment[a]	
	72 °C × 15 s	90 °C × 5 min
Milk composition		
Dry matter (%, w/w)	9.9	9.8
Total protein (g/kg)	3.7	3.7
Casein (%, w/w)	2.75	2.75
Whey protein (%w/w)	0.65	0.65
Denatured whey protein (% total)	2.5	70.0
Gel-forming protein (%, w/w)	2.80	3.20
Gel characteristics		
Storage modulus, G′ at 16 h (Pa)	100	20
Permeability coefficient (m^2)	2.56×10^{-13}	1.61×10^{-13}

Data presented in Figs. 16.11, 16.12 and 16.13 are for gels obtained from the above milks

[a]Guinee et al. (unpublished results)

wheying-off than acid-curd cheese from HHT milks. While the latter attributes are undesirable in many acid-curd cheese products including Quark, thermoQuark and Cream cheese, they are desirable in others where a slight curdy appearance and/or slight whey separation has appeal, e.g., traditional Quark (produced by straining the

broken gel through cheese cloth) and ripened acid-curd cheese varieties including Saurmilchkäse-types: Harzer, Mianzer, Olmützer (Schulz-Collins and Senge 2004). HHT treatment of milk is not suitable for the manufacture of Cottage cheese because it impairs syneresis and produces soft 'mushy', non-chewy curd granules (Farkye 2004a; see Sect. 16.7.3).

16.5.3 Incubation Temperature and Rate of Acidification

Increasing the acidification temperature of milk in the range 20–43 °C generally results in the following effects (Heertje et al. 1985; Kim and Kinsella 1989; Lucey et al. 1997a, b; Renault et al. 2000):

- the onset of gelation at a higher pH (e.g., pH 5.5 at 43 °C compared to pH 5.1 at 30 °C),
- the formation of coarser gels with a higher permeability,
- cheese products (following whey separation and gel concentration) that are firmer, more prone to sysnersis and have a rougher (less smooth) mouthfeel than those of acid-induced gels formed at lower temperature.

These effects are due to an increase in the extent of casein aggregation as promoted by (1) a more rapid rate of acidification associated with higher starter culture activity (provided that the increase in temperature does not become inhibitory to the starter culture used) or to a faster rate of hydrolysis of acidogen (e.g., glucono-δ-lactone, GDL, to gluconic acid), and (2) an increase in the extent of hydrophobic-induced protein interactions. Differing results have been found for the effect of incubation temperature on the rheology of the gel. Harwalkar and Kaláb (1981) investigated the effect of gelation (incubation) temperature on the firmness of milk gels formed by acidifying milk at 0 °C with GDL to a pH of 4.6 at 22 °C (after incubation and gelation) and heating quiescently to the gelation temperature in the range 40–90 °C. Firmness, as measured by the force required to penetrate the gel to a fixed distance using a 12.4 mm diameter probe, increased exponentially with gelation temperature. Similarly, Hashizume and Sato (1988) found that increasing the temperature of incubation from 60 to 80 °C for 30 min significantly increased the firmness of GDL-induced gels with a pH of 5.5. Lucey et al. (1997a) investigated the effect of temperatures in the range 20 to 40 °C on the gelation properties of sodium caseinate dispersions to which GDL was added. Increasing gelation temperature resulted in the onset of gelation after a shorter time, a more rapid development of G′ initially but a lower final (plateau) value of G′ after 70 h incubation (Fig. 16.15); shear deformation of the gels at 0.00185 s^{-1} indicated a 15-fold decrease in the magnitude of the fracture stress and an increase in the fracture strain, on increasing gelation temperature from 20 to 40 °C. These studies suggest differing trends for the effect of increasing the incubation temperature on the rheology of acid-induced milk gels formed using GDL, i.e., an increase in incubation temperature from 20 to 40 resulted in a lower plateau value of G′, a lower fracture stress and a higher

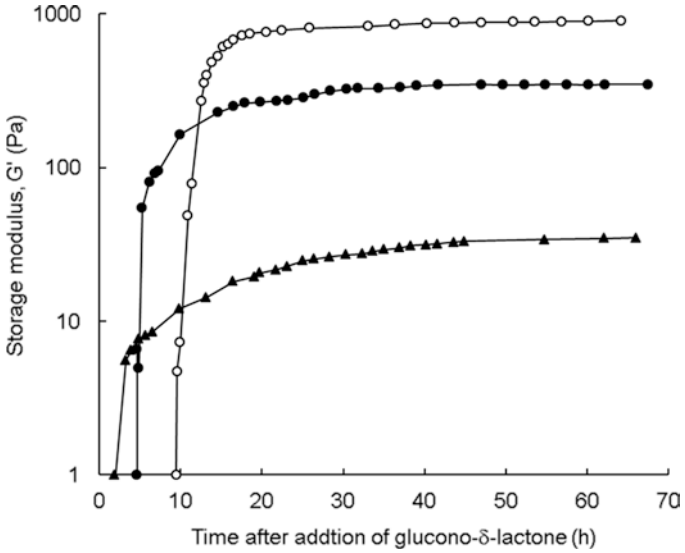


Fig. 16.15 Development of storage modulus as a function of time for acid-induced gels made from aqueous dispersions of sodium caseinate (~2.5 % protein) which were inoculated with 0.38 % (w/v) glucono-δ-lactone powder and incubated at 20 °C (*open circles*), 30 °C (*filled circles*) or 30 °C (*filled triangles*). Redrawn from Lucey et al. (1997a)

fracture strain in the aged gel (Lucey et al. 1997a), while an increase in gelation temperature from 40 to 90 °C led to a higher firmness of the aged gel (Harwalkar and Kaláb 1981; Hashizume and Sato 1988). However, it is likely that the positive correlation between gel firmness and gelation temperature (40–90 °C) is likely to be due, at least in large part, to the pro-rata increase in whey protein denaturation, their co-coagulation and incorporation into the gel with the casein.

In an extreme situation, rapid acidification to pH 4.6, for example by addition of acid at a temperature ≥20 °C, promotes rapid aggregation of casein with the formation of large dense aggregates which precipitate rather than form a gel. Unlike a gel, where the casein network forms a continuum entrapping the moisture and fat, expressed serum (whey) forms the continuous phase. Consequently, deformation of the latter requires relatively little stress compared to the former. Nevertheless, gel formation by rapid acidification is, however, possible when the tendency of micelles to coagulate is significantly impaired by acidifying to ~pH 4.6 at a low temperature (0–4 °C) and subsequent slow heating (~0.5 °C/min) under quiescent conditions to ~30 °C (Harwalkar and Kaláb 1981). Factors likely to contribute to the impedance of acid-induced gelation of milk at a low temperature include steric repulsion due to the protrusion of dissociating casein, especially β-casein (Fig. 16.4) from the micelle surface (Walstra and Jenness 1984) and decrease in calcium-induced cross-linking due to the solubilization of calcium phosphate (Fig. 16.3). The contribution of calcium is corroborated by the impairment of acid-induced gelation of milk as the

calcium content of milk is depleted through dialysis (Famelart et al. 2009) and by the more rapid onset of gelation and increase in storage modulus, G′, of the final gel on increasing the Ca^{2+} concentration of milk with $CaCl_2$ (Kim and Kinsella 1989).

16.5.4 pH of the Gel

The storage modulus, G′, and firmness of acid-induced milk gels increases with decreasing pH towards the isoelectric point and is maximal at pH ~ 4.6 (Fig. 16.8; Harwalkar and Kaláb 1980). These effects are due primarily to the concomitant reduction in negative charge of the casein and a greater degree of casein aggregation.

Lowering the pH of acid milk gels in the range 4.8–3.5 prior to cutting reduces the level of syneresis (Harwalkar and Kaláb 1980, 1983, 1986; Lucey et al. 1997a, b; Schkoda et al. 1999). The latter effect may be attributed to the increase in gel rigidity (G′), which reduces the ability of the casein network to rearrange and thereby the internal syneretic pressure on the enclosed serum. A lower degree of strand breakage of the stiffer gel affords less potential for new bonding sites and matrix contraction is thus less severe than otherwise. As a corollary, a decrease in pH of an acid milk gel during syneresis results in a higher level of syneresis than if the gel is brought to the same pH before cutting is initiated (Walstra et al. 1985). Hence, it is important that the pH of the acid-curd cheese is stabilized prior to packaging, to minimize the potential of spontaneous wheying-off during storage associated with damage (breaking) of the structure due to external stresses sustained during distribution/retailing.

16.5.5 Rennet Addition

It is common practice during the manufacture of some fresh cheese products, e.g., Quark and Cottage cheese, to add a small quantity of rennet to the milk shortly after culture addition (e.g., ~1–2 h), when the pH is ~6.1–6.3; typical levels of addition are 30–60 RU, or 0.5–1.0 ml single strength rennet, per 100 L milk. The addition of a low level of rennet increases the rate of casein aggregation and gel rigidity (G′) and enhances syneresis (Lucey et al. 2001; Niki et al. 2003). Therefore, a gel sufficiently firm for cutting and whey separation is obtained at a higher pH (e.g., 4.8 compared to 4.6); in the absence of added rennet, cutting is performed at pH ~ 4.6 so as to allow the gel to become more rigid and reduce its susceptibility to shattering and thereby prevent excessive loss of fines on whey separation. These effects are associated with rennet-induced hydrolysis of κ-casein and concomitant decreases in:

- the negative charge on the micelles (i.e., ζ-potential),
- extent of acid-induced casein dissociation from the micelles,
- casein hydration over the pH region 6.6–4.6.

Nevertheless, the addition of excess rennet (e.g., >2.0 ml of single strength rennet per 100 L) can lead to excessive hydrolysis of the casein (especially at the low pH of the milk; see Chaps. 12 and 13) which leads to a significant reduction in gel firmness (Niki et al. 2003) and increases the likelihood of a bitter flavour developing during storage.

16.6 Treatments of the Concentrated Gel after Whey Separation

In the production of fresh cheese products, the gel produced on acidification is subjected to whey expulsion and concentration, as discussed in Sect. 16.7. Exceptions include fresh cheese products produced from concentrated milk with a dry matter content equal to that of the finished cheese, for example when using ultrafiltered or microfiltered milk retentates (Hydamaka et al. 2001), recombined skim milk, or reformulated milks prepared by dispersion and blending dairy ingredients such as milk protein concentrates and whey protein concentrates/isolates in water (Guinee and Hickey 2009). Following whey separation, the curd (concentrated gel) for cold-pack varieties such as Quark, Fromage frais and low-fat Cottage cheese is cooled and held at this temperature during storage, distribution and retailing (Fig. 16.2; see Sect. 16.7). Hot-pack products such as Cream cheese, Neufchâtel and Mascarpone may be heated, blended with other materials (e.g., salt, stabilizers, flavouring materials, herbs), and heat-treated to a temperature of 75–85 °C, sheared, homogenized, hot-packed and stored at 4–8 °C. Little information is available on the effects of these treatments.

16.6.1 Cutting/Stirring the Gel

As for rennet-curd cheeses, the surface area available for whey release and gel concentration is increased by cutting into cubes (as in Cottage cheese) or by breaking into pieces using slow agitation (as in Quark, Cream cheese, Fromage frais). Stirring of the cut/broken gel exerts pressure on the curd matrix, leading to re-arrangement and aggregation of the protein network and enhancing syneresis.

Acid-induced gels are thixotrophic, undergoing shear thinning on shearing owing to breaking and alignment of the fragmented pieces of network. Shear thinning increases as the shear (stirring) rate increases. For a given degree of agitation, cooling of the gel to <20 °C (e.g., to retard a further decrease in pH before whey separation) results in a greater destruction of the gel matrix, as the contribution of hydrophobic bonding to the integrity of the protein network decreases (Kinsella 1984; Hayakawa and Nakai 1985; Bryant and McClements 1998). Hence, in the manufacture of Quark and Fromage frais-type products, rapid concentration of the

gel (using a mechanical separator) and cooling of the curd (using various type of heat exchanger such as scrape surface, tubular or plate to 4–8 °C) leads to smooth, soft, homogenous products. However, excessive shearing of the curd while cooling can result in products being too soft. In contrast, traditional manufacturing practices involving slower whey drainage at a higher temperature (e.g., 30 °C) and ambient cooling give products that are heavier and more curdy.

16.6.2 Heating the Gel or Curd

Heating the gel (e.g., in the range 25–30 °C) in the manufacture of Quark and Fromage frais-type products enhances protein aggregation and whey separation, making it easier to attain the desired dry matter content (~16–18 %, w/w). However, a higher temperature (>30 °C) is conducive to excessive protein aggregation and network shrinkage in the low pH environment. This can lead to defects in the final product such as chalkiness, astringency, sandiness or graininess; these textural defects are attributable to the presence of protein aggregates perceived during consumption. Hence, rapid cooling to <8 °C while shearing is normal practice to reduce the incidence of such defects.

In the manufacture of Cream cheese, the broken gel is heated to a much higher temperature (e.g., 75–85 °C) to promote greater whey separation and dry matter content (40–50 %, w/w). In contrast to Quark, the lower protein-to-fat ratio of Cream cheese (e.g., ~2.7 versus 24) and subsequent homogenization of the hot product prior to filling reduce the risk of defects such as graininess/sandiness and chalky mouthfeel in Cream cheese.

16.6.3 Homogenization of the Curd

Homogenization of the curd results in size reduction, to an extent depending on the magnitude of the shear, of large protein aggregates (e.g., >50 μm) formed during mechanical separation and heating of the curd. It thereby contributes to a more homogeneous size and spatial distribution of the matrix-forming material, and reduces the likelihood of a chalky or grainy texture in the final product.

In the manufacture of Cream cheese-type products, the hot curd at 75–85 °C may be held while sheared for a period of up to 1 h prior to packing. The final cheese becomes firmer and shorter (more brittle) as the holding time prior to cooling is increased, an effect attributed to an increase in hydrophobic-induced interactions between the proteins. Hence, variation of holding time and shearing of the curd at a high temperature is used commercially as a means of creating Cream cheese of different textures, i.e., soft, creamy texture after a short holding time and firm and brittle textures after a long holding time. Nevertheless, prolonged holding at a high temperature is conducive to the development of a chalky, powdery, gritty or grainy

mouthfeel. This defect may be attributed to excessive casein dehydration and the formation of large protein aggregates perceptible on the tongue and lips during consumption. Slow cooling accentuates this defect.

16.6.4 Addition of Stabilizers

A wide variety of plant and animal-derived hydrocolloids are allowed as stabilizers in some fresh cheese products, the type and level allowed depending on legislation (e.g., FAO/WHO 2011; CFR 2012; cf. Chap. 21). Typical hydrocolloids used include starches (native, modified) and gums (neutral gums, e.g., guar, locust bean gum; anionic gums, e.g., pectins, alginates, gellan, carrageenans) (Phillips and Williams 2000). They are generally added to the curd after whey separation and concentration under optimized conditions of shear and temperature to facilitate dispersion, hydration and functional expression. They may serve a number of interrelated functions (cf. Chap. 18), deepening on the type and level added:

- water binding, to increase the viscosity of the aqueous phase and thereby restrict the movement of protein/protein-fat particles;
- product structuring to give the desired degree of viscoelasticty and rheological behaviour by (a) forming a polymer gel network that reinforces and immobilizes the existing network, or (b) binding to the protein and thereby creating a particle network.

These functions in turn may serve to improve product quality by imparting the desired:

- physico-chemical stability and shelf-life, by controlling the propensities to wheying-off, and oiling-off
- rheological properties (e.g., viscoelasticity, stiffness, fracture properties, firmness)
- functionality, e.g., hardness/softness, sliceability, crumbliness and spreadability of the unheated cheese, and heat-induced flow or oiling-off of the heated cheese
- sensory properties of unheated and cooked/heated cheese, e.g., mouthfeel and texture of the unheated and heated/cooked product by limiting the degree of protein/protein-fat particle interaction.

Generally, a blend of hydrocolloids is best suited to these functions because of synergistic effects which optimize the blend functionality. Some anionic hydrocolloids (e.g., pectin, alginate, carrageenans, acyl gellan) may form networks that impart structure and rigidity to the unheated cheese matrix to varying degrees depending on type and composition of hydrocolloid (e.g. degree of sulphation of carrageenans), composition of cheese (e.g., concentrations and type of salt present, pH), and manufacturing conditions (e.g., severity of heat treatment, shear and rate of cooling of the curd-hydrocolloid mixture). Similarly, the thermo-reversibility of

these structures on heating the final cheese varies from irreversible (e.g., sodium alginate) to fully reversible (κ-carrageenan), and so affect the functionality of the heated cheese. Neutral hydrocolloids primarily affect stability and texture by increasing water binding and viscosity.

16.7 Major Fresh Acid-Curd Cheese Varieties

The manufacture and quality of these cheeses has been reviewed extensively (Guinee et al. 1993; Farkye 2004a, b; Schulz-Collins and Senge 2004; Özer 2006).

16.7.1 Acid-Coagulated Cheeses: Quark, Labneh and Related Varieties

Quark and Labneh represent the classical fresh cheese product, in that they are essentially fermented/acidified milks from which some whey is removed (traditionally by straining through cheesecloth) to recover the gelled solids in more concentrated form. Numerous other products of this type have evolved in different regions of the world, the most notable being Chakka (India). Nevertheless, these products differ somewhat in production details (~e.g., temperature, shear), which gives rise to sensory differences that lead to distinctive products appreciated in different markets.

16.7.1.1 Quark (Quarg)

Also referred to as Quarg or Tvorog in some European countries, Quark is a cheese of major commercial significance in Germany where annual per caput consumption is ~7.1 kg. Quark is a soft, homogeneous, mildly-supple white cheese with a smooth mouthfeel and a clean, refreshing, mildly-acidic flavour. The product is shelf-stable for 2–4 weeks at <8 °C; stability refers to the absence of:

- bacteriological deterioration, and
- wheying-off (syneresis) and the development of graininess and over-acid or bitter flavour during storage.

Quark is sometimes loosely referred to as the German equivalent of Cottage cheese. However, while these cheeses are related in the sense that both are fresh acid-curd products of similar composition, they are quite different from a production viewpoint and in sensory aspects; Cottage is a (dressed) granular cheese comprised of distinct, separate curd granules that are frequently coated with a light dressing of cream and have a chewy, meat-like texture.

Traditional Quark is normally made from pasteurized (72–85 °C × 15 s) skim milk which is cooled to 20–23 °C and inoculated with an O-type culture. The milk is then held for 14–18 h until the desired pH of 4.6–4.8 is reached; shortly after culture addition (e.g., 1–2 h), a small quantity of rennet (30–60 RU/100 L) is added when the pH is ~6.3–6.1. Rennet gives a firmer coagulum at a higher pH; its addition minimizes casein loss on subsequent whey separation and reduces the risk of over-acid products (in the absence of rennet, a lower pH is required to obtain the same degree of curd firmness). The fermented gelled milk is stirred gently (100–200 rpm) into a smooth flowable consistency and pumped to a nozzle centrifuge where it is separated into curd and whey, containing 0.65 % whey protein and 0.19 % non-protein N. The curd (Quark) is cooled immediately (<10 °C) *en route* to the buffer tank feeding the packaging machine.

Various methods have been used to reduce the loss of whey proteins and increase yield (Jelen and Renz-schauen 1989; Hinrichs 2001):

- **Westfalia Thermoprocess**, where the milk is typically heat-treated to 95–98 °C for 2–3 min, the gelled milk (pH 4.6) is heated to 60 °C for ~3 min and then cooled to the separating temperature (25 °C). In this process, 50–60 % of the whey proteins are recovered in the cheese;
- **Centriwhey process**, where the whey from the separator is heated to 95 °C to precipitate the whey proteins. The denatured whey proteins are recovered by centrifugation in the form of a concentrate (~12–14 % dry matter) which is added to the milk for the next batch of Quark;
- **Lactal process**, where the whey from the separator is heated to 95 °C to precipitate the whey proteins which are allowed to settle. A concentrated whey (~7–8 % dry matter) is obtained on partial decantation of the whey. A whey Quark (17–18 % solids), which is blended at a level of ~10 % with regular Quark, is produced on further concentration using a nozzle centrifuge.
- **Ultrafiltration** (UF) of the gelled milk is used on a large scale for commercial production of Quark and other fresh cheese varieties. This method gives full recovery of whey proteins in the cheese.

The levels of casein and whey protein in skim milk Quark produced by subjecting the acid-induced milk gel to centrifugal separation or ultrafiltration techniques are ~13.4 and 0.6 g/100, or 11.0 and 3.0 g/100 g, respectively. However, whey proteins in the native state do not gel under the cheesemaking conditions used for Quark made using either UF or centrifugation techniques. Hence, while the level of total protein in cheese produced by either of these methods is similar, the concentration of gel-forming protein differs, being lower in UF Quark. Therefore, suppliers of ultrafiltration units to the Quark industry recommend a high heat treatment (95 °C × 3–5 min) of milk prior to fermentation. The high heat treatment causes extensive denaturation of the native whey proteins and their complexation with the casein micelles (see Sect. 16.5.3), and thereby increases the level of gel-forming protein in ultrafiltration-produced Quark to a level similar to that in Quark produced by centrifugation. Otherwise, Quark produced by UF, while having the standard protein content (~13–14 %), has a relatively thin consistency due to the lower level

(11 versus 13.4 %) of matrix-building protein. Quark produced by the recommended UF-procedure (i.e., high heat milk treatment prior to culturing) has sensory characteristics similar to those of Quark produced using centrifugation.

Owing to its relatively high-moisture (~82 %) and low-protein (14 % g/kg) levels, the shelf-life of Quark is 2–4 weeks at <8 °C due to microbial growth, syneresis and off-flavour (especially bitterness) defects. Microbiological quality can be improved by various methods, including the addition of sorbates, modified atmosphere packaging, thermization (58–60 °C) of the broken gel prior to separation or high heat treatment of the product (containing hydrocolloids).

Addition of excessive rennet to the cheese milk, while increasing the yield of Quark, can cause an unacceptable bitter flavour and, thereby, reduce shelf-life. Sohal et al. (1988) found that increasing the quantity of single strength rennet (containing 88 RU/ml) from 0–4.4 ml/100 L milk increased the intensity of bitterness perceived in the cheese after storage for 1–4 weeks at 5 or 10 °C. However, a level of ≤0.45 ml single strength rennet/100 L gave an acceptable product which did not differ significantly from the control (with no added rennet) in terms of bitter intensity score.

Quark produced from lactose-hydrolysed milk is sweeter and has a yellower colour than that produced from control milk (Sheth et al. 1988).

16.7.1.2 Quark-Based Desserts

Further processing of Quark curd (e.g., heating, homogenization and/or aeration) and the addition of various ingredients (e.g., spices, herbs, fruit purees, cream, sugar, other fresh fermented products of different fat levels, hydrocolloids) give rise to a range of Quark-based products such as half-fat (20 % FDM) and full-fat (40 % FDM) Quark, fruit and savory Quarks, Shrikhand, dairy desserts and fresh cheese preparations.

16.7.1.3 Labneh and Related Varieties

Labneh, also referred to as Labeneh or Laban, is a very popular fresh fermented milk cheese consumed in large quantities in the Middle East and the Balkan Peninsula; related products include Torba (Turkey), Tan (Armenia), Stragisto (Greece) and Syuzma (Russia) (Özer 2006). In the Middle East, it is a daily stable food that is consumed at breakfast and is popular in cafes and food service outlets.

These products are versions of concentrated, natural stirred-curd yoghurt which represent the interface between the classical fresh cheeses (i.e., standard separator Quark and Cream cheese) and yoghurt. As for yoghurt, the milk is subjected to a high heat treatment (~85–90 °C × 5 min) so as to effect a high degree of β-lactoglobulin-κ-casein interaction, which in turn leads to a finer gel network which gives a product with a smoother mouthfeel and the ability to occlude more water. In contrast to yoghurt, the milk is not normally fortified and the coagulated milk is concentrated by various means (pouring into cloth bags, as for example in

traditional Labeneh manufacture, use of a nozzle-type centrifuge or ultrafiltration). Labneh typically contains ~25 % dry matter, ~10 % protein, and 12 % fat, although the composition can vary according to national legislation.

Conventional manufacture of these products generally involves:

- Standardization to the desired protein-to-fat ratio and heat treatment of the milk;
- Inoculation with a yoghurt-type starter culture (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*), incubation at ~40 °C, and fermentation/acidification to pH 4.4;
- concentration of the coagulated milk by drainage in cloth bags for 15–20 h at <10 °C, by mechanical separator (Özer 2006), or by membrane filtration involving either fermentation of the standardized milk prior to concentration, or concentration of the standardized fermented milk.
- smoothening of the separated curd by passing through shearing devices or by homogenization.

These products may be flavoured by the addition of sugar, fruit purees or other condiments which are blended in prior to homogenization. While acceptable products in their own right, they are (like Quark and Cream cheese) often blended with yoghurt or other fresh cheeses for the production of 'new' fresh cheese preparations with different compositional, textural and flavour attributes.

16.7.2 Acid-Coagulated Cheeses: Cream Cheese and Related Varieties

Cream cheese (hot pack) is a cream-white coloured, clean and slightly acid-tasting product with a mild diacetyl flavour; its consistency ranges from brittle, especially double Cream cheese (DCC) to spreadable, e.g., single Cream cheese (SCC). The product, which is very popular in North America, has a shelf-life of ~3 month at <8 °C. Reviews on the manufacture of cream cheese and related varieties, such as Neufchâtel, Petit Suisse, Mascarpone and Kajmak, include Schulz-Collins and Senge (2004, Phadungath (2005a) and Guinee and Hickey (2009).

Cream cheese is produced from standardized (typically to a fat: protein ratio of 2.85:1 for DCC, or 1.2:1 for SCC), homogenized, pasteurized (72–75 °C × 30–90 s) milk. Homogenization is important for the following reasons:

- it reduces creaming of fat during the fermentation/acidification stage and therefore prevents compositional heterogeneity of the resultant gel,
- it reduces fat losses on subsequent whey separation, and
- the reformed fat globules become coated with a protein layer consisting of casein micelles, sub-micelles and to a lesser extent whey protein, referred to as recombined fat globule membrane (RFGM). The RFGM enables the emulsified fat globules to participate in the formation of the casein network, thereby increasing the effective concentration of gel-forming protein. The incorporation of fat into

the gel structure by this means gives a smoother, firmer curd and therefore is especially important to the quality of cold-pack Cream cheese where the curd after whey separation is not treated further.

Following pasteurization, the milk is cooled (20–30 °C), inoculated with a mesophilic starter culture, and held at this temperature until the desired pH, ~4.5–4.8, is reached. The resulting gel is agitated gently, heated and concentrated by various methods:

- draining through muslin bags at 60–90 °C over 12–16 h, as in the traditional batch method;
- continuous concentration using a centrifugal curd separator at 70–85 °C, or
- ultrafiltration at 50–55 °C.

In the batch method, the curd is cooled to ~10 °C and salt (5–10 g/kg) and hydrocolloid (<5 g/kg; e.g., sodium alginate, κ-carrageenan, locust bean gum, guar gum) added. Then, the treated curd may be:

- packaged directly as cold-pack Cream cheese, which has a somewhat spongy, aerated consistency and a coarse appearance, or
- heated (70–85 °C) and sheared by batch (in a process cheese-type cooker at a relatively high shear rate for 4–15 min) or continuous (in scraped-surface heat exchangers) cooking. The degree of heat and shear and the duration of cooking have a major influence on the consistency of the final product; increasing the latter two parameters, while keeping the temperature constant, generally results in an increasingly more elastic and brittle texture. The hot, molten product, known as hot-pack Cream cheese, has a shelf-life of ~3 months at 4–8 °C.

In the continuous production method, curd from the separator is treated continuously with stabilizer via an on-line metering/mixing device, pumped through a scraped-surface heat exchanger, homogenized on-line, and fed to the buffer tank feeding the packaging machine.

Owing to the thick, viscous consistency of the curd, concentration by UF necessitates a two-stage process (stage 1: standard modules with centrifugal or positive displacement pumps; stage 2: high-flow modules with positive displacement pumps) in order to maintain a satisfactory flux rate and obtain the correct dry matter level.

The flavour diversity of Cream cheese may be increased by adding various flavours, spices, herbs and/or sterilized deboned-fish. Cream cheese-type products may also be prepared by blending two or more acid-curd products (e.g., fermented cream, Ricotta, Quark, cultured buttermilk), followed by pasteurizing and homogenizing the blend and hot packing. These cheeses compare well with commercial double Cream cheese in all quality aspects.

The manufacture and sensory attributes of other cream cheese-type products, such as Neufchâtel and Petit Suisse, are similar to double Cream cheese; they differ mainly with respect to composition. Mascarpone, however, differs from other Cream cheese-type products in that acidification and coagulation are brought about by a combination of chemical acidification (using food-grade organic acids, such as

lactic or citric) to ~pH 5.0–5.6 and heat (90–95 °C) rather than by starter fermentation at 20–45 °C. The hot, acidified cream (400–500 g/kg fat), which is Mascarpone cheese, is packed in cartons or tubs and stored at ~5 °C. The product, which has a shelf-life of 1–3 weeks, has a soft homogeneous texture and a mild buttery, slightly tangy flavour.

16.7.3 Acid/Rennet-Coagulated Cheeses

16.7.3.1 Cottage Cheese

Cottage cheese is a soft granular, unripened cheese in which the curd granules are lightly coated with a salted cream dressing; the flavour ranges from a cream-like blandness to mildly acidic with overtones of diacetyl. While coagulation is essentially acid-induced, as for Quark and Cream cheese, a small quantity of rennet (<3 % of that normally added to rennet-curd cheese for milk of similar protein content) shortly after starter inoculation (pH ~ 6.3). Hence, Cottage cheese may be classified as an acid/rennet-coagulated cheese. Rennet serves two main functions: it imparts the desired curdy, 'chewy' texture, and minimizes the risk of a soft gel which shatters on cutting. Even though the quantity of added rennet is small and the set temperature (~22 °C) is sub-optimal for rennet-induced coagulation, the long incubation time (between rennet addition and cutting the gel) prior to separation ensures casein hydrolysis and the formation of acidified *para*-κ-casein. The latter is more amenable than acidified casein to the formation of a curdy texture that resembles rennet-curd cheese and clearly distinguishes Cottage cheese from other acid- or acid/heat-coagulated cheeses. Studies on the manufacture and factors affecting the quality of Cottage cheese include Emmons (1963a, b), Sandine (1975), Emmons et al. (1976), Jensen (1983), Emmons and Beckett (1984), Grandison et al. (1986) and Farkye (2004a).

Cottage cheese is made from skimmed, pasteurized (72 °C × 15 s) milk which is inoculated with a mesophilic DL starter at a level depending on the set time: long set (21–23 °C for a 14–15 h incubation time using 5–10 g/kg starter) or short set (30–32 °C for 4–5 h using 4–50 g/kg starter) (Farkye 2004a). The starter normally consists of lactic acid (*Lc lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis*) and flavour producing bacteria (citrate-positive lactococci or *Leuconostoc mesenteroides* subsp. *cremoris*). The metabolism of citrate by the latter results in the production of the flavour compounds, diacetyl and acetate, and CO_2, which vapourizes on subsequent cooking (~55 °C) and forms gas bubbles which tend to cause floating of curd particles to the top of the whey. Excessive CO_2 production gives rise to the defect known as "floating curd", which reduces the yield; the curd is fragile and shatters on cutting and stirring to give fines which are lost in the whey and wash water. However, selection of a suitable starter with the correct balance of acid and flavour producers gives cheese of a satisfactory flavour while avoiding the above defect. Much of the diacetyl produced (>3.2 mg/kg) is lost in the whey. The risk of floating curd is mini-

mized by removing the flavour producing strains from the culture; instead, diacetyl may be added directly to the cream dressing or the creaming mixture may be cultured with a diacetyl-producing starter. Another starter-related problem in Cottage cheese manufacture is agglutination and its associated defect of "minor-sludge" formation, i.e., the formation of a layer of fragile, discoloured (yellowish) material at the bottom of the vat during acidification. Agglutination of starter lactococci is due to immunoglobulins which occur naturally in milk as part of the whey protein fraction and are at a particularly high level in colostrum and mastitic milk. On agglutination, the starter bacteria clump and settle to the bottom of the cheese vat, resulting in the localization of lactic acid production to give a pH difference of ~0.5 units between the milk at the top and the bottom of the vat after ~4 h incubation. Consequently, precipitation of casein (~4–8 % of the total casein) occurs to form a sludge, which shatters on subsequent cutting and stirring to produce fines that are lost on whey drainage and washing. The risk of starter agglutination is reduced by homogenizing the skim milk (e.g., at ~155 bar) or the bulk culture (e.g., at 176 bar) or by the addition of lecithin to the bulk culture. Homogenization of skim milk destroys agglutinins, while homogenization or addition of lecithin to the culture causes fragmentation of starter chains without affecting cell numbers or acid production. The defect known as "major sludge formation", whereby all the casein forms a precipitate (which cannot be made into satisfactory cheese) rather than a gel during acidification, is thought to be due to phage infection of starter after acid development is well advanced (i.e., at pH 5.2–4.9).

As for Quark, a small amount of rennet is added to the milk when the pH reaches 6.3 with the aim of increasing gel firmness at cutting. The optimum pH at cutting is ~4.8. At a constant cooking temperature in the range 50–60 °C, the firmness and dry matter of the final Cottage cheese increase with pH at cutting in the range 4.6–4.9. At a cutting pH > 4.8, the curds tend to coalesce and knit on cooking, giving rise to clumping of the curd granules. However, if the heat treatment is more severe than normal pasteurization (i.e., > 72 °C), the curds synerese poorly during subsequent stirring and cooking, giving a soft mushy product. Increasing the cutting pH to >4.9 (e.g., to 5.1) and the level of rennet added enhance the syneretic properties of curd from high heat-treated milk, without the risk of matting. In addition to heat treatment of the milk, other factors which influence the firmness and syneretic properties of the curd during cooking, determine the desired cutting pH:

- milk composition (stage of lactation)—at a given cutting pH, a higher casein level gives a firmer gel, which synereses better than those from low-protein milk;
- level of added rennet—higher levels of rennet addition promote firmer gels at a given pH; however, as for Quark, excess rennet causes bitterness.
- grain size: large curd granules, because they require a longer time to dehydrate on cooking and stirring, tend to be more susceptible than smaller grains to shattering and therefore generally necessitate a higher cutting pH and hence a coagulum which firms more rapidly on cooking.

All other factors being equal, reducing the pH from 4.8 to 4.6 tends to give a softer, more fragile coagulum, an effect which may be due to increased loss of

casein-bound calcium, which impairs the ability of the curd to synerese and become firm during stirring and cooking. The cut size depends on whether a large curd (~2 cm cut) or small curd (~1 cm cut) end-product is desired.

After cutting, the curds are allowed to settle for 5–15 min (depending on their firmness) to undergo "healing" of the cut surfaces, gently agitated and cooked slowly, i.e., at 1 °C/5 min to 40 °C and at 2 °C/5 min to 55 °C. Higher cooking temperatures enhance syneresis and consequently give a product in which the curd granules are more defined and stronger and have a more chewy, meat-like texture. When the curd particles have acquired the correct degree of resilience and firmness, the whey is drained off and the curds are washed to:

- prevent them from matting together,
- remove lactose and to minimize the growth of spoilage bacteria in the final product,
- remove lactic acid and therefore prevent the likelihood over-acid-tasting cheese.

Washing involves the addition of water (at a volume equivalent to the volume of whey drained) at ~25 °C to the curds, stirring for 2–3 min and then draining. This process is repeated 2–3 times using ice-water chlorinated to a level of 5–25 mg/kg. The wash water is drained and the cooled curd grains are trenched and allowed to stand for at least 1 h until all wash water has drained away.

A homogenized, pasteurized, salted (10–40 g/kg salt) cream dressing (90–180 g/kg fat) is mixed with the curds at a level which gives a finished product with the desired level of salt (8–10 g/kg) and fat, e.g., dry-curd cottage cheese (fat < 5 g/kg), low-fat Cottage cheese (5 g/kg < fat < 20 g/kg) and Cottage cheese containing not less than 40 g/kg fat. The dressing may be cultured or contain added starter distillate; the use of such a dressing is advocated when a plant is experiencing production difficulties as a result of "floating curd", as discussed above.

16.7.3.2 Baker's Cheese

Baker's cheese is soft, granular fresh cheese produced specifically for bakers and confectioners (Davis 1976; Kosikowski and Mistry 1997). It may be classed as a hybrid as it bears a resemblance to both Cottage cheese and Quark. Compared to Cottage cheese, it has a higher moisture content, is more acidic and the granules are softer, while it is less homogenous than Quark. A key characteristic is the absence of any graininess or sandiness. These characteristics are achieved by removal of most of the calcium (caseinate calcium and colloidal calcium phosphate), preferentially separating the broken gel in cloth bags (rather than in centrifugal separators which tend to give a more continuous, less granular product), and cooling the drained curd by packing ice around the cloth bags.

Manufacture essentially involves pasteurization and cooling of skim milk to ~30 °C, addition of a mesophilic starter culture and a small quantity of rennet (1–2 ml single strength rennet per 100 L), incubation until the pH reaches ~4.6 or until the gel is sufficiently firm to cut or break, pouring of the broken gel into cloth bags and

allowing the whey to drain, cooling the bags of drained curd on ice, addition of 1 % salt to the drained curd and gently mixing, packing the salted curd into tubs or cartons and storing at 0–1 °C. The product is used within a few days of manufacture.

16.7.4 Acid/Heat-Coagulated Varieties

16.7.4.1 Paneer

Paneer is an acid/heat-coagulated cheese that is consumed in large parts of southern Asia, including India, Afghanistan, Pakistan and Iran (Mistry et al. 1992; Aneja et al. 2002; Umer Khan and Asraf Pal 2011; Kumar et al. 2014). It is a product of antiquity, having been developed by nomadic tribes (e.g., Bhakhtiari) in the arid mountainous regions. Outside of India, Paneer-type products include Panir-Khiki, Panir-e-Shour (Iran), Zspiri (Himalayas) Kareish (Egypt), Armavir (Western Caucasus) and Queso Llanero (Latin America).

Typical Paneer cuisine includes fried dishes/snacks (e.g., Paneer Pakora, Paneer Tikka), sauce-based meals (e.g., Mattar paneer), curry (e.g., Shahi paneer) and salads. Hence Paneer is a vegetarian high protein-rich product in Indian cuisine, comparable in usage to meat in western dishes; it is also similar to Tofu in terms of cooking properties and many of its uses.

Despite several regional product variations, owing, *inter alia*, to differences in manufacturing practices, certain key quality characteristics are globally evident. It is typically marble white, has a slightly spongy texture, is slightly firm with good sliceability, possesses a sweetish-acidic nutty flavour, retains shape when heated (cooked) while yet being smooth and semi-soft. According to Indian regulations (PFA Act 1955), Paneer or Chhana complies with the following compositional standard: ≤70 % moisture and ≥50 % FDM; the fat content of skim milk Paneer ≤13 %.

Traditionally, Paneer was made from semi-skimmed buffalo milk, after removing some fat for the manufacture of ghee. The semi-skimmed buffalo milk is heat-treated to ~90 °C, to denature a high portion of the whey proteins and their complexation with the casein micelles, via thiol-disulphide interchange with κ-casein at the micelle surface. High heat treatment is critical in maximizing recovery of whey proteins and conferring the final product with the desired high-moisture content/water-holding capacity, and, above all, the non-melt characteristics which enables Paneer to be fried or grilled without loosing shape and identity. The heat-treated milk is then cooled to ~76 °C and acidified to ~pH 5.3–5.5 by the addition of food-grade acid (e.g., citric acid or lemon juice). These conditions promote rapid protein aggregation and the formation of a gel that synereses spontaneously, becomes denser and settles to the bottom of the coagulation tank. Values of pH and temperature are critical to ensure the formation of smooth coagula/gels with the desired water-holding capacities and syneretic properties, good microbiological quality and desired taste, while avoiding the development of protein precipitates/particles which would otherwise lead to graininess, a major potential defect in the

final product. The whey is drained after ~10 min; the curds are then moulded, lightly pressed for a short period and cooled quickly.

Owing to its relatively high-moisture level (~60–80 %) and low contents of protein (10–14 %) and calcium, Paneer is susceptible to uncontrolled post-manufacture protein aggregation and dehydration via mobility and re-arrangement of the protein network when subjected to stresses, for example during transport/distribution and retailing. This is the basis of major sensory defects including excessive wheying-off and the development of a sandy/grainy texture during storage. Avoidance of such defects requires optimization of the degree of casein-whey protein interaction during heating and protein interaction/aggregation at the different stages of manufacture through the use of appropriate means such as temperature, pH, ionic strength and cooling.

16.7.4.2 Ricotta/Ricottone

Ricotta is a soft, cream-coloured unripened cheese, with a sweet-cream and somewhat nutty/caramel flavour and a delicate aerated texture. The cheese, which was produced traditionally in Italy from cheese whey from ewes' milk, now enjoys more widespread popularity, particularly in North America and Western Europe, where it is produced mainly from whole milk (whole milk Ricotta) or partly skimmed bovine milk (Part-skim Ricotta) or whey/skim milk mixtures (Ricotta or Ricottone) (Kosikowski and Mistry 1997; Farkye 2004b). The USDA specifies three types of Ricotta in the US (USDA 1981), namely:

- Whole-skim Ricotta, which is made from whole milk, and has moisture and fat contents of ≤80 % (w/w) and ≥11 % (w/w), respectively;
- Part-skim Ricotta, which is made from reduced-fat milk, has a moisture content ≤80 % (w/w) and a fat content of <11 % (w/w) but ≥6.0 % (w/w);
- Ricotta (also known as Ricottone) made from whey, skim milk or a whey/skim milk blend, which has moisture and fat levels of ≤82.5 %(w/w) and <1 % (w/w), respectively.

In the traditional batch production method, the milk or milk-whey blend is directly acidified to pH ~ 5.9–6.0 by the addition of food-grade acid (e.g., acetic, citric, lactic), starter culture (~200 g/kg inoculum) or acid whey powder (~25 % addition). Heating of the milk to ~80 °C by direct steam injection, induces coagulation of the casein and whey proteins and results in the formation of curd flocs in the whey after ~30 min, at which stage direct steam heating is discontinued. The curd particles, now under quiescent conditions, begin to coalesce and float to the surface where they form into a layer. Indirect steam, applied to the vat jacket, together with manual movement of curd from the vat walls towards the centre, initiates the process of 'rolling' whereby the curds roll from the walls towards the centre of the vat where they form into a layer which is easily recovered by scooping (using perforated scoops). The curds are filled into perforated moulds and allowed to drain for 4–6 h at <8 °C.

The above procedure gives only partial recovery of the whey proteins. A secondary precipitation, whereby the whey from Ricotta cheese manufacture is acidified to pH 5.4 with citric acid, heated to 80 °C and treated as for Ricotta, is sometimes practiced in order to recover remaining whey proteins in the form of Ricottone cheese. Ricottone has a relatively hard and tough consistency and therefore is normally blended with Ricotta in an attempt to moderate its undesirable features.

Owing to its relatively high pH, high-moisture content (Table 16.1) and manual method of filling, Ricotta produced by the traditional method is very susceptible to microbial spoilage and hence has a relatively short shelf-life of 1–3 weeks at 4 °C. However, significant advances have been made in the automation of Ricotta cheese production with the view to improving curd separation, cheese yield and shelf-life. Excellent quality Ricotta has been produced using an ultrafiltration-based production method (Maubois and Kosikowski 1978). Whole milk is acidified with acid whey powder to pH 5.9 and ultrafiltered at 55 °C to 11.6 % protein (~29 % dry matter). The retentate is heated batchwise at 80 °C for 2 min to induce coagulation (without whey separation); the coagulum is hot-packed and has a shelf-life of at least 9 weeks at 9 °C. In another process based on ultrafiltration, milk and/or whey is standardized, pasteurized at pH 6.3, cooled to 50 °C and ultrafiltered to 30 % DM. The retentate is heated to 90 °C and continuously acidified to pH 5.7–6.0 at a pressure of 1.0–1.5 bar; the pressure is reduced to induce coagulation without whey separation and the curds are cooled to 70 °C and hot-packed. In a process developed by Modler (1988), a 20:80 blend of whole milk and concentrated whey (neutralized to pH 6.9–7.1) is heated from 4 to 92 °C, pumped to a 10 min holding tube (to induce whey protein denaturation) and acidified by on-line dosing with citric acid (250 g/kg), to induce coagulation. The curds are separated from the 'deproteinated' whey on a nylon conveyor belt. This process gives excellent recoveries of fat and protein (996 and 995 g/kg, respectively). Other methods used to increase yield and automate the production of Ricotta include filtration of whey after curd removal and the use of perforated tubes or baskets in the bottom of the curd-forming vat to collect the curds after whey drainage.

Ricotta cheese, in addition to being a very acceptable product itself, has many applications, including a base for whipped dairy desserts, use in confectionery fillings and cheesecake and as base for products such as Cream cheese and pasteurized processed cheese products.

16.7.4.3 Mascarpone

Mascarpone is a mild, sweet-nutty, smooth, spreadable creamy cheese, comparable in texture to clotted cream. Manufacture involves heating cream (30–40 %, w/w, fat) to ~90 °C and acidifying with acetic acid while stirring. The resultant coagulum is concentrated by a centrifugal separator or by pouring into suspended muslin bags or metal perforated cans.

16.7.4.4 Queso Blanco

Queso blanco (white cheese) is the generic name for white, semi-soft cheeses, produced in Central and South America and which can be consumed fresh; however, some cheeses may be held for 2–8 weeks before consumption (Torres and Chandan 1981a, b; Farkye 2004b). Elsewhere in the world, similar cheeses include Chhana and Paneer in India, Armavir in the Western Caucasus, Zsirpi in the Himalyas and low-salt (<10 g/kg), high-moisture (>600 g/kg) unripened cheeses (e.g., Beli sir types, Telemes) in the Balkans (Anifantakis and Moatsou 2006). Beli sir-type cheeses may also be salted and ripened in brine for up to 2 months to give white pickled cheeses usually known by local names, e.g., Travnicki sir and Sjenicki sir.

In Latin America, Queso blanco covers many white cheese varieties which differ from each other by the method of production (i.e., acid/heat- or rennet-coagulated), composition, size, shape and region of production. Examples include Queso de Cincho, Queso del Pais and Queso Llanero which are acid/heat-coagulated and Queso de Matera and Queso Pasteurizado which are rennet-coagulated. The use of a high temperature (80–90 °C) during the production of acid/heat-coagulated white cheeses was, traditionally, a very effective way of improving the keeping quality in warm climates.

In general, Queso blanco-type cheeses are creamy, highly salted and acid in flavour; the texture and body resemble those of very young high-moisture Cheddar and the cheese has good slicing properties. The average composition of the fresh cheese is 40–50 % moisture, 22–25 % protein and 15–20 % fat (Torres and Chandan 1981a; Kosikowski and Mistry 1997; Oliveira and Brito 2006).

Queso blanco may be categorized into two types based principally on means of coagulation, namely rennet-coagulated and acid/heat-coagulated. Type 1 Queso blanco, a rennet-curd cheese, may be subdivided into two types, namely Queso fresco (e.g., Minas Fescal) which is consumed fresh (shortly after manufacture) and Queso presna (Minas Padrão, Canastra), which is ripened. The manufacture of both types is fairly similar to that of all rennet-curd cheeses, as discussed in Chap. 2, and involves: milk standardization and pasteurization at 72 °C for 15 s, addition of a mesophilic starter culture, rennet addition and gelation (35–37 °C), cutting the gel, stirring of the curds-whey mixture for 20–30 min, whey drainage and moulding, pressing the moulded curds under their own weight, brine-salting of the curd at 10–12 °C until the salt content reaches the target level (1.4–1.6 %), removal from the brine and holding at 10–12 °C to dry and vacuum packing.

The production method for acid/heat-coagulated Queso blanco varies, but generally involves the following steps:

- standardization of milk to the required protein-to-fat ratio to achieve the desired end-product composition;
- heat treatment of the milk to ~82–85 °C, followed by holding for ~5 min. This heat treatment achieves partial denaturation (i.e., ~600–700 g/kg) of whey proteins which complex with the caseins and are therefore recovered with the casein on subsequent coagulation;
- acidification of the hot milk to pH 5.3 by adding food-grade acid (acetic, citric or tartaric acid, lime juice or lactic culture) to the milk while stirring gently. Citric

and/or acetic acid are used most frequently. The acids are diluted prior to addition, typically to a concentration of 50–100 g/L, to facilitate dispersion and prevent localized coagulation;
- curd formation: protein aggregation occurs rapidly under non-quiescent conditions, owing to the low pH and high temperature of the milk, resulting in the formation of curd particles and whey;
- curd recovery/salting/moulding/pressing. The curd particles are separated from the whey, dry stirred, dry-salted (at a level sufficient to give ~20–40 g/kg in the final cheese) and pressed. The pressed cheese is cut into consumer-size portions which are vacuum-packed and stored at 4–8 °C; the product is shelf-stable at these temperatures for 2–3 months.

Queso blanco is traditionally consumed fresh because, as a result of the processing conditions (i.e., high heat treatment during curd formation), very few biochemical changes occur during storage. However, starter bacteria (*Lactobacillus* spp.) and/or exogenous lipases may be added to the curd before salting and pressing to improve the flavour of the cheese during storage. Major volatile compounds that contribute to the flavour and aroma of Queso blanco include acetaldehyde, acetone, isopropanol, butanol and formic, acetic, propionic and butyric acids. The pH of Queso blanco decreases from ~5.2 to 4.9 during ripening, an effect which may be due to fermentation of residual lactose to lactic acid by heat-stable indigenous bacteria in milk which survive cheesemaking, or post-manufacture contaminating bacteria (Torres and Chandan 1981b).

One of the interesting properties of the cheese is its flow-resistance on heating, owing to the inclusion of whey proteins which gel on heating. This enables the cheese to be deep-fat fried in the preparation of many savoury snack foods, such as cheese sticks in batter.

16.8 Whey Cheeses

Brunost, meaning brown cheese, refers to a distinctive group of indigenous Norwegian, unripened 'cheese' varieties made from sweet (rennet casein/cheese) whey or skim milk to which cream may be added (Otterholm 1984; Kosikowski and Mistry 1997). The best known members of the group include Mysost and Gudbrandsdalsost (≥350 g/kg FDM; from bovine and caprine milk), Ektegeitost (≥33 % FDM; from goat's milk), Flotemysost (≥33 % FDM; from bovine milk) and Primost.

In the classical sense, Mysost is not a cheese but rather a fat-protein-enriched concentrated heated whey; however, being unripened and made from whey (a by-product of cheese manufacture) it may be loosely classed as a 'fresh cheese'. The cheese, characterized by a light golden to a dark brown colour, is produced by:

- standardization, by blending whey, milk and/or cream to give the correct end-product FDM. The whey is first filtered or decanted to remove casein particles, which otherwise occur in the product as black-brown specks;

- concentration of the whey, which is performed in two stages: the standardized whey is pre-concentrated in a multi-stage film evaporator to 50–60 % total solids; the viscous concentrate is then transferred to special steam-jacketed, conical kettles in which the second concentration step to 80–82 % (w/w) dry matter is achieved by heating, while agitating vigorously, under vacuum;
- working and kneading: The molten viscous mass is transferred to a vessel with a strong, rotary, swept metal agitator. Kneading over a 20 min period with slow atmospheric cooling helps to give the butter-like, plastic consistency and prevents the formation of large lactose crystals, and therefore grittiness, in the final product.

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Chapter 17
Processed Cheese and Substitute/Imitation Cheese Products

Summary The development of pasteurised processed (also called process) cheese products (PCPs) in the early 1900s was motivated by the need to develop cheese-like products that were stable (i.e., did not leak fat, 'sweat' or become greasy) at ambient temperatures (≤40 °C) and could be stored for a long time without a change in quality. Today, PCPs are used mainly as ingredient cheese products with customized functionalities, and to a lesser extent as table cheese products (e.g., processed cheese spreads and slices). They are produced by comminuting, melting and emulsifying, into a smooth homogeneous molten blend, one or more varieties of natural cheese and optional ingredients using heat, mechanical shear and (usually) emulsifying salts (ES). The optional ingredients permitted are determined by the type of PCP, as defined by national legislation. Manufacture involves formulation, size reduction of cheese and blending of ingredients, heating to 75–85 °C while continuously shearing until a hot uniform molten mass is obtained, hot-filling into packages and cooling. While the ES are not emulsifying agents *per se*, they solubilise the cheese protein which binds the free water and emulsifies the free fat released during processing (heating and shearing). The ES, usually sodium citrates or sodium phosphates, mediate protein solubilisation by upward adjustment of the pH and sequestering calcium from the cheese protein. PCPs are packaged in varying formats, for example retail products are available as foil-covered blocks or triangle portions, individually wrapped- or stacked-slices or tubs of spread, while products for the catering trade are available as sliceable blocks, slabs, sausage form or spreadable products filled into drums or buckets. The texture, cooking attributes and overall quality of PCPs are influenced by many parameters including characteristics of the cheese and optional ingredients used in formulation, processing conditions (heat, shear) and composition.

Cheese substitutes or imitations may be generally defined as products which are intended to partly or wholly substitute or imitate cheese and in which milk fat, milk protein or both are partially or wholly replaced by non-milk-based alternatives, principally of vegetable origin. Owing to the low cost of vegetable oils compared to butterfat, and the partial replacement of protein by starch, they are cheaper than natural cheeses or PCPs. They are used mainly as low-cost cheese-like ingredients in products such as frozen pizza. The main type of cheese substitute/imitations are analogue cheese (AC), typically formulated from milk proteins, such as rennet casein and caseinates, vegetable oil, water, ES and other ingredients. The method of

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manufacture of ACs is generally similar to that used for the production of PCPs: formulation, blending, heating and shearing, packaging and cooling. Similarly, their quality is influenced by composition, formulation and processing conditions.

Keywords Processed/ imitation • Manufacturing principles • Factors affecting quality

17.1 Introduction

In the broadest sense, this group of cheese products differ from natural cheeses in that they are not made directly from milk (or dehydrated milk), but rather from various ingredients such as natural cheese, skim milk, water, butter oil, casein, caseinates, other dairy ingredients, vegetable oils, vegetable proteins and minor ingredients. The two main categories, namely, pasteurized processed (also called process) cheese products and substitute or imitation products, may be further subdivided depending on composition and the types and levels of ingredients used (Fig. 17.1). The individual categories are discussed below (Guinee, 2011a, b).

17.2 Pasteurized Processed Cheese Products

Pasteurised processed cheese products (PCPs) are produced by comminuting, melting and emulsifying, into a smooth homogeneous molten blend, one or more varieties of natural cheeses, water, emulsifying salts (ESs) and optional ingredients using heat and mechanical shear. Optional ingredients permitted are determined by the product type, i.e., whether processed cheese, processed cheese food or processed cheese spread, and include dairy ingredients, vegetables, meats, stabilisers, flavours, colours and preservatives (Table 17.1). There are various types of PCPs, the category and standard of which depend on national legislation, as discussed in Sect. 17.2.2, below.

Although a product of recent origin (~1911–1918) compared to natural cheese, significant quantities of processed cheese products are now produced globally, ~1.5 million tonnes per annum versus ~17 million tonnes of natural cheeses (IDF 2005). Their popularity as products may be attributed to a number of factors including, *inter alia*:

- the diversity they offer in flavour, texture-based attributes (e.g., sliceability, shreddability, spreadability, consistency), and cooking properties (meltability, viscosity, flowability);
- easy customisation to cheese ingredient applications and adaptability to the fast food trade;

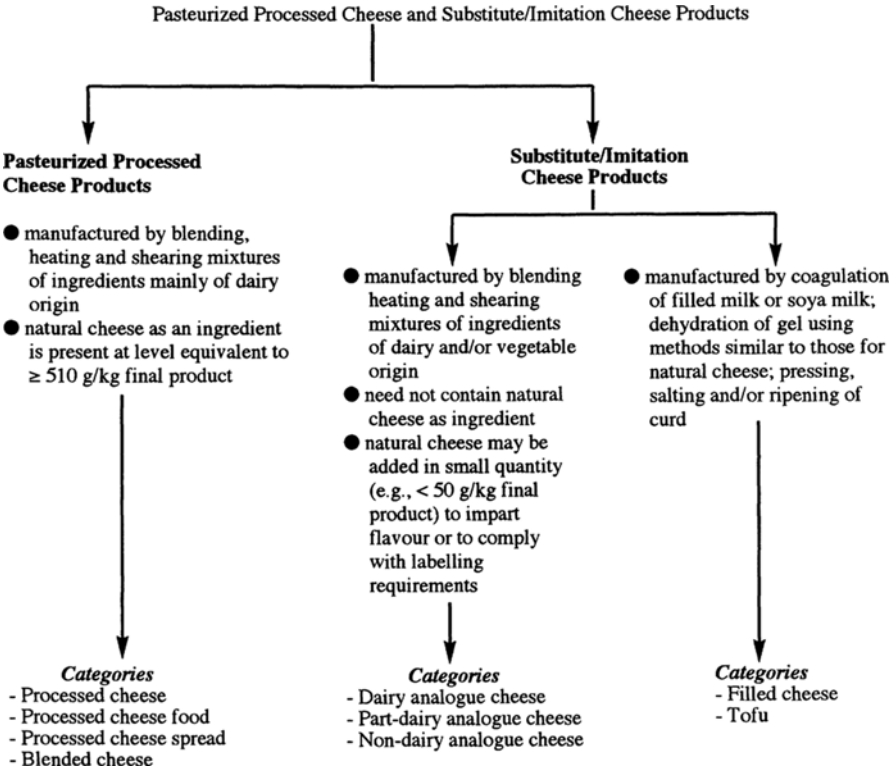


Fig. 17.1 Generalized classification scheme for pasteurized processed cheese and substitute/imitation cheese products based on manufacturing procedure and ingredients used

Table 17.1 Optional ingredients permitted in pasteurized processed cheese products

Dairy ingredients	Anhydrous milk fat, cream, milk, skim milk solids, whey solids, milk proteins, co-precipitates, milk ultrafiltrates
Stabilizers	Emulsifying salts, including sodium phosphates and sodium citrates
	Hydrocolloids: guar gum, xanthan gum, carageenans
	Organic emulsifiers: lecithin, mono- and diglycerides
Acidifying agents	Various food-grade acids, including lactic, acetic, phosphoric and citric acids
Sweetening agents	Sucrose, dextrose, corn syrup, hydrolyzed lactose
Flavours	Enzyme modified cheese (EMC), artificial flavours, smoke extracts, starter distillate
Flavour enhancers	NaCl, autolysed yeast extract
Colours	Annatto, oleoresin, paprika, artificial colours
Preservatives	Potassium sorbate, calcium and/or sodium propionates, nisin
Condiments	Cooked meats/fish
	Cooked or dried fruit or vegetables

- attractive packaging in convenient formats and shapes suited for both retail (retail foil-covered blocks or triangles, slices—stacked or individually wrapped in plastic film, fingers, tubes), or industrial and catering use (sliceable blocks, slabs, sausage form wrapped in various types of plastic film, dips/sauce or pastes filled into drums or buckets;
- popularity with young children owing to their safe ingestible consistency, mild flavours, and attractive packaging formats and shapes which are convenient for use in lunch boxes;
- lower cost relative to natural cheese due to the incorporation of lower-than-optimum-grade natural cheese, cheese off-cuts, and relatively cheap non-cheese solids (whey, skim milk powder, milk proteins and fat).

Such diversity is controlled by changes in formulation, processing conditions, and composition (Meyer 1973; Zehren and Nusbaum 1992; Guinee et al. 2004; Kapoor and Metzger 2008; Tamime 2011). Moreover, the development of companies specializing in the manufacture of equipment (production and packaging), stabilisers, other ingredients tailor-made to industry's needs, and packaging formats has greatly contributed to the innovation and success of PCPs as a group.

17.2.1 First Attempts at Stabilising Natural Cheeses by Heating and the Development of PCPs

PCPs were first developed during the early twentieth century, the main incentive being the creation of 'cheeses' that would be more stable (i.e., did not leak fat, 'sweat' or become greasy) at ambient temperatures (≤40 °C), could be stored for a longer time with little or no change in quality, and would have a longer shelf-life. Heating of the natural cheeses (and other ingredients) used in the preparation of PCPs was an obvious approach, as it would inactivate the indigenous microflora, enzymes and any spoilage microorganisms that were naturally present. Thereby, it would minimise changes in physico-chemical properties (e.g., hydrolysis of fat or protein, rheology), microbiology or sensory properties (e.g., flavour, texture) that otherwise normally occur during storage and maturation of natural cheese. In effect, a natural cheese after a desired level of maturation would be heat-treated to stabilise its properties. However, heating also is conducive to destabilisation. Most natural cheeses melt and flow on heating to 60–100 °C, but on cooling the molten cheese mass feels greasy and wet owing to the presence of free fat and exuded moisture (see Chap. 18). These changes are associated with increased hydrophobic-induced aggregation of the protein phase, which then contracts releasing free water and fat from the cheese matrix. The major changes induced by heating are summarised in Table 17.2 and include:

- increased hydrophobic-induced aggregation of the casein;
- pH reduction
- shrinkage of the casein/ *para*-casein network

Table 17.2 Changes in cheese during heating (e.g., to a temperature of 60–100 °C)

Changes	Effects	Factors affecting the extent of heat-induced change and effect
Increased hydrophobic-induced aggregation of the casein (in acid-curd cheeses) or para-casein (in rennet-curd cheeses)	• Shrinkage of the casein/para-casein network	• Cheese type (acid-curd or rennet-curd cheese) • Cheese composition (e.g., protein-in-moisture concentration, protein-to-fat ratio, pH and calcium level) • Cheese manufacturing process, e.g., – Extent of heat treatment and homogenisation of the milk prior to gelation in acid-curd cheeses – Degrees of protein aggregation and fat coalescence in the cheese • Extent of cheese maturation, which affects the level of intact casein • Extent of heating, as influenced by temperature and time
	• Expulsion of moisture (serum) previously entrapped within network (prior to heating the cheese)	
pH reduction (associated with the complexation of calcium and phosphate in the cheese serum as insoluble calcium phosphate and the release of hydrogen ions) leading to increased casein/para-casein aggregation	• Expulsion of moisture (serum)	
Compression and rupture of the fat globules (the protective membrane around the fat globules is perturbed) enclosed within the casein/para-casein networks of the cheese	• Exudation and release of free fat	

- rupture of the fat globules enclosed within the casein/*para*-casein network
- expression of moisture, previously entrapped by the *para*-casein fraction (network) as free moisture, and non-globular fat as free oil.

The extent of these changes during heating are influenced by various factors (Table 17.2), including the temperature to which the cheese is heated and sheared, composition of the cheese and the type of cheese (acid-curd or rennet-curd).

Hence, the industrial development of more 'stable' cheeses by heat treatment required means of circumventing the destabilising effects of heat *per se*. Nevertheless, the existence of traditional heated cheese dishes such as Kochkäse and Swiss Fondue indicated the potential of heat treatment and thereby provided impetus. The former product involved extending the use and shelf-life of cottage-type cheese (acid-curd cheese) which was nearing the end of its shelf-life by blending with sodium bicarbonate and other ingredients (e.g., salt, butter, flavourings), heating the blend until it melted into a homogeneous mass and pouring into containers to give Kochkäse which could be stored for a couple of weeks. In the preparation of Swiss fondue, a hot cheese dish, shredded mature rennet-curd cheese (typically Gruyere or Emmental) was blended with wine and optional ingredients (e.g., starch, condiments) and heated until a molten, creamy consistency was obtained. In both of these dishes, one or more of the added ingredients somehow stabilised the cheese to heat. The addition of sodium bicarbonate in Kochkäse increased the pH, which assisted in solubilising the protein, analogous to the solubilisation of acid casein by addition of NaOH in the manufacture of sodium caseinate. Likewise, protein solubilisation in Swiss fondue was assisted by the calcium-chelating properties of potassium sorbate (a frequently used preservative in white wine) and by starch which would absorb water expressed by the heated protein, increase the viscosity, reduce the overall mobility of the system, and thereby prevent visual precipitation.

However, many of the early attempts to extend the shelf-life of cheese on an industrial scale by application of heat to the cheese were unsuccessful. Eventually in 1911, Swiss workers (Gerber and Stettler) produced a stable heat-treated Emmental cheese, known as Schachtelkäse, by the addition of a 'melting salt', sodium citrate, to the comminuted cheese before heating and shearing. Subsequently, it was found that other cheeses, such as Cheddar could be also processed to form stable products by the addition of other 'melting salts' (e.g., sodium phosphates) or blends of different salts. The 'melting salts' were gradually referred to as emulsifying salts as the mechanism of their heat-stabilising effects became known: solubilisation of the heated protein by removal of bound calcium and increasing pH, and emulsification of free fat by the hydrated protein.

17.2.2 Classification of PCPs

There are various types of PCPs, the type/standard of which depends on national legislation. They generally differ with respect to specifications on composition (e.g., contents of fat and dry matter), minimum content of natural cheese and permitted

ingredients (e.g., cheese, dairy ingredients and stabilisers). The categories and related specifications vary between countries. Hence, in the UK, two categories of PCPs, namely Processed Cheese and Cheese Spread (or Cheese Food) were specified in the Cream Regulations 1995 (HMSO 1995). Cheese is the only dairy product that may be used in Processed Cheese, while other milk products may be used in Cheese Spread. In Germany, four categories of PCP are defined in the Deutsche Käseverordnung (Bundesministeriums der Justiz in Zusammenarbeit mit der juris GmbH, 2010: Schmelzkäse (processed cheese), Schmelzkäsezubereitung (processed cheese preparation), Käsezubereitung (cheese preparation) and Käsekomposition (cheese composition). In the USA, the Food and Drugs Administration (FDA 2012a) identifies three main categories, namely: pasteurized process cheese, pasteurized process cheese food and pasteurized process cheese spread. The criteria for classification include type and level of permitted ingredients, compositional parameters and minimum levels of added cheese; the main aspects of the different categories are summarized in Tables 17.3 and 17.4. In process cheeses, permitted ingredients include natural cheese, water, anhydrous milk fat at a level ≤5 % (w/w) of the finished product, ESs, flavouring, colouring agent and condiments. Based on the levels of non-cheese ingredients, the level of natural cheese can be as high 85 % (w/w) of the final product but varies depending on the processed cheese product being manufactured (e.g., processed Cheddar cheese, processed Edam cheese, processed cheese), composition of the cheeses used and the moisture content of the PCP. A minimum cheese content of ≥51 % (w/w) of the final product is required in pasteurised process cheese foods and spreads, in which non-cheese ingredients (e.g., dairy ingredients) can be used at a level up to ~15 %, depending on composition of the PCP. The legislation pertaining to cheese and cheese products, including PCPs is discussed in detail in Chap. 21.

Table 17.3 Classification of process(ed) cheese products (PCPs), and permitted Ingredients

Product category	Ingredients
Pasteurized process cheese	Cheese; emulsifying salts [sodium phosphates, sodium citrates; 3 % (w/w) of finished product], food-grade organic acids (e.g., lactic, acetic or citric) at levels such that the pH of the finished product is ≥ 5.3; cream, water, salt, colour and spieces or flavourings
Pasteurized process cheese food	As for pasteurized process cheese, but with the following extra optional ingredients: dairy ingredients (milk, skim milk, buttermilk, cheese whey, whey, milk fat—in wet or dehydrated forms); natural cheese content ≥51 %(w/w)
Pasteurized process cheese spread	As for pasteurized process cheese food but with the following extra optional ingredients: food-grade hydrocolloids (e.g., carob bean gum, guar gum, xanthan gums, gelatin, carboxymethylcellulose, and/or carageenan) at levels <0.8 % (w/w) of finished products; food-grade sweetening agents (e.g., sucrose, dextrose, corn syrup, glucose syrup, hydrolyzed lactose); natural cheese content ≥51 %(w/w)

Summarized from FDA (2012a). Code of Federal Regulations Title 21, Part 133: Cheese and Related Cheese Products

Table 17.4 Compositional specifications for pasteurized process(ed) cheese products

Product category	Moisture (%, w/w)	Fat (%, w/w)	Fat-in-dry matter (%, w/w)
Pasteurized blended cheese	≤43	–	≥47
Pasteurized process cheese	≤43	–	≥47
Pasteurized process cheese foods	≤44	≥23	–
Pasteurized process cheese spread	40–60	≥20	–

Summarized from FDA (2012a). Code of Federal Regulations Title 21, Part 133: Cheese and Related Cheese Products. Minimum temperature and time specified for processing is 65.6 °C for 30 s, so that when tested for phosphatase under defined conditions, the phenol equivalent ≤1.2 μg/g product

17.2.3 Manufacture of PCPs

Manufacture involves the following steps (Fig. 17.2):

- *Formulation:* This involves selection of the correct type and quantity of natural cheeses, ESs, water and optional ingredients, to impart the desired end-product composition and ensure compliance with standards. Formulation is typically performed using computer programmes (e.g., Microsoft Excel) in which ingredients and their compositions are input in worksheet format, and the effect of changes in the proportions of different ingredients (e.g., cheese type, diary powders) are immediately recognizable in terms of composition (e.g., levels of protein, fat, moisture, intact casein, calcium: casein ratio). Otherwise, formulation requires knowledge and experience of the potential impact of characteristics of different ingredients on the finished products, e.g., pH, intact casein content, calcium: casein ratio of cheese; casein: whey protein ratio, and lactose: protein ratio of dairy ingredient powders; type and level of ESs; gelling characteristics or water-binding characteristics of hydrocolloids or starches.
- *Size reduction of added cheese and other ingredients such as butter*: This involves an initial breaking of blocks/wheels (e.g., 5–20 kg) or barrels (~227 kg) of cheese using specialised equipment (curd 'breakers' consisting of claws or resolving shafts) into chunks which are then minced using specialised cheese grinders. This maximizes the surface area of the cheese and facilitates subsequent heat transfer and interaction of ingredients during subsequent processing of the formulation.
- *Blending of ingredients*: This involves mixing all ingredients (including cheese, butter, ESs, water and powders) prior to subsequent processing to ensure homogeneity of all materials and uniform end-product quality. This may be undertaken in the processed cheese cooker, or in specialized pre-blenders that typically consist of counter-rotating helix-shaped ribbons (e.g., twin ribbon blenders). Blending optimises interaction of all ingredients and uniformity of the composition of the blend being supplied to the cooker.

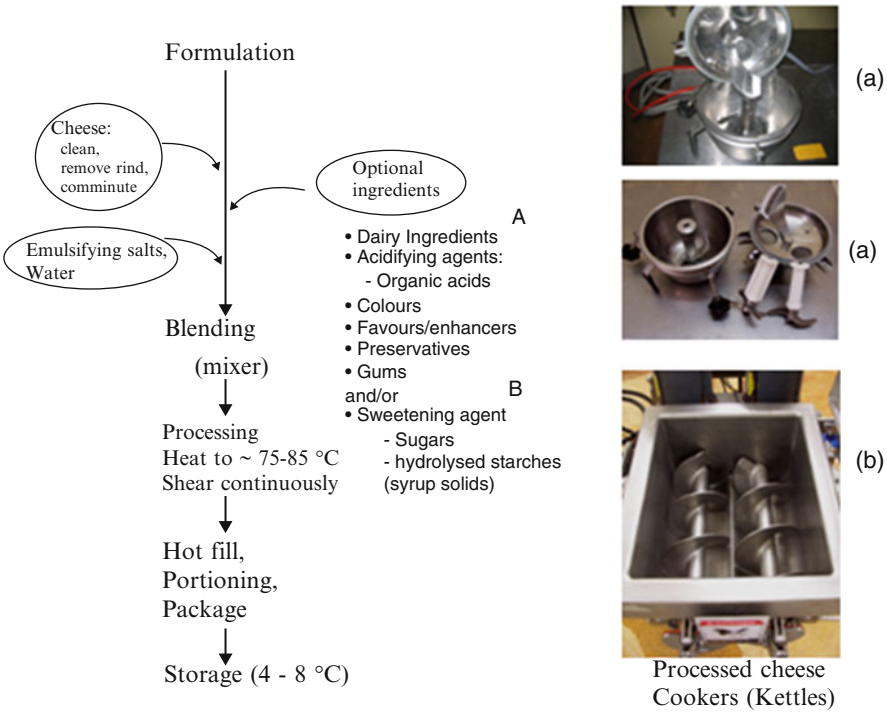


Fig. 17.2 Schematic illustration for the manufacture of processed cheese products, and two cooker types: (**a**) high shear, with blade insets, and (**b**) low shear, twin screw

- *Processing the blend*: This involves heating by direct or indirect steam injection in a cooker (kettle) typically to 75–85 °C for 1–5 min while constantly agitating/shearing until a uniform molten consistency is attained. The objectives of processing are:
  - kill any potential pathogenic and spoilage microorganisms, and thereby extend the shelf-life of the final PCP.
  - facilitate the physico-chemical and microstructural changes that transform the blend to a safe, stable PCP with the desired end-product characteristics. These changes essentially convert the insoluble casein (acid-curd cheeses) or sodium phosphate *para*-casein (rennet-curd cheeses) into a hydrated caseinate/*para*-caseinate which binds free water, emulsifies free fat released during the heating and shearing, and thereby creates a stable oil-in-water emulsion. Processing is discussed in more detail below.
- *Optional homogenisation of the hot molten blend*: Homogenisation (e.g., at first and second stage pressures of 150 and 50 bar, respectively) is an optional step that may be carried out in high-moisture (e.g., ≥60 %) processed cheese

spreads to promote 'creaming' and a thick creamy consistency. It promotes a finer dispersion of fat droplets, which leads to a smoother and creamier hot blend and thicker and firmer consistency in the final PCP.

- *Packaging, cooling and storage of the hot molten processed cheese*: The molten processed cheese product is usually hot-filled (>72 °C) and then cooled. Cooling leads to fat crystallisation and setting of the end-product. The rate of cooling has a major influence on end-product characteristics with slow cooled products being firmer and more sliceable, and fast-cooled products being softer and more spreadable. A more rapid cooling rate favours a lower degree of local structural organisation of both the protein phase (network) and crystalline fat network.

Processing refers to the heat treatment of the blend by direct or indirect steam, with constant agitation. Application of a partial vacuum during cooking is optional; it may be used to regulate moisture content when direct steam injection is used, and is also beneficial in removing air and thus preventing air openings in the finished set product. In batch processing, the temperature—time combination varies (i.e., 70–95 °C for 4–15 min), depending on the formulation, extent of agitation, the desired product texture, body and shelf-life characteristics. At a given temperature, the processing time generally decreases with agitation rate, which may vary, depending on the kettle (cooker) type, from 50 to 3000 rpm. In continuous cookers, the blend is pumped continuously through a steam-injection nozzle which effects instant agitation and heating to 85–95 °C. The heated product then continues to a holding tube, with dimensions and operating back pressure that ensure the desired residence time at high temperature. The cooked product is then flash-cooled to ~74–75 °C, hot-filled, and packaged, as discussed above.

Continuous cookers with direct steam injection are used in the manufacture of sterilised high-moisture PCPs with the consistency of dips, spreads or sauces. Typically, the products are heated to ~140 °C and held for ~10 s, vacuum-flash cooled to ~90 °C, pumped to a buffer tank (creaming tank) where the low viscosity product is held and agitated for sufficient time to develop the desired thick creamy consistency that facilitates hot-filling (without splashing) and gives the desired rich creamy texture to the end-product following cooling. The purpose of sterilisation is generally not to produce a sterile product (as filling equipment generally does not allow this) but rather to inactivate spores of Clostridia (e.g., *Clostridium tyrobutyricum*) potentially present in cheese or other ingredients. Such spores, if present, could be activated by conventional heat treatment during processing (75–85 °C), and grow during cooling or storage, especially in high-moisture PCPs (with a relatively high water activity) where they could induce spoilage.

In the manufacture of slices, the hot molten PCP, typically from a continuous cooker, is pumped through a manifold with 8–12 nozzles which extrude ribbons of the hot cheese onto rotating chill rolls or belts and are, thereby, cooled from 80–70 °C to ~15 °C. The ribbons are cut automatically into slices, which are stacked and packaged.

The reader is referred to Dixon (2011) for a comprehensive description of the various types of equipment currently used at the different stages of PCP manufacture.

17.2.4 Principles of Manufacture of Processed Cheese Products

Cheese manufacture involves controlled destabilisation of milk protein, mainly casein, to form of a gel which is dehydrated and concentrated (~6–12 fold) to form a concentrated casein network (~14–30 % protein) that occludes the milk fat, if present. Conversely, processed cheese manufacture deconstructs the casein network of natural cheese, with the aim of partially solubilising the protein thereby enabling it to bind the free water and emulsify the free fat released during processing (heating and shearing). The process represents a structural transformation from a concentrated fat-filled gel network to a concentrated oil-in-water emulsion. Consequently, the principles of manufacture of PCPs require an appreciation of the mechanisms that underlie the formation of natural cheese structure, and its transformation during processing.

17.2.4.1 Changes in Milk Protein and Fat During Manufacture of Natural Cheeses

A key step in the manufacture of all cheeses is the controlled destabilisation of the micelles to form of a gel by increasing their surface hydrophobicity either through enzymatic hydrolysis of the surface κ-casein layer by proteinases, referred to as rennets, for rennet-curd cheeses and/or by charge neutralisation (adjustment to isoelectric pH) for acid-curd cheeses. A controlled, moderate degree of contact between the surfaces of touching micelles promotes the formation of a gel which may be described as a network continuum that occupies the full volume of the milk and encloses the fat globules and serum, analogous to how a sponge holds water.

In rennet-curd cheeses, calcium-mediated cross-linkages are considered to be the major attractive force responsible for cementing the casein micelles together within the gel. These cross-links, also referred to as ‘calcium bridges’, are formed by Ca ions binding directly to aspartate and glutamate groups, or calcium phosphate nanoclusters attaching to serine phosphate groups, on different casein molecules. Following rennet-induced gelation of the milk, further casein aggregation occurs by hydrophobic interactions between the caseins, promoted by heating and acidification of the gel, and reducing its moisture content from about 88 % to 33–55 % in the final cheese, depending on the variety. Moisture reduction is achieved by subjecting the rennet-induced gel to the interactive effects of various dehydrating operations: cutting into pieces/particles, stirring, cooking and acidification of gel particles in

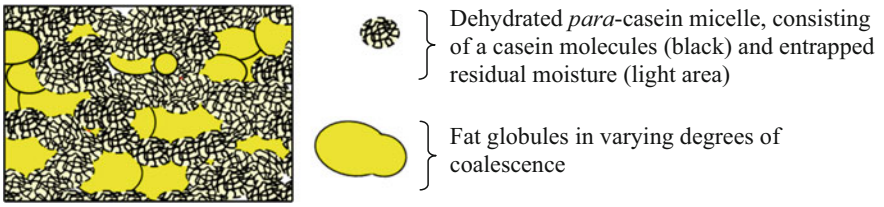


Fig. 17.3 Schematic representation of the structure of natural cheese, showing a matrix comprised of a network of fused *para*-casein micelles with entrapped residual serum (moisture) and fat globules entrapped with the network

whey; physical removal of whey (drainage); and, acidification, heating, salting, and/or pressing of the resultant curd mass. The cheesemaking process results in a significant increase in casein concentration, from ~2.5 % (w/w) in milk to 16–20 % in semi-hard chesses such as Feta, Camembert, and to 25–32 % in hard cheeses like Cheddar, Emmental and Parmesan. Simultaneously, the concentration of casein in moisture increases from 20 % in native casein micelles (in cheese milk) to ~30–50 % in the cheese depending on the cheese variety. Similarly, 60–80 % of the calcium and phosphate in the casein micelles is concentrated during manufacture, resulting in a calcium level ranging from ~0.50 % (e.g., Blue cheese) to 0.95 % (Emmnetal). The resultant cheese may be defined as a highly concentrated amalgam of dehydrated calcium phosphate *para*-casein micelles in the form of a network that encases the milk fat in the form of coalesced globules and/or pools of fat (Fig. 17.3). The *para*-casein has been rendered insoluble by the cheesemaking process, owing to the hydrolysis and removal of a highly hydrophilic casein macropeptide, the increased extent of calcium-mediated interaction between the caseins molecules and their dehydration. The shear forces applied during the various stages of cheesemaking (cutting the gel, stirring, cheddaring, moulding, pressing and/or curd treatments such as kneading and shearing in *pasta filata* cheeses such as Mozzarella and Provolone) and shrinkage of the casein network around the enclosed fat remove the protective milk fat globule membrane (MFGM) to varying degrees, resulting in partial coalescence and pooling of the fat. Hence, on subsequent heating of the finished cheese in cooking applications (e.g., toast, pizza), some free fat readily comes to the surface of the melting cheese mass where it protects against over-dehydration, facilitates the flow (spread) of individual cheese shreds into a cohesive molten mass, creates a desired glossy appearance, and gives a nice fatty mouthfeel on eating.

In the manufacture of acid-curd cheeses, such as Cottage cheese, Quark and Labneh, gelation is induced by slow quiescent acidification of the milk to pH 4.6 (the isoelectric pH of the caseins), following fermentation of lactose to lactic acid by the starter culture. The reduction of pH close to the isoelectric point of casein leads to a collapse in the stabilising role of κ-casein and a reduction in the intermicellar repulsive forces. These changes result in contact between neighbouring micelles and the formation of a gel; unlike rennet-curd cheeses, gelation involves charge neutralisation but does not involve the hydrolysis of κ-casein. Electrostatic (between positive and negative amino acid residues) and hydrophobic interactions

between the casein molecules are responsible for the integrity of the gel structure. Similar to rennet-curd cheeses, the gels are concentrated by cutting (stirring or breaking the gel), heating and whey drainage, the latter being performed by straining the gel through cheese cloth or subjecting it to membrane filtration or centrifugal force in a mechanical separator (Chap. 16). In contrast to rennet-curd cheeses, the degree to which casein, calcium and phosphate are concentrated is significantly lower in acid-curd cheeses, which typically have a protein content of 10–15 % and a calcium level of ~ <0.15 %. The relatively low calcium level (compared to rennet-curd cheese) is due to the solubilisation of micellar calcium phosphate in the whey prior to whey drainage and the low level of calcium binding by aspartic and glutamic acid residues which are largely protonated at pH 4.6. Consequently, the potential of the protein of acid-curd cheese (acid casein) to hydrate and bind water is much greater than that of the protein (calcium phosphate *para*-casein) in rennet-curd when the pH is readjusted to a value typical of that (5.8–6.0) in PCPs because of the presence of the intact κ-casein and the much lower level of casein-bound calcium. The extent of fat globule coalescence in acid-curd cheese is generally lower than in rennet-curd cheese; in some acid-curd cheeses (e.g., Cream cheese) the milk is homogenised prior to fermentation, thereby further stabilising the fat globules against coalescence during subsequent manufacture.

17.2.4.2 Destabilisation of Natural Cheese on Heating and Shearing in the Absence of Emulsifying Salts (ESs)

Application of heat (70–90 °C) and mechanical shear to natural cheese in the absence of stabilisers usually results in a heterogeneous, gummy, pudding-like mass which oils-off extensively and exudes moisture during processing and on subsequent cooling/storage. These defects arise from:

- liquefaction of fat and the coalescence of free fat into pools as a result of shearing and removal of the MFGM surrounding the fat, and
- dehydration, aggregation and shrinkage of the *para*-casein network as a result of
  - an increase in hydrophobic interactions between the casein molecules, as a result the combined effects of high temperature and low pH (pH 4.6–5.6),
  - precipitation of soluble (serum) calcium and phosphate, leading to further calcium phosphate-mediated interactions between the *para*-casein molecules (especially, in rennet-curd cheeses).

17.2.4.3 Role of Emulsifying Salt (ES) in Transforming Natural Cheese to a Stable Processed Cheese Product During Processing

The above defects can be prevented by the addition of ESs, at a level of 1–3 % (w/w), to the cheese blend prior to processing. The ESs most commonly used in the manufacture of PCPs are sodium citrates, sodium orthophosphates, sodium pyrophosphates,

sodium tripolyphosphates, sodium polyphosphates and basic sodium aluminium phosphates (e.g., Kasal). Today, ES are generally supplied as proprietary blends of phosphates (e.g., Joha C, Joha T, Joha SE, Joha S9S, Joha S9S230, Solva 35S) or phosphates and citrates (e.g., Solva NZ 10), tailor-made to impart desired functionalities (e.g., degree of sliceability, spreadability, meltability, thermal stability) (Berger et al. 1989). Other potential emulsifying agents include sodium gluconates, lactates, malates and tartarates. In all cases, the ES generally has a monovalent cation (i.e., sodium) and a polyvalent anion (e.g., phosphate or citrate). While these salts are not emulsifying agents *per se*, they promote, with the aid of heat and shear, a number of physico-chemical changes in the blend, which partially convert the insoluble casein of acid-curd cheeses or calcium phosphate *para*-casein in rennet-curd cheeses to hydrated sodium caseinate or sodium *para*-caseinate, respectively. The hydrated proteins then bind water and emulsify the free oil released during the heating and shearing, and thereby contribute to the formation of a smooth, homogeneous, physico-chemically stable PCP. These changes, which are discussed briefly below, include:

- upward adjustment and stabilisation (buffering) of the pH;
- calcium sequestration and casein demineralisation
- casein hydration
- emulsification of free fat;
- structure formation.

Displacement and Stabilisation (Buffering) of pH

The use of the correct blend of ESs usually shifts the pH of the cheese upwards, typically from ~5.0–5.5 in the natural cheese to 5.8–6.0 in the PCP, and stabilises it by virtue of their high buffering capacity. This change contributes to an enhanced dissociation and calcium-sequestering ability of the ESs, and an increased negative charge on the *para*-caseinate.

Calcium Sequestration and Demineralisation of the Cheese Protein

This involves the exchange of the divalent Ca^{2+} from the *para*-casein network for the monovalent Na^{+} of the ES. The pKa values for phosphoric acid are 2.15, 7.2 and 12.4, while those for citric acid are 3.1, 4.8 and 6.4. Hence, ESs are sufficiently dissociated at the typical pH of the processed cheese blend (~5.8–6.0) to sequester a large portion of the calcium attached to the casein, resulting in its removal from the *para*-casein and/or casein and its subsequent deposition as finely dispersed insoluble calcium phosphate and/or calcium citrate inclusions within the PCP. This is confirmed by recent studies (Guinee and O'Kennedy 2009, 2012; Shirashoji et al. 2006) which show that while most of the protein (~70–80 % of total) is soluble, most of the Ca (≥75 % of total) and P (≥66 % of total) are insoluble, in a water-soluble extract of the processed cheese prepared by blending PC and water at a weight ratio of 1:2.

Casein Hydration and Dispersion

The partial demineralization of casein at the elevated pH results in the removal of most of the intra- and inter-casein calcium cross-links and an increase in negative charge. Both these factors contribute to the partial conversion of insoluble casein in acid-curd or calcium phosphate *para*-casein network in rennet-curd cheeses to sodium caseinate/*para*-caseinate, which has superior water-binding and emulsification capacity. These changes are validated by the large increase in the level of water-soluble protein following processing (e.g., from ~5–20 % of total protein in the natural cheese to ~60–90 % in processed cheese depending on the type and level of ES), and by the low calcium-to-protein ratio of the solubilised protein (~5–10 mg/g soluble protein versus 21 mg/g protein in natural cheese) (Guinee and O'Kennedy 2009, 2012).

Fat Emulsification

During processing, the hydrated casein/*para*-caseinate coats the surfaces of dispersed free fat droplets, creating a stable oil-in-water emulsion. The increase in viscosity as a result of water binding by the *para*-caseinate also enhances emulsion stability by restricting the mobility of the emulsified fat particles and thereby their potential coalescence.

Structure Formation on Cooling

During cooling, the homogeneous, molten, viscous mass sets to form a characteristic body, which, depending on blend formulation, processing conditions and cooling rate, may vary from a firm sliceable product to a semi-soft spreadable consistency. Factors contributing to structure formation include crystallization of fat and interactions (e.g., hydrophobic) between the proteins during the slow cooling. Microstructural studies on PCPs indicate that the structure is an emulsion of discrete, rounded fat droplets of varying size uniformly dispersed in a protein network.

The network consists of relatively short strands that are finer (thinner) than those of natural cheese, with the degree of fineness increasing as the pH is raised from 5.5 to 6.1, an effect attributed to a change in the proportions of different types of protein interactions: hydrophobic, electrostatic, hydrogen bonds, and residual calcium cross-links. The size of the fat globules, which is inversely related to the degree of fat emulsification (DFE), varies (0.3–5.0 μm diameter) with characteristics of formulation (e.g., pH, Ca and intact casein content of natural cheese; type and quantity of ES; composition, protein content, casein:whey protein ratio, calcium:casein ratio, and solubility of dairy protein powders) and processing conditions (shear rate, temperature and time). The caseinate/*para*-caseinate layers surrounding the dispersed fat droplets (which may be considered as fat-filled protein particles) interact with the casein strands, thereby increasing the effective protein content of the network, and augmenting the extent of protein structuring.

The DFE in PCPs correlates positively with their firmness and elasticity, and inversely with the level of oiling-off and flow or spread they exhibit on heating (Rayan et al. 1980). The ability of the PCP to release some free fat when heated (baked or grilled) is generally desirable, as it limits drying-out of the PCP and thus contributes to the desired flow, succulence and surface sheen of the melted product. However, PCPs that exhibit little, or no, heat-induced flow (referred to as controlled melt PCPs) are preferable in some applications, e.g., inclusions of PCP in baked breads or co-extruded cheese-filled consumer products.

17.2.5 Properties of Emulsifying Salts (ESs)

The ESs most commonly used are sodium citrates, sodium orthophosphates and sodium polyphosphates (Table 17.5). Sodium citrates are used widely, typically in combination with phosphates. Generally, they have been found to give PCPs with a clean flavour, a relatively low DFE and ample oiling-off and flowability on heating. Trisodium citrate is used most commonly; the mono- and di-sodium forms ($NaH_2C_6H_5O_7$ and $Na_2HC_6H_5O_7$), when used alone, tend to give over-acid PCPs that are mealy, acid and crumbly and show a tendency to oil-off due to poor emulsification. The dissociation constants (pKa) of citric acid at the ionic strength of milk are 3.0, 4.5 and 4.9. Owing to their acidic properties, mono- and di-sodium citrates may be used to correct the pH of a processed cheese blend, for example when a high proportion of very mature, high pH cheese or skim milk solids are used.

Table 17.5 Properties of emulsifying salts for processed cheese products[a]

Group	Emulsifying salt	Formula	Solubility at 20 °C (%)	pH value (1 % solution)
Citrates	Trisodium citrate	$2Na_3C_6H_5O_7.1H_2O$	High	6.23–6.26
Orthophosphates	Monosodium phosphate	$NaH_2PO_4.2H_2O$	40	4.0–4.2
	Disodium phosphate	$Na_2HPO_4.12H_2O$	18	8.9–9.1
Pyrophosphates	Disodium pyrophosphate	$Na_2H_2P_2O_7$	10.7	4.0–4.5
	Trisodium pyrophosphate	$Na_3PO_2O_7.9H_2O$	32.0	6.7–7.5
	Tetrasodium pyrophosphate	$Na_4P_2O_7.10H_2O$	10–12	10.2–10.4
Polyphosphates	Pentasodium tripolyphosphate	$Na_5P_3O_{10}$	14–15	9.3–9.5
	Sodium tetrapolyphosphate	$Na_6P_4O_{13}$	14–15	9.0–9.5
	Sodium hexametaphosphate (Graham's salt)	$Na_{n+2}PnO_{3n+1}$ (n = 10–25)	Very high	6.0–7.5
Aluminium phosphates	Sodium aluminium phosphate	$NaH_{14}Al_3(PO_4)_8.4H_8O$	–	8.0

[a]From van Wazer (1971) and Caric and Kalab (1993)

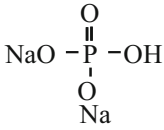
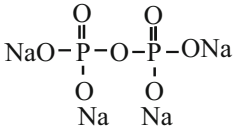
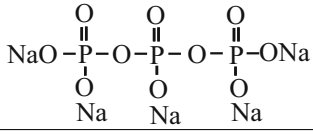
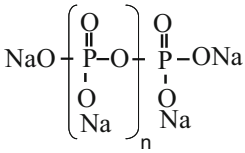
Orthophosphate (e.g., disodium orthophosphate)	
Pyrophosphate (e.g., tetrasodium diphosphate)	
Tripolyphosphate (e.g., pentasodium triphosphate)	
Polyphosphate (e.g., Calgon® 696, with average chain length, n -1, of ~ 25)	

Fig. 17.4 Types and formulae of sodium phosphate emulsifying salts used in manufacture of processed cheese products

The main phosphates used in cheese processing include sodium monophosphates (sodium orthophosphates) which contain 1 P atom (n = 1), linear condensed phosphates such as pyrophosphates (n = 2) and polyphosphates (n = 3–25; e.g., tripolyphosphate, n = 3; Calgon-polyphosphate, where n is ~25 on average) (Fig. 17.4), and proprietary phosphate blends (mixtures of orthophosphates and polyphosphates of varying chain length) designed to impart specific functionalities in the final PCP (e.g., spreadability, stringiness on cooking). Of the orthophosphates, disodium hydrogen orthophosphate (Na_2HPO_4) is the form normally used; when used alone, the mono- and trisodium salts tend to give over-acid and under-acid PCPs, respectively. While orthophosphates are the most widely used because of their good pH buffering and moderate levels of calcium sequestration, significant quantities of short-chain polyphosphates (pyrophosphates and tripolyphosphates) are used also. The buffering capacity of phosphates decreases with chain length, and consequently polyphosphates are generally used in conjunction with sodium orthophosphates and/or citrates to achieve the desired pH (5.8–6.0) in the finished PCP. The extent of polymerisation strongly affects the functionality (e.g., pH adjustment, calcium sequestration, protein hydration, DFE) and properties of the resultant PCPs.

Comparative studies have shown that the potassium salts of orthophosphates, pyrophosphates and citrates give processed cheeses with textural properties similar to those made with the equivalent sodium salts at similar concentrations. Hence, potassium ESs may have potential in the preparation of reduced-sodium formulations. However, the use of potassium salts is generally not favoured because they

Table 17.6 General properties of emulsifying salts in relation to cheese processing

Property	Citrates	Orthophosphates	Pyrophosphates	Polyphosphates	Aluminium phosphate
Ion exchange (calcium sequestration)	Low	Low	Moderate	High-very high	Low
Buffering action in the pH range 5.3–6.0	High	High	Moderate	Low-very low	–
para-Caseinate dispersion (peptization)	Low	Low	High	Very high	
Emulsification	Low	Low	Very high	Very high (n = 3–10)	Very low
Bacteriostatic effects	Nil	Low	High	High-very high	

Compiled using data from various sources: Templeton and Sommer (1936), Glandorf (1964), Roesler (1966), Scharf (1971), van Wazer (1971), Tanaka et al. (1986), Rayan et al. (1980)

tend to impart a bitter flavour and the association of high dietary potassium with hyperkalemia, a medical condition linked to an elevated level of potassium in the blood (Evans 2005). Owing to their aluminium content and the tentative association of aluminium with Alzheimer's disease (Gupta et al. 2005), sodium aluminium phosphates (e.g., Kasal) are used to a very limited extent.

The effectiveness of different ESs in promoting the various physico-chemical changes that occur during processing has been studied extensively. Reviews on the properties of different ESs in PCPs include those by Meyer (1973), Guinee et al. (2004), Kapoor and Metzger (2008) and Lucey et al. (2011). Discrepancies exist between these studies vis-a-vis the influence of ESs on different physico-chemical changes, probably reflecting differences in product formulation (e.g., intact protein, pH, ratio of ES to protein) and processing conditions (e.g., cooker type, degree of shear, time-temperature treatment). Nevertheless, these studies indicate definite trends that are summarized in Table 17.6, and discussed below.

17.2.5.1 Calcium Sequestration

Ion exchange is best accomplished by salts that contain a monovalent cation and a polyvalent anion and effectiveness generally increases with the valency of the anion. The general ranking of the calcium-sequestrating ability of the common ESs in cheese is in the order: polyphosphates > pyrophosphates > orthophosphates > sodium aluminium phosphate = citrates. However, the sequestering ability, especially of the shorter chain phosphates, is strongly influenced by pH. The increased ion-exchange function at higher pH values is attributed to more complete dissociation of the acid groups ($-P-O^-H^+$ in phosphates and $-COO^-H^+$ in citrates) resulting in the formation

of a higher valency anion. At low pH values (≤pKa), these groups are more protonated (in the –OH form), while at higher pH (>pKa), they are more dissociated (in the $–O^-$ form) and able to bind calcium ($^-OCa^{2+}O^-$). Thus, for the shorter chain phosphates, calcium binding increases in the order: $Na_4P_2O_7 > Na_3HP_2O_7 > Na_2H_2P_2O_7 > Na_2HPO_4 > NaH_2PO_4$.

17.2.5.2 Adjustment and Buffering of pH

The pH of PCPs is determined by:

- the types and proportions of different ingredients and their pH and buffering capacity, i.e., their ability to maintain pH by counteracting the acidity/alkalinity effects of other materials;
- the type, level and buffering capacity of the ES.

In terms of cheese and dairy ingredients, casein in its various forms (e.g., *para*-casein in cheese and rennet casein, casein in acid-curd cheeses, acid casein and caseinates) is in effect a large polyvalent anion containing a large number of potential dissociable groups (glutamate, aspartate and serine phosphate) that buffer strongly at the pH value (5.8–6.0) found in PCPs. Conversely, ingredients such as whey powders containing a relatively low level of whey proteins and a high level of lactose have lower ability to buffer. The ability of cheese to buffer pH is determined by its pH and levels of protein and calcium phosphate. Hence, a deviation from the normal formulation, involving a significant drop in the pH (e.g., from 5.4 to 5.0) of the cheese (which accounts for at least 51 %, w/w, of the blend) used, may necessitate the use of a pH-correcting salt (e.g., Na_3PO_4) to bring the pH of the PCP up to the target value. Conversely, a change involving the addition of high pH cheese (e.g., mature Emmental) and/or the incorporation of a relatively high quantity of casein powders (e.g., rennet casein, sodium or calcium caseinate) may require a change in the ES blend, favouring salts (e.g., NaH_2PO_4, disodium citrate) that buffer to low pH.

The buffering capacity of sodium phosphates at the pH value found during processing (i.e., 5.5–6.0) decreases with increasing chain length and is effectively zero for the longer-chain phosphates ($n > 10$). This decrease in buffering capacity with chain length is due to the corresponding reduction in the number of dissociable acid/base groups per P molecule, which range from 3 in orthophosphates, 2 in pyrophosphates, 1.7 in tripolyphosphates, to ~1.0 in polyphosphates ($n > 10$) (Fig. 17.4). The ortho- and pyrophosphates possess high buffering capacity in the pH ranges 2–3, 5.5–7.5 and 10–12; thus, in cheese processing they are not only very suitable as buffering agents but also as pH-correction agents. Within the citrate group, only the trisodium salt has buffering capacity in the pH range 5.3–6.0; the more acidic mono- and di-sodium citrates give over-acid, crumbly PCP with a propensity to oiling-off. The pH of PCP has been found to increase with ES concentration in the range 0–3 % for trisodium citrate, tetrasodium pyrophosphate, and sodium tripolyphosphate and disodium hydrogen phosphate (Templeton and Sommer 1936; Gupta et al.

1984; Cavalier-Salou and Cheftel 1991). Conversely, increasing the concentration of long-chain polyphosphate ($n \geq 25$) generally has little effect on pH because of their low buffering capacity (Swiatek 1964).

17.2.5.3 Hydration and Dispersion of Casein

The ability of the different groups of ESs to promote protein hydration and dispersion during cheese processing is in the following general order: polyphosphates ($n = 3–10$) > pyrophosphates > monophosphates ~ citrates. The greater protein hydrating effect of polyphosphates over citrates and orthophosphates can be explained in terms of the greater calcium-sequestering ability of the former. However, as discussed in Sect. 17.2.5.1, the calcium-sequestering efficacy of the different salts is pH-dependent, increasing with pH as the degree of dissociation of the $–O^-H^+$ groups becomes more pronounced. Hence, while polyphosphates have the highest potential calcium-sequestering ability, this generally requires that they are used in conjunction with orthophosphates (which buffer pH most effectively) for this influence to be manifested.

17.2.5.4 Ability to Promote Emulsification

The effectiveness of different ESs in promoting emulsification, as indicated by microscopy and oiling-off studies on PCPs, is in the following general order: sodium tripolyphosphates > pyrophosphates > polyphosphates ($P > 10$) > citrates ≈ orthophosphates ≈ basic sodium aluminium phosphates. This ability generally parallels their effectiveness in promoting hydration of the *para*-caseinate complex.

17.2.5.5 Hydrolysis (Stability)

During processing and storage of PCPs, linear condensed phosphates undergo varying degrees of hydrolysis to orthophosphates, to an extent that increases with processing time and temperature, product storage time and temperature, and with phosphate chain length (Glandorf 1964). Other influencing factors include the type of cheese, quantity of ES and the type of product being produced. In experiments with pasteurized processed Emmental, the level of polyphosphate (where $n > 4$) breakdown during melting at 85 °C varied from 7 % for block PCP (processed for 4 min) to 45 % for spreadable PCP (processed for 10 min) (Roesler 1966). While the breakdown of condensed phosphates to monophosphates was complete in the PCP spreads after 7 weeks, low levels were detectable in block PCP even after 12 weeks. The greater degradation of polyphosphates in spreadable PCPs is believed to be due to their generally higher pH and moisture content (Scharf 1971).

17.2.5.6 Bacteriostatic Effects

Cheese processing normally involves a temperature (75–95 °C) that is lower than those used for sterilization. Thus, processed cheese products may contain viable spores, especially of the genus *Clostridium*, which originate in the raw materials. Germination of spores during storage often leads to problems such as blowing of cans, protein putrefaction and off-flavours. While bacterial spoilage is minimized through the addition of preservatives, some ESs also possess bacteriostatic properties. The effects of ESs and other ingredients on the microbiology and safety of PCPs have been researched extensively (Eckner et al. 1994; Tanaka et al. 1986; ter Steeg and Cuppers 1995; Zottola et al. 1994; Loessner et al. 1997; Glass and Johnson 2004) and reviewed (Glass and Doyle 2005). Some of the major effects are discussed below.

Ortho- and polyphosphates are inhibitory to various microorganisms, including *Staphylococcus aureus*, *Bacillus subtilis*, *Clostridium sporogenes*, *Cl. botulinum*, and various *Salmonella* species. Orthophosphates have been found to inhibit the growth of *Cl. botulinum* in processed cheese. The inhibitory effect of sodium orthophosphates on *Cl. botulinum*, which has been found to be superior to that of sodium citrates in pasteurized processed cheese spreads with a moisture level in the range of 52–58 %, depends on the levels of moisture and NaCl and the pH of the processed cheese product (Tanaka et al. 1986). The general bacteriostatic effect of phosphates, which increases with chain length, may be attributed to their interactions with bacterial proteins and sequestration of calcium, which, generally, serves as an important cellular cation and cofactor for some microbial enzymes (Stanier et al. 1987). Compared to phosphates, citrates have been found to be less bacteriostatic and may even be degraded by bacteria, thus reducing product keeping quality (Glass and Doyle 2005). However, the growth of potential spoilage bacteria (e.g., *Clostridium tyrobutyricum*) and pathogens (e.g., *Listeria monocytogenes, Cl. botulinum)* in PCPs is affected by many factors including water activity, NaCl level, pH, organic acids (e.g., lactic), bacteriocins (e.g., Nisin), levels of fat and moisture, antimicrobials (e.g., sodium nitrite, lysozyme) (Glass and Doyle 2005). Predictive modelling on high-moisture processed cheese spread (58 % moisture) indicated that key determinants of product safety are pH and the concentration of salt (NaCl) and di-sodium phosphate (Na_2HPO_4) (Tanaka et al. 1986).

17.2.5.7 Flavour Effects

Phosphates can impart flavours characterized generally as chemical/metallic-like, especially when used at a high concentration; conversely, citrates have relatively little influence on overall flavour (Karahadian and Lindsay 1984). However, both citrates and phosphates, as anions, depress saltiness perception compared to chloride. Hence, sodium added in the form of sodium citrates or sodium phosphates is less effective than added NaCl in creating saltiness in PCPs.

17.2.6 Influence of Various Parameters on the Consistency of Processed Cheese Products

Numerous investigations have been undertaken to assess the effects of different variables (e.g., levels and types of ingredients, changes in processing conditions and composition) on the textural and functional characteristics of PCPs. Some discrepancies occur vis-à-vis the conclusions from different studies in which similar variables were investigated, probably due, in part, to differences in formulation and processing conditions. However, certain trends emerge, which are discussed below.

17.2.6.1 Cheese

Cheese is the major constituent of processed cheese products, ranging from a minimum of ~51 % in cheese spreads and cheese foods up to ~ 90 % in processed cheeses. Hence, both the type and degree of maturity of the cheese used have a major influence on the consistency of the product. While the gross composition (fat, protein and moisture contents) of the natural cheese is important in terms of achieving the required compositional specification of PCPs, critical characteristics of the cheese in terms of the physical properties of the resultant PCP include:

- calcium content
- intact casein level

The calcium content of acid-curd cheeses, with a pH value in the range 4.5–4.7, is relatively low (<0.15 %) and is almost completely soluble; protein insolubility is due primarily to hydrophobic-induced aggregation. In contrast, the calcium content of rennet-curd cheeses is high but shows both inter-variety (e.g., from ~18–24 mg/ protein in Camembert and Blue-type cheeses to ~30–34 mg/ g protein Paremsan and Emmental) and intra-variety (e.g., 25–30 mg/g protein in Cheddar) differences. These are associated with variations in pH at set (rennet addition), scald temperature, drain pH, pH and moisture content of the curd at moulding and degree of whey expressed from the moulded curd. Moreover, most of the calcium in rennet-curd cheeses is insoluble and attached to the *para*-casein, rendering it insoluble. A major function of ES in the manufacture of PCPs is to remove much of this Ca and thereby, solubilise the protein. A recent study (Guinee and O'Kennedy 2009) showed that reducing the level of calcium in Cheddar-type cheese (from 29.8 to 19.6 mg/g protein), while keeping the level of ES constant, had a marked impact on the functionality of the resultant PCP, leading to significant reductions in fracture stress, fracture strain and firmness of the unheated product, and increases in the extent of flow and fluidity (loss tangent) of the melted product. These changes coincided with the more complete removal of Ca from the protein as the level of ES was increased. Hence, there is a need to standardise the level of ES added to PCPs on the basis of the calcium content of the natural cheese used.

Another characteristic of natural cheese that varies with both variety and brand (of a given variety) is age, owing to differences in manufacture, composition and

ripening conditions (time, temperature) (cf. Chap. 12). During maturation of rennet-curd cheese, the *para*-casein is hydrolysed to peptides and free amino acids by proteinases and peptidases from various sources (cf. Chap. 12). Consequently, the level of intact casein (IC) decreases during maturation. Various studies have shown that decreases in the IC content (e.g., from ~95 % to 75 % of total casein in Cheddar cheese significantly reduces the firmness of the resultant PCP and increases both the extent of flow and fluidity on heating/grilling. These changes may be explained on the basis that peptides (produced on hydrolysis of the IC) are less capable of imparting structure and rigidity to the PCP and give a lower degree of fat emulsification than intact casein. Hence, it is generally recognised in practice that hard and semi-hard cheese varieties, such as Cheddar, Gouda and Emmental, which have a relatively high IC content, give firmer, longer-bodied (high fracture strain), more elastic processed products than mould-ripened varieties, such as Camembert and Blue cheese. The latter cheeses undergo more extensive proteolysis during ripening and have a low Ca:casein ratio. Likewise, experience indicates that block processed cheese with good sliceability and elasticity requires predominantly young cheese (75–90 % intact casein) whereas predominantly medium-ripe cheese (60–75 % intact casein) is required for processed cheese spreads.

17.2.6.2 Cheese Base

Cheese base (CB) is sometimes used as a substitute for natural cheese in the manufacture of PCPs, the main advantages being its lower cost and more consistent quality, i.e., intact casein content. Production generally involves ultrafiltration (UF) and diafiltration (DF) of milk, inoculation of the retentate with a lactic culture, incubation to a set pH (5.2–5.8), pasteurization and scraped-surface evaporation to ~60 % dry matter. Increasing the level of cheese substitution with CB generally results in PCPs which are longer-bodied, firmer and less flowable on re-melting. However, the effects vary depending on the method of CB preparation and subsequent heat treatment during processing (Guinee 2009):

- decreasing the pH of milk, in the range 6.6–5.2, prior to UF gives a lower Ca concentration in the CB and yields PCPs with improved meltability,
- rennet treatment of the UF retentate results in poorer flowability of the resulting PCPs, an effect attributed to the higher degree of interaction between β-lactoglobulin and *para*-κ-casein (than with native casein) during subsequent processing (Doi et al. 1983)
- treatment of the retentate with other proteinases (e.g., Savourase-A, proteinases from *Aspergillus oryzae* and *Candida cylindracea*) leads to higher levels of proteolysis in the CB, which in turn yields PCPs which are softer and more flowable than those containing untreated CB;
- increasing the processing temperature in the range 66–82 °C results in PCPs with reduced flowability, an effect attributed to the heat gelation of whey proteins at the higher temperatures, especially when rennet-treated CB is used.

17.2.6.3 Rework

"Rework" refers to PCP which is not packaged for sale. It is obtained from "leftovers" in the filling and cooking machines, damaged packs and batches which have over-thickened and are too viscous to pump. Rework may be ground and recycled into the PCP blend, at a typical level of 1–3 %. Its addition can be particularly useful in promoting creaming (thick, creamy consistency) in processed cheese spreads. Sometimes, the consistency of spreads following processing can be thin and 'liquidy' for a number of probable reasons: a high-moisture content of the PCP (e.g., >60 %), low IC content (e.g., ≪70 %) of the cheese and incorrect blend pH (e.g., <5.5). A low viscosity at this stage is undesirable as it makes hot-filling difficult (due to splashing) and usually coincides with a low viscosity, non-creamy consistency in the final product. However, the addition of rework cheese generally gives PCPs that are firmer, less spreadable, and flow less on heating.

17.2.6.4 Dairy Ingredients

Dairy ingredients are used extensively in PCPs, the types and levels depending on the legislation pertaining to the country of sale. The effects of different ingredients are summarised below; the reader is referred to reviews by Kapoor and Metzger (2008) and Guinee (2009) for more comprehensive insights on the topic.

Addition of skim milk powder at a level of 3–5 % of the blend results in a softer, more spreadable PCP but increases the propensity to non-enzymatic browning on storage. Higher levels (70–100 g/kg) are conducive to the development of textural defects, such as crumbliness.

Rennet casein, despite its insolubility, is frequently used in block PCPs or analogue/substitute pizza cheese. In both products, it behaves like fresh natural rennet-curd cheese, conferring a high degree of elasticity and firmness on the unheated processed cheese, and moderate meltability. Partial replacement of young Cheddar cheese (intact casein content, ~93 %) by commercial rennet casein (intact casein, ~99 %) results in a firmer cheese that melts/flows to a lower extent on heating. This effect is due to higher levels of IC and of calcium in rennet casein (~33 mg Ca/g casein compared to ~27 mg Ca/g in Cheddar cheese (Guinee 2009).

Acid casein is generally not used in processed cheese manufacture because of its insolubility, and especially its low pH which significantly reduces the pH of the PCP blend, the degree of dissociation of the ESs and their ability to sequester calcium from other proteins (e.g., cheese protein or rennet casein). Consequently, the use of acid casein at a level of 1–3 % (w/w) can significantly extend the product make-time, unless the pH of the blend is increased to its normal value (e.g. ~5.8–6.0) by addition of alkali and/or an ES blend with the desired pH-buffering effect. A comparison of the functionality of acid casein and rennet casein on the melt properties of model processed cheeses (made from the casein powder, vegetable fat, water and ESs) was made by Savello et al. (1989). The response of meltability to casein type

depended on the type of ES and the pH to which the acid casein was adjusted (upwards) during processing. Acid casein (pH adjusted to ~7.0–7.7) gave model processed cheeses with better meltability than those made with rennet casein, using disodium hydrogen orthophosphate or tetrasodium pyrophosphate, and an opposite effect when using sodium aluminium phosphate or trisodium citrate.

Owing to the above characteristics of acid casein, it is normally converted to sodium or calcium caseinate to improve its functionality. Caseinate manufacture essentially involves treatment of the curd with alkali solution and readjustment of the pH of the casein to a value (~6.8–7.0) similar to or slightly higher than that of the milk (Mulvihill and Ennis 2003). Caseinates (especially sodium) find most application in spreadable PCPs, where their high water-binding capacity and good emulsifying properties promote a desired viscosity and creaminess.

Whey proteins are commercially available in the form of whey protein concentrates (with a protein content of 35–75 %) and whey protein isolates (≥80 % protein). Inclusion in PCPs, even at a relatively low level (1–3 %), reduces the flowability of the heated PCP to an extent that increases as the level of substitution increases. Hence, they are useful in the manufacture of controlled melt PCPs.

Similarly, the addition of milk proteinate (co-precipitate of casein and whey proteins produced by high heat treatment of milk followed by acidification and calcium addition) at a level up to 5 % of the blend, yields pasteurized processed Cheddar which is firmer and less flowable on re-melting.

17.2.6.5 Processing Conditions

Increases in the processing time, shear and temperature (in the range 70–90 °C) during processing have generally been found to give firmer, more brittle, less spreadable PCPs that have a lower flowability and fluidity when heated (Rayan et al. 1980; Harvey et al. 1982; Glenn et al. 2003; Shirashoji et al. 2006; Guinee 2011c). These changes generally coincide with a higher degree of fat emulsification. Thus, for a given formulation, the properties of PCPs can be dramatically altered by adjusting processing conditions.

17.2.6.6 PCP Composition

Although the rheological attributes of processed cheese products with the same moisture level can differ significantly due to variations in blend composition and processing conditions, increasing the moisture content generally yields products which are softer, less elastic, more adhesive and spreadable. Product pH has a major effect on its texture. Low pH (4.8–5.2), e.g., due to the use of monosodium citrate, monosodium phosphate or sodium hexametaphosphate alone, gives a short, dry, crumbly cheese which shows a high propensity to oiling-off. High pH values (>6.0) give products which tend to be soft and exhibit excessive flow on heating. Reducing

the fat content, while holding the moisture content constant, significantly increases the firmness of the unheated PCP and reduces the flowability and maximum loss tangent (fluidity) of the melted PCPs (Guinee and O'Callaghan, 2013). These effects are associated with a concomitant increase in protein content, which leads to a higher volume fraction of the protein network.

17.3 Imitation and Substitute Cheese Products, and Tofu

Cheese substitutes or imitations may be generally defined as products which are intended to partly or wholly substitute or imitate cheese and in which milk fat, milk protein or both are partially or wholly replaced by non-milk-based alternatives, principally of vegetable origin. Included in this group of products are cheese analogues (CAs) and fat-filled-cheeses (Fig. 17.6).

In the USA, an imitation cheese is defined as a product which is a substitute for, and resembles, another cheese but is nutritionally inferior, where nutritional inferiority implies a reduction in the content of an essential nutrient(s) present in a measurable amount but does not include a reduction in the caloric or fat content, provided the food is labelled pursuant to the provisions of 101.9, "Code of Federal Regulations" (FDA 2012b). A substitute cheese is defined as a product which is a substitute for, and resembles, another cheese and is not nutritionally inferior (FDA 2012b). These products cannot be referred to as cheese if they replace in part, or in whole, milk, milk protein, milk fat or other milk solids with other constituents, and do not comply with the generic definition of cheese as defined in national legislation or by *Codex Alimenatarius* (FAO/WHO 2006). For pertinent information regarding designation and labelling, the reader should consult IDF (1989), McCarthy (1991), and current National Regulations and Codex Alimentarius.

A closely related food is Tofu, a vegan cheese-like product produced by coagulating the juice ('milk') from macerated soybeans; this product is traditionally produced and consumed in East- and South East Asia.

17.3.1 Analogue Cheeses (ACs)

ACs were introduced to the market in the USA in the early 1970s and constitute by far the largest group of imitation or substitute cheese products. Since then, the manufacture of analogues of a wide variety of natural cheeses (e.g., Cheddar, Monterey Jack, Mozzarella, Parmesan, Romano, Blue, Cream Cheese) and pasteurized processed cheese products has been reported in the trade literature. Based on feedback from the market place, current annual production of analogue cheese in the USA, the major producing region, amounts to ~300,000 tonnes, the major products being substitutes for, or imitations of, low-moisture Mozzarella, Cheddar and pasteurised

processed Cheddar. By comparison, European production is estimated to be relatively small (ca. 20,000 tonnes/annum), a fact that may be associated with the lower overall use of cheese as an ingredient in assembled and formulated foods. ACs find application mainly as toppings for frozen pizza pie and as slices in hamburgers; other applications include salads, sandwiches, spaghetti sprinkling, cheese sauces, cheese dips and ready-prepared meals. The success of AC products in the USA may be attributed to a number of factors, including their:

- lower cost of manufacture compared to natural cheese or PCP, principally because of the low cost of vegetable oils compared to butterfat, the absence of a maturation period and associated costs (compared to natural cheese);
- popularity in the industrial food sector as an ingredient that can readily deliver customised cheese-like functionalities (e.g., colour, texture, shreddability, heat-induced flow, melt resistance, shreddability);
- simplicity of manufacture compared to natural cheese;
- ability to be formulated to the nutrient requirements (e.g., lactose-free, low-calorie, low in saturated fat, vitamin enriched) of groups with special dietary needs, made possible by formulation changes and ready availability of customised ingredients.

17.3.1.1 Classification of ACs

ACs may be arbitrarily categorized as dairy, partial-dairy or non-dairy, depending on whether the fat and/or protein components are from dairy or vegetable sources (Shaw 1984; Fig. 17.5). Partial-dairy analogues, in which the fat is mainly vegetable oil, e.g., soya oil, palm oil, rapeseed or their hydrogenated equivalents, and the protein is dairy-based, usually rennet casein and/or caseinate, are the most common. Dairy analogues are not produced in large quantities because the cost is higher than that of natural cheeses with similar composition, owing to the extra cost associated with the preparation and reconstitution of ingredients such as casein and butter oil. The market for non-dairy analogues, in which both fat and protein are vegetable-derived, is probably very small but affords opportunity for vegan cheeses. Nevertheless, the preparation of experimental substitute/imitation cheese products, e.g., analogue pizza-style cheese (APC) and PCPs, from various vegetable proteins such as peanut and soya proteins or blends of these proteins with casein, has generally shown that the substitution of casein by vegetable proteins has resulted in products with impaired texture.

The following discussion pertains to partial-dairy, hard/semi-hard, shreddable ACs (especially analogue Pizza cheese, APC) that are used as ingredients in applications such as frozen pizza, salads, cheese-meat applications, ‘cheese’-filled co-extruded products, savoury snack toppings and sauces. The effects of various ingredients, processing conditions and low temperature storage on the quality of partial-dairy ACs have been reported extensively (see Yang and Taranto 1982; Marshall 1990; Cavalier-Salou and Cheftel 1991; Mulvihill and McCarthy 1994;

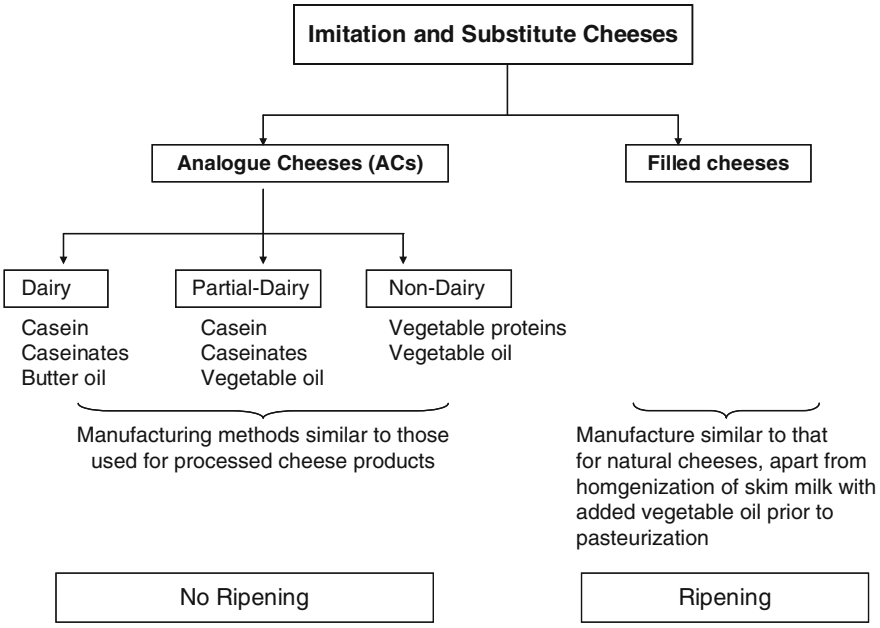


Fig. 17.5 Classification of imitation and substitute cheeses

Ennis and Mulvihill 1997; Mounsey and O'Riordan 2001, 2008; Noronha et al. 2008a, b). Pertinent reviews include Shaw (1984), IDF (1989), Bachmann (2001), Guinee (2011b) and O'Riordan et al. (2011).

17.3.1.2 Manufacturing Protocol for ACs

The manufacture of ACs is similar to that of PCPs and involves formulation, processing and packaging of the hot molten product. A typical formulation (Table 17.7) shows that it differs from PCPs in that cheese is not normally included, although some cheese may be introduced as a flavouring agent or as required by customer specifications for label declaration. While production methods vary somewhat, a typical manufacturing procedure (Fig. 17.6) involves the following steps:

- Formulation, involving selection of ingredient types and quantities to give the desired end-product characteristics (composition, flavour, texture and cooking properties)
- Blending of ingredients
- Processing (heating and shearing) the blend
- Hot packaging

Horizontal twin-screw cookers (e.g., Blentech, CheezeTherm), operating at typical screw speed of 40–80 rpm, are generally used in the manufacture of hard/

Table 17.7 Typical formulation for analogue Pizza cheese

Ingredient	Level added (g/100 g blend)
Casein/caseinates	23.00
Vegetable oil	25.00
Starch	2.00
Emulsifying salts	2.00
Flavour	2.00
Flavour enhancer	
Acid regulator	0.40
Colour	0.04
Preservative	0.10
Water	38.50
Condensate	7.00

On cooking the blend to ~85 °C using direct steam injection, condensate typically amounts to ~7.0 % (w/w)

semi-hard ACs. This design of cooker ensures adequate blending and a relatively low degree of mechanical shear. The sequence of ingredient addition and processing steps may vary with equipment, plant design/layout (Fig.17.6) but typically involves: the delivery of the water and dry ingredients (e.g., casein, starch, ES) to the cooker while agitating; heating to ~50 °C using direct steam injection and blending for ~2–3 min; addition of oil, heating to ~85 °C until a uniform homogeneous molten mass is obtained (typically 5–8 min); addition of flavouring materials (e.g., enzyme-modified cheese, starter distillate, hydrolysed butter oil) and acid regulator (e.g., citric acid, lactic acid); blending the mixture for a further 1–2 min and packaging the hot molten blend. These process conditions, together with the correct formulation, promote a low degree of fat dispersion and hence, relatively large fat globules (e.g., 5–25 μm). On subsequent baking of the AC, the relatively large fat globules ensure a sufficient degree of oiling-off, limit dehydration of the cheese topping and are, thereby, conducive to achieving the desired flow and succulence characteristics required in typical applications, e.g., pizza. As for PCPs, there is generally an inverse relationship between the degree of fat emulsification and the flowability of ACs (Neville 1998). The addition of flavours towards the end of the manufacturing process minimizes the loss of flavour volatiles in the dissipating steam. In contrast to PCPs, where the pH of the final product (e.g., 5.5–5.9) is adjusted by adding the correct blend of ES (and sometimes acidifying agent) prior to processing, the pH in ACs made using rennet casein as the main protein source are typically adjusted following casein hydration and emulsion formation by addition of food-grade acid to the hot molten blend. This protocol ensures a relatively high pH (typically > 7.0) during the manufacture of the AC, and in turn a higher negative charge to the casein, which is conducive to greater calcium sequestration (by sodium phosphate ESs) and casein hydration. Two factors necessitate this procedure:

- the high calcium content of rennet casein (e.g., 35 mg/g casein compared to ~25–30 mg/g casein for natural cheeses such as Cheddar used in PCPs),

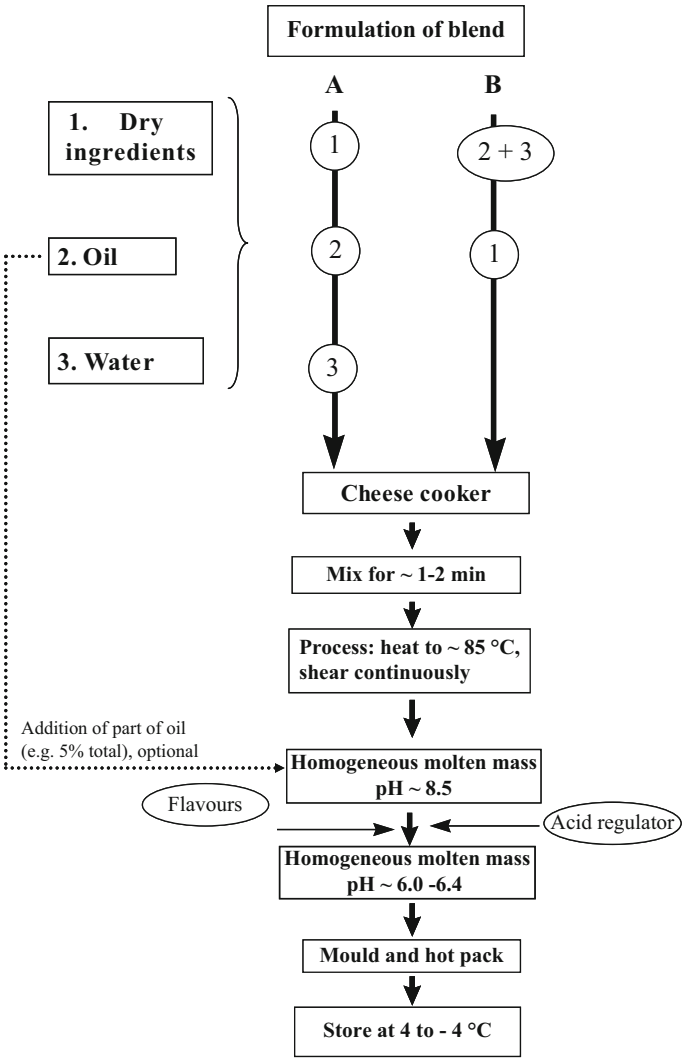


Fig. 17.6 Typical manufacturing procedures (A, B) for analogue cheese. The procedures differ with respect to sequence of ingredient addition. In procedure A, dry ingredients (e.g., casein, emulsifying salts) are added followed by oil and finally water, while being constantly agitated; in procedure B, oil and water are first added, followed by the dry ingredients

- rennet casein is in a dehydrated state whereas the casein in natural cheese is hydrated, to a degree dependent on the extent of proteolysis, pH and concentrations of NaCl and Ca in the cheese.

Both factors contribute to the efficient hydration of rennet casein and, hence, emulsification of added vegetable oil.

17.3.1.3 Principles of Manufacture of ACs

The principles of manufacture of ACs from rennet casein are similar to those for PCPs, involving the combined effects of ES, heat and shear to promote sequestration of Ca from the rennet casein (dehydrated *para*-casein which, in effect, is equivalent to dehydrated cheese protein), pH adjustment and buffering of the blend, casein hydration, fat dispersion and its emulsification by the hydrated *para*-caseinate and setting of the molten mass on cooling.

17.3.1.4 Composition and Functionality of Analogue Pizza Cheese, APC

Analogues of cheese used in pizza (e.g., low-moisture Mozzarella cheese, LMMC, or Cheddar) have been studied the most widely, primarily because of the size and financial importance of the pizza market. Comparison of the mean composition of commercial samples of APC and LMMC (Table 17.8) indicates that while many of the gross compositional parameters of APC are similar to those of LMM, the former generally has a lower level of protein and higher levels of fat-in-dry matter, Ca and P. While intra-varietal differences in composition occur for both products, they are more pronounced in the APC. Moreover, the sum of the mean percentages for moisture, fat, protein and ash account for only ~93 % of the dry matter (compared to ~99 % in LMMC), suggesting the addition of carbohydrate-based ingredients (e.g., lactose, maltodextrins, starch) during formulation of the APC. These materials may be added to impart certain functional characteristics to the end-product and/or as partial substitutes for rennet casein, thereby reducing formulation costs. The relatively large compositional variations exhibited by APCs probably reflect deliberate differences in formulation so as to achieve customized functionalities in the finished APCs.

Some important functional attributes of melted cheese on a cooked pizza pie are:

- melt time—an index of how rapidly the shredded cheese on a pizza pie melts and flows into a homogeneous molten mass showing no traces of shred identity;
- flowability—a measure of the degree of flow or spread on heating;
- stretchability—a measure of the tendency to form cohesive strings or sheets when extended;
- apparent viscosity—a measure of chewiness.

Upon baking on pizza pie, a good quality Pizza cheese melts relatively quickly, flows adequately to give the desired degree of surface coverage and possesses the desired degrees of chewiness and stretchability which, perhaps more than other functional properties, endow pizza pie with its unique culinary qualities (cf. Chap. 18). Comparison of the functional characteristics of commercial LMMCs and APC indicates that both cheeses have similar mean values for melt time, flowability and apparent viscosity (Table 17.9). However, the stretchability of APCs is generally inferior to that of LMMC. The differences in stretchability between LMMC and APC may be related primarily to differences in the degree of aggregation and microstructure of the *para*-casein, as affected by differences between the procedures

Table 17.8 Typical composition of low-moisture Mozzarella and analogue Pizza cheese

	Low-moisture Mozzarella (LMMC)	Analogue Pizza cheese (APC)
Moisture (g/100 g)	46.4	48.8
Protein (g/100 g)	26.0	18.5
Fat (g/100 g)	23.2	25.0
Fat-in-dry matter (g/100 g)	44.6	49.0
Salt-in-moisture (g/100 g)	3.1	3.5
Ash (g/100 g)	3.9	4.2
Ca (mg/100 g)	27.5	34.4
pH	5.5	6.1

Values presented are means of eight samples of each cheese type which were sourced in Ireland, UK and/or Denmark (modified from Guinee et al. 2000a)

Table 17.9 Functionality of low-moisture Mozzarella and analogue Pizza cheese

Functional attributes	Low-moisture Mozzarella (LMMC)	Analogue Pizza cheese (APC)
Aggregation index (-)	3.95	3.74
Melt time (s)	108	105
Flowability (%)	53	42
Stretchability (cm)	87	28
Apparent viscosity (Pa.s)	630	650

Values presented are means of eight samples of each cheese type which were sourced in Ireland, UK and/or Denmark (modified from Guinee et al. 2000 a)

used to manufacture the two products. During the manufacture of LMMC and other *pasta filata* cheeses, such as Provolone or Kashkaval, the cheese curd, at pH ~5.15, is subjected to a plasticization process, whereby it is heated to ~57–60 °C by kneading in hot water or dilute brine (e.g., 78–80 °C). These conditions promote a controlled degree of aggregation of the calcium phosphate *para*-casein and the formation of *para*-casein fibres with a relatively high tensile strength [see Figs. 17.7 and 18.8 (see Chap. 18)]; the cheese fat is physically entrapped between the *para*-casein fibres. In contrast, the conditions used during the manufacture of APC sequester calcium phosphate and increase the pH, thereby disaggregating and hydrating the *para*-casein aggregates of the rennet casein ingredient. The hydrated *para*-caseinate immobilizes large quantities of added water and emulsifies the added vegetable oil and thereby contributes to product formation and its physico-chemical stability. Hence, the protein in APC is mainly in the form of a concentrated hydrated dispersion of sodium *para*-caseinate compared to calcium phosphate *para*-casein fibres in LMMc (Fig. 17.7). Otherwise, both LMMCs and PCAs may exhibit marked intra-varietal differences in functionality, an occurrence which may be attributed to design differences in formulation (for APCs), or manufacturing conditions/degree of maturity (for the LMMC); the intra-varietal differences in functionality allow cheese manufactures to customize their cheese products to the requirements of different pizzerias.

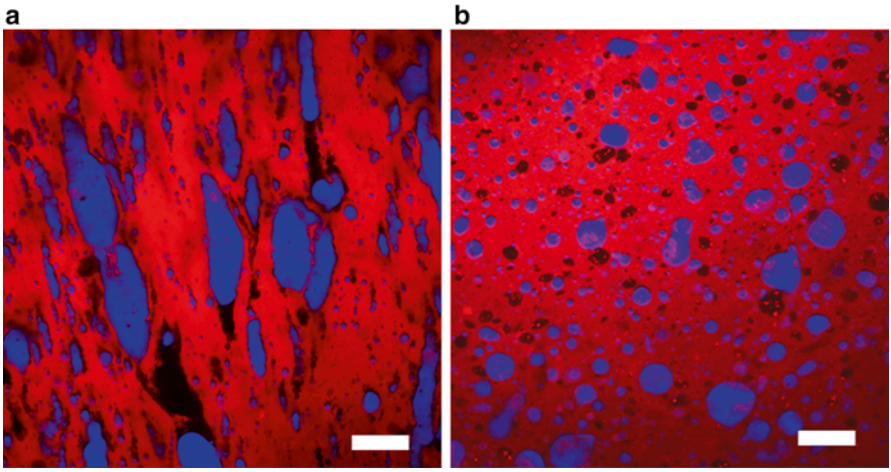


Fig. 17.7 Confocal laser scanning micrographs showing the microstructure of commercial samples of low-moisture Mozzarella (**a**) and analogue Pizza cheese (**b**). Fat is coloured *blue* and protein *red*; bar = 25 μm. In the low-moisture Mozzarella, the protein is in the form of elongated fibres and the fat in the form of elongated pools trapped between the protein fibres. In the analogue Pizza cheese the protein is not organized into fibres and the fat is mainly in the form of discrete rounded globules

17.3.1.5 Functionality Changes During Storage: Comparison of APC and LMMC

The functional properties of LMMC change markedly during storage at 4 °C (see Chap. 18). Initially, during the first 1–3 days, depending on make procedure and composition, the cheese is non-functional, as reflected by its limited flow/spread and exudation of free oil, and its tendency to crust or burn (develop charred black areas) during baking. After 5–10 days of ripening, the cheese acquires functionality, as reflected by decreases in melt time and apparent viscosity (chewiness) and increases in flowability and stretchability (see Chap. 18). Thereafter, these changes occur more slowly and the cheese maintains desirable functionality for ~40–50 days. However, prolonged ageing (e.g., to 75 days) of LMMC is associated with excessive flowability, loss of chewiness and a 'soupy' consistency in the grilled or baked cheese. Moreover, the uncooked shredded cheese develops an increased susceptibility to clumping or sticking, an occurrence which is undesirable as it leads to blocking of cheese dispensing units on pizza pie production lines and leads to non-uniform distribution of the cheese topping on the pizza pie. The main functional changes that occur during ripening appear to be mediated by:

- proteolysis of *para*-casein network (by plasmin and possibly residual coagulant)
- reduction in the level of insoluble Ca, possibly as a consequence of the release of serum-soluble phosphopeptides containing calcium, commensurate with proteolysis.

- a slow salting-in of the protein network over time, attendant with the equilibrium of the casein network and entrapped serum phase with respect to Na^+Cl^-. Unsalted LMMC is characterised by a low degree of casein hydration and low heat-induced flow compared to salted LMMC, despite a similar level of primary proteolysis in both products.

Various studies using microscopy, water-binding capacity and rheology (Guo et al. 1997; Paulson et al. 1998; Auty et al. 2001; Guinee et al. 2000b, 2001), have shown that these changes coincide in the *para*-casein network becoming less intact, more hydrated and swollen, and less rigid. Hence, on heating the cheese is softer (less chewy) and exhibits higher moisture retention, more oiling-off and higher flow.

Few studies have considered the changes in casein-based APCs during ripening. Mulvihill and McCarthy (1994) reported progressive proteolysis (e.g., pH 4.6-soluble N increased from ~3.5 % of total N at 1 day to 19.5 % total N after 51 weeks) and decreases in elasticity and chewiness on storage at 4 °C for 51 weeks. However, the changes during the first 4–6 week were relatively small; normally, analogues are used within 1 month after manufacture. Kiely et al. (1991) reported that casein-based APCs were more functionally stable than LMMC during storage at 4 °C for 28 days. In the authors' experience, casein-based analogues containing a high level of native maize starch (>4 %) may lose their functionality relatively rapidly (e.g., after 4 weeks) during storage at 4 °C, an effect which may be associated with the retrogradation of amylose. The loss of functionality is reflected by the increase in loose moisture on shredding, the loss of meltability and flowability and burning/crusting on baking. Added starch may undergo post-manufacture retrogradation during cold storage of the APC to form networks of aggregated amylose polymers. The degree of amylose aggregation depends on properties of the starch (e.g., ratio of amylose-to-amylopectin, native or modified starch, gelatinisation temperature), processing conditions (temperature, shear) which influence the integrity of starch granules and other materials such as casein fractions (Kett et al. 2013), which affect the gelatinisation temperature of the starch. This native starch-induced network, formed on cooling, appears to shrink during storage of the APC, resulting in the expulsion of moisture. It is envisaged that the free water renders the APC more susceptible to drying-out and crusting (puffing) during heating, while simultaneously the more aggregated starch networks impairs heat-induced flow. For a more thorough discussion on the uses of starch in CA, the reader is referred to Ennis and Mulvihill (1997) and O'Riordan et al. (2011).

17.3.2 Filled Cheeses

Filled cheeses generally differ from natural cheeses in that the milk fat is partly or fully replaced by vegetable oils, which may be partially hydrogenated to impart a melting profile similar to that of milk fat. However, filled cheeses may be categorized into two types depending on whether the base material is native skim milk or reformed skim milk; the latter is prepared by dispersing dairy ingredients, such as whey and total milk protein, in water. Preparation of the filled milk involves

dispersion of the vegetable oil in the native or reformed skim milk, using a high speed mixer, and subsequent homogenization of the blend. Dispersion and homogenization ensure emulsification of the added vegetable oils and thus prevent phase separation and/or excessive creaming during cheesemaking. The filled milk is then subjected to the conventional cheesemaking technology, with appropriate modifications to suit the particular type of cheese being substituted or imitated.

Homogenization of milk generally impairs curd syneresis and tends to give cheeses which have higher moisture content, lower yield stress and firmness, and lower flowability on melting compared to those from non-homogenized milk (Guinee et al. 2000b; Dejmek and Walstra 2004). Nevertheless, satisfactory syneresis and heat-induced flow can be achieved by preparation of a cream from the vegetable oil (in part of the skim milk) while minimising homogenisation pressures; this cream is then added to the bulk phase skim milk to attain the desired protein-to-fat ratio in the filled cheese milk.

17.3.3 Tofu (Soya Bean 'Cheeses')

Tofu, a staple food in the Orient for centuries, is a tough rubbery curd made from soya bean milk. Manufacture essentially involves soaking and swelling of the soya beans in water for a long period, addition of extra water, grinding and milling of the bean/water mixture into a smooth slurry and filtration of the slurry to obtain soya milk. The soya milk is boiled to induce protein denaturation, cooled to ~37 °C, and coagulated by the addition of a divalent salt such as calcium lactate and adjusting the pH to 4.5–5.0 using acetic acid or glucono-δ-lactone (Tharp 1986; McCarthy 1991). Following coagulation, the whey is drained off and the curd is moulded and lightly pressed to give Tofu, in which the levels of dry matter, protein, fat and carbohydrate are typically 15.2 %, 7.7 %, 4.2 % and 2.4 %, respectively. The moulded curd may be subjected to a high pressure and brine-salting to yield soya bean cheeses with a higher dry matter level than Tofu (e.g., 53 % dry matter; Abou-El-Ella 1980). Ras cheese made from soya bean milk was found to have a higher moisture level and received lower sensory scores for colour, flavour and body/texture characteristics than that made from cows' milk by conventional cheesemaking procedures (Abou-El-Ella 1980). A more comprehensive overview of Tofu is given in the *Book of Tofu* (Shurtleff and Aoyagi 1979, 1998).

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Chapter 18
Cheese as an Ingredient

Summary While most natural cheeses are consumed directly as table cheese, which can be eaten directly or with crackers or bread, they are also used extensively as ingredients in culinary dishes. Nevertheless, cheeses are in many cases manufactured specifically for use as an ingredient rather than as table cheese. The manufacture of ingredient cheeses involves protocols which impart specific functionalities, such as controlled textural/rheological properties (e.g., sliceability, shreddability or crumbliness) and cooking properties (e.g., Mozzarella with customized flow and stringiness suited to specific pizza brands). Ingredient cheeses are used in an array of culinary dishes, formulated food products and ready-prepared meals. The types and level of functional attributes required from ingredient cheeses depend on the application in which they are used. The functionalities of the unheated and heated cheese are key quality determinants of ingredient cheese. These are strongly influenced by micro- and macrostructure. At a microstructural level, rennet-curd cheese is a matrix comprised of a calcium phosphate *para*-casein network, which imbibes the cheese serum (moisture and dissolved solids) and encases the fat phase. The network may be viewed as a polymer network, in which the casein polymers are cross-linked mainly by calcium and calcium phosphate. The degree of polymer cross-linking and the relative proportion of fat in the network control the response of the unheated cheese matrix to stresses and strains encountered during the size-reduction processes involved in shredding, grating or eating, and the response of the heated cheese during baking and grilling. At the macrostructural level, cheese is an assembly of fused curd particles (microstructures), with the extent of fusion depending on both the microstructure of the curd particles and the processes to which the curd particles are subjected such as salting, moulding, texturizing and pressing. Hence, a key approach in designing ingredient cheeses with target functionalities is the control of cheese-making operations that affect the microstructure and macrostructure.

Cheese functionality is dynamic, changing with storage time owing to ongoing biochemical changes including proteolysis and lipolysis which affect flavour and rheology. Hence, there is a time window within which functionality is optimal, depending on the application.

Significant volumes of natural cheese are converted to processed cheese products which are used extensively as ingredient cheese products. Processing enables the functional characteristics of the natural cheese to be modified, extended and/or stabilized to varying degrees.

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In contrast to ingredient cheeses, cheese ingredients are products derived from cheese by subjecting it to secondary processing treatments; examples include cheese powders and enzyme-modified cheeses (EMCs). Cheese ingredients are used primarily as flavorings in an extensive array of snack and formulated food products.

Keywords Cheese as an ingredient • Cheese-derived ingredients • Functionality • Uses

18.1 Introduction and Definitions

There are at least 1000 different natural cheese varieties (IDF 1981). These are generally consumed as table cheese, which may be arbitrarily defined as cheese eaten on its own or as an accompaniment to bread or crackers during a meal. Many of these cheeses have long been used as ingredients in the preparation of culinary dishes in the home and hostelries, notable examples including toasted sandwiches, quiche, omelettes, pasta, pizza and lasagne (Fig. 18.1). In these applications, cheese imparts functionalities that contribute to the preparation and sensory properties of the food in which they are included. Pertinent functionalities of unheated cheese include crumbliness, sliceability, spreadability, shreddability or gratability, while those of the heated cheese include overall appearance, flavour, extent of flow, stringiness, fluidity, and oiling-off. The type and level of functionality required depends on the application (Tables 18.1 and 18.2).

The volume of cheese being consumed as an ingredient has increased greatly since the 1970s owing to rapid growth of the food service and prepared consumer food sectors. This trend has given rise to the commercial use of the terms, *ingredient cheese* and *cheese ingredients*. Ingredient cheese, as distinct from table cheese, may be defined arbitrarily as cheese manufactured with targeted functionalities designed to optimise its quality as an ingredient in specific applications, e.g., pizza. The production of ingredient cheese generally involves alteration of the manufacturing protocol so as to impart specific functionalities as specified in business-to-business relationships, and which typically include controlled cooking properties, e.g., low- or non-melt cream cheese or Cheddar cheese; customized degrees of flow, oiling-off and stringiness in pizza-style Mozzarella.

In contrast to ingredient cheeses, cheese ingredients are products derived from cheese by subjecting it to secondary processing treatments; examples include cheese powders and enzyme-modified cheeses (EMCs) (Fig. 18.1). The treatments applied during secondary processing may be minimal (e.g., grating and drying in the case of dried Parmesan) or more extensive (e.g., shredding, heating and structural transformation as in the manufacture of EMCs or cheese powders). Cheese ingredients are used primarily as flavourings in an extensive array of snack and formulated food products.

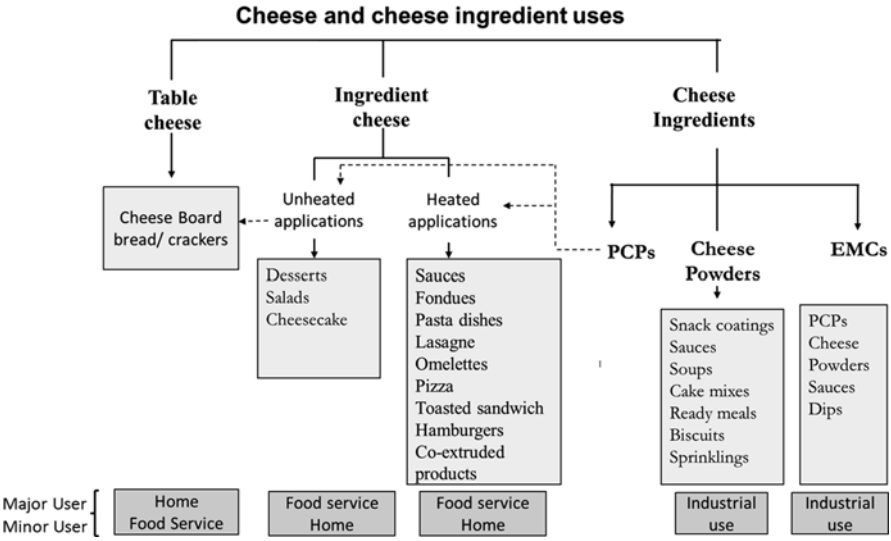


Fig. 18.1 Uses of cheese as a food ingredient

Processed cheese products, which involve significant secondary processing of natural cheese together with other ingredients (cf. Chap. 17), are used extensively as ingredient cheese products, because their functionalities are readily customized and stabilized by varying formulation, processing conditions and cooling conditions. Nevertheless, they are widely consumed as table products (e.g., spreads) in many parts of the world. Similarly, analogue cheese products (cf. Chap. 17) are widely used as substitute/imitation ingredient cheeses.

Ingredient cheese products are delivered in different formats to the consumer through two main sectors, namely:

- the retail sector, as an ingredient in prepared consumer foods such as frozen pizza, cheese-filled tortellini or ravioli, dried pasta dishes, cheesecake and savoury scones;
- the food service sector, as an ingredient in various dishes (e.g., lasagne, pizza, omelette, cheese panini, sandwiches);

Ingredient cheese is also used in the industrial sector in the manufacture of various assembled food products (e.g., frozen pizza, frozen cheese burgers, quiche), co-extruded foods (e.g., cheese-filled meat balls and sausages), formulated foods (e.g., gratins, prepared meals, dried soups) and cheese ingredients (e.g., cheese powders, dried cheese).

The current Chapter focuses on natural cheese and cheese-based products as an ingredient, drawing on relevant properties such as flavour, texture and cooking attributes as discussed in Chaps. 13 and 14.

Table 18.1 Functionalities of unheated ingredient cheeses

Functional requirements	Description	Cheese type displaying property	Usage application	Related rheological properties	Large strain deformation characteristics[b]
Shreddability	Ability to: – cut cleanly into long thin strips of uniform dimensions (typically cylindrically shaped; 2.5 cm long, 0.6 cm in diameter) – low susceptibility of cheese to fracture or to form curd dust during shredding – resistance of shreds to sticking, matting or clumping when stored in bins or retail packs	Low-moisture Mozzarella, Swiss-type, Gouda, Cheddar (young-medium), Provolone, some PCPs, ACPs	Shredded cheese for retail or catering, shredded cheese for pizza manufacture	High elasticity, longness (large displacement before fracture), firmness, springiness	High fracture strain (ε_f) Medium to high deformation force (σ_{max})
Sliceability	Ability of: – cheese to cut cleanly into thin slices without fracturing or crumbling or sticking to cutting implement – slices to resist breakage or fracture at edges (on contacting packing equipment), – slices to undergo a high level of bending before breaking – slices to resist drying-out and curling at ends on moderate exposure to atmosphere (e.g., under conditions of presentation in continental breakfast setting) – of slices to exhibit a high degree of peelability (e.g., from stacks of slice-on-slice in food service applications)	Swiss-type, Gouda, Cheddar, Provolone, some Quesco Blanco, some PCPs and ACPs	Slices for retail and food service	High elasticity, longness, springiness	High ε_f Medium-high σ_{max}

Gratability	Ability: – of cheese to fracture easily into small hard particles – of particles to resist matting during shearing, crushing, fluidization or piling – of particles to exhibit free flow	Hard brittle cheeses e.g., Parmesan, Romano-type	Dried cheese for sprinkling	Brittle, elastic fracturability, firm, low tendency to stick	High fracture force (σ_f) Low ε_f High σ_{max}
Spreadability	Ability: – of cheese to spread easily when subjected to a shear stress	Mature Camembert and Brie, some cream cheeses, processed cheese spreads, some ACPs	Cheese for spreading, e.g., on crackers and bread	Long, plastic fracturability, soft, adhesive	High ε_f Very low σ_f Very low σ_{max}
Crumbliness	Ability: – of cheese to fracture easily into small irregularly shaped pieces when rubbed between fingers	Feta, Blue, Stilton, Cheshire	Tossed salads, *crêpes au fromage*, soup garnishes	Medium-soft, brittle cheese which breaks into irregular shaped pieces	Low ε_f Medium-low σ_f Medium-low σ_{max}

Functionalities may be also referred to as attributes, properties or characteristics

PCPs processed cheese products, *ACPs* analogue cheese products

[a]Rheological terms relating to large strain deformation using uniaxial compression tests: ε_f fracture strain, σ_f fracture stress, σ_{max} firmness. See Chap. 14 for explanations of rheology-related properties and large strain deformation characteristics

Table 18.2 Functional requirements of heated cheese which affect its functionality as an ingredient

Types of properties	Description	Cheeses types display this property	Usage application
Meltability	Ability to soften on heating	Most cheeses (e.g., Cheddar, Gouda, Emmental, Mozzarella, …) after a given storage period, apart from low-fat and skim milk cheese and some fresh acid-curd cheeses Most processed and analogue cheeses	Most applications
Flowability	Ability of cheese (shredded, grated or sliced) to spread or flow on heating	Most cheeses after a given storage period, apart from low-fat and skim milk cheese and some fresh acid-curd cheeses Many processed and analogue cheeses, to a degree dependent on formulation and processing conditions	Most applications require some flowability, ranging from high in gratins, sauces, and *Cordon-blue* products, to moderate in pizza, hamburgers, toasted sandwiches and omelettes, to low in some bakery applications (e.g., savoury scones)
Flow-resistance	Ability of cheese to resist flow or spread, and to retain original shape and dimensions on heating	Customised rennet-curd ingredient cheeses: – with a high level of whey protein (e.g., from high heat-treated milk, especially if fortified with whey protein prior to heat treatment), – with a high intact protein content, as in young cheese, especially low-fat variants, – from homogenized milk Some fresh acid-curd and acid-heat coagulated fresh cheeses, especially those with a high whey protein content, low pH, and low-fat content. Example: include Paneer Some processed and analogue cheese products, especially those with a high whey protein content, high calcium-to-casein ratio, high protein-to-fat ratio, high protein content, and high degree of fat emulsification	Fried cheese, deep-fried cheese sticks, cheese for kebabs, cheese insets in burgers

Stretchability or stringiness	Ability of heated cheese to form strings and/or sheets when extended	Cheeses subjected to *pasta filata* process during manufacture (e.g., Mozzarella, Kachkaval, Halloumi, Provolone) or to other texturizing processes conducive to *para*-casein fibre formation, such as cheddaring (e.g., young Cheddar with a high intact casein content, >90 %) or extrusion at elevated temperature (e.g., string cheese)	Pizza
Oiling-off (surface sheen)	Ability of cheese to express a moderate level of free oil on heating, to impart gloss and succulence to the molten cheese mass	Most natural cheeses (provided that they are not over mature, when oiling-off may become excessive). Exceptions are low-fat cheeses, especially if prepared from homogenized milk Many processed and analogue cheeses (provided that the degree of fat emulsification is not too high or too low, where oiling-off level is deficient or excessive, respectively)	Most applications, ranging from moderate gratins to low for omelettes and pizza
Fluidity	Ability of cheese to attain desired fluidity on heating, and not to congeal too rapidly on cooling. Generally correlates positively with flowability and magnitude of loss tangent, tan δ.	Most natural cheeses tend to become more fluid during maturation, commensurate with degree of proteolysis	Depending on application, fluidity required may vary from very high (i.e. soupy) in gratins to moderate on pizza, to very low in flow-resistant applications such deep-fried breaded cheese sticks or fried Paneer.
Blistering	Ability of cheese to display a moderate to low level of blistering in some applications		Pizza

References used in compilation: Kindstedt and Rippe (1990); Rüegg et al. (1991); Guinee and O'Callaghan (1997), Fox et al. (2000), Guinee and Kilcawley (2004) and Guinee et al. (2015)

18.2 Overview of Functional Requirements of Cheese as an Ingredient

As an ingredient suited to specific food applications, cheese must possess one of more functionalities, some of which are listed in Tables 18.1 and 18.2. The functional properties of raw cheese are determined largely by its taste/aroma and rheological characteristics, which are discussed in Chaps. 13 and 14, respectively.

18.2.1 Organoleptic Characteristics

The organoleptic characteristics of cheese are perceived by the senses and include taste (sweet, salty, sour, bitter, umami), smell (aroma), sight (e.g., colour; smoothness; extent of free oil; surface dryness, glossiness or matt), and touch (e.g., mouthfeel, softness, tenderness, chewiness, stickiness, juiciness, moistness, 'fattyness' and mouth-coating) (Delahunty and Drake 2004). These are important in both unheated (e.g., sandwiches, salads) and heated (e.g., sandwiches, salads, spaghetti Bolognese, lasagna, pasta dishes) cheese applications. Flavour, which represents taste and aroma, may be considered as a *sine qua non*, with objectionable flavours generally resulting in rejection of the food (cheese) despite other organoleptic attributes (e.g., mouthfeel and tenderness), usage attributes (e.g., shreddability, sliceability) and cooking behaviour (e.g., colour, degree of melting and flow, stringiness) being satisfactory. The importance of cheese flavour is highlighted by the use of mature cheese and cheese products (processed cheese products, cheese powders and cheese sauces) containing added highly intense cheese flavour preparations (e.g., enzyme-modified cheeses, enzyme-hydrolyzed dairy ingredients) in many cheese ingredient applications, such as ready-prepared meals, snacks, soups and sauces. The increasing importance of cheese flavour is also highlighted by the increasing use of cheeses, such as mature Cheddar and Colby, which have poor stretchability compared to Mozzarella, in pizza cheese toppings. Cheese flavour is discussed extensively in Chap. 13, and will not be discussed further.

18.2.2 Size-Reduction Properties of Unheated Cheese

The primary stage of preparation of any food containing cheese requires that the cheese be reduced in size so as to facilitate:

- deposition onto surfaces (e.g., shredded Mozzarella cheese onto pizza base),
- layering/spreading onto a surface
- mixing/blending with other ingredients (e.g., shredded cheese with water and optional ingredients in the preparation of processed cheese products, cheese powders, cheese sauces, fresh cheese desserts)

Size reduction is achieved by cutting into relatively large pieces, crumbling, slicing, shredding, dicing, grating and/or shearing.

Hence, the behaviour of unheated cheese during size-reduction operations is a critical function. Depending on the application, the cheese may be required to exhibit particular size-reduction attributes (e.g., ability to be portioned, sliced, shredded, diced, grated or crumbled) that facilitate its use in the primary stages of preparation of various dishes, e.g., the ability of Parmesan to grate for lasagna; Feta to crumble easily into salads; Gouda to slice cleanly or to bend when in sliced form, Cream cheese or mature Camembert to spread on crackers. The size-reduction behaviour of cheeses is determined mainly by its rheology, which determines its response (fracture, deformation, flow, adhesiveness, springiness) to stresses and strains (e.g., cutting, shear, compression) applied during the size-reduction operations *per se*. The rheology of cheese (Chap. 14) is a function of:

- composition (e.g., levels of moisture, fat and protein), microstructure, which represents the spatial distribution of its compositional components and the level of intra- and intermolecular attractions between the components,
- macrostructure which represents the arrangement of, and attractions between, the different macro-components (e.g., curd particles, gas pockets, veins and/or rind) and determines the presence of heterogeneities such as curd granule junctions, cracks and fissures,
- the physico-chemical state of its components (e.g., ratio of solid-to-liquid fat as affected by temperature, degree of aggregation, hydration and hydrolysis of the protein).

In addition to size-reduction attributes, unheated cheese is generally required to contribute to the organoleptic characteristics, including colour, appearance, flavour, aroma and texture of the food in which it is included. Appearance criteria of the cheese used may include attributes such as the smoothness of slices, smoothness and dimensions of shreds, the extent of sticking and balling of shreds, the size and uniformity of crumbled cheese pieces, the level of curd fines, the sharpness and uniformity of appearance of cut edges and corners of portions, the degree of bending, drying or cracking of exposed slice sections (e.g., in sandwiches, baguettes), the opacity or translucence.

18.2.3 Cooking Properties

In most applications as an ingredient, the behaviour of cheese on heating and cooking is critical. The heated cheese, following grilling or baking, may be required to melt, flow, stretch, brown, blister, oil-off and/or stretch to varying degrees. It may also be expected to be chewy (as in pizza pie) and contribute to certain mouth-coating characteristics (as in sauces and pasta dishes). In many dishes, e.g., sauces, the cheese is required to have the ability to interact with other food components such as water, carbohydrates, proteins and fats during food preparation. In food

service, the cooked cheese may be expected to remain smooth and moist, without congealing, developing a 'skin' or becoming 'stodgy' over time or during cooling (during service and consumption) (Guinee et al. 2015).

18.3 Basis of Functional Properties in Cheese

As discussed above, many of the functional properties required in unheated cheese as an ingredient are based to a large extent on its structure and rheology. These determine its deformation and fracture behaviour when subjected to stresses and strains, as applied during size reduction. The rheological behaviour of the cheese during usage is ultimately determined by competition between internal forces responsible for the integrity of the matrix structure and external forces responsible for fracture and demolition. Hence, understanding the basis of functionality and how to tailor it requires us to ask the following question: what is the structure of the cheese matrix and what are the principal forces that are responsible for its integrity? This in turn necessitates an examination of cheese matrix, especially the casein network, in relation to its formation and the forces that bind the casein macromolecules within that network.

18.3.1 Formation of Rennet-Curd Cheeses

The manufacture of rennet-curd cheeses involves the formation of a gel in response to the enzymatic cleavage and removal, by the coagulant, of the stabilising surface layer of the casein micelles (κ-casein glycomacropeptide) (cf. Chaps. 4 and 7). This results in the surfaces of the resultant *para*-casein micelles interacting and forming a continuous gel network, which occludes the fat globules and moisture. During the cheesemaking process, the gel is subjected to several processes which interactively result in its dehydration: cutting, stirring, cooking, acidification, drainage of whey, moulding, salting and pressing. The moisture content decreases from ~88 % in the gel prior to cutting to 35–50 % in the curd after pressing, depending on the variety. The moisture content may decrease further during ripening depending on the presence/absence of packaging, the type of packaging, and the relative humidity of the environment. Simultaneously, the casein is concentrated from ~2.5 % (w/w) in the milk to 16–20 % in semi-soft chesses such as Feta, Camembert, and to 25–32 % in hard cheeses like Cheddar, Emmental and Parmesan. The moisture-to-protein ratio (g water/g protein) decreases from ~4 in the native casein micelles in milk to ~3.6, 2.4, 1.52, 1.4, 1.2 and 1.0 in Feta, Camembert, Cheddar, half-fat Cheddar and Parmesan, respectively. Consequently, the dehydration process results in removal of essentially all the bulk phase serum (moisture + dissolved solutes including lactose, soluble salts and non-protein nitrogen) and part of the serum entrapped within the

native casein micelles, as whey. The moisture remaining in the cheese curd following pressing corresponds to moisture that has not been removed from the *para*-casein micelles during the cheesemaking process.

18.3.2 Structure of Rennet-Curd Cheeses

The structure of rennet-curd cheeses may be considered at two levels, namely the microstructure and the macrostructure. Microstructure represents spatial distribution of the compositional components (casein, minerals, fat, moisture and dissolved solutes such as lactose, lactic acid, soluble salts and peptides) at the micro-scale level (e.g., >25× magnification) and the level of intra- and intermolecular attractions between the components. In the case of cheese, the microstructure represents the structure within individual curd particles; while it is usually observed directly using various forms of microscopy (e.g., transmission- and scanning electron microscopy, confocal laser scanning microscopy) other methods such rheometry, x-ray crystallography, fluorescence spectroscopy and differential scanning calorimetry may be used as indirect measures of the properties of the structure. The macrostructure represents the overall structure, as observed visually by the unaided eye or at very low levels of magnification (<10× magnification) with the aid of light microscopy. Macrostructure in the case of cheese may be considered as representing the gross aspects of structure within the moulded cheese, such as curd granule junctions, eyes and slits/cracks.

The microstructure of rennet-curd cheese may be defined as a highly concentrated matrix (Fig. 18.2), consisting of:

- a calcium phosphate *para*-casein network of extensively dehydrated, fused *para*-casein micelles
- a fat phase (in the form of globules, coalesced globules and/or pools) that is entrapped by the casein network
- a solvent phase consisting water and dissolved solutes (e.g., lactose, lactic acid, soluble salts, water-soluble peptides, enzymes), corresponding to residual serum retained by the *para*-casein micelles.

The fusion of casein within the network is mediated by various interactions, including:

- calcium bridges formed by divalent calcium ions which bind to dissociated acidic amino acid residues (glutamate and aspartate) on different molecules, and to colloidal calcium phosphate (attached to serine phosphate groups), and
- hydrophobic interactions between uncharged amino acid residues

Overall, the structure may be envisaged as a polymer network, comprised of chains of fused *para*-casein micelles, interconnected by numerous calcium and

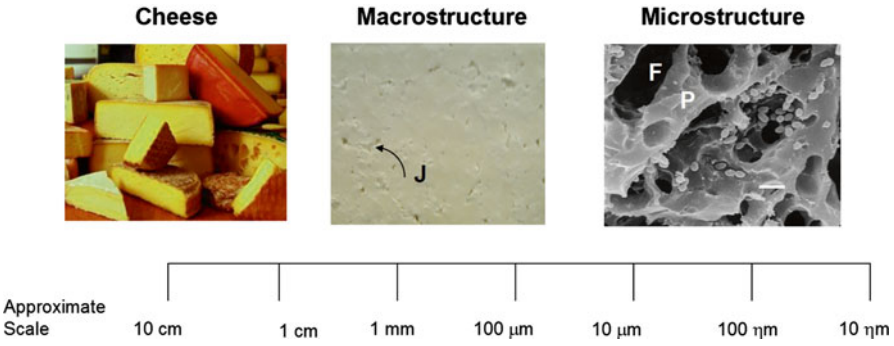


Fig. 18.2 Structural resolution of cheese showing its macrostructure and microstructure. The macrostructure consists of an assembly of curd particles or curd pieces (chips) fused through interaction of surfaces; the degree fusion at curd particle/curd chip junctions (J) depends on potential of microstructure to flow (deform under pressure and moulding) and conditions of moulding and pressing. The microstructure within curd particles or curd chips consists of a calcium phosphate *para*-casein network (P) which imbibes the serum phase (moisture and dissolved salts) within its pores and encases the fat (F) which occurs as globules in varying degrees of coalescence

hydrophobic cross-links to form a single macroscopic entity. The volume fraction of the network and degree of fusion (aggregation) of the *para*-casein particles forming the network increase as the levels of fat and moisture are reduced. Conversely, the volume fraction of the network decreases as the levels of the latter components, which may be considered as diluents, increase. This is clearly seen on comparing the structures of Cheddar cheese of varying fat content (Fig. 18.3). Hence, controlling the concentration and degree of fusion of *para*-casein through manipulation of the different manufacturing steps and their sequence, is critical in defining the properties of the final cheese, including rheological properties (e.g., ratio of viscous to elastic characteristics), physical properties (e.g., deformability, firmness, chewiness, ability to be slice, shred and grate) and opacity/translucence.

The macrostructure of cheese may be defined as an assembly of curd particles (in the case of brine-salted cheeses) or curd chips or pieces (in the case of dry-salted cheeses such as Cheddar and Stilton) that are pressed and fused together into a whole (moulded cheese) (Fig. 18.2). The knitting of individual curd particles or chips/pieces into a macrostructure is influenced by many factors which may be summarized as follows (Fig. 18.4):

- the microstructural-related properties of the particles or chips, which determine their potential to deform and flow into, and fuse with, other curd particles when subjected to moulding and pressing; critical factors include the degree of *para*-casein hydration (g water/g protein), level of calcium bound to the *para*-casein, protein-to-fat ratio of the matrix, and the salt content and pH of the moisture (solvent) phase which influence *para*-casein hydration.

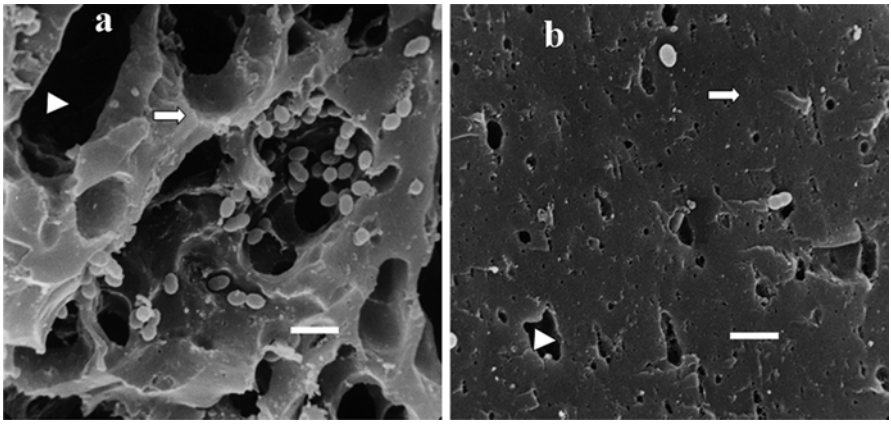


Fig. 18.3 Scanning electron micrographs of full (33 % fat, **a**) and low (3.0 %, **b**) fat Cheddar cheeses. The *arrows* correspond to the *para*-casein matrix and the *arrowheads* to the areas occupied by fat and free serum prior to their removal during sample preparation; bacteria (most likely starter lactococci) are visible in (**a**), being concentrated mainly at the fat-*para*-casein interface. Bar: 2 μm; 7000×. Modified from Fenelon et al. (1999)

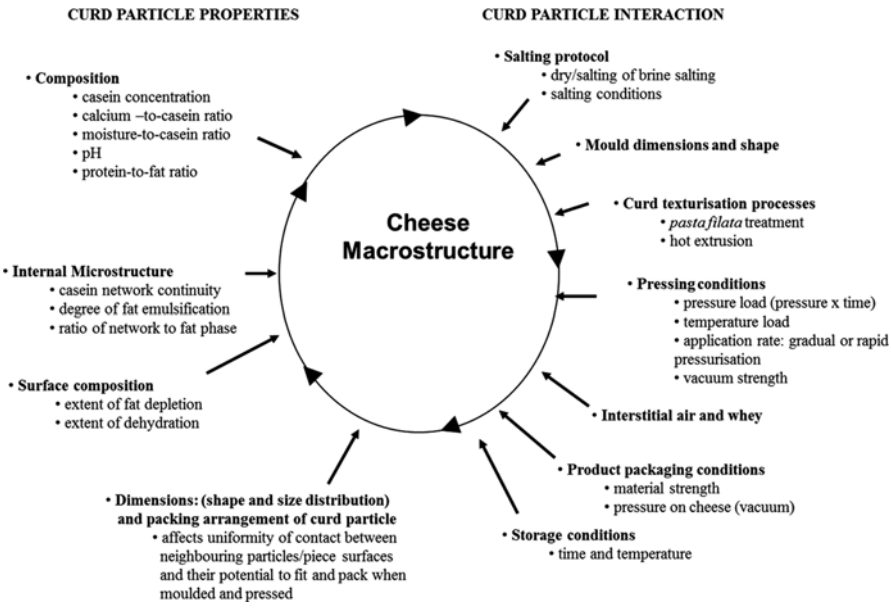


Fig. 18.4 Factors that affect the macrostructure of cheese: **curd particle properties** which determine the potential of curd particles to pack, flow and knit together, and **the interaction of curd particles** as affected by the processes to which curd particles are exposed. The effects of the various factors are interactive

- the curd particle surface properties such as composition (protein-to-fat ratio, salt content, moisture content); it is noteworthy that the surface layer of curd particles tend to have lower levels of moisture and fat than the interior, to a degree influenced by temperature of curd particle/whey mixture during scalding (cooking), temperature and duration of scalding and pH at whey separation.
- the size distribution of curd particles or pieces, which affects the packing arrangement, i.e., the neatness of fit, into a continuum.
- pressing conditions including temperature and pressure which affect factors such as the strength of hydrophobic interactions, the extent of protein solubilisation and fat crystallisation and the ability of curd particles to flow and knit into a seamless whole, without notable inter-particle microstructural discontinuities and junctions.
- the presence of interstitial air or air between curd particles.
- storage conditions (temperature, time) which affect the extent of age-related changes in pH, equilibrium between soluble and *para*-casein bound calcium, proteolysis and fat coalescence, and hence, the ability of curd particles to merge and lose their identity.

18.3.3 Structure-Function Relationships of Rennet-Curd Cheese

18.3.3.1 Unheated Cheese

As the concentration of *para*-casein (network) increases, its volume fraction increases and its structure becomes less particulate and more of a single continuum (rather than assembly of individual sub-structures). Simultaneously, the mobility of the system becomes increasingly restricted as the lubricating contribution of moisture diminishes, where mobility may be defined as the potential of contiguous planes of the cheese matrix to move or undergo displacement, when cheeses are subjected to a stress or strain. However, for a given casein concentration, the level of calcium, which may be envisaged as the binding 'glue' between the caseins, is a critical factor affecting the potential of the casein network to undergo displacement. Consequently, cheeses generally become more elastic, firm and chewy as the levels of protein (casein) and calcium increase. Simultaneously, the cheese becomes less opaque (less white) and more translucent as the volume fraction of the casein network increases, owing to the increasing degree of structural continuity and the reduction in the extent of structural interfaces (e.g., moisture/network interface) where light scattering occurs. However, the degree of opacity increases as the fat content increases, owing to the increase of light scattering interfaces between the fat globules/pools and the casein and the moisture phase.

18.3.3.2 Heated Cheese

Heating cheese to a temperature encountered during baking or grilling (90–98 °C), results in two major microstructural changes:

- contraction and shrinkage of the *para*-casein network (owing to a temperature-induced increase in the extent of hydrophobic interactions between the casein molecules) and the simultaneous expulsion of moisture;
- liquefaction and coalescence of fat globules, resulting in the formation of free fat which is readily observed as an oily layer at the surface of the melting cheese mass.

Consequently, the melting cheese mass becomes more fluid, as both the oil and moisture act as lubricants between adjoining layers of the cheese matrix. These heat-induced changes in microstructure form the basis of what are generally known as melt properties (Table 18.2), including softening of the melting cheese, flow and spread of the melting cheese mass under its own weight during grilling or baking, stretchability and stringiness of the molten cheese mass when extended, oil exudation and the formation of a free oil layer on surface of molten cheese mass.

18.3.4 Comparison of the Structure-Function Relationships in Different Rennet-Curd Cheese Varieties

18.3.4.1 Microstructural Effects

The effect of changes in microstructure, due to differences in the levels of protein and calcium, are readily apparent on comparing different cheese varieties. Hard cheeses (e.g., Cheddar, Mozzarella, Gouda) with high levels of casein and calcium are elastic, hard, chewy and moderately opaque in colour, have good shreddability and sliceability, and exhibit moderate and melt/flow on cooking. A very high casein level, as in low-fat hard Cheddar cheese (~1.3 % fat, 40 % protein, 52 % moisture), results in very low moisture-to-protein ratios (e.g., ~1.3 g/g). These cheeses tend to be very hard, rubbery, chewy and translucent. On cooking, they are prone to drying-out, which may result in blistering, 'puffing' and hardening of the surface and a low degree of flow; the melted cheese generally is not very fluid, is tough and chewy.

The adverse effects of a high concentration of protein on the properties of low-fat cheese may be attenuated by modifications of the cheesemaking process to reduce the levels of protein and calcium (hence calcium-to-casein ratio) and by increasing the levels of moisture and proteolysis during maturation. The latter has the effect of hydrolysing or 'chopping' the *para*-casein molecules, thereby making the insoluble *para*-casein network less rigid and more soluble. In contrast to the above, cheeses with a high moisture content and low levels of casein and calcium, such as mature Camembert and Blue-type cheeses, are relatively soft, adhesive, spreadable, easily mixed with other ingredients, and flow extensively on heating.

18.3.4.2 Macrostructural Effects

The effects of macrostructure on cheese functionality are probably most evident when comparing brine-salted and dry-salted hard cheeses. Brine-salted cheeses such as Gouda, Mozzarella and Emmental usually display excellent sliceability and shreddability, owing to the high degree of curd particle knitting into a seamless whole moulded cheese. The slices, which are usually retailed or used in food service as slice-on-slice (without the need of separating foils), are flexible, bendable and peel easily without breaking. In contrast, slices of dry-salted cheese such as Cheddar are generally more brittle and can break easily along curd chip junctions (Fig. 18.2, centre plate; see also Figs. 14.2 and 14.3, Chap. 14) which may be considered as 'fault lines', especially when chip-to-chip surface knitting is poor, for one or more reasons including a relatively high degree of dehydration of the *para*-casein network due to a salting-out effect at the high salt-in-moisture concentrations in the surface layer of the chip surfaces during dry salting (cf., Chap. 9), high concentrations of calcium and phosphate, low contents of moisture and fat or a low temperature during pressing. Nevertheless, the adverse effects of 'discontinuous' macrostructure of dry-salted cheeses on their sliceability and shreddability may be ameliorated by altering the microstructure and rheology of the curd chips which make them more amenable to flowing and knitting together (e.g., alteration of pH, calcium level, ratio of soluble-to-insoluble calcium) and modifying the conditions of salting. Another example, where the effect of macrostructure on functionality is evident is in Parmesan cheese. The manufacture of Parmigiano Reggiano is characterised by the use of partly skimmed milk (~2.5 % fat), cutting the gel into small curd particles, cooking (scalding) of curd to a high temperature (54–58 °C), whey drainage at pH > 6.0, moulding into large wheels and a long salting time (~14 days) in high concentration brines (e.g., ~26 % NaCl) (Davis 1976; Kosikowski and Mistry 1997; Gobbetti 2004). These conditions are conducive to:

- a very high degree of protein aggregation during manufacture, as promoted by temperature-induced hydrophobic interactions and the deposition of soluble calcium and phosphate as insoluble calcium phosphate;
- small, highly dehydrated curd particles that pack closely but do not kit very well when moulded and pressed;
- a curd with a relatively high protein-to-fat ratio and low-moisture content at salting.

The resultant cheese has a low-moisture content (<33 %, w/w), a very low moisture-to-protein ratio (~0.9, compared to ~1.7 in Gouda), a relatively high calcium-to-casein ratio and protein-to-fat ratio (~1.2–1.4, compared to 0.73–0.8 in Cheddar, and 0.9–1.0 in Emmental). Consequently, the cheese is very dry with a hard texture that fractures easily along its curd granule junctions (on application of a stress or strain) to yield to a 'mealy', granular/grainy texture on mastication as the poorly knitted curd particles (granules) come apart and are perceived as grains. Hence while the cheese is very hard, it is brittle and unsuited to slicing and shredding but ideally suited to grating for use as a sprinkling for dishes such as spaghetti Bolognese.

18.3.5 Formation of Acid-Curd Cheeses and Their Basic Microstructure

The manufacture of acid-curd cheese products such as Cream cheese, Quark and Labne(h) involves acid-induced gelation of milk, in response to the reduction of pH to the isoeletric point of the caseins (cf., Chap. 16). This results in the surface of the casein micelles becoming more hydrophobic and interacting to form a continuous gel network that encloses the fat globules and moisture. The gel is then dehydrated and concentrated to the desired dry matter of the final fresh cheese using various operations such as breaking/stirring the gel, heating, filtration of the broken curd through cheese cloth, ultrafiltration, and/or centrifugation (cf., Chap. 16). Fresh acid-curd cheeses generally have a high moisture content (~60–80 %) and low levels of protein (5–14 %) and calcium (<~0.18 %); the fat content varies from ~0.5 % in Quark to ~35 % in double Cream cheese and ~50 % in Mascarpone. The moisture-to-protein ratio is quite high compared to rennet-curd chesses, e.g., ~5.5 in double Cream cheese, 6.3 in Quark to ~8.5 in Labne(h).

While the structure of acid-curd cheeses is generically similar to that of rennet-curd cheese, in that it is essentially a polymer network of casein, it differs in the following respects:

- the polymer network is comprised of casein instead calcium phosphate *para*-casein;
- the degree of calcium bridging contributing to casein interconnectivity and casein network formation is significantly lower. Owing to the low pH (~4.5–4.8), all the colloidal calcium phosphate has been solubilised during gel formation and removed in the cheese whey during subsequent concentration. Only calcium attached directly to acidic amino acid residues (aspartate and glutamate) remains with the casein, and this decreases as the pH is reduced. Despite the overall low level, the quantity of calcium is, nevertheless, a critical determinant of texture (chewy or mushy) in the case of Cottage cheese, which may be described as a 'mainly acid-coagulated cheese' even though a small quantity of rennet is used in manufacture (Farkye 2004a).
- the volume fraction of the casein network is much lower and the degree of fusion between the casein particles (acidified casein micelles) making up the network is generally much lower because of the lower casein concentration and higher moisture content.

18.3.6 Structure-Function Relationships of Acid-Curd Cheese

Owing to the low volume fraction of the casein network and high moisture content, acid-curd cheeses generally have a soft, smooth consistency and mouthfeel. However, because of their high moisture-to-protein ratio, acid-curd cheeses tend to be susceptible to uncontrolled post-manufacture casein aggregation and dehydration *via*

mobility and re-arrangement of their casein network caused by external stresses on the product (for example during transport/distribution and retailing) or internal stresses induced by changes in temperature during storage. In extreme cases, this can lead to protein precipitation and extensive wheying-off. This tendency is more pronounced when the moisture content of the product is high, as this facilitates the sedimentation of protein aggregates.

Protein aggregation is the basis of major sensory defects including excessive wheying-off and the development of a sandy/grainy texture during storage, especially where the concentrated milk gel is heated and hot-filled post fermentation (e.g., as in Cream cheese) and where the product is required to have a long shelf-life (e.g., up to 6 month for hot-packed Cream cheese and some fresh cheese preparations). Avoidance of such defects requires optimisation of the degree of casein aggregation at the different stages of manufacture through the control of appropriate features such as milk composition (e.g., casein-to-whey protein ratio), heat treatment of the milk and level of whey protein denaturation, optimisation of product pH, treatment of gel post whey separation (temperature, homogenisation, cooling) and the use of a suitable stabiliser . Where permitted, suitable stabilisers, such as guar gum and locus bean gum, can increase the viscosity of the aqueous phase and, thereby, minimise casein aggregation by limiting mobility and re-arrangement of the gel structure. The options available depend on the product, which determines the steps involved in manufacture (including the heat load and degree of shear applied to the concentrated acidified milk gel), the sequence of steps, product composition and shelf-life.

18.4 Evaluation of the Rheological-Related Functional Properties of Cheese

18.4.1 Unheated Cheese

18.4.1.1 Sensory Tests

Several tests are available to assess the sensory properties of unheated cheese in a controlled environment (Delahunty and Drake 2004). These include:

- discrimination tests (e.g., triangle, paired comparison and ranking tests) to evaluate differences between samples in terms of overall quality or some specific attribute by trained panelists;
- descriptive sensory analysis to discern differences between samples based on quantitative scoring of a number of different attributes (e.g., harness, chewiness) according an agreed definition of attributes by a trained panel;
- Consumer acceptability testing by panelists who are untrained but who regularly eat cheese.

These tests are generally expensive to undertake because of the requirement for a dedicated sensory laboratory, training of a panel, or recruitment and payment of

consumer panels. Consequently, they are not used routinely in a factory environment for the measurement of functional properties of cheese.

In practice, the functional properties (e.g., shreddability, gratability, sliceability) are assessed by trained laboratory staff using empirical, in-house, sensory grading methods. Methods may include:

- visual assessment of shred (e.g., cleanliness of cut surface, length, balling, fines) or slices (smoothness, cleanliness, surface irregularities, gloss and drying-out or curling on standing) for appearance;
- manual pulling of slices to gauge elasticity, break force, cleanliness of break;
- squeezing of shredded cheese in the fist and observing the recovery, or lack of it, when released.

Nevertheless, instrumental tests may be also used to measure the functionality of the cheese. Such tests may be indirect such as large strain deformation (e.g., compression of a cheese sample, e.g., cube) testing on a texture analyzer, or direct (e.g., measuring shreddability by passing the shredded cheese through a vibrating stack of discs of varying aperture).

18.4.1.2 Large Strain Compression Tests

The rheological behaviour of cheese when subjected to large strain deformation (e.g., compression to>30–40 % of the original sample dimensions) and the magnitude of the outputs (e.g., fracture stress, σ_f; fracture strain, ε_f; firmness, σ_{max}; gumminess, adhesiveness) may be indicative of how a cheese may shred, slice, grate or spread (cf. Table 18.1 and Chap. 14, O'Callaghan and Guinee 2004). Consequently, it is used commercially for this purpose, especially as a tool to monitor changes in fracture properties if a cheese recipe is being redesigned .

Cheeses with low fractures stress (σ_f) and firmness (σ_{max}) are generally soft and adhesive and are not used in shredded/diced cheese applications, such as pizza pie, because of their tendency to ball and clump and to adhere to surfaces. Such cheeses include those with high moisture, a high degree of primary proteolysis, a low calcium-to-casein ratio, a low protein-to-fat ratio and include products such as mature Camembert, Chaumes, Havarti, Blue-type cheeses or Esrom. However, the adhesiveness and ability of these cheeses to flow under shear (i.e., spread) makes them ideal for blending with other materials such as butter, milk or flour in the preparation of fondues and sauces (Tables 18.1 and 18.2). Conversely, cheeses with a very high fracture stress and firmness and low facture strain (ε_f) tend to be hard and brittle and undergo elastic fracture on compression, i.e. fractures into distinct pieces with little tendency to adhere to one another (Chap. 14 and O'Callaghan and Guinee 2004). Such cheese, which include varieties such as Parmesan and Romano, generally display excellent gratability, e.g., when crushed between rollers, and are suitable for sprinkling onto dishes such as spaghetti Bolognese. However, these properties render the latter cheeses unsuitable in food applications that require sliced or shredded cheese, e.g., pizza, sandwiches or cheeseburgers. On the other

hand, varieties with moderate to low firmness and low fracture strain, such as Feta or medium-mature Blue-type cheeses, tend to be relatively soft and short (crumble under low displacement). These are particularly suited for easy inclusion into mixed salads because of their crumbliness, their breaking into irregularly shaped, curd-like particles that are visually appealing to the consumer as they convey an image of 'real' cheese. Cheeses such as Emmental-type, Gouda and Maasdammer are ideal for slicing very thinly and are, therefore, particularly suited to applications such as continental breakfast cheese and filled cheese slices. This is because of their high degree of elasticity, springiness (high recovery from deformation following removal of deforming force) and "long" body (high fracture strain). Similarly, the springiness of low-moisture Mozzarella cheese (LMMC) endows it with good shreddability (low tendency to fracture or form curd fines) and non-stick properties that facilitate uniform distribution on the surface of pizza pies (Tables 18.1 and 18.2).

18.4.1.3 Empirical Instrumental Tests

In addition to large strain deformation analysis, empirical tests are sometimes used. The suitability of cheese for shredding may be assessed directly by determining the tendency of the shredded cheese to aggregate or clump when vibrated under controlled conditions similar to those used on commercial pizza production lines. Cheese, after storing at 4 °C for ≥12 h, is cut into cubes of fixed dimensions (e.g., 2.5 cm) and a fixed weight (W1) of shredded cheese is placed immediately on the top sieve of a stack of sieves ranging in aperture from 9.5 to 1 mm (Guinee et al. 2000a). The stack is vibrated at fixed amplitude for a given time, resulting in the cheese shreds passing through the stack to a degree dependent on their susceptibility to stick/clump or fracture. The cheese on each sieve is then weighed (W) and an aggregation index (AGI) is calculated:

$$\mathrm{AGI} = 100 \times \left[\Sigma \left(\mathrm{W} \times \mathrm{SA} \right) \right] / \mathrm{W1}$$

where SA is the sieve aperture. A higher AGI value corresponds to a higher susceptibility to aggregation and clumping. Similar approaches are also used commercially, e.g., hand vibrating cheese in a colander and determining the quantity retained after a fixed time.

18.4.2 Heated Cheese

18.4.2.1 Sensory Tests

Similar to unheated cheese, the cooked cheese is routinely assessed using in-house, sensory tests. Typically, a defined weight of shredded cheese sample is distributed uniformly on a surface (e.g., heat-resistant glass plate, tin foil of given dimensions

or pizza base) and placed in an oven under defined conditions (type of oven, temperature and heating time). The cheese is withdrawn and inspected visually for attributes such as uniformity, shred identity, degree of shred coalescence, degree of spread or flow, level of oiling-off, surface appearance (colour, moistness, oiliness, puffing, glossiness, scorched/black spots, blister coverage). The cheese may be assessed orally for parameters such as succulence, oiliness, dryness, moistness, mouth-coating, chewiness. Additionally, it may be checked for stringiness by inserting a fork and noting the appearance and length of the sheet or strings formed, and how readily they break. Depending on the application, several attributes are scored and noted.

18.4.2.2 Instrumental Tests: Empirical

Flowability

The degree of flowability on heating is frequently assessed by placing a sample of cheese (e.g., disc, cylinder) of fixed dimensions (e.g., disc, 45 mm diameter, 4.0 mm high) on a surface (e.g., heat-resistant glass plate, glass petri dish, glass cylinder with bung) and putting it in a controlled temperature environment (e.g., oven, water bath) under defined conditions of temperature and time. The sample is then withdrawn, allowed to cool and the change in a particular sample dimension (e.g., height of vertical cylinder, length of horizontal cylinder, diameter of disc) is measured. The flowability is the change in dimension, expressed as a percentage of the dimension in the unheated sample. Various tests have been described in the literature to measure flowability, also frequently known as the meltability, e.g. Arnott test, Schreiber test, Price-Olson test (Park et al. 1984).

Melt Time

Shredded cheese is distributed uniformly on a polished surface (e.g., tin foil, steel), marked out in rectangular grids (e.g., 9×9 cm), typically at a rate of 1.73 kg cheese/m^2 and placed in an electric fan oven (e.g., at 280 °C) for a time that is sufficient for melting (e.g., ~3 min for Mozzarella) (Guinee et al. 2000a). The sample is then allowed to cool to room temperature and inspected for the coalescence of shreds into a homogeneous molten mass or the presence of individual shreds. The sample is then scored (e.g. on a scale of 1–5). A low score (e.g., 1) is assigned to samples showing individual shreds, especially where the shreds are hard, dried-out and scorched (brown); a high score (e.g., 5) is awarded when no traces of shred identity are evident and where the cheese mass is uniform and molten; and an intermediate scores assigned where some traces of shred identity remain and where the individual shreds are swollen and moist. In applications where a very flowable and fluid cheese is required (e.g., pasta dishes), a high score is desirable, whereas a low score is more suitable for controlled melt applications (e.g., for cheese slices in burgers).

A variation of the above test involves measurement of the time required for all the shreds to disappear completely to form a uniform molten mass, as assessed by viewing the melting samples through the glass door of an interior-lit oven. Obviously, this is suitable only for cheeses that melt within a reasonable time (e.g., 180–240 s at 280 °C at a cheese loading of 1.73 kg cheese/m^2.

Stretchability

Stretchability relates to the ability of the cooked cheese to form strings when extended, the dimensions of the strings (e.g., length, thickness), and/or the energy or work required to extend a given distance. The most basic method of assessment involves lifting the cooked cheese (on a pizza base or other surface) with a fork and sensorially gauging/scoring the string profile (thickness, number), length, smoothness and sheen (Fig. 18.5a).

More quantitative methods typically involve uniformly distributing a defined weight of shredded cheese on a surface, melting and instrumentally extending the molten cheese mass uniaxially under defined conditions of speed and measuring various aspects of the stretched cheese, including the distance at strand/string breakage, force required to extend a fixed distance and/or dynamic image analysis of forming strings (Apostolopoulos 1994; Pagliarini and Beatrice 1994; Guinee and O'Callaghan 1997). In the latter study, a defined weight of shredded cheese was loaded onto a pizza base (or other surface), pre-cut in halves and baked under defined conditions. The baked pizza is then loaded onto the cell of a stretching unit, comprising fixed and mobile elements. One half of the baked pizza is clamped to the fixed element, while the other half sits astride the mobile element. The mobile element is drawn at a fixed speed along a rail system stretching the molten cheese mass into strings or sheets until complete failure of the extended strings/sheets (Fig. 18.5b). Coupled with an overhead mounted mobile camera, both the dimensions of the strings and the length at breakage are measured (Guinee and O'Callaghan 1997).

A more recent development involves dynamic measurement of the force required to extend the molten cheese a fixed distance using a cheese extensibility rig on a texture analyzer, e.g., from Stable Microsystems (Fig. 18.5c). Shredded cheese is placed in a microwaveable plastic container (box) fitted with a comb (fork) that sits snugly inside it and has an adapter for rapid attachment to the load cell of the texture analyzer (Fig. 18.6). The cheese is loaded on top of the comb and the container/comb assembly is placed in a microwave oven under defined conditions and heated to 90–98 °C. The assembly is then withdrawn from the oven, the comb is attached to the cross-head of the texture analyzer and the molten cheese is extended a fixed distance (e.g., 38 cm) at a constant velocity (1 cm/s). The work required for extension is determined from the area of the resultant force/distance curve (Fig. 18.6).

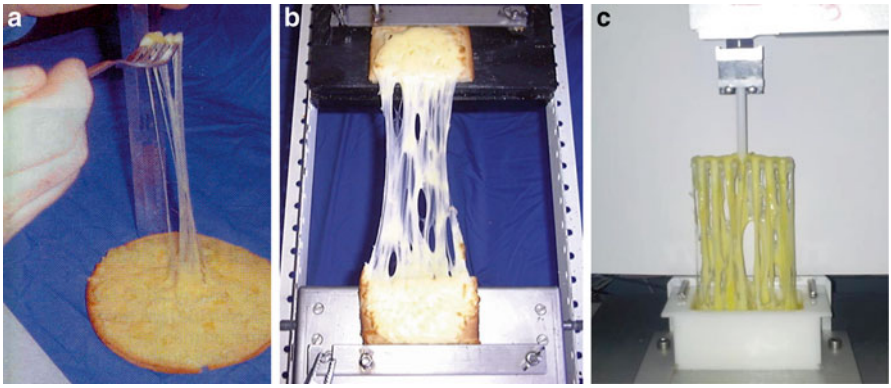


Fig. 18.5 Measurement of stretchability of molten cheese using different methods: (**a**) manual extension, (**b**) horizontal extension at a fixed velocity of 0.066 m/s along a twin rail (Guinee and O'Callaghan 1997; Guinee et al. 1999) and (**c**) vertical uniaxial extension to a distance of 0.38 m at a velocity of 1 cm/s on a TAHDi Texture Profile analyzer (Stable Micro Systems, Godalming, UK) with simultaneous measurement of force

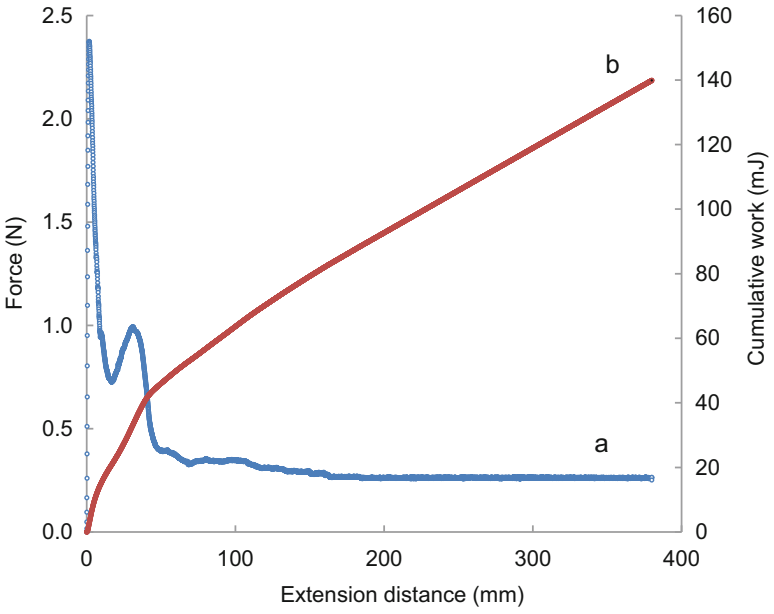


Fig. 18.6 Force (**a,** *blue line*) and cumulative work/energy (**b,** *red line*) required to extend 180-day-old Cheddar cheese at a velocity of 1 cm/s on a TAHDi Texture Profile analyzer (cf. Fig. 18.5c). McCarthy and Guinee (unpublished results)

Oiling-Off

Moderate release of free oil from cheese during heating is desirable in most cooking applications. Free oil forms a protective 'apolar' layer on the surface of the melting cheese mass, reducing the extent of dehydration and minimizing the risk of associated defects, i.e., crusting, puffing and extensive blistering. Conversely, excessive release of free oil is undesirable, being unaesthetic and creating the impression of excessive fat content. Several approaches have been used to estimate free oil release, some more empirical than others.

An early method involved placing a cheese disc of specified dimensions on a circular filter paper, heating under defined conditions of time and temperature and cooling. Free oil released on heating was absorbed by the filter paper forming a ring, the diameter of which was indicative of the extent of oiling-off. More recent methods involve placing a defined weight of shredded cheese in glass centrifuge tubes/containers, heating in a boiling water bath, adding distilled water and centrifuging to recover the free oil. The addition of a water:methanol mix to the supernatant followed by recentrifugation allows the formation of defined fat layer and enables accurate quantitative determination (Kindstedt and Rippe 1990; Kindstedt and Fox 1991).

18.5 Effects of Different Factors on the Functionality of Unheated Cheese

18.5.1 Rheological-Based Functional Characteristics

The functionality of unheated cheese is to a large extent determined by its flavour and rheological properties (e.g., fracture stress, fracture strain, firmness/hardness), which are discussed in detail in Chaps. 13 and 14. The key factors influencing the rheological characteristics are:

- composition (moisture, fat, protein, calcium) (cf. Chap. 14)
- pH (cf. Chap. 14)
- degree of proteolysis (cf. Chap. 14)
- cheese variety and structure (Sect. 18.3).

18.5.2 Cheese Shreddability

Good shreddability is characterized by free flow of the shredded cheese and a low tendency of the cheese shreds to stick together to form balls or clumps. It is essential to minimize 'irreversible' sticking and clumping in situations where the shredded cheese may be, in effect, compressed temporally, for example when holding

in storage bins prior to being fed to cheese depositor/applicator units (e.g., pizza production lines) or when retail or catering packs of shredded cheese are stacked during distribution. While some clumping may occur in the latter situations, it is important that clumps are easily broken up by vibration/fluidization or, simply, by shaking (retail packs). Poor shreddability leads to clumping of the cheese shreds, which leads to blocking of cheese dispensing units on pizza pie production lines, poor distribution of cheese on pizza pies and matting of shredded cheese when placed in retail packs.

18.5.2.1 Shreddability of Retail Cheeses

The shreddability of a range of commercial cheeses, as measured using the aggregation index (AGI), indicate that variety has a marked influence (Fig. 18.7). Such a trend is expected owing to inter-varietal differences in elasticity as affected by moisture content, casein content, degree of proteolysis, pH, calcium-to-casein ratio and protein-to-fat ratio. Hence, cheese such as Emmental and Gruyere with relatively low moisture, high protein, low proteolysis and a high calcium-to-casein ratio tend to have a better shreddability (lower AGI) than cheeses such as Tetilla and Fontina which have relatively high moisture, high degree of proteolysis, low calcium and low protein-to-fat ratio.

18.5.2.2 Changes in Shreddability of Mozzarella During Storage

Studies on the shreddability of Mozzarella show that it changes during storage at a low temperature (4–8 °C) and that there is a time window where it is optimal (Kindstedt 1995). Young (1–5 days old) Mozzarella cheese tends to have poor shred, as reflected by the tendency of the cheese shreds to ball/or clump, especially when the shredded cheese is under pressure, as occurs in practice where shredded

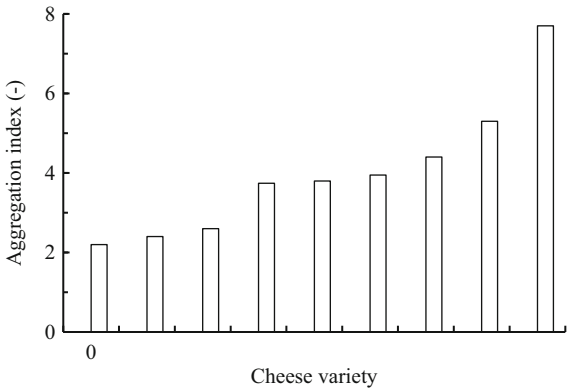


Fig. 18.7 Susceptibility of shredded cheese to clumping (as measured by aggregation index) in different cheese types: Gruyère (A), Emmental (B), Appenzeller (C), substitute Pizza cheese (D), Kackaval (E), low-moisture Mozzarella (F), Cheddar (G), Tetilla (H), Fontina (I). From Guinee et al. (unpublished results)

cheese is stored temporally in bins (e.g., up to 100 kg) prior to use in pizza pie manufacture. Thereafter, shreddability improves, being optimal after ~3weeks storage at 4 °C, but deteriorates progressively on further storage and the cheese becomes soft and sticky. These changes are associated with corresponding changes in moisture distribution and water-binding capacity of the cheese. In fresh curd, free moisture retained in the cheese after plasticization has not yet been absorbed. On ageing, free water is absorbed as the water-binging capacity of the *para*-casein increases due to calcium solubilisation and proteolysis. However, on prolonged ripening excessive proteolysis leads to excessive solubilization of the *para*-casein, a loss of matrix elasticity and an increase in stickiness.

18.5.2.3 Other Factors That Affect Shreddability

Apart from composition, other factors which are conducive to clumping of shredded cheese include:

- longer shred length and shred diameter which increase the chance of shred entanglement,
- free fat content

Most rennet-curd cheeses contain non-globular fat or free fat, formed as a result of disruption of the native milk fat globule membrane due to shear forces applied during cheesemaking operations, e.g., cutting, stirring, pressing, casein network shrinkage and concentration. The presence of free fat is confirmed by microscopy which shows the presence of non-spherical clumps (e.g., in cheese such as Cheddar) or pools (e.g., in Mozzarella) of fat (see Fig. 18.3). The effect of non-globular fat on shreddability depends on the ratio of solid-to-liquid fat, which decreases as the temperature is raised. At temperatures where milk fat is largely in the liquid state (60 % of total fat at 20 °C), free fat exudes to the surface of the cheese shreds where it acts as an adhesive for other shreds. This defect is compounded by temperature fluctuations during storage, e.g., cooling after holding at ambient temperature (~ 20 °C) leads to solidification of exuded fat which makes the breaking-up of clumps of cheese shreds more difficult as the solid fat forms a rigid bridge between neighbouring shreds. Hence, in practice, cheese is usually maintained at low temperature prior to, and after, shredding, e.g., at <2 °C before use in pizza manufacture and <8 °C in retail outlets.

18.6 Effects of Different Factors on the Functionality of Heated Cheese

The behaviour of cheese on cooking is important in most applications, e.g., grilled cheese sandwiches, pizza pie, cheeseburgers, pasta dishes and sauces. The functionalities or attributes required depend on the cooking application (Table 18.2). Hence, heated cheese for pizza pie is generally required to exhibit high stringiness,

moderate chewiness, flow and oiling-off and mild to medium flavour (much of the flavour being supplied by the tomato sauce and toppings other than cheese). Conversely, such attributes are undesirable in pasta dishes which require a high melt and flow, soft mouthfeel and medium to strong flavor. The functionality of heated cheese, frequently referred to as cooking properties, is influenced by many factors.

18.6.1 Effect of Cheese Type

A comparison of the cooking properties of different cheeses indicates inter- and intra-varietal differences in melt time, flowability, stretchability and apparent viscosity (Table 18.3). The inter-varietal differences in functional properties reflect differences in conditions of manufacture (e.g., pH at whey drainage, texturisation), composition (e.g., pH, calcium-to-casein ratio, protein content, moisture-to-protein ratio, protein-to-fat ratio), degree of maturity (e.g., pH 4.6-soluble N) (Table 18.4). These, in turn, influence the volume fraction of the *para*-casein network, its structure, water-binding capacity and rheological response when subjected to heating (cf. Sects. 18.3.1–18.3.4).

The *pasta filata* cheeses were differentiated from all other varieties by their superior stretchability, relatively high apparent viscosity, moderate flowability and melt

Table 18.3 Effect of variety on the functional attributes of heated cheese

Cheese type	Sample size	Melt time (s)	Flowability (%)	Stretchability (cm)	Apparent viscosity (Pa s)
Pasta filata type					
Low-moisture Mozzarella	8	108 (6)	53 (8)	83 (21)	623 (303)
Kashkaval	2	96 (11)	67 (4)	87 (13)	522 (330)
Provolone dolce	3	86 (6)	64 (21)	80 (13)	950 (–)
Provala fumica	1	99	67	77	–
Provala	1	92	71	76	–
Cheese with eyes					
Gruyère	1	105	78	67	391
Jarlsberg	1	82	52	35	371
Emmental	1	81	74	35	269
Cheddar	8	100 (7)	69 (9)	23 (10)	349 (129)
Analogue pizza cheese	8	105 (13)	42 (19)	27 (8)	668 (307)

Compiled from Guinee et al. (2000a) and Guinee (unpublished results)
Note: Where the sample size ≥ 2, mean values are presented; values in parentheses are standard deviations
Melt time measured at 280 °C; flowability using the Schreiber test at 240 °C for 4 min; stretchability using uniaxial extension following heating at 280 °C or 4 min and viscosity using heliopath viscometry (Guinee and O'Callaghan 1997)

Table 18.4 Compositional analyses of different commercial cheese varieties

Cheese type	Source	Sample size	Moisture (%)	Protein (%)	Fat (%)	FDM (%)	MNFS (%)	S/M (%)	Ash (%)	Ca (mg/g cheese protein)	P (mg/g cheese protein)	pH4.6 SN (% of total N)	PTAN (% of total N)	pH
Pasta filata type														
Low-moisture Mozzarella	Ireland	8	46.4	26.0	23.2	44.6	60.4	3.1	3.8	27.2	20.6	4.7	0.5	5.53
	UK													
	Denmark													
Kashkaval	Yugoslavia	2	44.1 (0.7)	25.3 (0.3)	25.6 (1.2)	45.8 (1.7)	59.6 (0.4)	4.9 (0.2)	4.3 (0.0)	31.0 (5)	22.2 (18)	6.1 (3.9)	0.7 (0.2)	5.37 (0.02)
Provolone dolce	Italy	2	38.6 (3.2)	276 (5)	29.4 (37)	47.7 (3.6)	54.6 (1.7)	5.7 (0.7)	–	–	–	10.4 (9.8)	–	5.54 (0.14)
Provola fumica	Italy	1	43.4	278	24.3	428	57.3	4.7	–	–	–	7.5	–	5.29
Provola	Italy	1	40.2	281	28.5	476	56.2	4.4	–	–	–	10.8	–	5.29
Cheese with eyes														
Gruyère	Switzerland	1	34.1	27.7	36.8	558	54.0	4.9	–	–	–	10.8	–	5.83
Jarlsberg	Norway	1	40.4	27.7	31.3	524	58.7	2.8	–	–	–	8.6	–	5.72
Emmental	Switzerland	1	34.3	24.9	38.0	578	55.3	1.3	–	–	–	9.2	–	5.64
Cheddar	Ireland	8	37.2 (1.1)	25.4 (0.8)	33.1 (2.0)	52.6 (2.4)	55.6 (1.0)	4.5 (0.7)	3.7 (0.3)	28.0 (1.4)	20.6 (1.3)	20.3 (3.7)	4.6 (2.5)	5.14 (0.12)
Analogue pizza cheese	Ireland	8	48.9 (3.7)	18.4 (1.9)	25.0 (1.9)	49.0 (3.4)	65.1 (4.4)	3.5 (0.4)	4.2 (0.3)	34.4 (2.1)	281 (44)	2.3 (0.8)	0.2 (0.1)	6.3 (0.1)

Source: Guinee (unpublished data)

Note: Where sample size > 1, means values are presented; values in parenthesis are standard deviations

Key to abbreviations: *FDM* fat-in-dry matter, *MNFS* moisture-in-non-fat substance, *S/M* salt-in-moisture, *pH4.6 SN* nitrogen soluble at pH 4.6, *PTAN* nitrogen soluble in 5 % phosphotungstic acid

Units for Ca (mg/g cheese protein); P (mg/g cheese protein); pH 4.6 SN (g/100 g total N); PTAN (g/100 g total N)

time. These attributes make them ideally suited for pizza pie, i.e., sufficiently rapid melt and desirable levels of stringiness, chewiness and flow. Some *pasta filata* cheeses, e.g., Provolone dolce and string cheese have a very high viscosity, which undoubtedly is associated with over-chewiness on pizza pie. While the functional requirements of the pizza market varies according to brand, Mozzarella cheese with the following characteristics is generally acceptable: melt time, <120 s; flowability, ≈40–55 %; stretchability, >75 cm; apparent viscosity, ≈800–400 Pa.s; AGI ≈ 3.5–4.5 (Table 18.3, Figs. 18.7 and 18.8). While the degree of browning required on pizza pie appears to depend very strongly on the pizzeria, generally a low degree is desirable.

The superior stringiness of *pasta filata* cheeses, compared to other cheese varieties, may be attributed primarily to plasticization of the curd during the kneading/stretching process. In this process, the milled curd (pH ~5.2) is heated to ~57–60 °C and kneaded, in hot water or dilute brine (e.g., 4 % NaCl) at ~78–82 °C. The combined effect of high temperature, low pH and kneading are conducive to the formation of *para*-casein fibres of high tensile strength. These fibres are orientated linearly and separated by pools of fat (columns) with the same orientation (McMahon and Oberg 2011). Confocal laser scanning micrographs of the curd before and after texturization clearly demonstrate the formation and linearization of protein fibres (Fig. 18.9). Conversely, stringiness, which is typical for LMMC and other *pasta filata* cheeses, such as Kashkaval and Provolone, is an undesirable attribute for applications such as sauces, pasta dishes, gratins, fondues or toasted sandwiches. Cheeses such as mature Cheddar, Emmental, Raclette and Gouda are much more satisfactory because of their excellent flowability and flavour and the absence of stringiness on baking or grilling.

In some applications, melt (softening of the melted cheese mass) is essential but very limited, or no, flow is required so as to preserve the shape and identity of the cheese. Commercially, the absence of heat-induced flow is frequently referred to as flow resistance and cheeses designed with flow resistance as 'controlled flow' cheeses. Examples of applications where flow resistance is required include fried Paneer, fried Halloumi, grilled or fried burgers containing cheese insets, deep-fried breaded cheese sticks and some baked cheese applications. In such applications, most mature natural cheeses are unsuitable owing to excessive flow and oiling-off and disintegration of the cheese during cooking. In the case of cheese insets in deep-fried burgers, such attributes would result in the cheese permeating the interstices of the coarse meat emulsion and losing its shape and visual effect in the cooked product. Nevertheless, flow resistance may be engineered into rennet-curd natural cheeses by a number of processes

- high heat treatment of the milk;
- addition of whey proteins to the milk followed by high heat treatment;
- homogenization of the cheese milk, e.g., at a pressure ≥15 MPa.

Similarly, high heat treatment of the cheese milk (e.g., ≥85 °C for 5 min) at a low pH in the manufacture of acid-heated coagulated varieties (Farkye 2004b) gives cheeses such as Paneer and Quesco Blanco, which are flow resistant. In the case of Cream cheeses, high heat treatment of milk and/or the curd (e.g., >85 °C for 5–15

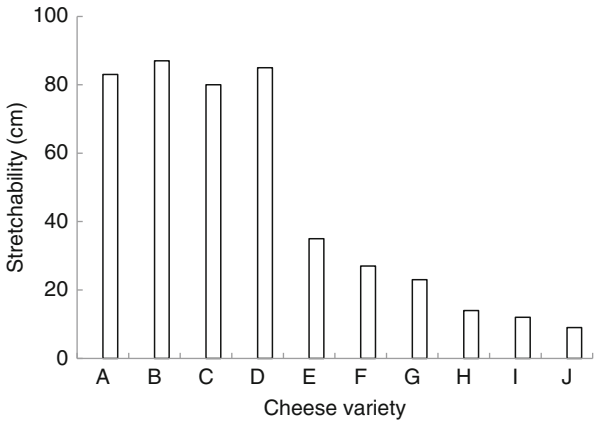


Fig. 18.8 Stretchability of different cheese types after baking at 280 °C for 4 min: low-moisture Mozzarella (A), Kachkaval (B), Provolone (C), String cheese (D), Emmental (E), analogue Pizza cheese (F), Cheddar (G), Parmesan (H), Raclette (I) and Appenzeller (J). From Guinee et al. (unpublished results)

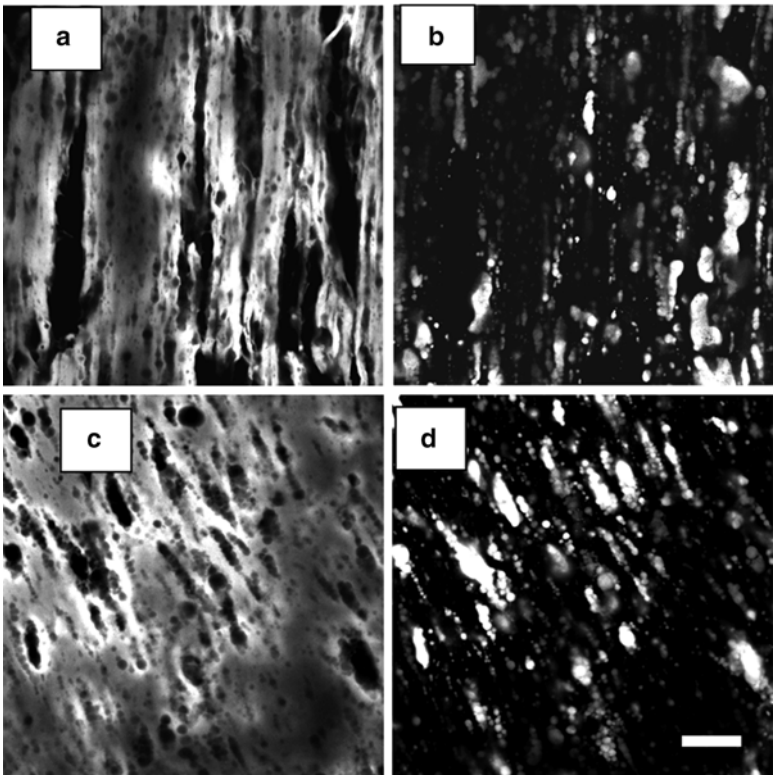


Fig. 18.9 Confocal laser scanning micrographs showing protein (**a**, **b**) and fat (**c**, **d**) as *light grey* areas against a *dark background* in unheated Mozzarella cheeses after storage for 1 (**a**, **b**) or 20 (**c**, **d**) days at 4 °C. The 1-day-old sample shows extensive linearization of *para*-casein into fibres (**a**) and of fat into pools (**b**). The 20-day-old sample shows that the *para*-casein fibres have swollen and expanded as a consequence of hydration (**c**), forcing the occluded fat into smaller pools. Bar = 25 μm. Modified from Guinee et al. (2000b)

min) and high pressure homogenization of the milk and/or curd result in flow-resistant products. In the latter products, high heat treatment of milk results in a high level of whey protein denaturation (e.g., ~60 % of total whey proteins) and their complexation with casein. On subsequent baking or grilling of the cheese, the included whey proteins appear to undergo gelation and thereby impede the flow of the heated cheese mass.

Controlled-flow processed cheese products (PCPs) can be easily designed through control of formulation (e.g., inclusion of whey protein at a level ≥ 2 %, addition of a high level of sodium ortho- or polyphosphates, use of natural cheese with a high content of intact casein and a high calcium-to-casein ratio) and processing conditions (e.g., increasing processing shear and time) (Guinee 2009, 2011; Chap. 17). Such approaches provide a convenient means of converting natural cheeses into PCPs with varying degrees of flow resistance. A major problem with natural rennet-curd cheese varieties is that the functionality of the heated and unheated cheese tends to be unstable, changing as protein hydrolysis and hydration continue to increase during maturation. There is a time window in which the physico-chemical (e.g., casein hydrolysis and hydration) and functional attributes are optimal for specific applications; prolonging ripening outside this window alters the magnitude of the different attributes (e.g., stretchability, flow, viscosity) and results in deterioration of the overall functional acceptability of the cheese.

18.6.2 Effect of Protein Concentration and Proteolysis

It is difficult to determine the direct effects of altering the concentration of any one compositional component, including protein, on the functionality of melted cheese since the levels of the different components tend to vary simultaneously, e.g., fat reduction is accompanied by increases in the levels of protein and moisture and decreases in the levels of moisture-in-non-fat substances and fat-in-dry matter. Nevertheless, results on the functionality of reduced-fat cheese provide insights on the effects of protein. Increasing the protein content of cheese (e.g., from 26 % to 40 % in Cheddar-type cheese while reducing fat from ~32 % to 5 %) generally results in significant increases in apparent viscosity and melt time and reductions in flowability and stretchability of the heated cheese (Fig. 18.10). Based on analysis of cheese microstructure and structure-function relationships (Sects. 18.3.2–18.3.3), these changes are due to:

- increases in the concentration and volume fraction of the *para*-casein network (Fig. 18.9) (cf. Sects. 18.4.2–18.4.4)
- reduction in the moisture-to-protein ratio (from ~1.5 to 1.2)
- reduction in fat content
- reduction in the lubricating effects of moisture and fat.

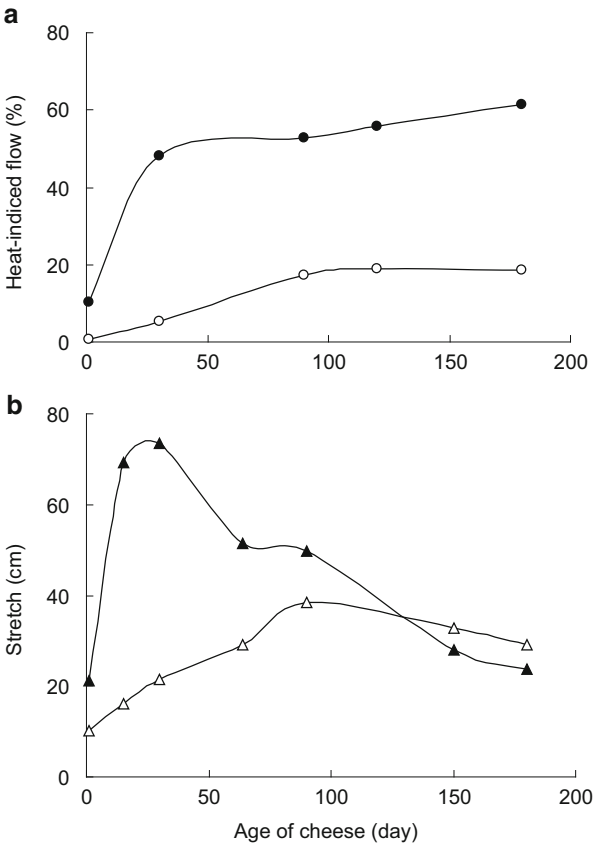


Fig. 18.10 Effect of fat and protein content on age-related changes in the flowability (**a**) and stretchability (**b**) of full-fat (*filled circle*, *filled triangle*; 26 % protein, 31 % fat) and reduced-fat (*open circle*, *open triangle*; 39 % protein, 7 % fat) Cheddar cheese, baked at 280 °C for 4 min. Modified from Guinee et al. (2000b)

For a given protein content, increasing the level of protein hydrolysis results in higher flowability, lower apparent viscosity and a reduction in stretchability. This is readily apparent when observing the changes in the latter attributes over the course of maturation in any cheese. The effect of proteolysis and maturation may be envisaged as a reduction in the concentration of intact *para*-casein molecules constituting the protein network (cf., Sects. 18.4.2–18.4.4). Proteolysis during maturation results in hydrolysis and solubilization of the *para*-casein, as indicated by the increases in the level of pH 4.6-soluble protein which does not contribute to the structure and integrity of the *para*-casein network.

18.6.3 Effect of Ripening Time

Studies on the cooking properties of various cheeses (Cheddar, Mozzarella and Swiss) have shown that functionality is dynamic, with the various functional attributes undergoing age-related changes to a degree depending on the composition and cheese variety(Figs. 18.8, 18.9, and 18.10).

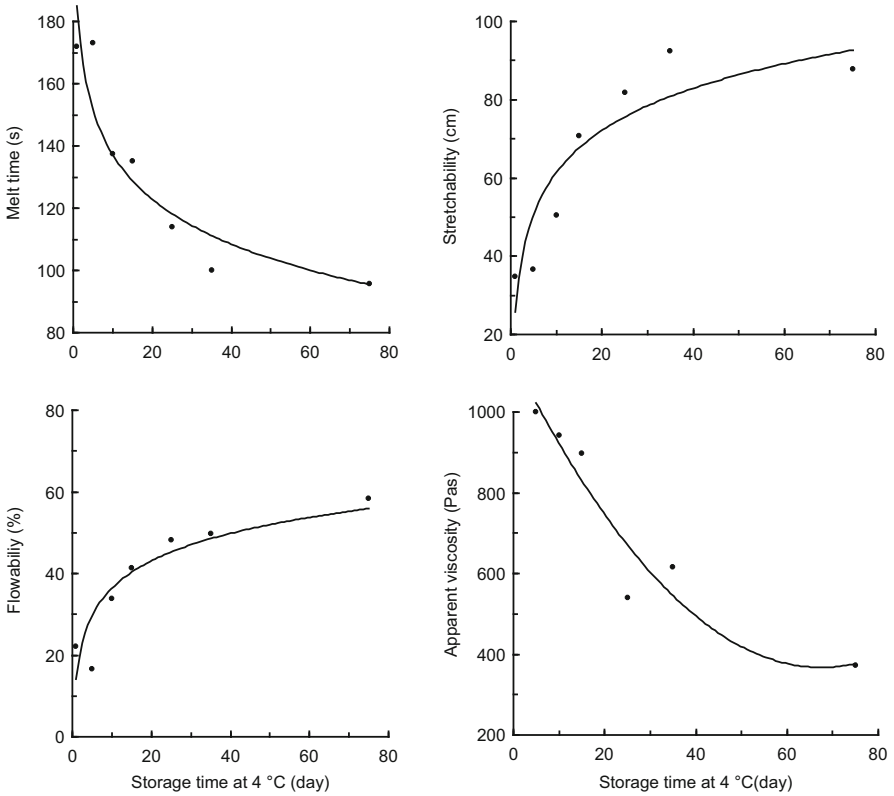


Fig. 18.11 Typical changes in functional attributes of low-moisture Mozzarella cheese during storage at 4 °C: melt time, flowability and stretchability after heating at 280 °C and apparent viscosity at 70 °C. From Guinee et al. (1997)

Low-moisture Mozzarella cheese (LMMC) is the most intensively studied, because of its very large production (~1.7 million tonnes in 2013) and it being the principal cheese used in pizza pie. The functionality of LMMC improves markedly during the first 2 weeks of storage at 4 °C, as reflected by decreases in melt time and apparent viscosity and increases in flowability and stretchability; this status is maintained until ~40–50 days (Fig. 18.11). The improved functionality is due to increases in protein hydrolysis, ratio of soluble to casein-bound calcium and *para*-casein hydration (Fig. 18.12) (Kindstedt and Guo 1997; Guinee et al. 2000b; McMahon and Oberg 2011). The vapour pressure of water bound by the *para*-casein is lower than that of free water and thus has a lower propensity to evaporate during baking. The exudation of free oil from the shredded cheese during baking also limits dehydration; the free oil forms an apolar surface layer, which impedes the escape of water vapour. The changes in protein hydration appear to be the result of a number of factors, including:

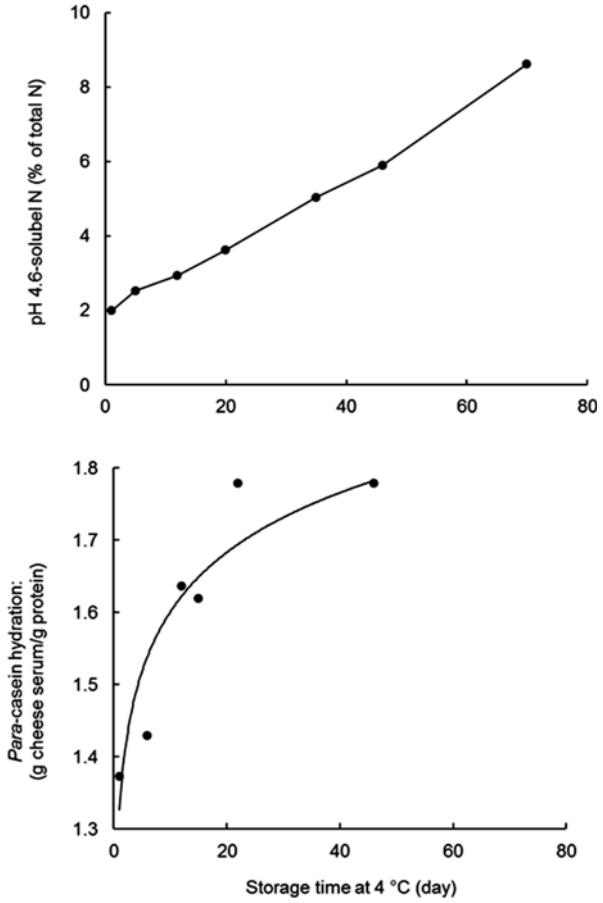


Fig. 18.12 Typical changes of low-moisture Mozzarella cheese during storage at 4 °C: primary proteolysis, as measured by pH 4.6-soluble nitrogen, and *para*-casein hydration. Modified from Guinee et al. (2002)

- a small increase in pH (from ~5.15 to ~5.35–5.40 at 5 days), as a result of solubilisation of calcium phosphate to replenish calcium removed from the aqueous phase of the curd during plasticization in hot water.
- an increase in primary proteolysis of *para*-casein by residual rennet and/or plasmin, and the ensuing solubilisation of some of the *para*-casein

On prolonged storage, up to 75 days, the unbaked cheese generally becomes too soft and sticky while the baked cheese becomes excessively flowable and 'soupy' and lacks the desired chewiness, which is reflected by the relatively low apparent viscosity. These changes in functionality are attributed to excessive proteolysis. However, stretchability remains relatively constant even when the product is stored for up to 4 months at 4 °C, suggesting that the level of primary proteolysis in the

cheese at this time (i.e., pH 4.6-soluble N ~ 12 % total N) is insufficient to significantly impair stretchability. Indeed, experiments show that young Cheddar cheese (i.e., 15–35 days; with a pH 4.6-soluble N level of <12 % total N) has good stretchability, similar to that of LMMC. In contrast, Cheddar with a pH 4.6-soluble N level >15 % of total N has inferior stretchability compared to LMMC (Guinee 2003).

Maillard browning on pizza pie results from heat-induced reactions between the carbonyl group of reducing sugars (lactose and galactose) and the amino groups of peptides and amino acids. The degree of browning is related to the sugar-fermenting and proteolytic characteristics of the starter culture used (Kindstedt 1993; McMahon and Oberg 2011). Most strains of *Streptococcus thermophilus* and *Lacobacillus delbrueckii* ssp. *bulgaricus*, which are commonly used in the manufacture of LMMC, are unable to metabolize galactose. Hence, cheese made solely with these cultures is susceptible to browning. Attempts to control the level of browning in pizza cheese include control of residual sugars and/or proteolysis products via:

- adjusting the ratio of *Lb. helveticus* to *Sc. thermophilus* in the starter culture,
- the use of galactose-positive strains of *Lb. delbrueckii* ssp. *bulgaricus,*
- the use of proteinase-negative starter strains to limit the formation of free amino groups,
- the use of curd washing to remove lactose from the cheese curd.

The propensity of LMMC to brown on baking changes markedly during ripening. Fresh cheese curd (<2–5 days) shows a high propensity to browning; this decreases markedly during the first few weeks of ripening due to the metabolism of lactose and/or galactose by galactose-fermenting starters but increases progressively thereafter owing to the accumulation of small peptides and amino acids.

18.6.4 Cheese Composition

For rennet-curd cheeses, the following factors tend to give melted cheese with high flowability, higher fluidity (as measured by phase angle or loss tangent), and lower apparent viscosity:

- higher moisture content,
- lower calcium content,
- lower pH in the range 5.2 to 6.0,
- lower protein-to-fat ratio,
- lower calcium-to-casein ratio
- higher moisture-to-protein ratio

The effects of these parameters are largely due to reductions in the concentration and volume fraction of the *para*-casein network or degree of the calcium-induced cross-linking of the *para*-casein molecules in the network, associated with the lower calcium concentration, or the reduction in the ratio of colloidal (casein-bound) calcium to soluble calcium as the pH is reduced (cf., Sects. 18.3.2–18.3.3). Reducing

the fat content of some cheeses, such as low-fat or reduced-fat variants of different varieties (e.g., Cheddar), causes significant impairment of the functional properties (e.g., reduced flow, reduced stretchability, very high viscosity, low fluidity; Fig. 18.10) despite the increase in moisture content. This effect is due the higher volume fraction of the casein network, but also the lower moisture-to-casein ratio which is indicative of a greater degree of fusion of the *para*-casein polymers constituting the network above.

18.6.5 Effect of Homogenization

Homogenization of milk is an integral part of the manufacturing process for soft, high-fat, acid-curd cheeses such as Cream cheese and Neufchatel, as it prevents creaming (flocculation and floatation of fat globules) during the relatively long gelation time (e.g., >4 h), and contributes to the formation of a homogeneous, thick, creamy texture in the end-product. The resulting textural characteristics ensue from the participation of the homogenized fat particles in the formation of a composite acid gel, which has a greater number of protein-protein interactions and is stiffer and more uniform than the corresponding gel from unhomogenized milk. Homogenization of cheese milk causes shearing of the native protein-phospholipid membrane of the fat globules and its replacement by a protein layer consisting of casein micelles, sub-micelles and whey proteins; this layer around the newly-formed fat globules is frequently denoted the recombined fat globule (RFG) membrane. The RFG membrane causes the fat globules to behave as fat-filled protein (FFP) particles, which can become an integral part of the gel network during acid- and rennet-induced gelation of milk.

In contrast, homogenization of milk or cream is rarely practiced in the manufacture of rennet-curd cheeses because it leads to defects in the resultant cheeses:

- Poorer ability of the curd particles to knit and mat during manufacture;
- Increased tendency of moulded curds to break/crack easily, making curd handling more difficult (in the case of Cheddar cheese, the curd tends to shatter during milling, and because of its larger surface area-to-volume ratio absorbs more of the added salt);
- Increased moisture content (e.g., 1–2 % at a total homogenization pressure of ~20 MPa);
- Altered curd rheology and texture, with the cheese being more easily fractured (lower facture strain), less elastic, 'shorter' and 'bitty';
- Impaired cooking properties of the melted cheese, as reflected by its lack of surface sheen, markedly lower degrees of flow/spread and stringiness, and increased tendency to dry out/burn.

A comparison of flow and fluidity of full-fat cheeses made from control (unhomogenized) or homogenized milks clearly demonstrates the effect of homogenization (Fig. 18.13). While the effects of homogenization are undesirable in most cheese applications requiring moderate-high flow, fluidity and oiling-off, they, nev-

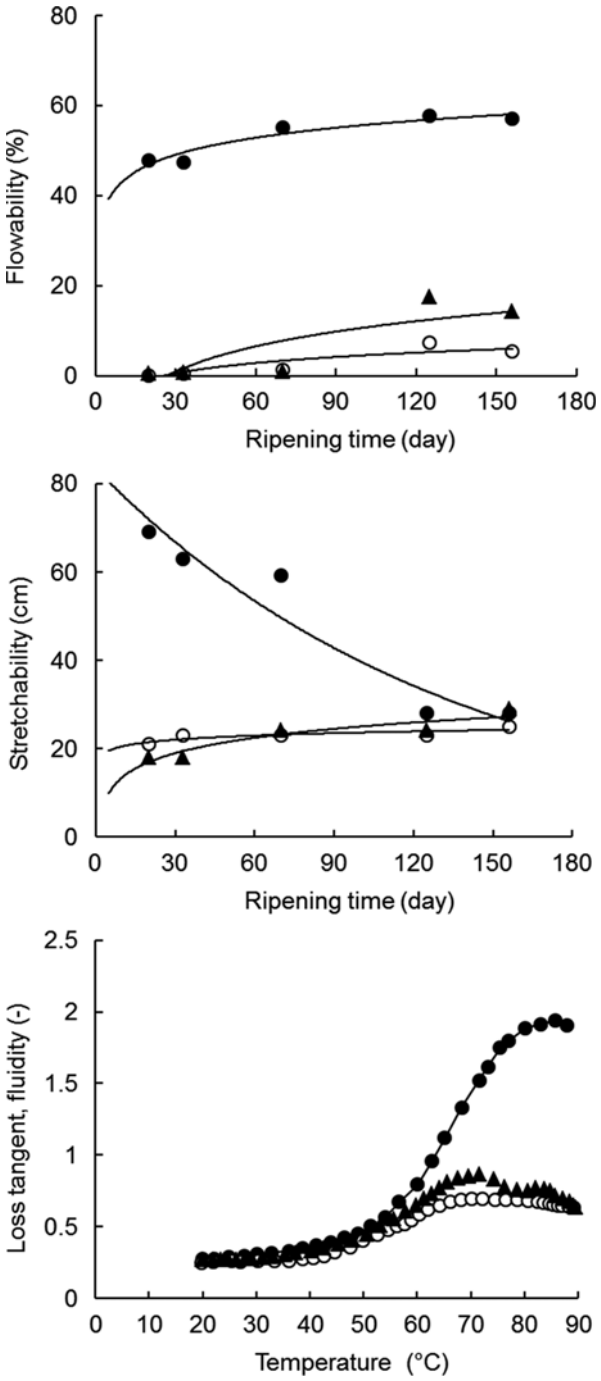


Fig. 18.13 Effect of fat content and homogenization on the functional properties of Cheddar-type cheeses: flowability and stretchability during ripening at 8 °C, and the loss tangent of the 5-day-old cheeses as a function of temperature on heating from 20 to 90 °C. The cheeses were: full-fat Cheddar from unhomogenized milk (control, 30 % fat; *filled circle*), full-fat Cheddar from milk homogenised at first and second stage pressures of 25 and 5 MPa, respectively (30 % fat, *open circle*), and low-fat Cheddar from unhomgenized milk (1.4 % fat, *filled triangle*). Modified from Guinee et al. (2000c)

ertheless, are advantageous in applications where a high degree of flow resistance is required (cf. Sect. 18.6.1). The effect of homogenization probably resides mainly in its formation of a recombined fat globule membrane, which stabilizes the fat globules to heat and thereby prevents free oil formation during cooking (baking/grilling). The lack of free oil, which may be considered as a lubricant facilitating relative displacement of the adjoining layers of the cheese matrix during melting, predisposes the cheese to dehydration, crusting, burning, and an inability to flow or form into a fluid, succulent, molten mass.

18.6.6 Cheesemaking Conditions

The functionality of cheeses can be s changed significantly by altering cheesemaking conditions. Several parameters can be varied:

- Milk standardization, including the level of casein (e.g., which can be altered by ultrafiltration of the milk) and protein-to-fat ratio;
- Milk pre-treatments, including pasteurization temperature and time, and homogenization pressures, which affect whey protein content and stability of the fat to heat-induced coalescence and destabilization;
- Type of starter culture, which influences the extent and type of proteolysis, sugar metabolism and propensity to browning;
- pH at different stages of manufacture including at set (rennet addition), whey drainage, curd salting and plasticization, owing to its effects on the calcium-to-casein ratio and moisture-to-protein ratio;
- Gel cutting, stirring and cooking conditions (including gel firmness during cutting, rate of heating, stirring speeds, holding time prior to whey drainage), which influence pH and moisture content;
- Heating, temperature of the curd during cooking and stretching (plasticization) of *pasta filata cheeses*, which influences residual rennet activity and, ultimately, the level of rennet-induced proteolysis in the cheese;
- Curd pH at plasticization of *pasta filata* cheeses, which influences the level of casein-bound calcium and the water -binding capacity of the casein;
- Salting method of *pasta filata* cheeses.

Alterations in the above parameters exert their effect on functionality by virtue of their impact of key compositional parameters (e.g., moisture, moisture-to-protein ratio, calcium-to-casein ratio), microstructure (e.g., volume fraction of the casein network, extent of calcium phosphate mediated of cross-linking and aggregation of casein), and macrostructure (e.g., degree to which curd particles flow and knit together into a cohesive structural whole).

Other factors that influence the composition and functionality of cheese include the stage of lactation and diet of the cows, and plant design.

18.7 Dried Cheese Products

Dehydrated cheese products are industrially-produced cheese-based ingredients which were developed for the US Army during World War II as a means of preserving cheese solids under conditions to which natural cheese would not normally be subjected, e.g., a temperature >21 °C for long periods. Since then, they have become ingredients of major economic importance owing to their widespread use as flavouring agents and/or nutritional supplements in a wide range of foods. These include bakery products, biscuits, dehydrated salad dressings, sauces, snack coatings, soups, pasta dishes, savoury baby meals, cheese dips, *au gratin* potatoes and ready-prepared dinners. They are also included in processed and analogue cheese products as flavouring agents or as a functional ingredient in powdered instant cheese preparations, which can be reconstituted by the consumer for the preparation of instant functional cheeses, e.g., pizza-type cheese, for domestic use. Advantages over natural cheese as an ingredient in the above applications include:

- convenience of use in fabricated foods. Cheese powders can be applied easily to the surface of snack foods (e.g., popcorn, potato crisps, nachos) or incorporated into food formulations, e.g., by dry mixing with other ingredients such as skim milk powder (e.g., for dried soup, sauce or cake mixes) or by blending into wet formulations. In contrast, natural cheeses require size reduction prior to their use in these applications.
- their longer shelf-life, because of their lower water activity (a_w) than natural cheese. The a_w of natural cheeses ranges from ~0.99 for Quark to 0.917 for Parmesan (see Chap. 9); for processed cheese products, it ranges from ~0.93 to 0.97 and from ~0.2–0.3 for various dairy powders. Owing to their relatively high stability, cheese powders may be stored for a long period without alteration or deterioration of quality. In contrast, the changes which occur in natural cheese during storage may influence its usage/functionality, e.g., the ease with which it can be size-reduced or its interaction with other ingredients, and its flavour profile and intensity. Hence, cheese powders are more amenable than natural cheese to inventory management and the formation of end-products (e.g., sauces, soups) with more consistent quality in large-scale manufacturing operations.
- the greater diversity of flavours that can be provided by cheese powders, made possible by the use of different types of cheese, EMCs and other ingredients in their preparation.

Dehydrated cheese products may be classified into four categories, depending on the ingredients used:

1. Dried grated cheeses, e.g., Parmesan and Romano
2. Natural cheese powders, made using natural cheeses, emulsifying salts and natural cheese flavours (optional)
3. Extended cheese powders, incorporating natural cheese and other ingredients such as dairy ingredients (e.g., skim milk solids, whey, lactose), starches, maltodextrins, flavours, flavour enhancers and/or colours;
4. dried EMCs

18.7.1 Dried Grated Cheeses

Dried grated cheeses are normally used as highly flavoured sprinklings, e.g., on pasta dishes and in bakery products, e.g., biscuits. Essentially, the production of these products involves finely grating hard cheeses and drying the grated cheese, usually in a fluidized bed drier by exposure to low humidity air (15–20 % relative humidity) at an inlet temperature <30 °C. Under these conditions, the cheese is dehydrated rapidly and evaporatively cooled, thereby reducing the risk of fat exudation and the tendency to ball or clump. The dried, grated cheese (typically containing 17 % moisture) is usually packed under nitrogen to reduce the risk of oxidative rancidity during distribution and retailing.

Certain properties of cheese are required for the production of dried grated cheeses, e.g., relatively low levels of moisture (30–34 %) and fat-in-dry matter (39 %), brittleness and elastic fracture characteristics (cf. Chap. 14). These properties lend themselves to efficient size reduction on grinding, minimal susceptibility to fat exudation or to sticking of the cheese particles, and contribute to efficient drying to a homogeneous product, free of clumps. An intense cheese flavour is also a desirable characteristic. Generally, dried grated cheeses are used in small quantities, as sprinklings, to impart strong cheese notes to pasta dishes, soups and casseroles. The cheese varieties which best meet these criteria are Parmesan and Romano, because of their composition, fracture properties and their strong, piquant, lipolyzed flavour. For Romano, pregastric esterase (from kid, goat or lamb) added to the cheese milk, preferentially hydrolyses the short-chain fatty acids (especially butanoic acid) from milk fat triglycerides during maturation; a high level of butanoic acid (1500–2000 mg/kg cheese) is responsible for the peppery, piquant flavour of Romano cheese.

Owing to their lower firmness and higher levels of moisture and FDM, hard cheeses such as mature Cheddar (moisture and FDM, ~37.0 and 52.0 %, respectively) or Gouda (moisture and FDM, ~41.0 and 48.0 %, respectively) are more difficult to dry than hard grating cheeses, owing to their potential to fat exudation and clumping during grinding and drying. Nevertheless, processes have been developed which allow them to be dried successfully, e.g., two-stage drying at temperature <63 °C (Sanders 1946). In the first (preliminary) stage, the cheese is exposed to a strong flow of dehumidified air at room temperature during shredding and depositing uniformly on trays or screens. This results in rapid removal of moisture and case hardening of the shred surfaces, and minimizes fat exudation and coalescence/matting of curd shreds during subsequent drying. The moisture which is typically reduced to ~33 % in the preliminary drying stage is further reduced to ~8 % by conveying the trays of shredded cheese through a drying tunnel where they are exposed to hot air which heats the cheese particles to 63 °C and reduces the moisture content. A further process (Cornwell and Foster 1969) involves conveying cheese cubes (~1 cm) into slowly-rotating (1 rpm) drum dryers through which heated air (~38 °C), preferably dehumidified, is circulated at high velocity (~12 m^3/min). The dried cubes are then milled to a powder. High moisture (82.0 %) cheeses, such as Cottage cheese, may also be dried directly to 3 - 4 % moisture in specialized

spray driers (e.g., silo spray drying using the Birs Dehydration Process; Kosikowski and Mistry 1997). These low-moisture, dried natural cheeses are generally used for nutritional supplementation of foods, e.g., dried baby meals.

18.7.2 Cheese Powders

18.7.2.1 Manufacture

The manufacture of cheese powders essentially involves the production of a pasteurized processed cheese slurry (40–45 % dry matter) which is then spray dried (Fig. 18.14). The production steps include formulation of the blend, processing of the blend to form a slurry, homogenization and drying of the slurry.

The blend usually consists of comminuted natural cheese, water, emulsifying salts, flavouring agents, flavour enhancers, colours, antioxidants, and perhaps filling materials such as whey or skim milk solids, starches, maltodextrins and milk fat. The types and levels of ingredients included are determined by the type of cheese powder, e.g., natural or extended, the flavour required, and its application, e.g., whether intended for use in a sauce, soup, snack coating or cheese dip. Antioxidants, such as propyl gallate and butylated hydrozyanisole, may be added at a level of 0.5 to 1.0 g/kg fat to retard potential spoilage due to oxidative rancidity. Typical formulations of the slurries required for the production of natural and extended cheese powders with different levels of cheese solids are given in Table 18.5.

The flavour profile and intensity of the final cheese powder is determined by the type(s) of cheese used, the types and levels of other flavouring agents, e.g., EMC, hydrolyzed milk fat, starter distillate and flavour enhancers, e.g., NaCl, monosodium glutamate, autolyzed yeast extract. Generally, mature cheese with an intense flavour is used so as to impart a strong flavour to the final product. Apart from their lack of flavour-imparting properties, young cheeses with a high level of intact casein are unsuitable as they result in slurries that are very viscous and difficult to atomize and dry efficiently. Filling materials in extended cheese powders are usually added to replace cheese solids and reduce formulation costs. However, they may also influence flavour, wettability and mouth-coating characteristics of the product in which the cheese powder is used.

Processing principles and technology are similar to those used for the manufacture of processed cheese products (cf. Chap. 17). Processing typically involves batch heating of the blend to ~80 °C in high shear (1500–3000 rpm) processing vessels using indirect steam injection. The blend is processed until the hot molten slurry is homogeneous in colour and consistency and free of lumps or non-hydrated material. The maximum processing temperature should be maintained below 85 °C to minimize the loss of volatile flavour compounds in the dissipating steam and to minimize the risk of browning, especially for formulations containing a high level of lactose or high dextrose (glucose) equivalent (DE) malto dextrins.

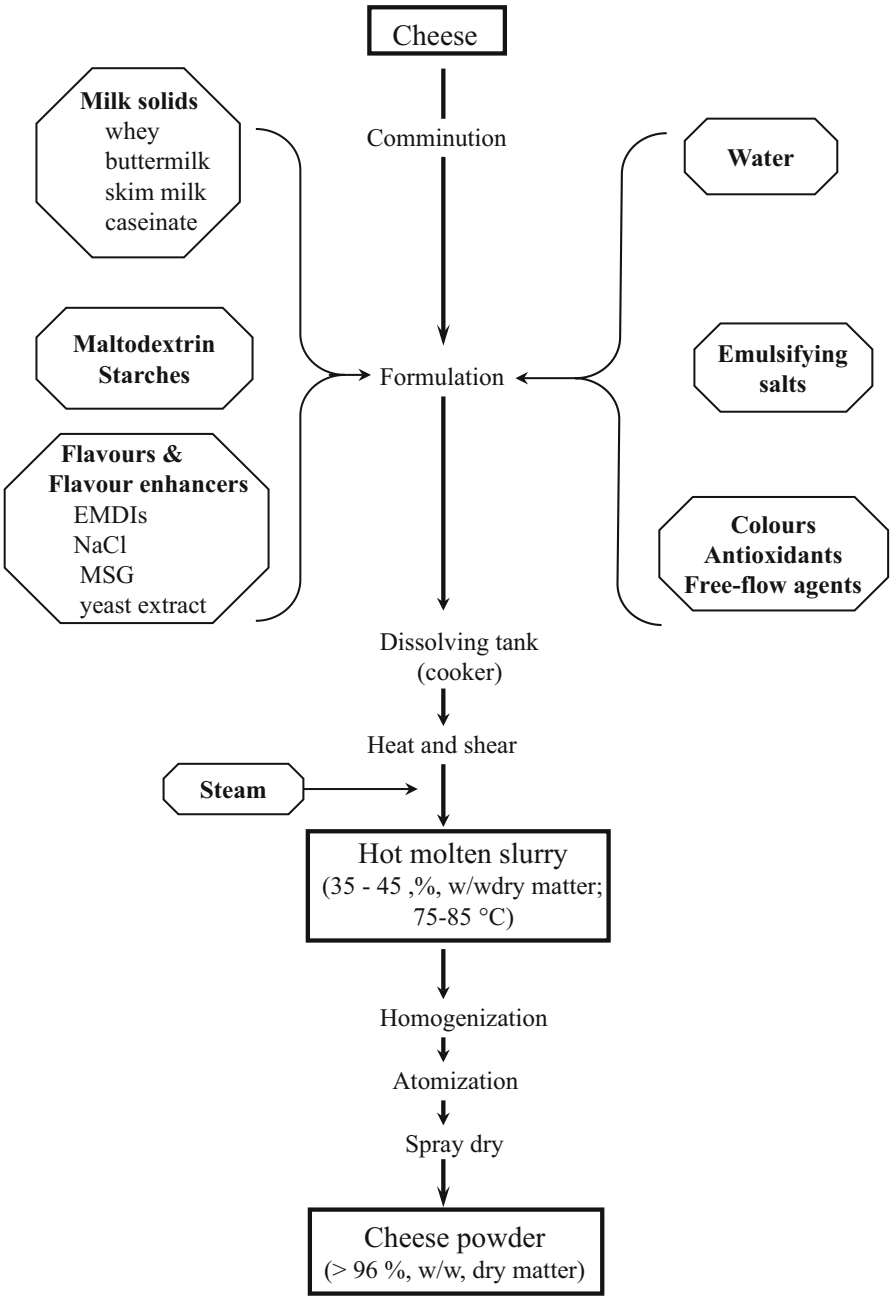


Fig. 18.14 Production process for cheese powder

Table 18.5 Typical formulations of cheese slurries for the production of cheese powders with different levels of cheese solids

Formulation	Ingredients used (% of total formulation weight)		
	Low solids (26 %)	Medium solids (53 %)	High solids (95 %)
Medium aged Cheddar	–	19.0	–
Mature Cheddar	20.0	17.0	63.5
‡EMC paste	0.5	0.2	1.0
‡EMC powder	0.5	2.0	–
Whey powder	12.0	5.0	–
Skim milk powder	8.0	3.8	–
Maltodextrin (DE 17)	1.7	11.0	–
Emulsifying salts	1.5	2.5	1.5
Butyl-hydroxyanisole (BHA)	0.05	0.05	0.05
Sodium chloride	1.5	1.0	0.5
Water and condensate 39.0	39.0	38.5	33.5

‡EMC, enzyme-modified cheese
Modified from Guinee et al. (1994)

The viscosity of the cheese slurry has a major influence on its tendency to foam and, therefore, on the level of air in the resultant powder, which affects the stability of the product to oxidative rancidity during storage. Owing to its effects on the air content of the powder, the viscosity of the cheese slurry influences the physical characteristics (bulk density and wettability) of the resultant powder and its susceptibility to oxidative rancidity and flavour deterioration during storage. High viscosity slurries (≥3.0 Pa.s) have a lower propensity to foam and therefore yield a powder with a lower level of air compared to low viscosity (<0.3 Pa.s) slurries. The viscosity of the cheese slurry is determined by its dry matter content and the characteristics of its ingredients, e.g., density of the different ingredients, levels of fat and protein, pH and degree of ingredient hydration. The air content of the cheese powder is also influenced by the levels of formulation ingredients which tend to promote (undenatured whey proteins) and/or depress (fat, food-grade anti-foaming agents) foaming of the slurry during preparation and drying.

Homogenization of the slurry is optional but is commonly practiced to ensure homogeneity and the absence of free fat. The pressures applied (typically 15 and 5 MPa, in the first and second stages, respectively) have a major effect on the viscosity of the slurry, with higher pressures generally imparting higher viscosity for a given level of dry matter.

Several spray-drying processes (e.g., single stage or two stage) and dryer configurations (e.g., tall-form, filtermat, silo-form) may be used. The design of the drier and operating conditions, e.g., atomizer type and pressure, direction of air flow, air inlet and outlet temperature and air humidity influence the physical (e.g., bulk density, wettability and solubility) and the flavour characteristics of the cheese powder. The physical properties are important in applications that require reconstitution of the cheese powder, e.g., ready-prepared soups, sauces and baby foods.

In all cases, the homogenized cheese slurry is pumped to the dryer where it is atomized and dried, typically at an inlet air temperature of 180–200 °C and an outlet air temperature of 85–90 °C, depending on the type of dryer. The powder is then cooled from ~55 to ~20 °C, separated from the air and packaged. The moisture content of the dried powder is typically 3–4 % and generally decreases with increasing outlet air temperature. However, an elevated outlet temperature, e.g., >95 °C, may be detrimental to product quality, owing to:

- increased Maillard browning,
- reduced product wettability and solubility (due to denaturation of ingredients),
- loss of volatile flavour compounds, and
- greater susceptibility to oiling-off and free fat formation; free fat in the cheese powder leads to lumpiness, flow problems and flavour deterioration.

Commercially, cheese powders are normally manufactured using two-stage drying systems; filtermat (box) dryers are used frequently in the USA whereas tall-form dryers with an integrated fluidized bed are used widely in Europe. While the operating conditions of these dryers influence the quality of the cheese powder, the tall-form drier is generally considered to give better flavour retention, larger powder particles and better powder flowability. Conventional single-stage tall-form dryers are rarely used because of the high outlet air temperature (e.g., >95 °C) necessary to achieve the low-moisture content required. However, single-stage silo-dryers (with a 60–70 m drying tower compared to ~10 m for the tall-form dryer) may be used, as in the Birs Dehydration Process (Kosikowski and Mistry 1997). In this process, the drying air is dehumidified but not heated; the main advantages over conventional two-stage drying are improved colour stability and enhanced flavour retention, especially in mildly flavoured products, the flavour of which is dominated by a few compounds, e.g., Cottage cheese.

18.7.2.2 Composition

The composition of cheese powders varies considerably, depending on the formulation ingredients; typical values are shown in Table 18.6.

18.7.2.3 Some Applications of Cheese Powder

Cheese powders are generally used as flavouring ingredients in a wide variety of foods, especially snack coatings (e.g., pop corn, nachos, tortilla shells), cheese sauces, soups, savoury dressings and savoury biscuits. In snack foods, the powder is dusted after the snack has been sprayed lightly with vegetable oil. In cheese sauces, the level of cheese powder is typically 5–10 %, depending on the flavour intensity of the cheese powder and the types and levels of other flavouring ingredients in the formulation. Generally, at these levels, the cheese powder has little influence on the rheological properties of the sauces which are controlled mainly by the types and levels of starch used (Guinee et al. 1994).

Table 18.6 Composition of cheese powders with different levels of cheese solids

	Low solids (26 %)	Medium solids (53 %)	High solids (95 %)
Dry Matter (%, w/w)	97.0	97.0	96.0
Protein (%, w/w)	20.1	23.0	36.1
Fat (%, w/w)	14.5	21.9	38.8
Lactose (%, w/w)	26.4	12.3	0.3
Ash (%, w/w)	8.9	10.4	10.4
pH	6.4	6.5	6.3

Modified from Guinee et al. (1994)

18.7.3 Enzyme-Modified Cheeses

Enzyme-modified cheeses (EMCs) are used principally as flavouring agents in industrially-produced cheese products/ingredients, such as pasteurized processed cheese products, cheese substitutes/imitations, cheese powders, and ready-prepared meals. Natural cheeses have certain limitations as flavour ingredients:

- low flavour stability due to ongoing biochemical and microbiological changes during storage
- flavour inconsistency, e.g., due to changes in cheese composition
- insufficient flavour intensity, considering the small quantity incorporated into many foods (e.g., soups, sauces, analogue cheeses)
- high cost due to the relatively long ripening time required for most cheese varieties

These deficiencies led to the development, in the 1960s, of enzyme-modified cheeses with flavours which are 5–20-fold more intense than those of the corresponding natural cheeses (Guinee and Kilcawley 2004; Wilkinson et al. 2011). Essentially, manufacture involves the addition of exogenous enzymes (proteinases, peptidase, lipases and/or esterases) and/or lactic acid bacterial cultures to a dairy-based substrate, usually cheese curd, and incubation under controlled conditions to achieve a paste with a predicted flavour profile and intensity. Reactions contributing to flavour generation include proteolysis and lipolysis (cf. Chaps. 12 and 13). Heat treatment of the substrate prior to addition of flavor-generating agents is essential to inactivate the contributions of varying microbial and enzymatic activities present in curd, to direct and control flavour development, and to, thereby, achieve consistently the desired flavour profile and intensity.

The production of EMCs generally involves the following production steps (Fig. 18.15):

- *production of a cheese curd,* as for conventional cheese
- *formation of a paste* (typically 40–50 % dry matter) by blending the curd with water and emulsifying salts. The addition of sodium phosphate or citrate salts assists in adjusting the pH of the paste to a value which is optimal for subsequent

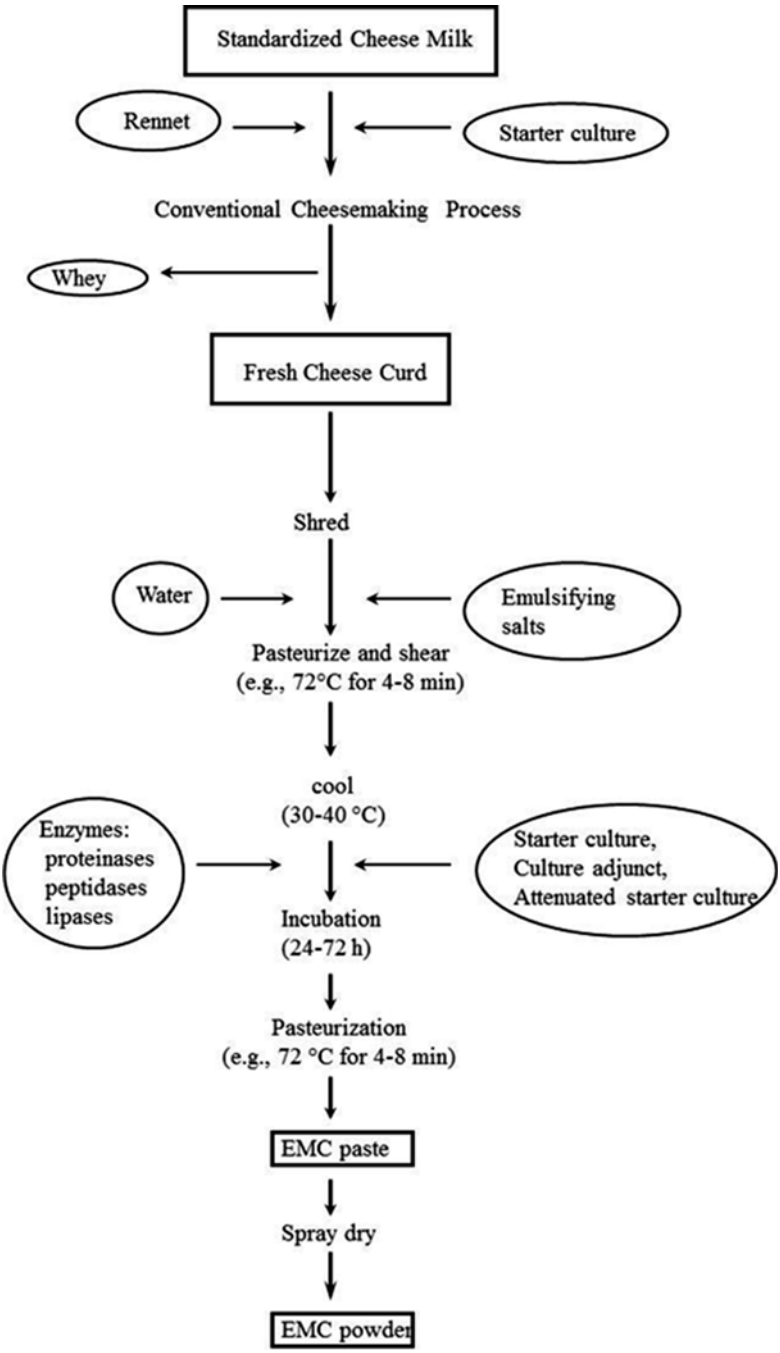


Fig. 18.15 Production process for enzyme-modified cheese

enzymatic reactions, and hydrating the curd protein, enabling it to emulsify free fat formed during subsequent heating and shearing of the paste.

- *pasteurization* of the cheese paste to inactivate the existing cheese microflora and enzymatic activities. Pasteurization reduces the risk of flavour unpredictability and inconsistency associated with variations in bacterial microflora (populations and strain composition of starter and non-starter lactic acid bacteria) and enzyme activities (e.g., chymosin, plasmin, esterase and lipase) in curds obtained from different suppliers.
- *treatment of the pasteurized curd* with the desired cocktail of enzymes and bacterial cultures to give the required flavour profile and intensity in the EMC. Added enzymes may include proteinases, peptidases and lipases, chosen based on knowledge of the enzymology and flavour profile of the cheese which is being simulated. Some commercial EMCs are prepared using a combination of added enzymes and bacterial culture systems. The advantage of using starter culture systems is that each starter cell is essentially a sack of enzymes which are known to contribute to balanced flavour production in any given cheese variety; hence, it is generally easier to simulate a particular cheese flavour by using cultures rather than enzyme cocktails (cf., Chaps. 12 and 13).
- *incubation of the slurry* at 30–40 °C for ~24–72 h. During this period, the added enzymes, or those released from starter cells during growth and/or autolysis, act on the casein and fat in the paste to produce the correct balance of peptides, amino acids, amines, aldehydes, alcohols, ammonia, fatty acids, ketones and alcohols.
- *pasteurization of the enzyme-treated paste* to inactivate enzymes and thereby preserve the flavour characteristics generated with minimum change during storage.
- *homgenization* of the hot pasteurized paste to reduce the risk of phase separation during storage and ensure product homogeneity. The homogenized cheese paste, known as EMC paste, may be packaged and stored at refrigeration temperature, usually in opaque materials to minimize the risk of oxidative rancidity and off-flavour development.
- *drying*. The paste may be dried to give an EMC powder, which has a longer shelf-life than the paste and is more suitable for applications involving dry blending with other ingredients.

EMC variants of many natural cheeses, e.g., Cheddar, Blue cheese, Romano and Emmental are commercially available. The development of EMCs requires elucidation of the flavour compounds and/or enzyme activities in the cheese being simulated, followed by testing of different blends of enzymes and/or bacterial cultures on curds under various conditions, e.g., age of curd, composition of curd (dry matter, protein-to-fat ratio, pH), incubation temperature, until the desired flavour characteristics are obtained. The composition, flavour-forming reactions and flavour components of EMCs have been reviewed extensively (see Kilcawley et al. 1998a, b; 2000).

18.8 Conclusions

Cheese is a highly versatile dairy ingredient which can be used directly in an array of culinary dishes, formulated food products and ready-prepared meals. In these applications, added cheese performs a number of functions, i.e., it contributes to structure, texture, flavour, mouth-feel, cooking properties and/or nutrition. The current chapter focused primarily on the use of rennet-curd cheese, mainly because rennet-curd cheeses such as Cheddar, Mozzarella, Gouda and Emmental dominate the ingredient cheese market; processed cheeses are also very significant, and are discussed separately in Chap. 17.

The use of cheese as an ingredient relies heavily on the texture/rheological characteristics of the unheated cheese and the cooking/rheological characteristics of the heated cheese. For both unheated and heated cheese, the structure at both the micro- and macro-structural levels is a major determinant. Microstructure of cheese refers to the assembly of the cheese components (protein, fat, serum, minerals) in a structure at the nano-/micro-meter scale, while macrostructure refers to the overall assembly of the curd particles into a moulded cheese. The microstructure controls the proportions of serum (moisture + dissolved solutes), fat, and calcium phosphate *para*-casein network. The *para*-casein network in most rennet-curd cheeses is highly concentrated (~20–55 % protein-in-moisture), extensively dehydrated (0.9–2.5 g water/g casein) relative to the native casein (micelles) in milk (~4.0 g water/g casein), and relies heavily on calcium-/calcium phosphate-mediated cross-linking of the constituent casein molecules for its integrity and rigidity. Consequently, the volume fraction of the casein network, the extent of cross-linking and the degree of hydrolysis of the *para*-casein, are key determinants of its rheology, the stress and strain required to fracture, the level of displacement in the form of flow or spread on cooking, its viscoelasticity (ratio of viscous to elastic behaviour, cf. Chap. 14) and, consequently, its overall functionality. The above network characteristics (volume fraction, etc.) can be controlled through manipulation of the overall cheesemaking process to control casein concentration, moisture-to-casein ratio, calcium-to-casein ratio, residual rennet activity and pH of the curd. Apart from the *para*-casein network, the volume fraction of the fat phase and the degree to which the fat is emulsified are also critical elements in functionality control. In cheese, fat exists as discrete globules, coalesced globules and/or as pools, the proportions of which depend on the level of fat and manufacturing steps. At the temperatures involved in cheese usage (e.g., ≥ room temperature) much of the fat is liquid (e.g., ≥60 % of total fat). It thereby creates liquid 'soft spots' within the matrix during compression, shearing or extension and acts as a lubricant on the surfaces of adjoining layers of the cheese matrix during displacement. Additionally, free oil in the heated cheese (due to heat-induced calescence) acts a protectant apolar layer which prevents dehydration and blistering.

The functionality of cheese is also affected by its macrostructure, although little work has been reported on quantifying its effects. It is affected by the microstructure, which determines the rheological properties of the constituent curd particles

and their ability to flow and knit together, and by the processes to which the curd particles are subjected (e.g., degrees of salting, texturisation, heating, moulding and pressing). Operations which reduce the ability of curd particles to flow (e.g., high protein, low moisture, high pH, and high calcium) or cause excessive surface dehydration of curd particles (e.g., salting-out or depletion of fat and moisture) impair the knitting of curd particles into a uniform structural continuum. Such processes alter the rheological properties (reduce fracture stress and strain and elasticity) and the functionality (e.g., brittleness, shreddability and sliceability). Hence, engineering of cheese functionality ultimately resides in the control of cheesemaking operations that affect both the microstructure and macrostructure and their inter relationships.

Despite the fact that cheese as a product is versatile in terms of the functionality (including flavour) it potentially affords, cheese is expensive and its functionality is not always consistent. It is unstable, changing to a greater or lesser degree during storage depending on storage time, cheese type, composition, structure and storage temperature and humidity; proteolysis is a key factor contributing to the age-related changes in functionality. Variability in functionality, even within a given cheese type from the same supplier, can arise owing to batch-to-batch variations in cheese composition (cf. Chap. 15). Moreover, the consistency of natural cheese is unsuitable for some applications such as those involving dry blending with other ingredients in the manufacture of cake mixes, dried soups and baby meals. These deficiencies in the properties of cheese as an ingredient led to the development of cheese ingredients, most notably processed cheese products (PCPs), dried cheeses, cheese powders and EMCs. PCPs, the properties of which can be conveniently customized for given applications by altering manufacturing variables (e.g., formulation, composition, processing conditions), are used mainly as an ingredient cheese product for cooking applications (cf. Chap. 17). Cheese powders and EMCs are used mainly as flavouring agents, the former as a snack coating and the latter in processed cheese products and formulated foods including sauces, gratins, soups and pet foods.

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Chapter 19
Pathogens in Cheese and Foodborne Illnesses

Summary This chapter summarises foodborne illness due to cheese. Since 1980, there have been 53 outbreaks of food poisoning due to the consumption of cheese during which time almost 250,000,000 tonnes of cheese were produced. The most common organisms involved were *Listeria monocytogenes*, enteropathogenic *Escherichia coli*, particularly 0157:H7, *Salmonella* and *Staphylococcus aureus*. Of these, listeriosis is the most serious since many outbreaks have resulted in fatalities. The factors controlling the growth of pathogens are the same as those controlling the growth of starters and non-starter lactic acid bacteria in cheese (see Chap. 6). Generally, soft cheeses are more likely to be involved in cheese-associated outbreaks of foodborne illness than hard and semi-hard cheese. Generally, no details of the compositional data of the cheese involved in an outbreak are given, e.g., pH, salt and moisture; such information could be important in understanding how outbreaks occurred. Each of the four groups of bacteria involved is considered in some detail regarding their origin, the symptoms of the illness and the experimental evidence for their growth in cheese. Many cheeses are made from raw milk and confounding factors other than the use of raw milk were involved in these outbreaks.

Keywords Food-poisoning outbreaks • Listeriosis • Enteropathogenic • *Escherichia coli* • *Salmonella* • *Staphylococcus aureus* • Raw milk cheese

Milk is a highly nutritious medium of almost neutral pH and, therefore, many bacteria, including spoilage and pathogenic ones, can grow in it, when the environmental conditions, e.g., temperature, are right. Numerous outbreaks of foodborne illness have been traced to milk. However, milk is not as important as other foods in causing foodborne illness; a recent review (De Buyser et al. 2001) of foodborne disease in France, Belgium, Italy, Canada, UK, Denmark and the US concluded that milk and milk products cause ~5 % of the total outbreaks of foodborne disease.

Although cheese is equally nutritious, it has been responsible for relatively few foodborne illnesses. Since 1980, there have been 53 outbreaks of food poisoning due to the consumption of cheese (Table 19.1), during which time almost 250,000,000 tonnes of cheese were produced worldwide. These data were compiled mostly from the PubMed website (www.ncbi.nlm.nih.gov/**pubmed**) and some outbreaks may have been missed. Nevertheless, the data suggest that cheese is a very safe product,

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even when made from raw milk. Earlier outbreaks have been comprehensively reviewed by Johnson et al. (1990a, b, c).

Several organisms were involved in causing these outbreaks, with the most common being *Listeria monocytogenes*, enteropathogenic *Escherichia coli*, particularly 0157:H7, *Salmonella* and *Staphylococcus aureus*; other microorganisms, e.g., *Brucella* spp. and *Clostridium botulinum* were responsible for occasional outbreaks. In many outbreaks, cheese and human isolates had identical Pulsed Feld Gel Electrophoresis (PFGE) patterns, confirming that the cheese was the source of the organism. Several conclusions can be drawn from Table 19.1. Salmonella outbreaks were the most common and almost invariably involved cheeses made from raw milk; fatalities were associated only with *L. monocytogenes* outbreaks, *S. aureus* has not been involved in any outbreak in the last 20 years, except for the outbreak in Greece in 2005. *Campylobacter jejuni*, which is the most common cause of foodborne illness, was involved in only one cheese-associated outbreak.

The infectious dose (ID) of some pathogens is low, e.g., *E. coli* 0157:H7, *S. enterica* and *L. monocytogenes* have IDs of 5–50, 100–500 and, perhaps, 1000 cells, respectively. Fat is thought to reduce the ID dramatically. In contrast, the infective dose of *S. aureus* is high because the actual cause of the foodborne illness is not the organism itself but a number of closely related, protein toxins produced by it, which are heat stable, withstanding 100 °C for >30 min. *S. aureus* must reach numbers of ~10^6 cfu/g to produce sufficient toxin (~1 ng of toxin per g of cheese) to cause foodborne illness. It is not clear that the outbreak in Greece was definitively caused by *S. aureus*, since the presence of enterotoxins in the cheese was not measured and the number of *S. aureus* in the cheese was only ~3000/g. The strains of *S. aureus* present in raw milk and therefore presumably also in raw milk cheese are primarily those that cause mastitis, assuming no contamination from personnel; 20–50 % of mastitis strains produce enterotoxin.

The dominant cheeses involved were soft, surface-ripened and Mexican-style varieties; the latter also usually contain a low level of lactic acid and, therefore, a high pH value. Hard cheese is less likely to support the growth of pathogens, nevertheless several outbreaks involved Cheddar produced from pasteurised or raw milk, one of which was traced to a farm where a single cow was shedding ~200 cfu of salmonella/ml of her milk (Wood et al. 1984). Cows may also shed listeria in their milk. Recently a cow was identified in a herd in Ireland shedding 280 *L. monocytogenes*/ml from one quarter. The infected cow showed no clinical signs of disease and the milk no sign of any abnormaility (Hunt et al 2012).

In Table 19.1, the pH of the cheese was reported on only two occasions and the moisture and salt levels appeared not to have been measured. Compositional analysis of the cheese, particularly salt, moisture and pH, and determination of indicators of hygiene should always be measured in outbreaks of foodborne disease in the hope that manufacturing procedures can be improved and the number of future outbreaks reduced. This is rarely done in practice, because the cheese involved is often no longer extant. Efforts should also be made to measure hygiene in the plant in which the cheese was made and the numbers of the causative organisms involved. Samples of the washed-rind cheese involved in the Japanese listeria outbreak con-

Table 19.1 Outbreaks of food poisoning involving cheese 1980–2013

Country of origin	Variety of cheese	Year	No. of cases	No. of deaths	Sero-group	Milk	Comments	Reference
Listeria monocytogenes								
US	Asadero (Mexican-style) cheese	2009	8			Past	Post-pasteurisation contamination from a vat gasket; plant closed down	Jackson et al. (2011)
Austria and Germany	Quargel (washed rind) 1st outbreak	2009	14	4	1/2a		20/63 cheese samples positive for *L. monocytogenes*; one sample contained 2.1×10^6 cfu/g	Fretz et al. (2010a)
	2nd outbreak	2009	20	3	1/2a		Two different clones of *L. monocytogenes*	Fretz et al. (2010b)
Canada	Washed-rind cheese	2008	38	2		Past	Brine a likely source; extensive cross contamination at retailer level; $>10^4$ *L. monocytogenes*/g of cheese	Gaulin et al. (2012)
Belgium	Goats' cheese	2005				Raw	Asymptomatic sheddar in the goat herd	Delhalle et al. (2012)
Switzerland	Tomme	2005	10	3	1/2a		*L. moncytogenes* was widespread throughout the factory	Bille et al. (2006)
US	Queso Fresco, home-made	2000	13			Raw	Poor hygiene; improved teat and milking machine washing reduced contamination	MacDonald et al. (2005)
Italy	Gorgonzola		1		1/2a & 1/2b	Past	Same isolate persisted for at least 5 months; 1,200 cfu/g in the cheese; pH 7.4	Gianfranceschi et al. (2006)
Japan	Washed-rind cheese	2001	38		1/2b		Poor hygiene; *L. monocytogenes* ranged from <3 to 4.6×10^7 cfu/g of cheese	Makino et al. (2005)
Belgium	Camembert	1997	1		1/2a			Gilot et al. (1997)
France	Brie de Meaux	1995	20	1		Raw		Goulet et al. (1995)

(continued)

Table 19.1 (continued)

Country of origin	Variety of cheese	Year	No. of cases	No. of deaths	Sero-group	Milk	Comments	Reference
US	Mexican style	1985	142	48	4b	Past	Poor hygiene; inadequately pasteurised milk	Linnan et al. (1988)
Switzerland	Vacherin Mont d'Or	1983/87	122	34	4b	Raw	Plant closed down	Bille (1990)
Enteropathogenic *E. coli*								
US	Aged Gouda	2010	41		O157:H7	Raw	Poor hygiene	McCollum et al. (2012)
France	Fresh Goats' cheese	2004	3		O157	Raw		Espié et al. (2006)
Canada	Gouda	2002	13		O157:H7	Raw	Minor sanitation problems	Honish et al. (2005)
US	Fresh curds	1998	8		O157:H7	Raw	Curd incorrectly labelled as pasteurised	Durch et al. (2000)
UK	Hard cheese	1997	2		O157	Raw		Anon (1997b)
France	Fromage frais	1992	4	1	VTEC	Raw	Mixture of cows' and goats' milk; isolate was VTEC but not O157:H7	Deschenes et al. (1996)
US	French Brie	1983	169		O27:H20		Coliform numbers ranged from 10^2 to 10^8/g of cheese	MacDonald et al. (1985)
Salmonella enterica								
France	Goats' cheese	2008	25		Münster	Raw	Artisanal producer	van Cauteren et al. (2009)
US	Mexican-style cheese	2007	2		Typhimuium	Raw	Poor hygiene; bird & rodent infestation; unlicensed producer	Lind et al. (2007)
US	Mexican-style cheese	2006/07	85		Newport	Raw	Illegal sale of raw milk to grocery store where cheese was made	Austin et al. (2008)

Netherlands	Hard cheese	2006	224		Typhimurium	Raw	Poor hygiene; artisanal producer; 4.2 salmonella per kg in two cheeses; four cows excreting in faeces	van Duynhoven et al. (2009)
France	Washed-rind cheese	2006	23		Montevideo	Raw	Milk from one farm contaminated; origin of contamination not identified	Dominguez et al. (2009)
Switzerland	Soft cheese	2006	82		Stanley	Thermised		Pastore et al. 2008
France	Cantal	2001	199		Enteridiidis	Raw	One manufacturer who knew cheese contained salmonella; cows excreting	Haeghebaert et al. (2003)
Austria	Alpine farmhouse cheese	1999	16		Oranienberg	Raw	Poor hygiene; cooking temperature 46–48 °C; pig and chicken faeces incriminated	Allerberger et al. (2000)
Italy	Mascarpone	1998	9		Enteridiidis		Actual cause was tiarmisu held at RT for 1 day before eating	Panico et al. (1999)
Canada	Cheese in a lunch pack	1998	800		Enteridiidis	Raw		Ratnam et al. (1999)
France	Morbier	1997	113		Typhimurium	Raw	No deficiencies in hygiene	deValk et al. (2000)
US	Mexican-style cheese	1997	54		Typhimurium	Raw	Poor hygiene; raw milk stored overnight	Villar et al. (1999)
US	Mexican-style cheese	1997	110		Copenhagen	Raw	Two outbreaks; home-made cheese mainly sold by street vendors	Cody et al. (1999)
Canada	Koch Kase	1994	82		Berta	Raw	Poor hygiene; cross contamination from chickens	Ellis et al. (1998)
Switzerland/ France	Cows' milk cheese	1995	25	5	Dublin	Raw		Vaillant et al. (1996)
UK	Cheddar	1994	>84		Gold-coast	Past	Pasteurisation failure; recovered from a dairy herd supplying the factory	Anon (1997a)

(continued)

Table 19.1 (continued)

Country of origin	Variety of cheese	Year	No. of cases	No. of deaths	Sero-group	Milk	Comments	Reference
France	Goats' milk cheese	1993	273	1	Paratyphi B	Raw	1/40 farm milks were positive for salmonella	Desenclos et al. (1996)
US	Mozzarella	1989	164		Javiana & Oranienberg	Past	Poor hygiene; two cheeses contained 0.36 and 4.3 salmonella/100 g	Hedberg et al. (1992)
UK	Irish soft cheese	1989	42		Dublin	Raw	Artisanal producer	Maguire et al. (1992)
Switzerland/ France	Vacherin Mont d'Or	1985	>10		Typhimurium	Raw	Poor hygiene; hand-borne contamination from piglet carriers	Sadik et al. (1986)
Canada	Cheddar	1984	>1500	1	Typhimurium	Past/Therm	Salmonella levels ranged from 0.36 to 9.3/100 g cheese; pH of cheese ranged from 4.97 to 5.40.	D'Aoust et al. (1985)
Canada	Cheddar	1982			Münster	Raw	One cow shedding 200 salmonella/ml of milk	Wood et al. (1984)
US	Cheddar	1976	339		Heidelberg	Past	Poor hygiene in milking, storage of milk and manufacture of cheese	Fontaine et al. (1980)
Staphylococcus aureus								
Greece	Grated cheese (variety not specified)	2005	>600				*S. aureus* ranged from 10^3 to 10^4 cfu/g in the two samples of cheese tested; enterotoxins not measured	Jelastopulu et al. (2006)
UK	Sheeps' milk cheese	1984	>13				Poor hygiene; artisanal cheese;	Bone et al. (1989)
UK	Cheddar	1983	2					Sharpe (1987)
Canada	Cheese curd	1980	62					Todd et al. (1981)

Other microorganisms								
US	Soft cheese	2007	19		*C. jejuni*	Raw	No starter used	Hunt et al. (2009)
Italy	Mascarpone	1996	8	1	*Cl. botulinum*		pH, a_w and E_h were conducive to growth of *Cl. botulinum*	Aureli et al. (1996)
Malta	Soft cheese	1995	135	1	*Br. melitensis*	Raw		Anon (1995)
Greece	Home-made, unripened	1983	23		*Brucella*			Sharpe (1987)
US	Queso Blanco	1983	16		*Str. zooepidemicus*		Home-made cheese from a 7 cow herd	Espinosa et al. (1983)
Scandinavia	Brie from France	1982	>50		*Shigella sonnei*			Sharpe (1987)

tained from <0.3 to 4.6×10^7 *L. monocytogenes*/g of cheese. In the Austrian/German outbreak, 11 samples of cheese had <100 cfu of *L. monocytogenes*/g and 9 had >100/g while one sample contained $>2 \times 10^6$ *L. monocytogenes*/g. Poor hygiene was incriminated in 13 of the outbreaks.

The EU criteria for the levels of various pathogens in cheese, made from raw and pasteurised milk, are summarised in Table 19.2. These criteria apply at different stages of the production process, e.g., at the time during manufacture when the count is expected to be highest, at the end of manufacture or when the product is placed on the market; in the case of listeria, different standards apply whether the cheese is ready to eat or whether it has left the manufacturing premises. There are standards for coagulase-positive staphylococci and salmonella but not for *E. coli* in raw milk cheeses.

19.1 Pathogens in Cheese

Cheeses can be hard, semi-hard or soft, mainly reflecting an increasing level of moisture in the cheese and consequently a higher a_w value. In addition, in many surface-ripened cheeses, the pH, particularly at the surface, increases during ripening and many of them are frequently handled during ripening, e.g., smear cheeses. Consequently, such cheeses are more prone to growth of pathogens.

Recent surveys of the microbiological quality of cheeses at retail show that the level of pathogens in cheese is very low. None of 512 retail cheeses in Ireland (Anon 2004) contained salmonella, 1 sample contained *L. monocytogenes* at 5.7×10^3 cfu/g, 3 samples had *E. coli* counts between 10^4 and 10^5 cfu/g and 16 samples had *S. aureus* counts $>10^4$ cfu/g. In a UK survey (Little et al. 2008) of 1819 retail samples of cheese made from raw or thermised milk and 2618 samples of cheese made from pasteurised milk, for the four common pathogens, 96 % were of satisfactory microbiological quality, 2 % were of borderline quality and a further 2 % were of unsatisfactory quality, due to high levels of *S. aureus* (13 samples, ranging from 1.6×10^5 to $>10^7$ cfu/g) and/or *E. coli* (25 samples, ranging from 1.1×10^5 to 4.6×10^6 cfu/g) and/or *L. monocytogenes* (present in one sample at >100 cfu/g). Salmonella were not found in any sample. None of 41 aged, raw milk US cheeses contained *E. coli* O157, *L. monocytogenes*, *Salmonella* or *Campylobacter* (Brooks et al. 2012). Coliform levels were extremely low in most cases and two samples contained ~200 *S. aureus*/g and one >30,000 cfu/g.

Pathogens in raw milk have been discussed in Chap. 5.

19.1.1 Listeriosis

Listeriosis is caused by *L. monocytogenes* and contamination of cheese with this organism is a serious problem since many outbreaks involve fatalities (Table 19.1); the main serotypes involved were the 1/2a and 1/2b types. Listeriosis mainly affects

Table 19.2 European Commission Regulations for the microbiological criteria for foods (Anon 2004)

Category	Microorganism	Sampling plan		Limits		Stage where the criterion applies
		n	c	m	M	
Ready-to-eat foods able to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes	*Listeria monocytogenes*	5	0	100 cfu/g (5)		Products placed on the market during their shelf-life
		5	0	Absent in 25 g		Before the food has left the immediate control of the food business operator, who has produced it
Cheeses, butter, cream made from raw milk or milk that has undergone a lower heat treatment than pasteurisation	Salmonella	5	0	Absent in 25 g		Products placed on the market during their shelf-life
Cheeses, milk powder and whey powder	Staphylococcal enterotoxins	5	0	Not detected in 25 g		Products placed on the market during their shelf-life
Cheeses made from milk or whey that has undergone heat treatment	*E. coli*	5	2	100 cfu/g	1000 cfu/g	At the time during the manufacturing process when the *E. coli* count is expected to be highest
Cheeses made from raw milk	Coagulase-positive staphylococci	5	2	10,000 cfu/g	100,000 cfu/g	At the time during the manufacturing process when the number of staphylococci is expected to be highest
Cheeses made from milk that has undergone a lower treatment than pasteurisation and ripened cheese made from milk or whey that has undergone pasteurisation or a stronger heat treatment	Coagulase-positive staphylococci	5	2	100 cfu/g	1000 cfu/g	At the time during the manufacturing process when the number of staphylococci is expected to be highest
Unripened soft cheese (fresh cheese) made from milk or whey which has undergone pasteurisation or a stronger heat treatment	Coagulase-positive staphylococci	5	2	10 cfu/g	100 cfu/g	End of the manufacturing process

n = the number of units comprising the sample
c = the number of sample units giving values between m and M
m = the threshold value; the result is satisfactory if the number in all sample units does not exceed this value
M = the maximum value; the result is **un**satisfactory if the number exceeds M in ≥1 sample units

pregnant women, immuno-compromised people (e.g., HIV positive and patients recovering from chemotherapy after treatment for cancer) and the elderly. Prominent symptoms include vomiting and diarrhoea, which may lead to meningitis and death in the case of young children, and bacteremia. Infection of the blood stream, the central nervous system, the foetus *in utero* and infants by mothers, who show no obvious signs of infection, during birth, are common. In healthy individuals, the symptoms are usually vomiting and diarrhoea, which usually resolve themselves.

There have been several fairly major outbreaks of listeriosis due to cheese. The two most serious ones, involved >100 patients and >30 fatalities and occurred in California (Linnan et al. 1988) and Switzerland (Bille 1990). Mexican-style and Vacherin Mont d'Or cheese were implicated, respectively. Poor hygiene was a major factor in both of these outbreaks while improper pasteurisation was also implicated in the case of the Mexican-style cheese. The fact that both cheeses also had a low salt level and that the Mexican-style product is a low-acid cheese, made without the deliberate addition of a starter culture, while the Vacherin is a washed-rind variety made from raw milk, in which the pH increases during ripening, were likely to be contributory factors. The complete DNA sequence of the strain causing the listeriosis epidemic in Switzerland has been determined (Weinmaier et al. 2013) and showed 99.9 % similarity with that of the strain causing the outbreak in California. *L. monocytogenes* in cheese has also been implicated as a cause of meningitis; numbers of the organism in the cheese were 30–40 million/g (Azadian et al. 1989).

L. monocytogenes is widespread in the environment, including soil, water, silage, mud and human and animal faeces. Sometimes, the organism is found inside phagocytes (neutrophils and macrophages) in milk and this was thought to protect the cells from inactivation during pasteurisation but the general consensus now is that the organism is inactivated by pasteurisation, whether the cells are inside the phagocytes or not. It is a Gram-positive rod which can grow from –0.4 to 45 °C, at pH values of 4.4–9.4 and in the presence of 10 % NaCl. These properties make this microorganism particularly problematical, especially on surface-ripened cheeses, where the surface pH can often be >6.0 and the temperature of ripening 15 °C. At refrigeration temperatures, a one degree difference in temperature has a considerable effect on growth parameters since the generation time at 0 and 1 °C are 131 and 62 h and the lag times are 33 and 3 days, respectively. The optimum pH and temperature are 7.0 and 37 °C, respectively. The survival of listeria in adverse environments and the mechanisms involved such as biofilm formation, quorum sensing and antimicrobial resistance have been reviewed by Gandhi and Chikindas (2007).

19.1.2 Pathogenic Escherichia coli

The normal habitat of *E. coli* is animal faeces from where it can contaminate raw milk, particularly if the animals have been lying in their own dung and the udders and teats have not been properly washed before milking. However, recent studies show

that in modern milk production, little contamination of raw milk comes from faeces (Kagkli et al. 2007). Numbers of *E. coli* can exceed 10^8 cfu/g in human faeces.

Strains of *E. coli* are differentiated from each other on the basis of the serological detection of somatic (outer membrane) (O), flagellar (H) and capsular (K) antigens. At least 174 O, 56 H and 80 K antigens have been detected. Most strains of *E. coli* are harmless, commensal organisms but some are pathogenic. The latter are divided into enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and enterohaemorrhagic *E. coli* (EHEC), reflecting how they cause infection; EHEC are also called shiga toxin-producing *E. coli* (STEC) or verotoxin producing *E. coli* (VTEC) because the toxin produced by these strains is cytotoxic to African green monkey kidney (vero) cells. One of these toxins is structurally and immunologically indistinguishable from the shiga toxin of *Shigella dysenteriae*, which causes dysentery. How *E. coli* O157:H7 acquired the shigella toxin gene is not clear. The most common serotype causing foodborne disease is *E. coli* O157:H7, which is an EHEC strain. The significance of pathogenic *E. coli* in dairy products has been reviewed by Farrokh et al. (2013).

Enteropathogenic *E. coli* was first recognised as a cause of disease (infantile diarrhoea) in 1945. The onset of the disease and its symptoms are different for each of the subgroups, e.g., an average of 11 h and watery diarrhoea, low-grade fever, abdominal cramps, malaise and nausea, in the case of ETEC strains to an average of 4 days and haemorrhagic colitis (sudden onset of severe abdominal pain, grossly bloody diarrhoea and vomiting but no fever) and haemolytic uraemic syndrome (HUS), characterised by acute renal failure, haemolytic anaemia especially in young children under 5 years of age in the case of VTEC strains (Bell and Kyriakides 1998). Oral challenges, using human volunteers, suggest that 10^5–10^{10} cells of EPEC, 10^8–10^{10} ETEC, 10^8 cells of EIEC are required to produce diarrhoea. In contrast, only 5–50 cells of VTEC strains are required to produce the illness.

E. coli is a Gram-negative, rod-shaped, non-spore forming, motile or non-motile organism. *E. coli* strains normally do not tolerate low pH values but *E. coli* 0157:H7 is an exception and can grow in media adjusted to pH 4.5 with HCl but not if the pH is adjusted with lactic acid. The organism does not grow in cheese at a pH $\leq$ 5.4. *E. coli* O157:H7 has a minimum growth temperature of 8 °C, an optimum of 37 °C and a maximum of 44 °C and does not withstand pasteurisation. *E. coli* O157:H7 is unusual in being slow to ferment sorbitol and this property has been used to develop media on which to enumerate it.

Several outbreaks of food borne disease due to pathogenic *E. coli* have been traced to both soft and semi-hard cheeses; VTEC (O157:H7) strains were the dominant strain involved but an ETEC strain (O27:H20) was also implicated (Table 19.1). Several cheeses were poorly described, e.g., fresh curds, farmhouse cheese and fresh goats' milk cheese. Most were made from raw milk and in one case it is not clear whether the cheese was made from raw or pasteurised milk. Poor hygiene was incriminated in three of the outbreaks but no information was given on hygienic aspects in the other outbreaks. The ETEC strain (*E. coli* O27:H20) outbreak was a

major one involving 169 persons and two different batches of Brie, made 46 days apart, suggesting that contamination was intermittent (MacDonald et al. 1985). Another major outbreak occurred in 1971. This involved an EIEC strain (O124:B17) and three different cheeses (Camembert, Brie and Coulommiers) produced in the same plant in France, over a 2-day period (Marier et al. 1973). These cheeses were widely distributed since outbreaks occurred in 14 US states from Connecticut in the East to California in the West; 387 people developed food poisoning.

19.1.3 Salmonella

Salmonella are Gram-negative, non-spore forming rods, and their normal habitat is the intestinal tract of man, animals and birds. Like *E. coli*, salmonella, are distinguished by somatic, flagellar and occasionally capsular antigens. They have also been called after the disease they cause, e.g., *S. typhi*, the cause of typhoid, or the place they were first isolated, e.g. *S. arizona*. Serology and phage-typing are used to differentiate strains. The results of DNA hybridizations and PFGE analyses of their macrorestricted DNA suggest that there are only two species, viz., *S. bongori* and *S. enterica*, which is itself divided into six subspecies (*enterica*, *arizonae*, *diarizonae*, *houtenae*, *indica* and *salamae*). The old "species," *S. typhi*, *S. paratyphi*, *S. cholerasuis* and *S. typhimurium*, are all now considered to be *S. enterica* subsp. *enterica* (Tindall et al. 2005). Within each species and subspecies there are more than 2,500 serovars and those involved in the different outbreaks are shown in Table 19.1. The minimum and maximum temperatures permitting growth are 2 °C and 46 °C, while the minimum and maximum pH values are 3.8 and 9.5; the minimum a_w is 0.94. Thus, they may grow in cheese.

The illnesses caused by salmonella include gastroenteritis, enteric (typhoid) fever, septicaemia and chronic sequelae. Septicaemia is an infection of the blood stream while sequelae are uncommon but include arthritis, appendicitis, endocarditis, meningitis and urinary tract infections (Bell and Kyriakides 2002). Gastroenteritis has an incubation time of 12–17 h and lasts 2–7 days. The symptoms include diarrhoea, vomiting and fever. Prolonged excretion of the organism may occur. In contrast, enteric fever has an incubation time of 7–28 days and involves high fever, malaise, nausea, abdominal pain, constipation (early stages) and diarrhoea (later stages). Convalescence may take 8 weeks and the carrier state can last for months or years. The ID for gastroenteritis is usually >10,000 cells but in high-fat foods like cheese, it may be <100 cells. The ID for enteric fever is <1000 cells.

Foodborne illnesses due to salmonella were almost invariably due to raw milk cheeses. In many cases, no comments were made on hygiene but where it is reported, poor hygiene was a factor in all outbreaks except the one involving Morbier cheese (deValk et al., 2000). High numbers of salmonella in cheese are not a prerequisite to infection by salmonella (D'Aoust et al., 1985).

19.1.4 Staphylococcus aureus

In the past 30 years, *S. aureus* has been incriminated in only five foodborne outbreaks associated with cheese (Table 19.1). This is not an excuse for complacency because *S. aureus* is very salt tolerant, growing in the presence of 10 % salt, which is a much greater concentration than that found in most cheeses. The actual cause of the food poisoning is not the organism itself but a series of heat-stable, protein enterotoxins which are produced by the organism during growth in the cheese before consumption. There are nine enterotoxins, with an average MW of ~27 kDa, corresponding to 229 amino acid residues. They are called staphylococcal enterotoxin A (SEA), SEB, SEC etc., (there is no SEF). SEA and SEE are produced during exponential growth while SEB and SEC are produced during the stationary phase of growth. Those produced during the exponential phase are the more common causes of food poisoning. SEA is the most common toxin and the gene for its production is carried on a temperate phage while that for SEB is chromosomal in clinical isolates from food-poisoning cases; in other strains it is carried on a plasmid (Balaban and Rasooly 2000).

The symptoms of staphylococcal food poisoning include vomiting, nausea and diarrhoea and the time from ingestion to onset is short, ~6 h. A useful rule of thumb is that at least 10^6 cfu/g of cheese are required to cause vomiting, corresponding to the production of ~1 ng of enterotoxin/g of cheese; however, the amount required varies depending on the toxin involved, e.g., ~20 μg of staphylococcus enterotoxin B are required to cause vomiting. In a monkey assay, the emetic dose varied from ~5 to 20 ng/animal. Staphylococcal enterotoxins are not inactivated to any great extent by gastrointestinal proteases, like trypsin and pepsin; the effect of chymosin on them does not appear to have been studied but could be important in contaminated cheese ripened for a long time.

Staphylococci are Gram-positive, facultatively anaerobic, coagulase-positive cocci, which occur mainly in clumps. Staphylococci are found mainly on the skin, skin glands and mucous membranes, particularly the anterior nares, of man and other warm-blooded animals. This implies that personnel working in the milking parlour or cheese production plant are potential sources. They are also found in cheese (Chap. 11) and in soil, air and water. *S. aureus* also produces coagulase and this correlates very well with the ability to produce enterotoxins; other coagulase-positive species include *S. hyicus*, *S. intermedius*, and *S. schleiferi* subsp. *coagulans*. The virulence of *S. aureus* is impaired by growth in mixed cultures of *Lc. lactis* (Even et al. 2009).

S. aureus is the most common cause of mastitis, an inflammation of the udder, in dairy cows. Consequently, it is a common contaminant of milk and of raw milk cheese; it is inactivated by pasteurisation. About 50 % of isolates from raw milk produce enterotoxins (D'Amico and Donnelly 2011).

Many cheeses, especially surface-ripened varieties, also contain coagulase-negative staphylococci (CNS), especially *S. equorum*, *S. xylosus*, *S. saprophyticus* and *S. succinus* subsp. *casei*, as part of the natural surface microflora. These species

are generally considered not to cause disease. A recent study (Even et al. 2010) of 129 CNS including *S. equorum* and *S. xylosus*, the dominant species isolated from foods, and *S. epidermidis* and *S. saprophyticus*, the common species isolated from both food and clinical sources, showed that most strains did not contain genes encoding enterotoxins; however, 71 % of isolates possessed at least one gene encoding antibiotic resistance.

19.1.5 Mycobacterium avium subsp. paratuberculosis

This organism, commonly called MAP, causes Johne's disease (paratuberculosis) in cattle. It does not cause food poisoning *per se* but it is important to consider since there is conflicting evidence for its involvement in Crohn's disease in humans. Raw milk can become contaminated with the organism either directly in the udder or indirectly through faecal contamination. In the former case, the contamination level is low with <100 cfu/ml in symptomatic cows and 0.04–1 cfu/ml in asymptomatic cows. No viable MAP were found in 143 raw milk cheeses from Switzerland, although 4.2 % of the samples were positive using a real-time PCR method, suggesting that the organism was present in the raw milk and had been inactivated by cooking during manufacture (Stephan et al. 2007). Whether the organism survives pasteurisation is unclear with some studies showing that it does and others that it does not. MAP levels decrease in artificially contaminated cheese during ripening, with a D value (time to reduce the levels by 90 %) of 27.8, 45.5 and 90–107 days in Emmental, Tilsit and Cheddar, respectively (Spahr and Schafroth 2001; Donaghy et al. 2004).

19.2 Growth of Pathogens in Cheese During Manufacture

The major factors responsible for the control of microbial growth in cheese are the decrease in pH and in water content, the high cooking temperature of many hard cheeses and the level of salt (Chap. 11). These factors are also involved in controlling the growth of pathogens. The main reason for the low incidence of foodborne disease caused by cheese is that most milk used in making cheese is pasteurised, which kills all pathogens present in the raw milk. However, significant amounts of cheese are still made from raw milk in many countries, particularly France (~15 % of all French cheese is made from raw milk), Switzerland and Italy. Pasteurisation is normally carried out at 72 °C for 15 s but a lower heat treatment, called thermisation (e.g., 65 °C for 16–18 s), will destroy all the likely pathogenic microorganisms which are commonly found in cheese except, perhaps, *L. monocytogenes*. Such sub-pasteurisation heat treatments are used in some countries (e.g., Canada) for milk for cheesemaking. The reason for this practice is that better flavoured cheeses are produced from raw or sub-pasteurised milk compared with cheese made from fully

pasteurised milk, due to less inactivation of indigenous enzymes and bacteria present in the raw milk.

The cooking temperature during cheese manufacture can vary from 33 °C for Camembert (essentially no cooking), 36 °C for Dutch type cheese, 38 °C for Cheddar and 52–54 °C for many Swiss and Italian cheeses, and plays a major role in controlling the growth of pathogens in cheese. A temperature of 25–40 °C is conducive to the growth of pathogens, if they are present. The cooking temperature for many Swiss- and Italian-type cheeses is ~54 °C; in addition, the curd is held at this temperature for >60 min after which a slow decrease in temperature occurs in the large wheels of cheese produced (Chap. 11). These conditions kill all pathogens. This has been shown for several pathogens in Swiss Emmental (Fig. 19.1), a raw milk cheese. All of the pathogens tested decreased during cheese manufacture and none of them were subsequently recovered after 90 days of ripening (Spahr and Schafroth 2001).

Data for the growth of *E. coli* 0157:H7, *L. monocytogenes* and *S. aureus* during Cheddar cheese manufacture are shown in Fig. 19.2. *E. coli* and *S. aureus* multiplied during manufacture but *L. monocytogenes* did not. When interpreting these data, one must remember that the moisture content in the curd decreases at each stage of manufacture which will result in an apparent increase in bacterial numbers due to the concentration effect. Based on this argument, a small decrease in the number of listeria occurred during manufacture. Considerable growth of *E. coli* occurred between the beginning of manufacture and cutting the coagulum, when little acid would have been produced; no data were reported for *S. aureus* between these two

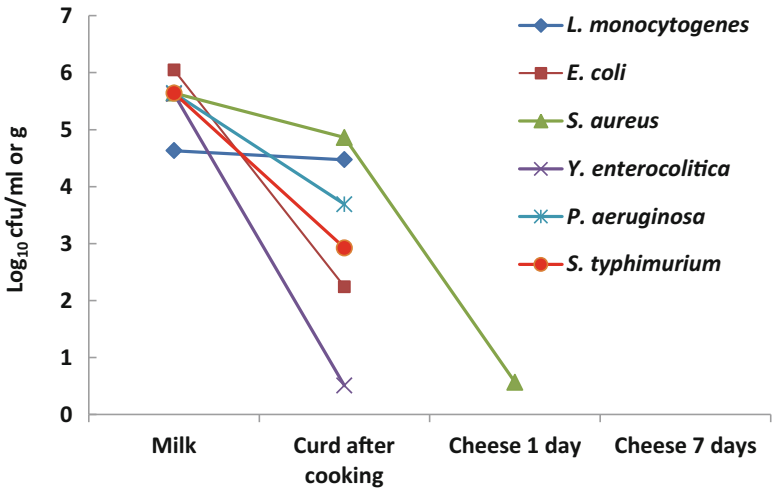


Fig. 19.1 Survival of *L. monocytogenes*, *E. coli*, *S. aureus*, *Y. enterocolitica*, *P. aeruginosa*, and *S. typhimurium* in Emmental cheese during ripening at 12 °C. None of the organisms was detected in the cheese after 1 day of ripening. *C. jejuni* and *A. hydrophila* were not detected at any stage in the milk or cheese. Redrawn from Bachmann and Spahr (1995)

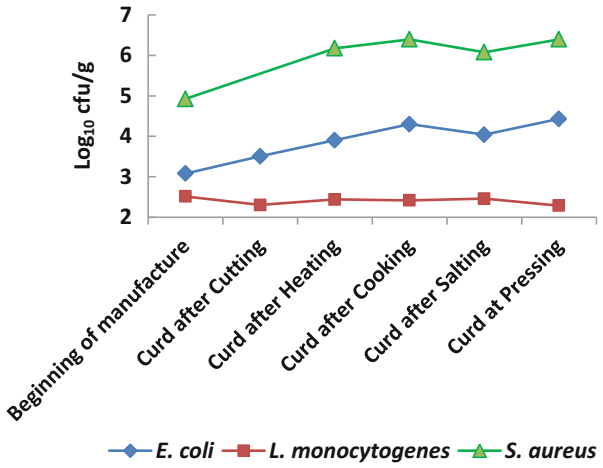


Fig. 19.2 Growth of *E. coli* 0157, *L. monocytogenes* and *S. aureus* in Cheddar cheese during manufacture. Redrawn from Reitsma and Henning (1996), Ryser and Marth (1987) and Tuckey et al. 1964)

stages, but growth of this organism is also likely to occur. These data refer to Cheddar cheese which was cooked to ~39 °C and in which acid production is relatively rapid (acid production will also have a major rate-limiting effect on growth). Growth of pathogens in the curd of most other hard varieties would probably be greater than in Cheddar, because of slower acid production.

In Belgium, an outbreak of listeriosis due to the consumption of a goats' milk cheese was shown to be associated with one of the goats in the 350 strong herd shedding milk containing 400 *L. monocytogenes*/ml (Delhalle et al. 2012). Growth of *L. monocytogenes* in the milk during cooling and in cheese made from that milk was modelled (Fig. 19.3). The milk was cooled very gradually from 39.5 °C to 10 °C overnight, a practice which is NOT to be recommended, and the number of listeria increased from log 0 to log 2.2 cfu/ml (i.e., from 1 to 160 cells/ml) during cooling the milk for 14.5 h. For cheese manufacture, a commercial starter was used, the maximum cooking temperature was 22 °C and the curd was held at this temperature for 24 h. The numbers of *L. monocytogenes* increased from log 2.2 to log 3.4 cfu/ml (i.e., from 160 to 2550 cfu/ml) during cheese manufacture and slowed down significantly once the pH had decreased to 5.5. Acid production during cheesemaking was also relatively slow, e.g., the pH of the curd, 5 h after adding the starter, was 6.2; as a comparison, Cheddar curd would have a pH 5.5, 5 h after adding the starter.

The relationships between the growth of *E. coli* O157:H7 and the development of pH and temperature during manufacture of a laboratory made, smear-ripened cheese (variety not stated), is shown in Fig. 19.4 (Maher et al. 2001). The cheese was made from raw milk and the cooking temperature was 37 °C. Growth occurred

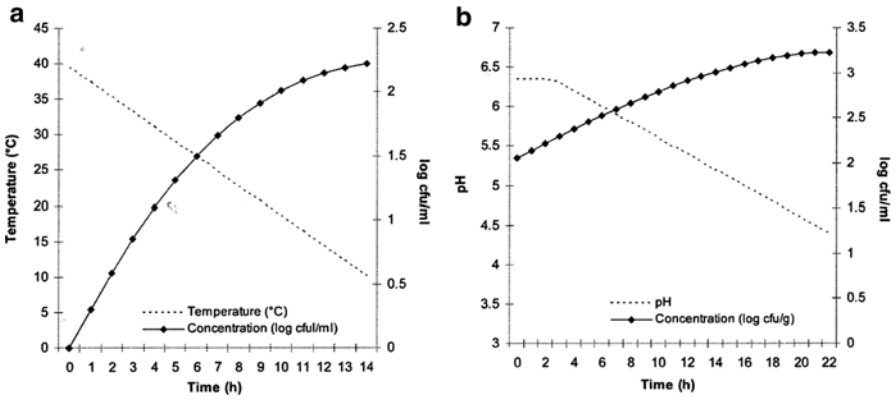


Fig. 19.3 (**a**) Growth of *L. monocytogenes* in goats' milk during gradual cooling and (**b**) growth during the manufacture of a goats' milk cheese from that milk. From Delhalle et al. (2012)

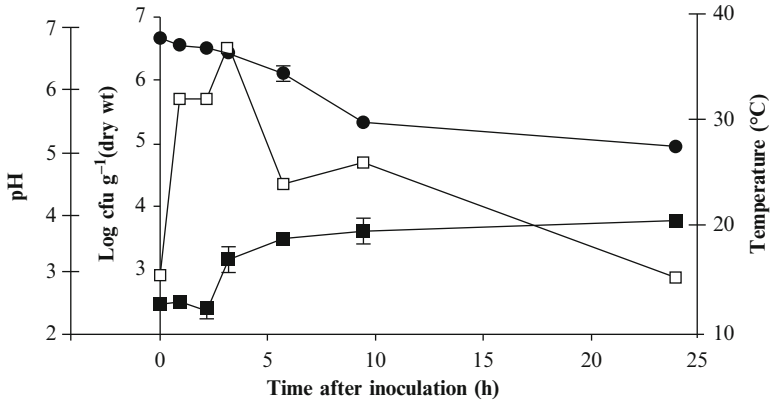


Fig. 19.4 Relationships between the growth of *E. coli* O157:H7 (*filled squares*), decrease in pH (*filled circles*) and temperature (*open squares*) of the curd during the manufacture of a smear-ripened cheese. From Maher et al. (2001)

from an initial level of 2.44log cfu/g dry weight (equivalent to 34 cfu/ml of milk) to 3.71log cfu/g dry weight in 24 h and the pH had fallen to 5.3. The number of cells was expressed as cfu/g dry weight of curd to allow for the decrease in moisture during cheese manufacture.

As already indicated (see Chap. 11), several factors are involved in the control of the growth of bacteria in cheese: pH, temperature and the level of salt are probably the most important. The same factors are involved in controlling the growth of pathogens and combinations of these so-called "hurdles" are more restrictive on bacterial growth than each individually. Several models have been developed

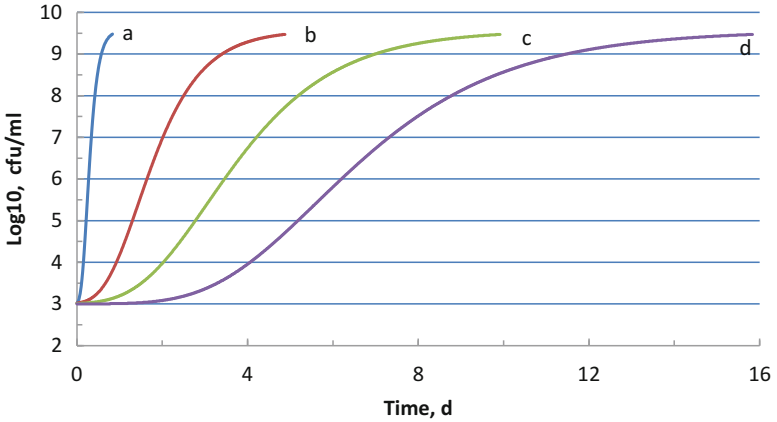


Fig. 19.5 Growth of *L. monocytogenes* under conditions simulating cheese ripening. Curve *a*, the control, is for growth at 30 °C, pH 6.5 and 0.5 % NaCl. Curve *b* is for growth at 13 °C, pH 6.5 and 0.5 % NaCl. Curve *c* is for growth at 13 °C, pH 5.4 and 0.5 % NaCl while curve *d* is for growth at 13 °C, pH 5.4 and 4.1 % NaCl, which simulate growth of a smear cheese during ripening. The data were obtained from the Pathogen Model developed by the USDA and available online at http://pmp.errc.ars.usda.gov/PMPonline.aspx

to predict the growth of pathogens in food based on their growth in different combinations of salt, temperature and pH. These predictive models have been developed mainly from experiments carried out in complex media and foods other than cheese; but the results reflect the worst case scenario in foods since growth in foods at the same temperature, salt concentration and pH value is generally less than in model systems. One of the most complete ones is that developed by the Agricultural Research Service of the USDA (http://pmp.ars.usda.gov/PMP.asps) which is simple to use and available on line. An example showing the growth of *L. monocytogenes* under different combinations of temperature, pH and salt concentration is shown in Fig. 19.5. Curve a, the control, shows rapid growth at 30 °C, pH 6.5 and 0.5 % salt, with a generation time (GT) of 0.42 h and a lag phase (LP) of 2.4 h. Reducing the temperature from 30 to 13 °C (curve b) increased the GT to 2.44 h and the LP to 15.0 h. Decreasing the temperature to 13 °C and simultaneously reducing the pH from 6.5 to 5.4 (curve c) further increased the GT to 4.90 h and the LP to 30.2 h while increasing the level of salt from 0.5 % to 4.1 % and maintaining the temperature at 13 °C and the pH at 5.4 (curve d), conditions which simulate the surface of a smear cheese during ripening, led to further increases in GT and LP of 7.31 h and 65.3 h (2.7 days), respectively. These data are from the model for the growth of *L. monocytogenes* in broth under aerobic conditions and the actual GT and LP in cheese would be much greater. Nevertheless growth would occur and in interpreting this figure one should also remember that the pH of smear cheese increases during ripening which would result in a decreased GT and a shortened LP.

19.3 Growth of Pathogens in Cheese During Ripening

What happens during ripening of the cheese depends on the variety. Each cheese is a unique microbial ecosystem and should be considered individually. Nevertheless, broad generalisation can be made. Hard and semi-hard cheeses, if made properly, are safe since almost all pathogens die off during ripening; in contrast, significant growth of pathogens can occur in surface-ripened cheeses due to the rise in pH of the surface during ripening. This mainly reflects the moisture content of the various cheeses and the increase in pH which occurs in surface-ripened cheese during ripening.

19.3.1 Hard and Semi-Hard Cheeses

The fate of several pathogens in Emmental and Cheddar cheese during ripening is shown in Figs. 19.4 and 19.6. Both Emmental and Cheddar are hard cheeses which have similar pH values (~pH 5.2) immediately after manufacture. None of the pathogens, except *S. aureus* at very low levels, was detected in the Emmental cheese within one day of manufacture, due to the high cooking temperature (~53 °C) used in the manufacture of this cheese. In Cheddar cheese, *S. aureus*, *E. faecalis*, *E. coli* and a *Salmonella* spp. all decreased during ripening at 12 °C and the Gram-negative bacteria decreased at a faster rate than the Gram-positive organisms (Fig. 19.6). One of the problems with *S. aureus* is that even though the numbers of the organism decrease significantly during ripening, high numbers may have been present during the early stages of ripening, and produce sufficient enterotoxin to cause food poisoning. The enterotoxins appear to be quite stable during cheese ripening and are not hydrolysed to any great extent by the chymosin or bacterial proteineases present

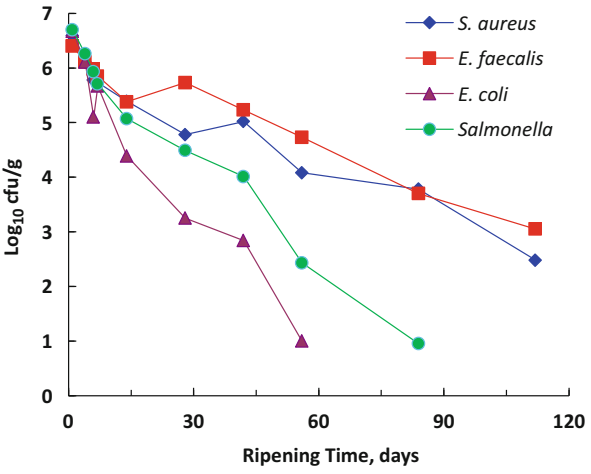


Fig. 19.6 Decrease in numbers of *S. aureus*, *E. faecalis*, *E. coli* and *Salmonella* in Cheddar cheese during ripening at 12 °C. Redrawn from Bautista and Kroll (1988)

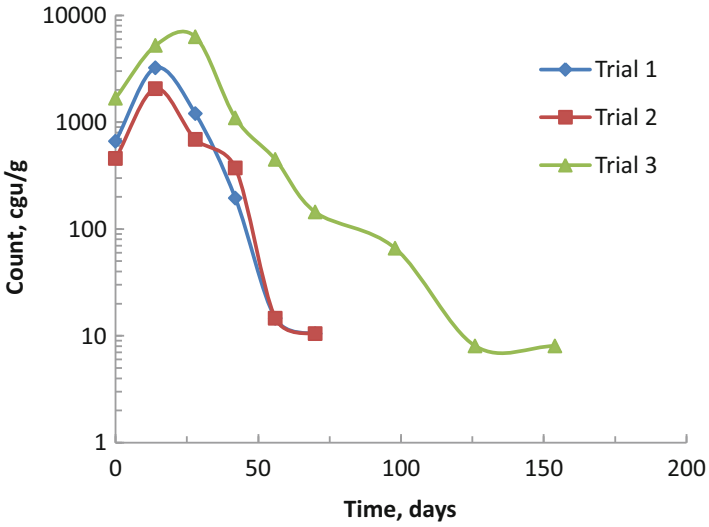


Fig. 19.7 Growth and die off of *L. monocytogenes* Scott A in three trials of Cheddar cheese during ripening at 6 °C. Redrawn from Ryser and Marth (1987)

in the cheese during ripening and may still be present in the cheese at the time of consumption. Therefore, it is possible that a cheese with a low level of *S. aureus* may contain a high level of enterotoxin.

The numbers of *L. monocytogenes* in Cheddar cheese also decrease during ripening at 6 °C (Fig. 19.7), but variation in individual trials occurs (Ryser and Marth 1987). Nevertheless significant die off occurs. There was also some variation in the rate of die off of *L. monocytogenes* in cheese ripened at 13 °C but generally the differences were small (data not shown). Recently, Dalmasso and Jordan (2014) found that *L. monocytogenes* did not grow in naturally contaminated raw milk Cheddar cheese. Very low levels of contamination were present necessitating enrichment to detect them; nevertheless, 11 different PFGE patterns were identified amongst the isolates, one of which was isolated from the farmyard, from the floor in the processing area and from the cheese, implying a possible route of contamination.

The survival of a 5 strain cocktail of *E. coli* 0157:H7 in raw milk Cheddar cheese ripened at 7 °C for 1 year was thoroughly examined by Schlesser et al. (2006) Three different levels of the organisms were used, viz., 10^5, 10^3 and 10 cfu/ml of raw milk and three trials were undertaken at each level. The results (Fig. 19.8) showed that the numbers decreased slowly at the three different levels of the cocktail used and that the US regulation that cheese made from raw milk hard cheese should be aged at >1.7 °C for 60d before releasing it to the market is not sufficient to ensure that a cheese is free of pathogens. The data for the intermediate level were variable at the beginning which affected the slope and regression coefficient; however, the slopes for the high and low levels were essentially the same, implying that the rate of decrease is the same regardless of the initial level of the cells used. Similar results

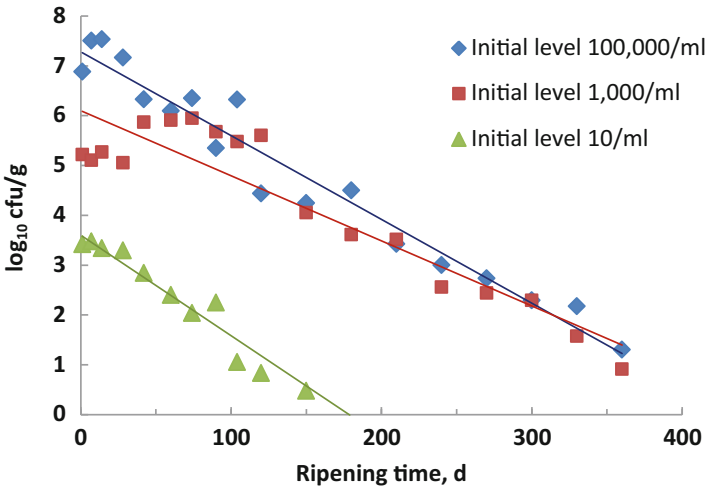


Fig. 19.8 Decrease in numbers of a 5 strain cocktail of *E. coli* O157 during the ripening of raw milk Cheddar cheese. Three different initial levels were used. Redrawn from Schlesser et al. (2006)

were found by Peng et al. (2013) for the survival of 5 non-O157 strains of *E. coli*, including three Shiga toxin-producing strains, in a semi-hard cheese, ripened at 13–14.5 °C for 16 weeks. An increase of approx 3.5 logs in the number of *E. coli* which occurred on day 1 was attributed to growth and the concentration effect. This was followed by a slow continuous decrease in all strains over the 16 week ripening period. The rate of decrease of *E. coli* in the Cheddar cheese in Fig. 19.6 is 4.5 times more rapid than that in Fig. 19.8. The reasons for this are not clear. In the US, cheeses made from raw milk must be held for a minimum of 60 days at 1.7 °C or otherwise the milk must be pasteurised. The above results show that this regulation needs to be readdressed.

In Tilsit, a semi-hard cheese, the numbers of all the pathogens tested, except *L. monocytogenes*, which remained fairly constant, decreased during ripening at 12 °C for 30 days, after which a gradual decrease of about 1 log cycle occurred over the following 2 months (Fig. 19.9). The stability of *L. monocytogenes* in this cheese was attributed to the relatively low cooking temperature and short cooking time (42 °C for 15 min), which were bacteriostatic rather than bactericidal. pH and the temperature of ripening may also be important; the pH increased from 5.2 at day 1 to 5.8 at day 90 during ripening; commercial samples normally have a pH of ~6.2 at 90 days. *L. monocytogenes* can grow over a wide range of growth temperature, from −1 to 45 °C, and this property may also be important for its survival in Tilsit cheese.

The pH of the cheese is also critical; data for salmonella in Cheddar cheese are shown in Fig. 19.10. At pH 5.03 and 5.23, salmonella died off quite rapidly, while at pH 5.7 they did not die at all. A pH of 5.23 within 1 day of manufacture is typical of a well-made Cheddar while a pH of 5.7 could indicate poor starter activity, either as a result of phage contamination or antibiotic residues in the milk or a combination of both.

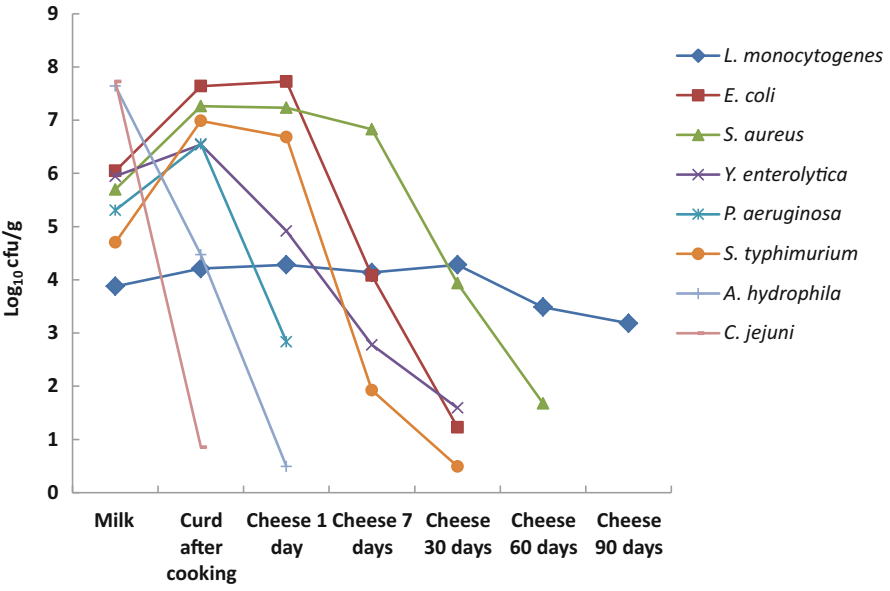


Fig. 19.9 Growth of *L. monocytogenes*, *E. coli*, *S. aureus*, *Y. enterocolitica*, *P. aeruginosa*, *S. typhimurium*, *A. hydrophila* and *C. jejuni* in Tilsit cheese during ripening at 12 °C. Redrawn from Bachmann and Spahr (1995)

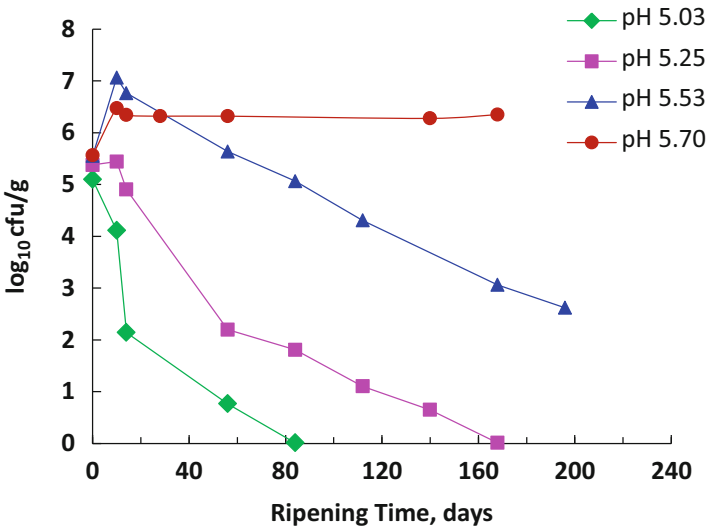


Fig. 19.10 Effect of pH on the survival of *Salmonella* in Cheddar cheese during ripening. Redrawn from Hargrove et al. (1969)

19.3.2 Surface-Ripened and Soft Cheeses

The situation in surface-ripened varieties like Camembert, Brie and Limburger, is quite different and many pathogens can grow readily in such cheeses. The reasons for this are:

- these cheeses have a relatively high moisture content
- they are ripened at a temperature (10–15 °C) at which significant bacterial growth can occur over a relatively short ripening time
- the pH increases during ripening, especially on the surface due to the metabolism of lactate by the surface microflora to a point where growth of bacterial contaminants can occur.

The growth/non-growth of *L. monocytogenes*, *Hafnia* strain 14–1, enteropathogenic *E. coli* and *Enterobacter aerogenes* in Camembert cheese during manufacture is shown in Fig. 19.11. *Hafnia* are closely related to coliform bacteria and only one species, *H. alvei*, which occurs in water and in human and animal faeces, is recognised. The numbers of *L. monocytogenes* decreased initially during ripening but increased again once the pH rose above 6, reaching final cell numbers of 10^8 cfu/g. The increase also took place in the interior of the cheese but not to the same extent, because the pH increased more slowly in the interior than in the exterior of the cheese (data not shown). In contrast, the numbers of *E. coli* and *Ent. aerogenes* decreased during ripening. This is probably true for all coliforms but *Hafnia* strain 14–1 is an exception to this rule. Numbers of *Hafnia* strain 14–1 increased during the early weeks of ripening and then remained constant. The rate of increase in the pH of the 4 Camembert cheeses varied. This was probably due to differences in manufacturing procedures and differences in the rates of growth of the different strains of yeast and *P. camemberti* used in cheesemaking. Of course, it is the combined effect of the temperature of ripening, the salt concentration and the decrease in pH that really determine the extent of growth of pathogens.

A pH gradient exists in surface-ripened cheese, with the pH of the surface being higher than that in the interior of the cheese. This also affects the growth of pathogens. An example is shown in Fig. 19.12 where numbers of *E. coli* O157 decreased faster (D value 7 days) on the surface than in the interior of the cheese (D value 14 days). This may be due to bacteriocin production by some of the adventitious flora on the cheese surface, particularly *Staph. epidermidis* and *Staph. warneri* (Gori et al. 2010). In this figure the time the cheese enters the market and its best-before date are also indicated.

There is little information on the growth of pathogens in soft, non-surface-ripened varieties. Hicks and Lund (1991) found that the numbers of *L. monocytogenes* in artificially contaminated cheese decreased during ripening and the decrease was lowest in the cheese of highest pH. Blue cheese is another soft, non-surface-ripened variety but the pH increases from about 4.6 at day 1 to 6.2 after 10 days due probably to metabolism of lactate by *P. roqueforti*; *L monocytogenes* dies out during ripening. The death of listeria in Blue cheese has been attributed to inhibition of

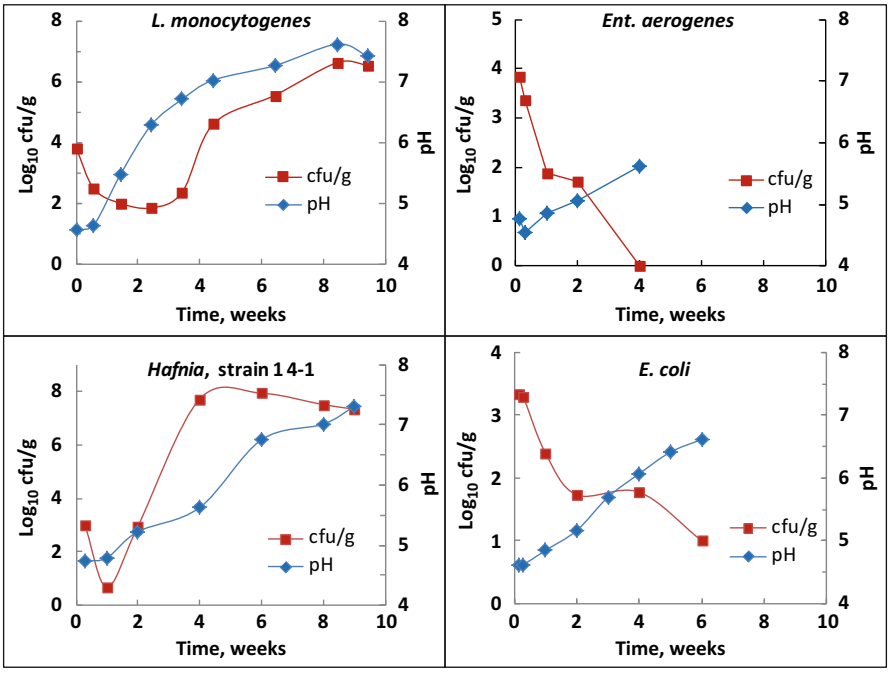


Fig. 19.11 Growth of *Listeria monocytogenes*, *Enterobacter aerogenes*, *Escherichia coli*, and *Hafnia* strain 14.1 and the increase in pH in Camembert cheese during ripening. Redrawn from Frank et al. (1977), Rutzinski et al. (1979) and Ryser and Marth (1987)

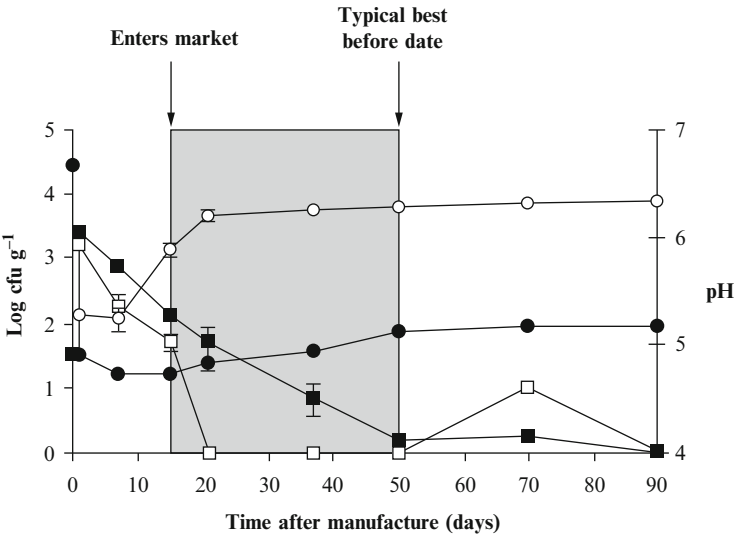


Fig. 19.12 Behaviour of *E. coli* O157:H7 on the rind (*open squares*) and in the core (*filled squares*) and the development of pH in the rind (*open circles*) and the core (*filled circles*) during ripening of a smear-ripened cheese. Cheeses were ripened at 15 °C for 18 days and then at 6 °C for the remaining time. The points at which the cheese is put on the market and a typical best-before date are indicated. From Maher et al. (2001)

their growth by the high level of salt (~10 % S:M) in these cheeses. The growth of other pathogens on the surface of soft cheeses does not appear to have been investigated.

Cheese brines should be considered as potential sources of pathogens, especially since salmonella and *E. coli* O157:H7 survive for several weeks in both model and commercial brines (Ingham et al. 2000).

19.4 Raw Milk Cheeses

Many great cheeses, e.g., Parmigiano Reggiano, Grana Padano and Swiss Emmental are made exclusively from raw milk. In these cheeses the cooking temperature is ~54 °C and this has a significant effect on inactivating any pathogens that might be present (Fig. 19.1). About 15 % of the cheeses made in France are also made from raw milk, including Comtè, which is also cooked to a high temperature, but also Roquefort, Reblochon, Brie de Meaux and Camembert de Normandie, which are cooked to ~35 °C. The latter cheeses also have high moisture contents and so are more prone to the growth of any pathogen that might be present in the raw milk.

Cheese made from raw milk has a much better taste than the same cheese made from pasteurised milk because no inactivation of the indigenous enzymes in the milk occurs and some bacteria, particularly LAB and PAB, present in the raw milk play a positive role in ripening. This is an important marketing advantage for raw milk cheeses. Nevertheless, it is clear from the foregoing that soft cheeses can be problematic and those made from raw milk particularly so. *S. aureus* is a common cause of mastitis in dairy cows and, therefore, is probably present in most raw milks. Many *S. aureus* strains present in raw milk produce enterotoxin. *E coli* 0157:H7 is also likely to be present in raw milk, as its major source is bovine faeces. Despite this, relatively few foodborne outbreaks have been traced to cheese containing *E. coli* 0157:H7 (Table 19.1). Small numbers of *L. monocytogenes* may also be present in raw milk. *S. aureus*, *E. coli* and *L. monocytogenes* will grow during cheese manufacture and create potential problems in cheese made from raw milk. In addition, soft mould- and smear-ripened cheeses with a high moisture content and in which the pH increases, especially on the surface, during ripening, are potentially hazardous, especially when they are made from raw milk.

Donnelly (2001) has comprehensively reviewed the outbreaks of foodborne illness due to cheese made from raw milk and concluded that confounding parameters other than the use of raw milk contributed to the presence of pathogens in the majority of cheese-related outbreaks of human illness. This author also stated that there are no compelling data to indicate that mandatory pasteurisation would lead to a safer product, especially since post-pasteurisation contamination of aged cheese accounts for much of the documented contamination of cheese.

There is a need for stringent control of the microbiological quality of the milk for cheesemaking, especially where raw milk cheese is being made. To produce a good quality raw milk cheese, free from pathogens, the raw milk should be:

- of good quality, with a bacterial count <20,000 cfu/ml and a somatic cell count <400,000/ml
- produced under extremely hygienic conditions
- free of pathogens
- held at a low temperatures (<4 °C) until cheesemaking begins
- made with an active (fast acid-producing) starter

In addition, good quality control procedures should be in place, including a Hazard Analysis and Critical Control Point (HACCP) plan. Key critical control points in any HACCP plan should include the temperature to which the milk is cooled and the time it took to reach this temperature, the milk storage time (1 day at most) and the pH at a predetermined time after starter addition, e.g., 6–10 h, to indicate how active the starter is. Comparisons of the latter data will allow one to determine if starter activity is normal.

In the EU standards for cheese (Anon 2004), a distinction is made between soft cheeses produced from raw milk and those made from pasteurised milk, with more stringent standards being set for the former (Table 19.2). This reflects the fact that pasteurisation inactivates all pathogens likely to be present in the milk.

19.5 Control of Growth of Pathogens

Pathogens arise mainly in milk, from the udder, e.g., *S. aureus*, as the cause of mastitis, or from contamination by dung, e.g., salmonella and *E. coli* or from the environment e.g., *L. monocytogenes*. The criteria for producing a pasteurised milk cheese are the same as those for producing raw milk cheese. Personnel involved in milking and cheesemaking may also be a source of pathogens. To reduce contamination of the milk and cheese one must pay particular attention to hygiene in production and storage. To-day, much cheese is made in automated systems but small-scale artisanal production involves manual manipulation of the curd during manufacture, moulding and ripening. Good hygiene is critical at each of these steps. Environmental sampling, particularly for listeria, should be practiced, especially cheese contact surfaces, drains, seals and brines. Implementation of HAACP systems is also effective in reducing the growth of pathogens in cheese. The use of an active, phage-free starter, pasteurisation and determining the pH of every batch of cheese at a pre-set time after starter inoculation e.g. 6–10 h, respectively, particularly in the case of soft cheeses, are major critical control points.

If an active starter is used, the pH will decrease quicker and the growth of pathogens will not be as rapid; the reverse is also true. If growth of the starter is slow, due to phage contamination and/or antibiotic residues in the milk, considerable growth of pathogens can occur. Therefore, a fast acid-producing starter is one of the best

ways of controlling the growth of pathogens in cheese. The growth of most pathogens will eventually cease due to the decrease in pH as significant amounts of lactic acid are produced.

Soft cheeses are small and will cool quickly; therefore, keeping the ambient temperature high is important when the cheese is in the moulds, e.g., by covering them with a clean towel, to promote acid production by the starter.

Good hygiene is also important, particularly in smear cheeses, and especially where the old-young method of smearing is used to inoculate fresh cheeses. Old smear can often be contaminated with *L. monocytogenes* and all cheeses are therefore potentially infected. Efforts are being made to develop defined-strain smear starters to overcome this problem. Much attention is being focused on identifying smear bacteria which produce bacteriocins active against *L. monocytogenes* and applying them directly to the cheese surface. *Lb. plantarum* WHE 92, also known as ALC (anti-listerial culture) 01, which produces pediocin AcH, has been shown to be effective in controlling the growth of *L. monocytogenes* in model cheeses (Loessner et al. 2003). This strain was isolated originally from an artisanal Münster cheese which showed low levels of contamination by *L. monocytogenes*. The activity spectrum of the bacteriocin is relatively wide and it lyses susceptible cells by binding to the cytoplasmic membrane, inserting the bacteriocin molecule into the membrane and forming a poration complex, which leads to dissipation of the proton motive force. *Enterococcus faecium* WHE 81, isolated from the same source, has also shown potential to inhibit the growth of *L. monocytogenes* on the cheese surface (Izquierdo et al. 2009). Recently, three strains of facultatively anaerobic halophilic and alkaliphilic bacteria, viz., *Alkalibacterium kapli*, *Marinilactobacillus psychrotolerans and Faclamia tabacinasalis*, isolated from the smear of Racclette cheese, have been shown to exert strong anti-listerial activity in situ on cheese (Roth et al. 2011).

Starter bacteria have also been evaluated. Application of a strain of *Lc. lactis* producing Lactocin 3147 reduced the numbers of *L. moncytogenes* in artificially contaminated cheese 1000-fold compared with the control cheese (O'Sullivan et al. 2006). In addition, efforts are being made to identify yeasts with anti-listerial activity (Goerges et al. 2006). In this case, much greater inhibition occurred on agar plates than on cheese. This was thought to be due to reduced expression of the inhibitory substance by the lactate present in the cheese.

An interesting experiment was conducted by Guillier et al. (2008). Co-cultures of harvested biofilms from wooden shelves, used in smear cheese production, and 2 strains of *L. monocytogenes* were conducted on glass fibre filters deposited on sterile smear cheese models, incubated at 15 °C and 98 % RH, to simulate smear cheese production. Growth of each listerial strain stopped as soon as the biofilm bacteria entered the stationary phase of growth. No inhibitors of listerial growth were found and it was suggested that the inhibition was due to nutrient exhaustion by the bacteria present in the biofilm.

The use of broad host range phage (1×10^9 phage particles) has also been shown to be useful in reducing the numbers of *L. monocytogenes* and salmonella on com-

mercial Limburger and Camembert cheese by at least 3 log cycles; repeated application of the phage further delayed re-growth (Modi et al. 2001; Guenther and Loessner 2011). Such products are available commercially.

19.6 Stresses and Survival of Pathogens

When bacteria are exposed to mild stresses, they can adapt, become resistant to, and survive under harsher conditions. These are called stress responses and the important ones in cheese are the acid and salt (osmotic) stress responses; in addition the heat shock stress response is likely to operate in cheeses like Emmental and Parmigiano Reggiano which are cooked to high temperatures. All of these are important in determining the ability of foodborne pathogens to survive in foods. The molecular mechanisms involved in the stress responses in *L. monocytogenes* and *E. coli* have been reviewed by Gandhi and Chikindas (2007) and Peng et al. (2011), respectively.

In *E. coli*, the master regulator of the general stress response is the sigma subunit of RNA polymerase (σ^s). In an acidic environment maintenance of the proton motive force, which is important in supplying energy to the cell, can become problematic. The influx of protons can disturb the intracellular pH. *E. coli* has four decarboxylase systems, glutamate, argine, lysine and ornithine decarboxylases, the function of which is to replace the carboxyl group of the substrate with a proton from the cytoplasm, generating CO_2 and the corresponding end-product, and maintain the intracellular pH. The osmotic response mechanisms involve the synthesis of compatible solutes, e.g., glycine, betaine and carnithine, outer membrane compatibility and K^+ uptake. Compatible solutes are highly soluble compounds with no net charge which can accumulate to high concentrations within a cell without affecting its function.

L. monocytogenes also has a general stress factor, called the σ^B factor (how different it is from the *E. coli* one is unclear) and produces increased levels of several proteins when exposed to acid and osmotic stress. *L. monocytogenes* easily forms biofilms, particularly in drains and on food processing equipment; however, strains may vary in their ability to form biofilms. In the biofilm, the cells are enclosed in a matrix predominantly made up of polysaccharide and the levels of at least 19 intracellular proteins are increased. Biofilms are more resistant to disinfectants and sanitizers than the free planktonic cell.

19.7 Biogenic Amines

Biogenic amines can be formed through decarboxylation of amino acids by some strains of non-starter lactic acid bacteria, particularly *Lb. buchneri*, during cheese ripening. Tyramine, produced from tyrosine is probably the most important one. These amines can cause food intoxication within a few hours of ingestion and are discussed further in Chap. 20.

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Chapter 20
Nutritional Aspects of Cheese

Y.C. O'Callaghan, T.P. O'Connor, and N.M. O'Brien

Summary Cheese is widely regarded as a nutritious food and this has contributed to its enduring popularity amongst consumers. The nutritional composition of cheese varies between different types but, in general, cheese is high in fat, saturated fat and also contains cholesterol. Conjugated linoleic acid (CLA) and phytanic acid are two fatty acids found in cheese which have reported health benefits. The protein content of cheese ranges from 3 to 40 %. Several peptides, formed by the hydrolysis of proteins during cheese production, have demonstrated biological activities including antihypertensive, antioxidant and anti-inflammatory effects. Cheese is a good source of vitamin A, riboflavin and vitamin B_{12} and, due to its high-fat content, cheese is suitable for fortification with vitamin D. Cheese is a particularly good source of bioavailable calcium and has also been successfully fortified with iron, zinc and selenium. There is an association between cheese consumption and a reduction in dental caries which has been attributed to the high content of calcium, phosphate and casein in cheese. Mycotoxins are fungal metabolites which can arise in cheese as a result of direct or indirect contamination and have been shown to be cytotoxic and carcinogenic in animals. The presence of mycotoxins can be limited by reducing the contamination of animal feedstuffs and maintaining good sanitation throughout cheese manufacture and storage. Biogenic amines, such as histamine and tyramine, are produced by decarboxylation of amino acids during the normal cheese maturation process but are more often associated with the presence of spoilage microorganisms. Histamine and tyramine may induce poisoning in susceptible individuals and their presence in cheese should be controlled.

Keywords Fat • Cholesterol • Vitamins • Dental caries • Mycotoxins • Biogenic amines

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20.1 Introduction

While the nutritional merits of any food should be considered in the context of overall dietary intake, nevertheless, it is accurate to describe cheese as a nutritious and versatile food which can play an important role in a healthy diet, as outlined in current guidelines (Table 20.1). Although per caput consumption of most dairy products has declined worldwide, cheese is a notable exception, with consumption increasing worldwide; current consumption in a number of countries is shown in Table 1.3. World cheese production is projected to increase by 19 % between 2008 and 2020 due to a continual development of the range of products available and an increase in the consumption of a western style diet (OECD-FAO 2011). The popularity of cheese is enhanced by its healthy and positive image, the variety of cheeses available and the compatibility of cheese and cheese containing products with modern trends for convenience and prepared consumer foods. Recent developments in cheese manufacturing processes have focused on the production of reduced-fat cheese and the development of cheese as a functional food. There has also been a substantial increase in the availability and variety of artisan cheeses.

Cheese is a nutrient-dense food, the precise nutritional composition of which is determined by multifactorial parameters, including the type of milk used (species, breed, stage of lactation, fat content) and the manufacturing and ripening procedures. In general, cheese is rich in the fat and casein constituents of milk which are retained in the curd during manufacture and contains relatively small amounts of the water-soluble constituents (whey proteins, lactose, water-soluble vitamins) which partition mainly into the whey. The composition of selected cheeses is shown in Table 20.2.

20.2 Fat and Cholesterol

Fat plays several important functions in cheese, e.g., it affects cheese firmness, adhesiveness, mouthfeel and flavour (see Chaps. 13, 14 and 15). It also contributes significantly to the nutritional properties of cheese as most cheeses contain significant amounts of fat. For example, a 50 g serving of Cheddar cheese provides 17 g fat, in which approximately 66 % of the fatty acids are saturated, 30 % are monounsaturated and 4 % are polyunsaturated. A typical Western diet providing 2000 kcal (8400 J) per day, with 40 % of energy derived from fat, contains approximately 88 g fat. Thus, cheese contributes a significant amount of both saturated fat and total fat to the diet.

Table 20.1 Dietary guidelines for Americans issued by the US Departments of Agriculture and Health & Human Services (2010)

• Balance calories with physical activity to manage weight
• Consume more of certain foods and nutrients such as fruits, vegetables, whole grains, fat-free and low-fat dairy products, and seafood
• Consume fewer foods with sodium (salt), saturated fats, trans fats, cholesterol, added sugars, and refined grains

Table 20.2 Composition of selected cheeses, per 100 g[a]

Cheese type	Water (g)	Protein (g)	Fat (g)	Carbohydrate (g)	Cholesterol (mg)	Energy	
						kcal	kJ
Fromage frais	48.6	19.2	26.9	Tr[b]	100	319	1323
Caerphilly	41.8	23.2	31.3	0.1	90	375	1554
Camembert	50.7	20.9	23.7	Tr[b]	75	297	1232
Cheddar (normal)	36.0	25.5	34.4	0.1	100	412	1708
Cheddar (reduced fat)	47.1	31.5	15.0	Tr[b]	43	261	1091
Cheshire	40.6	24.0	31.4	0.1	90	379	1571
Cottage cheese	79.1	13.8	3.9	2.1	13	98	413
Cream cheese	45.5	3.1	47.4	Tr[b]	95	439	1807
Danish blue	45.3	20.1	29.6	Tr[b]	75	347	1437
Edam	43.8	26.0	25.4	Tr[b]	80	333	1382
Emmental	35.7	28.7	29.7	Tr[b]	90	382	1587
Feta	56.5	15.6	20.2	1.5	70	250	1037
Fromage frais	77.9	6.8	7.1	5.7	25	113	469
Gouda	40.1	24.0	31.0	Tr[b]	100	375	1555
Gruyère	35.0	27.2	33.3	Tr[b]	100	409	1695
Mozzarella	49.8	25.1	21.0	Tr[b]	65	289	1204
Parmesan	18.4	39.4	32.7	Tr[b]	100	452	1880
Processed cheese[c]	45.7	20.8	27.0	0.9	85	330	1367
Ricotta	72.1	9.4	11.0	2.0	50	144	599
Roquefort	41.3	19.7	32.9	Tr[b]	90	375	1552
Stilton	38.6	22.7	35.5	0.1	105	411	1701

[a]Adapted from Holland et al. (1989)
[b]*Tr* trace
[c]Variety not specified

Many expert groups worldwide have issued dietary guidelines which recommend reductions in the intake of both total and saturated fat by Western populations (Table 20.1). The WHO and the 2010 Dietary Guidelines for Americans have recommended that less that 10 % of energy should be consumed as saturated fat. While some experts dispute the merit and efficacy of these guidelines, the vast body of expert opinion supports the concept that dietary intake does significantly influence the risk of chronic disease. This message has been, in large part, accepted by consumers and the food industry has responded by producing foods low in fat and cholesterol to meet market trends. A range of "light" cheese products with a reduced level of fat has been developed.

Palmitic, myristic and stearic acid are the predominant saturated fatty acids present in cheese; the principal unsaturated fatty acid is oleic acid. Blood lipid profiles and cholesterol levels are influenced by the type of fatty acid consumed and it has been found that cheese can result in a favourable lipoprotein profile (Parodi 2006). Cheese with a healthier fatty acid profile may be developed by enriching animal feeds with vegetable oils (Gómez-Cortés et al. 2009).

In addition to short-chain fatty acids, cheese and other dairy products are also a rich source of conjugated linoleic acid (CLA) and phytanic acid. CLA is a trans fatty acid found in cheese and other dairy products with reported health benefits including anti-carcinogenic, anti-atherogenic and anti-inflammatory effects. Phytanic acid is a C20 saturated fatty acid with four methyl-branches which has demonstrated the ability to enhance glucose uptake in hepatocytes and may have the ability to improve glucose-homeostasis and protect against the development of the metabolic syndrome and type-2 diabetes (Hellgren 2010).

The cholesterol content of cheese varies from approximately 10 to 100 mg/100 g, depending on the variety (Table 20.2). It is well established that dietary cholesterol intake exerts a much smaller influence than the intake of dietary saturated fat on blood cholesterol level, which is a significant risk indicator for coronary heart disease (Keys 1984). Most individuals (80 %) show little change in blood cholesterol level in response to a change in dietary cholesterol intake in the range 250–800 mg/day. However, a minority of adults do exhibit an increased level of blood cholesterol in response to increased dietary intake of cholesterol (McNamara 1987).

In recent years, there has been considerable research interest in the role of cholesterol oxidation products (COPS) in the diet in the etiology of chronic diseases. UV treatment of milk for use in the manufacture of Cheddar cheese resulted in a 36 % decrease in the cholesterol content but there was no corresponding increase in 7-ketocholesterol, the major COP formed in foods (Cilliers et al. 2014). Under normal conditions of manufacture, ripening and storage, negligible amounts of COPS are formed in cheese.

20.3 Protein and Carbohydrate

The concentration of protein in cheese varies from approximately 3–40 %, depending on the variety (Table 20.2). Cheese protein is predominantly casein, as the vast majority of the whey proteins are lost in the whey. As casein is slightly deficient in sulphur-containing amino acids, the biological value of cheese protein is slightly less than that of total milk protein. If the essential amino acid index of total milk protein is assigned a value of 100, the corresponding value for cheese protein varies from 91 to 97, depending on the variety. If whey proteins are incorporated into cheese, e.g., by use of ultrafiltration, the biological value of cheese protein is similar to that of total milk protein.

Cheese ripening typically involves the progressive breakdown of casein by indigenous milk enzymes, rennet and bacterial enzymes into water-soluble and -insoluble peptides and amino acids. This process, which is essential for the development of flavour and texture (see Chaps. 11–14), also increases the digestibility of cheese protein to almost 100 %.

Cheese and other dairy products are a source of numerous bioactive peptides with anti-microbial, anti-inflammatory, antihypertensive and antioxidant properties (Pritchard et al. 2010). Bioactive peptides are inactive within protein molecules but

may be released by digestion in the gastrointestinal tract or by the fermentation of milk with proteolytic starter cultures. The degree of maturation influences the content of bioactive peptides in cheese, therefore cheese manufacturers have the potential to optimise the content of bioactive peptides in their product.

Cheese contains only trace amounts of residual carbohydrates, primarily lactose. The residual lactose in cheese curd is, normally, fermented to lactic acid by starter bacteria during manufacture and ripening. Thus, cheese can be safely consumed by persons deficient in the intestinal enzyme, β-galactosidase, which is involved in the digestion of lactose.

20.4 Vitamins and Minerals

Since ~90 % of the milk fat is retained in the cheese curd, it follows that the fat-soluble vitamins in milk also partition into the curd. Most (80–85 %) of the vitamin A in milk fat is present in cheese fat. Conversely, most of the water-soluble vitamins in milk partition into the whey during curd manufacture. However, some microbial synthesis of B vitamins may occur in cheese during ripening. Significant quantities of vitamin B_{12} are produced in Swiss cheeses by propionic acid bacteria. The vitamin content of a range of cheeses is indicated in Table 20.3. In general, most cheeses are good sources of vitamin A, riboflavin, vitamin B_{12} and, to a lesser extent, folate. Unfortified whole milk and cheese provide only 1 % of the daily value of vitamin D which is insufficient for proper nutrition. However, cheese has a high-fat content which aids the incorporation of lipophilic vitamins and is therefore a suitable vehicle for fortification with vitamin D (Ganesan et al. 2011). Cheese contains negligible amounts of vitamin C.

Cheese is also an important source of several nutritionally important elements, including calcium, phosphorus and magnesium (Table 20.4); it is a particularly good source of bioavailable calcium, with most hard cheeses containing approximately 800 mg calcium/100 g cheese. Acid-coagulated cheeses (e.g., Cottage cheese) contain significantly lower levels of calcium than rennet-coagulated varieties. Unal et al. (2005) reported that the bioavailability of calcium from cheese is comparable to that from milk. Osteoporosis, which may lead to debilitating bone fractures, is a common condition in Western societies. While it is a disease of multifactorial etiology, there is widespread agreement that adequate calcium intake during childhood and teenage years, especially by girls, is important in assuring the development of optimum peak bone mass and reducing the risk of subsequent osteoporotic fractures. Cheese can play a positive role in the context of overall diet in supplying highly bioavailable calcium.

As discussed in Chap. 9, sodium chloride plays several important roles during cheese manufacture. The amount of salt added during the manufacture of different cheese varies significantly, resulting in large differences in the concentration of sodium in cheese (Table 20.4). There is substantial evidence that adults in Western societies consume, on average, above optimum levels of sodium. Elevated sodium intake is recognised as a risk factor for hypertension, particularly in those members of the population who are genetically salt-sensitive. Hypertension, in turn, is an important risk factor for coronary heart disease. Health authorities recommend that

Table 20.3 Vitamin content of selected cheeses, per 100 g[a] (Holland et al. 1989)

Cheese type	Retinol (μg)	Carotene (μg)	Vitamin D (μg)	Vitamin E (μg)	Thiamine (μg)	Riboflavin (μg)	Niacin (μg)	Vitamin B_6 (μg)	Vitamin B_{12} (μg)	Folate (μg)	Pantothenate (μg)	Biotin (μg)
Brie	285	210	0.20	0.84	0.04	0.43	0.43	0.15	1.0	58	0.35	5.6
Caerphilly	315	210	0.24	0.78	0.03	0.47	0.11	0.11	1.1	50	0.29	3.5
Camembert	230	315	0.18	0.65	0.05	0.52	0.96	0.22	1.1	102	0.36	7.6
Cheddar (normal)	325	225	0.26	0.53	0.03	0.40	0.07	0.10	1.1	33	0.36	3.0
Cheddar (reduced fat)	165	100	0.11	0.39	0.03	0.53	0.09	0.13	1.3	56	0.51	3.8
Cheshire	350	220	0.24	0.70	0.03	0.48	0.11	0.09	0.9	40	0.31	4.0
Cottage cheese	44	10	0.03	0.08	0.03	0.26	0.13	0.08	0.7	27	0.40	3.0
Cream cheese	385	220	0.27	1.0	0.03	0.13	0.06	0.04	0.3	11	0.27	1.6
Danish blue	280	250	0.23	0.76	0.03	0.41	0.48	0.12	1.0	50	0.53	2.7
Edam	175	150	0.19	0.48	0.03	0.35	0.07	0.09	2.1	40	0.38	1.8
Emmental	320	140	N[b]	0.44	0.05	0.35	0.10	0.09	2.0	20	0.40	3.0
Feta	220	33	0.50	0.37	0.04	0.21	0.19	0.07	1.1	23	0.36	2.4
Fromage frais	100	Tr[c]	0.05	0.02	0.04	0.40	0.13	0.10	1.4	15	N[b]	N[b]
Gouda	245	145	0.24	0.53	0.03	0.30	0.05	0.08	1.7	43	0.32	1.4
Gruyère	325	225	0.25	0.58	0.03	0.39	0.04	0.11	1.6	12	0.35	1.5
Mozzarella	240	170	0.16	0.33	0.03	0.31	0.08	0.09	2.1	19	0.25	2.2
Parmesan	345	210	0.25	0.70	0.03	0.44	0.12	0.13	1.9	12	0.43	3.3

Processed cheese[d]	270	95	0.21	0.55	0.03	0.28	0.10	0.08	0.9	18	0.31	2.3
Ricotta	185	92	N[b]	0.03	0.02	0.19	0.09	0.03	0.3	N[b]	N[b]	N[b]
Roquefort	295	10	N[b]	0.55	0.04	0.65	0.57	0.09	0.4	45	0.50	2.3
Stilton	355	185	0.27	0.61	0.03	0.43	0.49	0.16	1.0	77	0.71	3.6

[a]Adapted from Holland et al. (1989)
[b]The nutrient is present in significant quantities but there is not reliable information on the amount
[c]*Tr* trace
[d]Variety not specified

Table 20.4 Mineral content of selected cheeses, mg per 100 g[a]

Cheese type	Na	K	Ca	Mg	P	Fe	Zn
Brie	700	100	540	27	390	0.8	2.2
Caerphilly	480	91	550	20	400	0.7	3.3
Camembert	650	100	350	21	310	0.2	2.7
Cheddar (normal)	670	77	720	25	490	0.3	2.3
Cheddar (reduced fat)	670	110	840	39	620	0.2	2.8
Cheshire	550	87	560	19	400	0.3	3.3
Cottage cheese	380	89	73	9	160	0.1	0.6
Cream cheese	300	160	98	10	100	0.1	0.5
Danish blue	1260	89	500	27	370	0.2	2.0
Edam	1020	97	770	39	530	0.4	2.2
Emmental	45	89	970	35	590	0.3	4.4
Feta	1440	95	360	20	280	0.2	0.9
Fromage frais	31	110	89	8	110	0.1	0.3
Gouda	910	91	740	38	490	0.1	1.8
Gruyère	670	99	950	37	610	0.3	2.3
Mozzarella	610	75	590	27	420	0.3	1.4
Parmesan	1090	110	1200	45	810	1.1	5.3
Processed cheese[b]	1320	130	600	22	800	0.5	3.2
Ricotta	100	110	240	13	170	0.4	1.3
Roquefort	1670	91	530	33	400	0.4	1.6
Stilton	930	130	320	20	310	0.3	2.5

[a]Adapted from Holland et al. (1989)
[b]Variety not specified

sodium intake should not exceed 2000 mg per day, which corresponds to 5 g of NaCl intake (WHO 2012). However, even among populations with a high cheese intake, cheese contributes only about 5–8 % to total sodium intake.

Mechanisms for reducing the sodium content of cheese through the use of different mineral salt replacers including potassium, magnesium or calcium chloride have been investigated but they have a negative impact on the flavour of the cheese (WHO 2012).

Cheese, along with other dairy products, is a poor source of dietary iron. Iron deficiency anemia is a major worldwide nutrition-related problem, both in developed and developing countries. In an attempt to alleviate this problem, there is considerable interest in fortifying commonly-consumed foods with iron. Cheddar and processed cheeses have been fortified successfully with iron. Cheese has also been fortified with zinc which is essential for normal growth and development and has an important role in immune function (Kahraman and Ustunol 2012). The content of selenium in cheese was shown to be increased by incorporating selenium into the feed of dairy cows. Selenium deficiency is associated with cardiomyopathy and joint abnormalities and selenium supplementation is associated with a reduced risk of certain cancers (Phipps et al. 2008).

20.5 Additives in Cheese

Preservatives are added occasionally during the manufacture of certain cheeses. Growth of yeasts and moulds on hard and semi-hard cheeses may be inhibited by the addition of sorbic acid or its salts.

Nitrate may be added to milk prior to the manufacture of certain types of cheese. It is reduced to nitrite which inhibits the growth of *Clostridium* species which can cause late gas blowing and flavour defects (see Chaps. 11 and 12). However, nitrite does not persist well in cheese and the contribution by cheese to total intake of nitrite is negligible.

In recent years, there has been considerable research interest in the efficacy of natural bacterially-produced preservatives in cheese. Most interest has focused on the bacteriocins, nisin and natamycin. Nisin is a peptide produced by some strains of *Lactococcus lactis*. It has been exploited commercially in Swiss-type and processed cheeses to prevent late blowing by *Clostridium* species, the spores of which survive pasteurization. Natamycin is produced by *Streptomyces natalensis* which prevents yeast and mould contamination and surface spoilage of cheese (Koontz et al. 2003).

20.6 Cheese and Dental Caries

Dental caries are a commonly occurring problem which, in simple terms, involves degeneration of tooth enamel due to acid produced by oral microorganisms during the metabolism of sugars. The progress and extent of dental caries are influenced by a variety of dietary parameters and nutrient interactions, including the composition, texture, solubility and retentiveness of food and by its ability to stimulate saliva flow. In recent years, considerable work has been conducted on the cariostatic effects of cheese.

Early work demonstrated that dairy products reduced the development of dental caries in rats and also *in vitro*. These effects were attributed to the high concentrations of calcium and phosphate in milk and to protective effects of casein. This early work was supported by further detailed work on rats which indicated that both casein and whey proteins had protective effects, the former being the more effective, and also confirmed the protective effects of calcium and phosphate.

The first evidence that cheese had an anticariogenic effect in humans was reported by Rugg-Gunn et al. (1975). Consumption of Cheddar cheese after sweetened coffee or a sausage roll increased plaque pH, possibly due to increased output of saliva which can buffer the effect of acids formed in plaque. Some early work suggested that the consumption of cheese resulted in reduced numbers of *Streptococcus mutans,* which is involved in acid production, in the mouth. However, later work suggested that the cariostatic effects of cheese may not be related directly to effects on *Sc. mutans* but could be explained primarily by mass action effects on soluble ions, particularly calcium and phosphate (Jenkins and Harper 1983).

Further investigation of the cariostatic effects of cheese in humans was reported by de Silva et al. (1986) who measured the demineralization and hardness of enamel slabs which were fastened to a prosthetic appliance made specifically for each human subject to replace a missing lower first permanent molar. Each subject chewed 5 g of cheese immediately after rinsing his/her mouth with a 10 % (w/v) solution of sucrose. Chewing cheese resulted in a 71 % decrease in demineralization of the enamel slabs, increased plaque pH, but did not significantly affect the oral microflora compared to controls.

Further trials on humans have confirmed that the consumption of hard cheese results in significant rehardening of softened enamel surfaces (Gedalia et al. 1991; Jenkins and Hargreaves 1989). Consumption of cheese as part of a six week study increased calcium and phosphorus and pH levels in the plaque of both caries-active and caries-free human subjects (Ravishankar et al. 2012). While more research is required to define precisely the mechanism(s) involved in the cariostatic effects of cheese, it is reasonable to recommend the consumption of cheese at the end of a meal as an anticaries measure.

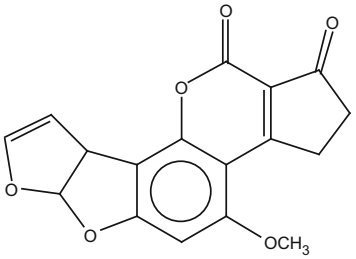
20.7 Mycotoxins

Mycotoxins (Fig. 21.1) are fungal metabolites which have been shown to be cytotoxic, mutagenic, teratogenic and carcinogenic in animals. Certain mycotoxins, e.g., aflatoxin, are among the most potent animal toxins known, hence giving rise to concerns regarding their potential effects in the human food supply.

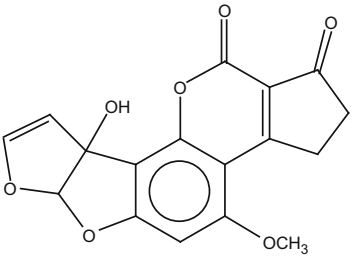
The consumption of aflatoxin-contaminated food, particularly in conjunction with hepatitis B infection, is a key risk factor for liver cancer which is the principal form of cancer reported in less developed countries. Mycotoxins may be present in milk and dairy products, such as cheese, due to indirect contamination (contamination of the cows' feedstuffs) or direct contamination (growth of mycotoxin-producing fungi in the milk or dairy products).

20.7.1 Indirect Contamination

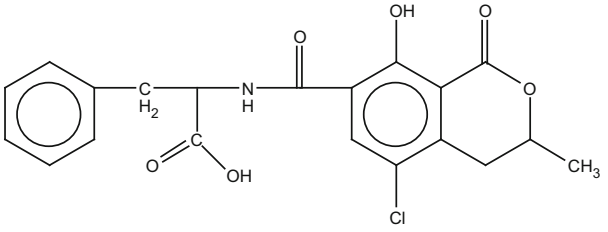
It has been known for almost 50 years that the intake of feedstuffs contaminated with aflatoxin B_1 by dairy cows may result in the excretion of toxic factors (principally aflatoxin M_1) in their milk within a few hours (Allcroft and Carnaghan 1962). On average, 1–2 % of ingested aflatoxin B_1 is excreted in milk as aflatoxin M_1. Cheese prepared from cow's milk has been associated with higher contents of aflatoxin M_1 than that of other animals possibly due to differences in their digestive systems and mechanisms of aflatoxin B_1 assimilation or as a consequence of the greater likelihood of cattle fodder contamination with aflatoxin B_1 (Anfossi et al. 2012). It has been shown subsequently that indirect contamination of milk with other mycotoxins, such as ochratoxin A, zearalenone, T-2 toxin, sterigmatocystin



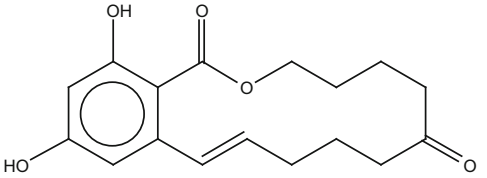
Aflatoxin B1



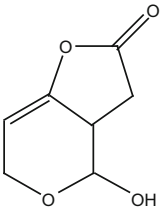
Aflatoxin M1



Ochratoxin A



Zearalenone



Patulin

Fig. 21.1 Structures of selected mycotoxins

and deoxynivalenone, does not represent a major public health issue (for a comprehensive review of mycotoxins in dairy products, see van Egmond 1989).

van Egmond (1989) summarized the results of surveillance programmes in many countries for aflatoxin M_1 in milk and milk products. The incidence and levels of aflatoxin M_1 in milk and milk products have decreased significantly over the years, which can be attributed primarily to implementation of regulatory limits on the contamination of feedstuffs with aflatoxin B_1. A notable exception was the significant increase in the level of aflatoxin M_1 in US dairy products in 1988/1989. This resulted from feeding aflatoxin B_1-contaminated maize products due to the severe drought in the US Midwest in 1988 which created ideal conditions for the growth of, and aflatoxin production by, the producing organism, *Aspergillus flavus*. Results reported in surveillance programmes for cheese have, in general, indicated that aflatoxin M_1 was not detected or occurred at concentrations below legal limits (0.2–0.25 μg/kg).

Studies have been conducted on the fate and stability of aflatoxin M_1 in milk during cheese manufacture and ripening. These studies indicate that aflatoxin M_1 partitions between the curd and whey in both acid-coagulated and rennet-coagulated cheeses and that aflatoxin M_1 is very stable during cheese manufacture. The partition coefficient for aflatoxin M_1 in water suggests that most of the toxin should partition into the whey. This anomaly can be explained by findings that aflatoxin M_1 tends to associate hydrophobically with casein micelles, resulting in a greater than expected partitioning into the cheese curd.

In general, it appears that aflatoxin M_1 is stable in several cheese varieties during ripening.

20.7.2 Production of Toxic Metabolites in Mould-Ripened Cheese

Penicillium roqueforti and *P. camemberti* are used in the manufacture of various types of blue-veined and white surface mould cheeses. These moulds can produce a range of toxic metabolites. Some strains of *P. roqueforti* can produce PR toxin, patulin, mycophenolic acid, penicillic acid, roquefortine, cyclopiazonic acid, isofumigaclavine A and B and festuclavine. *P. camemberti* strains produce cyclopiazonic acid which has been detected in commercial samples of Camembert and Brie. It occurs primarily in the rind at a level <0.5 mg/kg whole cheese but may occur at a level up to 5 mg/kg if the storage temperature is too high. However, evaluation of available toxicological data for cyclopiazonic acid, together with potential human exposure estimated from consumption data for Camembert and Brie, suggest that this metabolite causes no appreciable public health risk (Engel and Teuber 1989).

P. roqueforti can produce a range of toxins, as outlined above. Patulin, penicillic acid and PR toxin have not been detected in commercial samples of cheese. Mycophenolic acid has been detected in commercial cheese samples but at levels well below those which pose a risk to human health. Roquefortine and isofumigaclavine

A and B have been detected at low levels in commercial blue cheese and their toxicity is low. Compelling evidence that the consumption of mould-ripened cheeses is not hazardous to human health was provided by studies on rats and rainbow trout which consumed levels of mould equivalent to a daily human intake of 100 kg cheese with no apparent signs of toxicity. Mould-ripened cheeses have been consumed for several hundred years without apparent ill-effects.

20.7.3 Direct Contamination of Cheese with Mycotoxins

Cheese is a good substrate for the growth of adventitious moulds given suitable conditions of temperature, humidity and oxygen. Mycotoxin-producing moulds require oxygen for growth and hence are very unlikely to grow on vacuum-packed or wax-coated cheese, particularly if there is good plant sanitation during manufacture and handling and if the storage temperature is low.

Unintentional growth of moulds on cheese during ripening and/or storage results in financial loss, reduces consumer appeal and may necessitate trimming. However, the production of mycotoxins may also represent a health risk. Cheese on which unintentional growth of mould had occurred has been reported to contain mycotoxins that are nephrotoxic (ochratoxin A, citrinin), teratogenic (ochratoxin A, aflatoxin B_1), neurotoxic (penitrem A, cyclopiazonic acid) or carcinogenic (aflatoxin B_1 and G_1, ochratoxin A, patulin, penicillic acid, sterigmatocystin) (Ueno 1985). A tolerable weekly intake of 120 ng/kg bodyweight has been established for ochratoxin A (EFSA 2006).

Penicillium species are the mycotoxigenic fungi most frequently isolated from cheese; *Aspergillus* and other species are encountered only occasionally. However, the presence of mould growth does not imply that mycotoxins are present in cheese. A large body of work (see van Egmond 1989) has been conducted on the occurrence of mycotoxins in mouldy cheese. The overall incidence was low in most studies. Furthermore, less than 50 % of *Penicillium* species were toxigenic in animal studies. Work has also been conducted on the incidence of mycotoxins in cheeses contaminated with *Aspergillus* species. There is very little evidence that significant levels of aflatoxins are produced in cheese by these species.

Work has also been undertaken on the ability of mycotoxins to migrate from the surface of cheese into the interior. These data are significant in making decisions on whether to trim or discard mould-contaminated cheese. Some evidence has shown that patulin and ochratoxin A can be detected in the interior of a very limited number of handmade semi-hard Italian cheeses matured under certain storage conditions (Pattono et al. 2013). The Health Protection Branch of the Department of Health and Welfare, Canada, has recommended that if a hard cheese is contaminated with a patch of mould, the cheese can be salvaged by removing the infected portion to a depth of 2.5 cm.

20.8 Biogenic Amines in Cheese

The term "biogenic amines" refers to non-volatile, low molecular weight aliphatic, alicyclic or heterocyclic amines, such as histamine, tyramine, tryptamine, putrescine, cadaverine and phenylethylamine which may be present in cheese or other foods (Linares et al. 2011). In cheese, biogenic amines are produced by decarboxylation of amino acids during ripening by enzymes released by the microorganisms present. Levels produced vary as a function of ripening period and microflora, with the highest levels most likely in cheeses heavily contaminated with spoilage microorganisms. An increase in the content of histamine was detected in cheese following grating due to the higher surface/volume ratio which promotes bacterial growth (Ladero et al. 2009). Renner (1987) reported average values of histamine and tyramine in some cheeses (Table 20.5).

Consumption of foods containing significant levels of biogenic amines may cause food poisoning. However, for most individuals, consumption of even large amounts of biogenic amines does not elicit toxicity symptoms since they are converted rapidly to aldehydes by mono- and di-amine oxidases and then to carboxylic acid by oxidative deamination. However, if these enzymes are impaired, due either to a genetic defect or inhibitory drugs, toxic symptoms may result. There has been a recommendation to include non-production of biogenic amines as a condition of bacterial strains selected as starter cultures (EFSA Panel on biological hazards (BIOHAZ) 2011).

Histamine is a normal body constituent formed from histidine by a pyridoxal phosphate-dependent decarboxylase. Its concentration in blood is tightly regulated and orally administered histamine results in toxicity only following ingestion of a very high dose or impairment of histidine metabolism. Toxic symptoms generally become apparent within 3 h of ingestion and include, initially, a flushing of the face and neck, followed by an intense, throbbing headache. Other symptoms are observed occasionally, including cardiac palpitations, dizziness, faintness, rapid and weak pulse, gastrointestinal complaints, bronchospasms and respiratory distress.

Consumption of fish, particularly of the Scombroidae family, has been associated with most cases of histamine poisoning. However, some instances have been reported to be related to cheese consumption. Gouda cheese containing 85 mg histidine/100 g was

Table 20.5 Average tyramine and histamine contents of some cheese varieties[a]

Cheese variety	Tyramine, µg/g	Histamine, µg/g
Cheddar	910	110
Emmental	190	100
Blue	440	400
Edam, Gouda	210	35
Camembert, Brie	140	30
Cottage	5	5

[a]Adapted from Renner (1987)

implicated in an outbreak in the Netherlands. Incidences in the US resulting from the consumption of contaminated Swiss cheese have also been reported (Taylor et al. 1982).

Tyramine is normally present at low levels in the body. In humans, monoamine oxidase (MAO)-catalysed oxidative deamination to *p*-hydrophenylacetic acid is the main degradative pathway for tyramine. However, if a genetic deficiency of MAO exists or if MAO-inhibitory drugs are administered, toxicity symptoms may be manifest. These include a hypertensive crisis, often accompanied by severe headache and, in certain cases, intercranial hemorrhage, cardiac failure and pulmonary edema. The main dietary sources of tyramine, besides cheese, include marinated herring, dry sausages and marmite. The tyramine content of cheese is generally greater in long-ripened varieties, such as extra-mature Cheddar, than in young cheese. Patients prescribed MAO-inhibitory drugs should be advised to avoid intake of tyramine-rich foods. Tyramine poisoning in the absence of MAO-inhibitory drugs has not been reported. The toxicity threshold for tyramine has been estimated at 400 mg and, therefore, healthy individuals can tolerate intakes of large amounts of tyramine-rich cheese.

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Chapter 21
Current Legislation on Cheese

Michael Hickey

Summary It is generally accepted that cheese has been manufactured since prehistoric times as a means of preserving the main solid components of milk. Many different individual varieties evolved, which derived their names from the town, district, region, monastery and the like where they originated. As population groups migrated to another country or region, they tended to bring their cheesemaking skills with them, which they utilised in their new homelands. Oftentimes, the original manufacturing techniques were adapted to meet the needs and demands of their new homeland and thus the resultant cheeses, though retaining traditional names, are not necessarily identical to the original. Until about the middle of the nineteenth century, cheese was intended for local consumption. Then larger and more commercial cheese factories were established especially in Europe and the United States during the latter decades of that century.

Over the years, individual countries developed, and still maintain, their own national cheese legislation and standards. Some early efforts at greater harmonisation among its members were made by the International Dairy Federation, founded in 1903, with some limited success. In 1953, the Stresa Convention on the naming and compositional requirements of certain varieties of cheese was signed; however, only nine countries, all European, were involved (Austria, Belgium, Denmark, France, Italy, Netherlands, Norway, Sweden and Switzerland). It was the establishment of the Codex Alimentarius in 1963 by the Food and Agricultural Organisation of the United Nations and the World Health Organisation to develop and harmonise international food standards, guidelines and codes of practice to protect the health of consumers and ensure fair practices in the food trade, that the gave real momentum to the elaboration of international standards. Yet these standards, though important influences, have not replace the existing national legislation, which remains relevant and important.

This chapter details and reviews in a general way, the evolution and legislation on cheese in Codex Alimentarius, the European Union, the United Kingdom, Ireland, and the United States of America, and gives the titles and sources of specific cheese legislation in 11 other countries.

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Keywords Historical aspects of food legislation • Codex Alimentarius • European and US legislation on cheese

21.1 Background

Cheese evolved since prehistoric times as a means of preserving the main solid ingredients of milk. For most of its history cheese was either for home or local consumption until larger and more commercial cheese factories were established in Europe and the United States during the latter decades of the nineteenth century.

Given its local origins it should not come as a surprise that many individual varieties derived their names from a town, district, region, monastery and the like where they originated (see Table 21.1). This is not the case with all cheeses, however, for example cream cheese and cottage cheese are names of a more generic or descriptive nature. Over the centuries, as individuals or groups of individuals migrated to another country or region, they tended to bring their cheesemaking skills with them, which they utilised in their new homelands. This is particularly so as regards immigrants to North America, where cheese varieties were produced and standardised that clearly originated in their native countries or regions. Sometimes, the original manufacturing techniques were adapted, or changed, to meet the needs and demands of their new homeland and thus the resultant cheeses, while retaining traditional names, are not necessarily identical to the original.

One such example is Neufchâtel/Neufchatel cheese, where the original cheese is a ripened cheese, as produced, and indeed protected by Apellation d'Origine Contrôlée (AOC), in France, while the cheese with this name produced in the US and Canada is an unripened cream cheese; one common feature, however, is that

Table 21.1 Examples of the origin of certain cheese variety names

Cheese variety	Origin of name
Cheddar	The village of Cheddar in Somerset, England
Camembert	The commune of Camembert in Normandy, France
Emmental	The valley of the Emme river in Switzerland
Gouda	The city and municipality of Gouda near Rotterdam, the Netherlands
Gorgonzola	The commune of Gorgonzola, near Milan, in the Lombardy region of Italy
Port Salut	The Trappist Abbaye du Notre Dame du Port du Salut, Entramme in Brittany, France
Grana Padano	The Cistercian monks of Chiaravelle Abbey near Milan, Italy—with the name Padano derived from Pianura Padano, i.e., Po Plain in Italian
Parmigiano Reggiano	The Italian adjectives for Parma and Reggio Emilia in Lombardy, Italy
Neufchâtel	The town of Neufchâtel-en-Bray in the region of Haute Normandie, France
Monterey Jack	Originally made by the Mexican Franciscan friars of Monterey, California, during the nineteenth century but commercialised by David Jacks

both in France and North America this cheese variety is made from milk enriched with cream.

Local rules regarding the composition and manufacture of certain foodstuffs can be traced back to the Roman Empire and medieval Europe, the best known of which is probably the Bavarian Reinheitsgebot (the Pure Beer Law), promulgated by Duke Wilhelm IV of Bavaria on 23rd April 1516 in Ingolstadt, which specified that the only permitted ingredients for the manufacture of beer were barley, hops and water (Rieck 2008). In the twentieth century, some changes were made to the Reinheitsgebot, including a provision that yeast was a permitted ingredient in brewing, a distinction between beers brewed with "top-fermenting" yeasts and those brewed with "bottom-fermenting" yeasts, and some exceptions and variations in the law were specified that can be applied under certain circumstances. Some German beers to this day claim that they are manufactured according to the (modified) Reinheitsgebot.

By the middle of the nineteenth Century concerns about the adulteration, purity and wholesomeness of many foods led to the development of food legislation in different jurisdictions. In the United Kingdom in the 1850s there was increasing concern on the issues of food purity and food adulteration based on the identification of such issues by analysts and medical doctors. This led to the adoption of three separate pieces of legislation addressing of food adulteration, one such was the Adulteration of Food and Drugs Act, 1860 (HMSO 1860). Although this was ineffective, it paved the way for the enactment of the Sale of Food and Drugs Act 1875 (HMSO 1875). Although it was not without its critics, this Act, with subsequent amendments, enlargement and consolidation, remained in force for the next 60 years (Monier-Williams 1951). Then, in the early 1930s, a Departmental Committee on the Composition and Description of Foods was established to look into the whole area of definitions, standards, labelling and advertising. This Committee were in favour of a limited number of standards, the main aim of which would be to inform consumers of what they were purchasing (Monier-Williams 1951). Their report, in 1934, resulted in a new consolidated Sale of Food and Drugs Act, 1938 (HMSO 1938). The 1938 Act remained in place until it was replaced by the Sale of Food and Drugs Act, 1955 (HMSO 1955). Then, as the result of a number of food scares in the 1980s, due to salmonella, listeria and Bovine Spongiform Encephalopathy (BSE), the Food Safety Act, 1990 was enacted (HMSO 1990). This was a broad measure which created a more systematic structure of UK food law and tightened up on offences, enforcement powers and penalties. The first pieces of UK legislation that set standards for dairy products were the Butter and Margarine Act, 1907 (HMSO 1907) (French and Phillips 2000), the Condensed Milk Regulations, 1923 (HMSO 1923a), and the Dried Milk Regulations, 1923 (HMSO 1923b). However, as we shall see later, it was another 43 years before the UK had equivalent regulations for cheese.

Another example is that of the United States, where, from the early 1880s the U.S. Department of Agriculture (USDA) Division of Chemistry (renamed the Bureau of Chemistry in 1901), under Harvey Wiley who had been appointed its chief chemist in 1883, began researching the adulteration and misbranding of food

(and drugs) on the market. Though without any regulatory powers, the Division published its findings in a ten-part series entitled Foods and Food Adulterants. Based on these results, Wiley started to lobby for a federal law to set standards for food and drugs in interstate trade. In this, he was assisted and supported by state regulators, consumer bodies, medical doctors, pharmacists and certain journalists. Their efforts coincided with a general trend towards increased federal regulations in all matters pertinent to safeguarding public health. State laws provided varying degrees of protection against practices such as misrepresenting the ingredients of food products or medicines (Swann 2008). In the early 1900s the food industry strongly supported national food legislation in order to obtain national uniformity in regulatory requirements and to build credibility for the food supply (Porter and Earl 1992). These are the origins of the US Food and Drugs Administration, which today is responsible for approximately 80 % of food on the US market. The responsibility for remaining 20 % lies with the USDA and covers the safety, wholesomeness and labelling of meat, poultry and certain egg products.

Despite considerable debate on the issue of constitutionality surrounding the rights of the individual States, Congress enacted the Food and Drugs Act 1906 (Pub. L. No. 59-384 34 STAT. 768 (1906), sometimes called the "Wiley Act" in honour of its chief advocate (see http://www.fda.gov/opacom/laws/wileyact.htm). This act was aimed at "preventing the manufacture, sale or transportation of adulterated or misbranded or poisonous or deleterious foods, drugs, medicines and liquors, and for regulating traffic therein, and for other purposes".

Nowadays, the basis for food legislation is usually given as for ensuring food safety, providing consumer protection and promoting and facilitating fair trade. To a large extent, while the words may differ, the fundamental reasons have not really changed.

Because of the diversity of varieties and types, with local and regional variations, and indeed even among the more generic varieties, the development of international standards for cheese has proved to be a challenge. The concept of the protection of the authenticity and diversity of certain traditional food products may be traced to Article 6 of the Convention of Paris for the Protection of Industrial Property in March 1883 (Bertozzi and Panari 1993; McSweeney et al. 2004). An amended version of this Convention can still be found on the World Intellectual Property Organization (WIPO) Database of Intellectual Property (Anon. 2008b). This became the basis for the Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS), which is now administered by the World Trade Organization (for more on these matters go to http://www.wto.org/english/docs_e/legal_e/legal_e.htm#TRIPs). It probably developed from the Apellation d'Origine Contrôlée (AOC) system in the legislation of France, which evolved from the Law for the Protection of the Place of Origin in May 1919. This system specified the place (e.g., region or department) in France where certain products must be manufactured. Roquefort was the first cheese granted an AOC under the law of 26 July 1925 (Anon. 2001, 2008a) and many others followed.

The concept behind the AOC system also spread to other European countries. An important landmark was the international convention, signed at Stresa, north of

Milan, Italy, on 1 June 1951, and ratified by France, Italy, Switzerland, Austria, Denmark, Sweden and Netherlands, on the use of designations of origin and names of cheeses. This was a series of multilateral agreements and is commonly referred to as The Stresa Convention (Anon. 1952). This stated that "*only cheese manufactured or matured in traditional regions*, *by virtue of local*, *loyal and uninterrupted usages*" may benefit from designations of origin governed by national legislation. Article 1 of the Convention prohibits the use of descriptions that contravene that principle.

Further details of the Stresa Convention, and some other bilateral agreements, may be accessed (Ministero dell'Economia e delle Finanze di Italia 1954; Rieke 2004). It should be noted, however, that in an important 1988 ruling by the European Court of Justice (ECJ) in the "Deserbais" case, which related to the fat content of Edam cheese, the ECJ noted that the Stresa Convention was signed before the EEC Treaty entered into force and only some Member States were party to it, hence it did not feel that it was bound to take its requirements as limiting (ECJ 1988). Probably as a consequence of this ruling, the European Community developed and adopted regulations on Protected Designations of Origin, Protected Geographical Indications and Traditional Speciality Guarantees in 1992. These established schemes to promote and protect the names of quality agricultural products and foodstuffs throughout the Member States. These will be discussed in detail later.

Of course many individual countries developed their own national legislation and standards over the years. In the US, the Code of Federal Regulations (CFR) contains the official and complete text of departmental and agency regulations of the US Federal Government in an organized fashion in a single publication. In covering all aspects of legislation, it encompasses standards for foods, including cheese, and this will be discussed later. Such legislation on cheese shall be discussed, with particular reference to the legislation in the European Union, the United Kingdom, Ireland and the United States of America. A table will show the titles and sources of the legislation regarding cheese in Austria, Australia and New Zealand, Canada, Denmark, France, Germany, Greece, Italy, Spain and Sweden.

Before addressing national legislation, a word of warning should be given on the challenges and difficulties of using such legislation. Not alone is legislation published in the official languages of the countries concerned, which has difficulties in itself, but furthermore how that legislation is applied and administered differs from country to country. Therefore, when introducing products into the markets of countries other than one's own country, it is wise to get advice and information, ideally from competent local sources, on the principles, intent, details, interpretation and market place practices of the relevant countries. Finally, in this regard, there is the further additional challenge of ensuring that one has the most up-to-date version of the relevant legislation. This is not necessarily a simple task. In Ireland, the Foods Safety Authority of Ireland (FSAI) has a legislation section on its website and is a good and up-to-date source (see at http://www.fsai.ie/legislation/food_legislation.html). In the UK Statutory Instruments are available at http://www.legislation.gov.uk/uksi but one needs to know the number, year and title of the particular legislation. Contacting competent local sources can save a lot of time and effort in accessing

individual country legislation and dealing with the relevant challenges as regards their use and interpretation.

Now, the evolution and development of International Standards for cheese, and in particular the work done in the Codex Alimentarius, will be considered.

21.2 International Standards for Cheese Developed by the Codex Alimentarius Commission

The International Dairy Federation, which was founded in Brussels in 1903, from its early days worked on the development of standards for the major commodity milk products. In 1954, IDF highlighted the need for international agreement on terminology for milk and milk products to protect consumers and producers from misleading descriptions, with special reference to foodstuffs which sought to benefit from the recognition and good name of milk and products made from milk. In 1957, this was discussed at the 9th Session of the Conference of the Food and Agricultural Organization of the United Nations (FAO) and a resolution was agreed that its Director-General, in consultation with IDF and other interested organizations, should invite all member governments to nominate experts to a committee to develop standards for certain milk and milk products. The resultant body became known as the Joint FAO/WHO Committee of Government Experts on the Code of Principles Concerning Milk and Milk Products (the CGECPMMP) (FAO/WHO 1968b). This body remained in place until 1994 when it was re-established and renamed as the Codex Committee on Milk and Milk Products (CCMMP). The Codex Alimentarius Commission was established in 1962 to implement the joint FAO/WHO foods standards programme. Through the CGECPMMP and its successor the CCMMP, the Codex Alimentarius Commission developed, *inter alia*, international standards for cheese in general, unripened cheese and 35 individual cheese varieties between 1963 and 1978.

The original Codex General Standard for Cheese was adopted in 1963 (FAO/WHO 1968a). Work started on revision of this standard in the early 1970s. In 1978, the earlier standard was replaced. A further revision of the standard was completed in 1999 and amended in 2010 (FAO/WHO 2007b, 2011a). This contains the following definition of cheese that is relevant to all cheeses:-

> Cheese is the ripened or unripened soft, semi-hard, hard, or extra-hard product, which may be coated, and in which the whey protein/casein ratio does not exceed that of milk, obtained by:

(a) coagulating, wholly or partly the protein of milk, skimmed milk, partly skimmed milk, cream, whey cream or buttermilk, or any combination of these materials, through the action of rennet or other suitable coagulating agents, and by partly draining the whey resulting from the coagulation, while respecting the principle that cheesemaking results in the concentration of milk protein (in particular, the casein portion), and that consequently, the protein content of the cheese will

be distinctly higher than the protein level of the blend of the above milk materials from which the cheese was made; and/or

(b) processing techniques involving coagulation of the protein of milk and/or products obtained from milk which give an end-product with similar physical, chemical and organoleptic characteristics as the product defined under (a).

The definition in (a) above represents the traditional method of manufacture, while that outlined in (b) is intended to encompass and facilitate the use of evolving methods of manufacture. It should be mentioned that it is unclear how the term "similar", as used in (b), applying to characteristics of the cheese, would be interpreted in the event of any future trade disputes.

The Codex General Standard for Cheese also describes (defines) unripened cheese, including fresh cheese, as:-

> Cheese that is ready for consumption shortly after manufacture.

A Codex group standard for unripened cheese, including fresh cheese, was also elaborated and adopted in 2001 (CODEX STAN 221-2001); in addition to specific provisions contained in this standard, it is a requirement that unripened cheese should conform with the general definition for cheese outlined above. There is also a group standard for ripened cheeses in brine adopted in 1999 and amended in 2010 (CODEX STAN 208-1999, Amd-2010) (FAO/WHO 2011b).

The 35 individual cheese standards were adopted between 1963 and 1978. A review of these was commenced in the early 1990s. Following discussions that focussed on those varieties significant in international trade, 19 of the standards were deleted, due to their limited, if any, involvement in such trade (Codex Committee on Milk and Milk Products 1996, 1999). Fifteen of the remaining standards were revised thoroughly and were adopted in 2007. The 16th standard, that for Extra-hard Grating Cheese, was retained, but not revised, and one new standard (for Mozzarella) was also adopted (FAO/WHO 2007a, 2011a).

There are a number of differences between the new standards and the older ones; one such difference relates to the compositional requirements related to the fat content of certain varieties. A lower fat content is now allowed for cheeses such as Cheddar and Cream Cheese. In such cases, a reference fat level is specified and the relevant variety name may be used together with a qualifying term such as "reduced fat". Other varieties do not allow a lower fat content, e.g., Emmental, Provolone, Saint-Paulin, Brie, Camembert and Coulommiers. An absolute minimum fat content is specified for all cheeses and use of the relevant variety name is not permitted below the specified level, even when used with qualifying terms.

In 1978, three standards were adopted for processed cheese and processed cheese preparations. By the early 1990s, it was realised that the 1978 standards had become out of date due to product and process innovations and changes. The national legislation of some major producing countries reflected some of the aspects of the standards, but none had incorporated or adopted them in their entirety. For the last 20 years, Codex has endeavoured to redraft its standard(s) for Processed Cheese but has failed to make any significant progress. The growing importance of Codex Standards as a significant reference point in the WTO has contributed to this, with

countries reluctant to accept a standard that differs from their own legislative requirements. The differences outlined in discussing national legislation above, serve to outline the problems—mainly due to definitions; the need for a minimum cheese content and maximum levels of non-cheese dairy ingredients; the use of the food additives that function as stabilisers and thickeners and the related functional ingredients, starch and gelatine; and the product names that would be listed in the labelling section of the standard being among the main contentious issues.

The three Codex standards were finally revoked by the Codex Alimentarius Commission in 2010 but, at the insistence of a number of countries, work continues at Codex level to see if a revision is possible. A total of 43 member countries of Codex are involved in the present work. A number of countries, especially those in Central and South America, Africa and the Middle East are keen that there should be a Codex standard or standards, but others believe that such a standard is either not justified, or continues to prove impossible to achieve sufficient agreement to allow progress.

A list of all Codex cheese standards is given in Table 21.2 and the standards may be accessed on the Codex Alimentarius website (FAO/WHO 2014). The format of Codex standards is outlined in the Codex Procedural Manual and the sections headings are shown in Table 21.3 (FAO/WHO 2013).

Codex standards were originally intended to be adopted by the member countries of the Codex Alimentarius Commission, however, this could only be encouraged and they were not binding in law. The formal recognition of Codex Standards, as reference points for facilitating international trade and resolving disputes in the World Trade Organisation (WTO) has increased their significance, role and profile.

21.3 European Legislation Pertaining to Cheese

There is no specific European legislation on cheese or individual cheese varieties. However, in 1992 three EU schemes known as PDO (Protected Designation of Origin), PGI (Protected Geographical Indication) and TSG (Traditional Speciality Guaranteed) were elaborated to promote and protect names of quality agricultural products and foodstuffs. The original Regulation 2081/92 was replaced by Regulation 510/2006, and Regulation 1107/96 by Regulation 1898/2006. A new Regulation on quality schemes for agricultural products and foodstuffs (Regulation 1151/2012) entered into force at the beginning of 2013, replacing the earlier regulations (EU 2012). This was done to provide a simplified regime for several quality schemes by putting them under a single regulation. In addition, it aims to create a more robust framework for the protection and promotion of quality agricultural products.

The key elements of the new Regulation include:

- Providing more coherence and clarity to the EU quality schemes.
- Reinforcing the existing scheme for Protected Designations of Origin and Geographical Indications (PDOs and PGIs).

Table 21.2 List of Codex general standards for cheese and named cheese variety standards

Codex standard title	Codex standard number[a] (CODEX STAN)	Latest revision	Latest amendment
General Standard for Cheese	283-1978	1-1999	2008
Standard for Whey Cheeses	284-1971	2-2006	2010
Group Standard for Cheese in Brine	208-1999		2010
Group Standard for Unripened Cheese including Fresh Cheese	221-2001		2010
Standard for Mozzarella	262-2007		2010
Standard for Cheddar	263-1966	1-2007	2010
Standard for Danbo	264-1966	1-2007	2010
Standard for Edam	265-1966	1-2007	2010
Standard for Gouda	266-1966	1-2007	2010
Standard for Havarti	267-1966	1-2007	2010
Standard for Samsø	268-1966	1-2007	2010
Standard for Emmental	269-1967	1-2007	2010
Standard for Tilsiter	270-1968	1-2007	2010
Standard for Saint-Paulin	271-1968	1-2007	2010
Standard for Provolone	272-1968	1-2007	2010
Standard for Cottage Cheese including Creamed Cottage Cheese	273-1968	1-2007	2010
Standard for Coulommiers	274-1969	1-2007	2010
Standard for Cream Cheese	275-1973	1-2007	2010
Standard for Camembert	276-1973	1-2007	2010
Standard for Brie	277-1973	1-2007	2010
Standard for Extra-Hard Grating Cheese	278-1978		

[a]The Codex standard numbering system was revised in 2007, to have numbers only; prior to that time an A- prefix was used for general or group standards (e.g., Standard A-6 for the General Standard for Cheese), and a C- prefix for individual variety standards (e.g., Standard C-1 for Cheddar)

- Overhauling the Traditional Speciality Guaranteed scheme (TSGs).
- Setting down a new framework for the development of additional optional quality terms.
- It also creates and protects the optional quality term "mountain product".

As of May 2014, there are 181 cheeses registered as PDO (with a further 23 applications in the system) and 35 registered as PGI (with a further eight applications in the system).

The Traditional Speciality Guaranteed scheme has five registered cheeses—Mozzarella (Italy), Boerenkaas (Netherlands), Hushållsost (Sweden), Ovčí salašnícky údený syr and Ovčí hrudkový syr—salašnícky (both sheep milk cheeses from Slovakia). There are a further two cheese applications in the system for Heumilch (literally hay milk cheese from Austria), and Rögös túró (a cottage cheese from Hungary).

Table 21.3 Overall structure of sections of Codex Milk Product Standards

Section number	Section title	Sub-section number	Sub-section title
1	Scope		
2	Description		
3	Essential composition and quality factors		
		3.1	Raw materials
		3.2	Permitted ingredients
		3.3	Composition
4	Food additives		
5	Contaminants		
6	Hygiene		
7	Labelling[a]		
		7.1	Name of the food
		7.2	Declaration of milk fat
		7.3	Declaration of milk protein
		7.4	List of ingredients
		7.5	Labelling of non-retail containers
8	Methods of sampling and analysis		
Annex[b]	Annex		
Appendix[b]	Appendix		

[a]Not all labelling sub-sections are used in individual standards; those used are numbered sequentially 7.1, 7.2 etc.

[b]Annexes and Appendices are included only in certain cases and the wording is intended for voluntary application by commercial partners and not for application by governments

Under the old TSG legislation, the variety name of TSG cheeses was not necessarily protected, but use of the TSG mark was confined to cheeses that met the requirements of the system. However, under the new TSG scheme, the registration of a TSG cheese without the variety name being protected is no longer possible. To qualify for a TSG, use for at least 30 years in a domestic market is required to qualify for registration. Products already registered with the variety name protected under the old scheme will be transferred automatically to the new TSG register. For products registered without the name being protected, the relevant Member State has to request a status change before January 2016 but the product names may continue to be used until January 2023. Product names for which no transfer request is submitted will be deleted from the TSG register.

The main interest in this area is for Mozzarella, which was registered under the old TSG scheme without the name being protected. If Italy wants to apply for TSG status, under the new scheme, it must initiate the change of status procedure described above. Even if it does so, it is believed to be extremely unlikely that such an application would survive the opposition as Mozzarella that does not comply with the TSG specification is produced by several other Member States. Additionally, the administrative costs related to compliance with the TSG rules often outweigh

the economic benefits which, according to the same Commission official, make it uncertain that Italy will even apply for protection of the name Mozzarella.

As a consequence of the legislation protecting names of registered cheeses, a cheese in Europe (that is in the EU Member States and other European countries that recognise such protection by mutual agreements) could not be designated as, say, Neufchâtel, as this is a protected designation, unless it is produced in the designated region, in accordance with French legislation; however, a cheese such as Cheddar or Emmental could be so designated, under European law, unless the name of a protected designation of such varieties is used, e.g., West Country Farmhouse Cheddar, or Emmental de Savoie.

Furthermore, decisions of the European Court of Justice (ECJ) have ruled that Feta and Parmesan (i.e., Parmigiano Reggiano) are protected designations and cannot be used for cheeses other than those that are produced in accordance with the relevant requirements (ECJ 2005, 2007). Indeed the variety name Parmesan alone is not permitted in the EU.

The EU is keen to get international recognition of PDO and PGI protection to other agricultural and food products other than wine and spirits. Initial efforts through the WTO, were unsuccessful and it is understood that this led to the revision of the original EU legislation, to incorporate reciprocal arrangements with countries outside Europe. Though these efforts continue, no agreement has yet been reached; some countries are believed to be agreeable to an extension but other are strongly opposed.

European legislation addressing food hygiene, food additives, packaging materials, certain aspects related to labelling, including nutrition and health claims, contain provisions relating to cheese in general. In the EU, legislation which applies to a particular topic, such as those listed above, across most foods is often referred to as “horizontal legislation”.

21.4 EU Food Additive Legislation

The evolution of EU legislation on food additives has been a slow and difficult task. From the 1960s through to the mid-1970s, the EU established a series of basic directives addressing the use of colours, preservatives, antioxidants, emulsifiers, stabilisers and thickeners, which were amended over the years. During that time, specific additive provisions were included in EU legislation applicable to certain foods, or food groups but in other cases authorisation for their use was left to Member States. Inevitably, this led to differences between the legislative provisions of Member States, which hindered the free movement of foodstuffs in intra-Community trade; therefore, harmonisation of this area became a major priority.

With the move towards more horizontal legislation, as proposed in the White Paper on the Completion of the Internal Market in 1985, an initiative was started to address additives in a common comprehensive manner covering most foods (EU 1985). In 1988, the Flavourings Directive 88/388 (EU 1988) was adopted and this

was followed, in 1989, by the initial additive framework Directive 89/107/EEC (EU 1989). Then, in 1994 and 1995 specific additive directives were adopted addressing colours in Directive 94/36 (EU 1994b), sweeteners in Directive 94/35 (EU 1994a) as amended, and food additives other than colours and sweeteners Directive 95/2 (EU 1995), as amended. For simplicity, the latter was sometimes referred to as the Miscellaneous Additives Directive. These remained in place until a new framework regulation was adopted in 2008, followed by an amending regulation in 2011 that completed the 2008 Regulation, which specified the food additive provisions for foods grouped under the new food categorisation system (EU 2008a, 2011a). EU food additive provisions are regularly updated and it is best to consult the latest consolidated version of the 2008 Regulation, for example by using the EUR-LEX website at http://eur-lex.europa.eu/homepage.html (EU 2013).

In the consolidated legislation, the food additives permitted in cheese are listed under the relevant food categories, which are 01.7.1 unripened cheese, 01.7.2 ripened cheese, 01.7.3 edible cheese rind, 01.7.4 whey cheese, 01.7.5 processed cheese and 01.7.6 cheese products. Unfortunately, full descriptors or definitions of the food categories are not yet published and, as a consequence, it is not always fully clear under which category certain products fall. Though a similar food category system is used in Codex provisions on the use of food additives, it cannot be assumed with certainty that the EU food category system descriptors, when adopted, will follow the Codex ones.

The EU also adopted new Food Flavouring Regulation in 2008 (EU 2008b). This replaced the earlier 1988 directive mentioned earlier. As this is unlikely to have much impact on cheese, other than as regards enzyme-modified cheese, it shall not be discussed further, but it should be taken into consideration if flavourings are used in cheese.

21.5 EU Food Labelling Legislation

Since first introduced in 1979 under Directive 112/1979, European labelling requirements for foods have been replaced twice; firstly by the EU Labelling Directive 2000/13 (EU 2000), then, in late 2011, by Regulation 1169/2011 on the provision of food information to consumers (EU 2011b).

This legislation requires certain information to be provided on product labels, the main requirements being the name of the food, the net weight, a list of ingredients, information as regards the presence of specified allergens (milk being one of those specified), a date of minimum durability, indications of special conditions of storage or use, including any which relate to the date of minimum durability, the name and address of the packager or seller and the country of origin, if its absence would be likely to mislead consumers to a material degree. Regulation 1169/2011 also makes the provision of nutritional information compulsory on the labelling of food, including cheese, placed on the market or labelled after 13 December 2016. In practice many cheeses supply this information already.

A list of ingredients, a provision which has been there since 1979, and remains in the new regulation, is not required in cheese "*to which no ingredient has been added other than lactic products, food enzymes and micro-organism cultures essential to manufacture, or in the case of cheese other than fresh cheese and processed cheese the salt needed for its manufacture*." This has led to a situation where added ingredients are shown, e.g., the addition of colour to cheese, or the addition of other permitted food additives, or food ingredients in some cheeses. However, in practice, many cheeses already have full ingredient information and also follow the new requirement to have milk labelled in bold font in the ingredient list, following the new requirement of having all allergenic ingredients so listed. Indeed many cheeses already state on the label that they "*contain milk*".

In addition, Chapter IV of Annex III, Section IX of EU Regulation 853/2004 on the hygiene of foods of animal origin requires that in the case of products made from raw milk the labelling must clearly show the words "*made with raw milk*". For this purpose raw milk is defined as milk produced by the secretion of the mammary gland of farmed animals that has not been heated to more than 40 °C or undergone any treatment that has an equivalent effect (EU 2004).

Also under the requirements outlined in Article 5.1(a) and (b) and Annex II Section I of EU Regulation 853/2004, products such as cheese must be labelled with an identification mark giving the approval number of the plant where the final processing or packaging of the product took place. This requirement is for traceability purposes. In practice, this allows for the plant where the final processing of cheese is carried out to be identified from its unique identification mark. The lists of approved establishments for all Member States may be obtained by the appropriate links at http://ec.europa.eu/food/food/biosafety/establishments/list_en.htm . This website also has the links to approved establishments in Norway and Iceland, which are European Economic Area (EEA) countries, and also countries with which the EU has special agreements, namely Switzerland, Faroe Islands, Greenland and San Marino.

21.6 Cheese Legislation in the United Kingdom

The first UK cheese legislation was contained in the Cheese Regulations 1965 (HMSO 1965; Davis 1966). Actually, these regulations applied to England and Wales; Scotland and Northern Ireland had separate but identical legislation. A definition of cheese was laid down and the requirements for hard, soft and whey cheese, processed cheese and cheese spread were specified; it subdivided soft cheese into full-fat, medium-fat, low-fat and skimmed milk cheese based on cheese composition. These standards were amended on a number of occasions and in 1970 were replaced by the Cheese Regulations 1970 (HMSO 1970), which re-enacted, with some amendments, the 1965 Regulations, and so contained many of the earlier provisions. These 1970 regulations specified compositional requirements for 28 cheese varieties and variants.

Specific ingredients were allowed in cheese in general and in certain types such as soft cheese. The ingredients that could be used in various cheese types were specified and the list included food additives. European food additive legislation now supersedes this list; its interest at this time is that it may be taken as an indication of the additives in these categories that were in use at the time the legislation was in force.

The 1970 Regulations were amended on a number of occasions (HMSO 1974, 1984; The Stationery Office 1984, 1995) and were repealed by the short-lived Cream and Cheese Regulations 1995, which applied to England, Wales and Scotland (HMSO 1995). The 1995 Regulations had a new definition of cheese, retained the old definitions of processed cheese and cheese spread, and also retained compositional requirements for Cheddar, Blue Stilton and 10 other UK territorial cheeses. However, the compositional requirements for other cheeses such as Edam, Gouda, cream cheese, double cream cheese and soft cheeses were not retained. The 1995 Regulations were repealed and replaced by the Food Labelling Regulations 1996, which retained the definition of cheese and the compositional requirements of the cheeses contained in the 1995 Regulations, but did not retain the definitions of processed cheese and cheese spread (HMSO 1996). The compositional requirements for the 12 UK cheeses listed in the Food Labelling Regulations 1996 are shown in Table 21.4. For the cheeses, the composition of which is specified in the Food Labelling Regulations, the use of nutritional claims in conjunction with the variety name, such as Reduced-fat Cheddar, is not allowed at this time. However, based on nutritional concerns as regards fat intake from full-fat cheeses by the UK authorities, discussions are ongoing with industry and other groups on this subject; no agreement has yet been reached on it and the outcome remains a matter of conjecture.

Table 21.4 Cheese varieties and compositional requirements for maximum moisture and minimum fat in dry matter established in current UK Legislation (1996 Food Labelling Regulations)

1970 Cheese Regulations	1996 Food Labelling Regulations	Max. moisture	Min. fat in dry matter (FDM)
Cheddar	Cheddar	39	48
Blue Stilton	Blue Stilton	42	48
Derby	Derby	42	48
Leicester	Leicester	42	48
Cheshire	Cheshire	44	48
Dunlop	Dunlop	44	48
Gloucester	Gloucester	44	48
Double Gloucester	Double Gloucester	44	48
Caerphilly	Caerphilly	46	48
Wensleydale	Wensleydale	46	48
White Stilton	White Stilton	46	48
Lancashire	Lancashire	48	48

Complied from the (UK) Food Labelling Regulations 1996 (SI 1996 No. 1499)

The current definition of cheese given in the UK Food Labelling Regulations 1996 is as follows:-

> Cheese means the fresh or matured product intended for sale for human consumption, which is obtained as follows—
>
> (a) in the case of any cheese other than whey cheese, by the combining, by coagulation or by any technique involving coagulation, of any of the following substances, namely milk, cream, skimmed milk, partly skimmed milk, concentrated skimmed milk, reconstituted dried milk, butter milk, materials obtained from milk, other ingredients necessary for the manufacture of cheese provided that those are not used for replacing, in whole or in part, any milk constituent, with or without partially draining the whey resulting from coagulation;
>
> (b) in the case of whey cheese—
>
> (i) by concentrating whey with or without the addition of milk and milk fat, and moulding such concentrated whey, or
> (ii) by coagulating whey with or without the addition of milk and milk fat;

The legal status of other varieties of cheese, especially those for which minimum compositional standards were established in the Cheese Regulations 1970, is unclear. It is likely that they have become "customary names" in the UK (i.e., names customary in the Member State in which it is sold). Probably the best advice is still contained in 1997 LACOTS (the UK Local Authority Coordination Body on Trading Standards) opinion on cheese names, which indicates that customary names could be used if they did not depart from the original compositional profile. They also indicated that use of the terms full-fat, medium-fat, hard and soft in relation to a cheese would indicate the true nature of the food as required by Regulation 8 of the Food Labelling Regulations 1996 (LACOTS 1997). LACOTS is now known as LACORS (the Local Authorities Coordinators of Regulatory Services). Their advice is now intended for guidance for their local council colleagues only. Furthermore, if terms such as reduced fat or other such nutritional claims are used, then these should be in line with the relevant EU regulation in this area (EU 2006).

In the UK labelling regulations, customary names are not given preferential status and may be used but so also can designations which are a description of the product sufficiently precise to inform the consumer of the true nature of the product and to enable the food to be distinguished from products with which it could be confused. However, the existence of definitions in legislation from 1966 to 1996 are understood to change the position somewhat, particularly in cases of what could be construed as misleading consumers.

21.7 Ireland

In Ireland there is not, and never has been, specific legislation on cheese. There is one Irish cheese, Imokilly Regato, protected as a PDO by the EU. Cheeses produced in Ireland and labelled to meet UK legislation have not had problems when sold on the Irish market. This facilitates both producers and consumers; indeed the size of

the home market could create compositional and labelling problems if this were not the situation. However, in the case of reduced-fat variants of common cheese varieties, where these have specific compositional requirements in UK legislation, these can be designated reduced fat with the variety name on the Irish market but not in UK, e.g., Reduced-Fat Cheddar is acceptable in Ireland, provided it meets the relevant EU legislation on nutrition and health claims as regards a reduced-fat claim.

Another possible approach for products intended for the Irish market alone would be to manufacture in accordance with the relevant Codex Standards for the variety of cheese. In such instances it would be wise to seek prior approval of the competent Irish Authority, which at present is the Department of Agriculture, Food and the Marine, as regards its manufacture, composition and proposed product labelling. However, if very similar products on the market used different product names, this could be seen to be misleading or confusing to consumers.

21.8 US Legislation on Cheese

The Code of Federal Regulations (CFR) is the consolidated source of all US federal legislation and published in the Federal Register. It is divided into 50 titles that represent all broad areas that are subject to federal regulation. Title 7 of the CFR addresses Agriculture, which is administered by the USDA, and Title 21 deals with Food and Drugs and is administered by the FDA. The first edition of the CFR was published in 1938 and included all finalized regulations that were published in the Federal Register from 14th March 1936, when the Federal Register begun, to 1st June 1938. Each volume of the CFR is updated once each year and is issued on a quarterly basis. It is published by the Office of the Federal Register, an agency of the National Archives and Records Administration. The latest CFR is also available online at http://www.access.gpo.gov/nara/cfr/cfr-table-search.html#page1.

Each title of the CFR is divided into chapters and each chapter is subdivided into parts that cover specific regulatory areas. Large parts of the CFR are subdivided into subparts. All parts are organized in sections, and most citations in the CFR are provided at the section level.

Part 133 of the CFR addresses standards of identity for cheese; at this time there are 76 standards of identity for 36 varieties of cheese (see Table 21.5), and a further 12 standards for pasteurized processed cheese, pasteurized cheese spreads or pasteurized blended cheeses (see Table 21.6). For example, the reference to the standard of identity for Cheddar Cheese is 21 CFR §133.113. The standards of identity for cheese are addressed typically under four main headings:-

(a) Description—which can be detailed and lengthy;
(b) Optional Ingredients—usually subdivided into provisions on milk ingredients, clotting enzymes and other optional ingredients, which include additives provisions;
(c) Nomenclature—the name of the food;

Table 21.5 US cheese varieties or types with standards of identity specified in the 21 CFR part 133, excluding processed cheese and processed cheese products

Variety/type[a]	CFR ref.	Variety/type[a]	CFR ref.	Variety/type[a]	CFR ref.
Asiago	133.102; 133.103; 133.104	Gammelost	133.140	Nuworld	133.162
Blue	133.106	Gorgonzola	133.141	Parmesan and Reggiano	133.165
Brick	133.108; 133.109	Gouda	133.142	Provolone	133.181
Caciocavallo and Siciliano	133.111	Granular and Stirred-Curd Cheese	133.144; 133.145	Soft Ripened Cheeses	133.182
Cheddar	133.113; 133.114; 113.114	Grated Cheeses	133.146; 133.148	Romano	133.183
Colby	133.118; 133.119; 113.120	Gruyere	133.149	Roquefort and other sheep's milk blue-mould cheeses	133.183
Cold Pack	133.123; 133.124; 113.125	Hard Cheeses	133.150	Samsoe	133.185
Cook Cheese/ Koch Kaese	133.127	Limburger	133.152	Sap Sago	133.186
Cottage Cheese	133.128; 133.129	Monterey Monterey Jack	133.153; 133.154	Semi-soft Cheeses	133.187; 133.188
Cream Cheese	133.133; 133.134	Mozzarella and Scamorza	133.155; 133.156; 133.157; 133.158	Skim Cheese for manufacturing	133.189
Washed-Curd and Soaked-Curd Cheese	133.136; 133.137	Meunster and Munster	133.160; 133.161	Spiced Cheeses	133;190; 133.191; 133.193
Edam	133.138	Neufchatel	133.162	Swiss and Emmentaler	133.195; 13.196

[a]Some cheeses that have similar variety names are grouped together for convenience but all individual CFR references for standards of identity of cheeses are given

(d) Label Declarations which state that each of the ingredients used in the food shall be declared on the label as required by the applicable sections of 21 CFR §101 and §130, except that enzymes of animal, plant, or microbial origin may be declared as "enzymes"; and the dairy ingredients may be declared, in descending order of predominance, by the use of the terms "milkfat and nonfat milk" or "nonfat milk and milkfat", as appropriate.

The use of claims on nutrient content in conjunction with the names of standardised products, including cheeses, is permitted. However, in certain specific

Table 21.6 US standards of identity specified in the 21 CFR Part 133 for pasteurized processed cheese and pasteurized processed cheese products

Standard of identity name	CFR ref.
Grated American Cheese Food	133.147
Pasteurized Blended Cheese	133.167
Pasteurized Blended Cheese with fruits, vegetables and meats	133.168
Pasteurized Process Cheese	133.169
Pasteurized Process Cheese with fruits, vegetables and meats	133.170
Pasteurized Process Pimento Cheese	133.171
Pasteurized Process Cheese Food	133.173
Pasteurized Process Cheese Food with fruits, vegetables and meats	133.174
Pasteurized Cheese Spread	133.175
Pasteurized Cheese Spread with fruits, vegetables and meats	133.176
Pasteurized Neufchatel Cheese Spread with other foods	133.178
Pasteurized Process Cheese Spread	133.179
Pasteurized Process Cheese Spread with fruits, vegetables and meats	133.180

standards of identity nutritionally modified cheeses are addressed, e.g., low-sodium Cheddar (21 CFR §133.116) and low-sodium Colby (21 CFR §133.121). Other general requirements for foods named by use of a nutrient content claim are given in CFR §101.10.

Content claims are based on reference amounts customarily consumed per eating occasion; a list of such reference amounts is to be found in CFR §101.12—for most cheeses this reference amount is 30 g but different amounts apply for cottage cheese (110 g), certain cheeses used mainly as an ingredient (55 g) and extra-hard grating cheeses (5 g). Fat-related claims on nutrient content are contained in §101.62, with the requirements for light (or "lite") addressed in §101.56. The nutrient claims "reduced" and "light" are recognised as comparative nutrient claims and the appropriate reference food must be specified. There are also requirements for labelling statements to be used on foods that make claims based on nutrient content.

In standards of identity for cheeses, food additives, or their functional uses, may be listed as optional ingredients (e.g., stabilisers). A total of 32 food additive functional uses are defined in 21 CFR §170.3(o). Specific food additives are not necessarily all listed in the standards of identity. The US definition of a food additive is given in the Food, Drug and Cosmetic Act 1938, as amended, in Section 201(s) and (t) and in the CFR (21CFR§170.3).

There are four parts of the CFR that list and define the substances for food use:-

- 21 CFR §181—Prior-sanctioned Food Ingredients;
- 21 CFR §182—Substances Generally Recognized As Safe;
- 21 CFR §184—Direct Food Substances Affirmed As Generally Recognized As Safe;
- 21 CFR §186—Indirect Food Substances Affirmed As Generally Recognized As Safe

A list of all the ingredients and substances are given at the start of each of the above parts, giving the section reference for each compound. Substances prohibited from use in human food are listed in 21 CFR §189.

A further useful reference point on substances for use in food in the US is the Food Additive Status List on the FDA website (FDA 2006). This lists substances alphabetically and outlines their status and limitations for use. For a brief overview on this topic, a useful document is a short publication of the International Food Information Council, prepared with the assistance of the FDA (International Food Information Council 2005).

21.9 Cheese Legislation in a Selection of Other Countries

Many countries throughout the world have their own legislation on cheese, care must be exercised as regards the implications of such national legislation for cheese labelling in the individual countries of retail sale. However, in the EU, based on the decision on the landmark Cassis de Dijon case by the European Court of Justice in 1979, the principle of mutual recognition in the Community was established, when the court held that there was no valid reason why a product lawfully marketed in one member state should not be introduced in another member state. As a consequence, most Member States apply this principle to cheeses lawfully manufactured in another Member State, when sold in their country. Nonetheless, this principle is often qualified by the proviso that if such a cheese is so different, as regards its composition or manufacture, from the cheese known under the same name in the Member State where it is sold, and other information on the labelling of the cheese cannot ensure that adequate information is given for consumers in this regard, then the mutual recognition shall not apply. In practice it is often difficult to establish in advance if or when this requirement is actually met.

It is beyond the scope of this chapter to address the individual legislation of further countries in any detail. However, Table 21.7 lists the titles and sources of specific legislation that addresses cheese in 11 other countries, Austria, Australia and New Zealand (which, though separate countries, have developed a common food code), Canada, Denmark, France, Germany, Greece, Italy, Spain and Sweden. The legislation is in the official language of the country concerned.

21.10 Summary

In this chapter, the standards for cheese elaborated by the Codex Alimentarius, some relevant legislation of the European Union and the legislation of the United Kingdom, Ireland and the United States of America have been reviewed in a general way; but it should be stated that it does not purport to address all the minutiae of the full legislation of even those countries. Information on the sources of the legislation

Table 21.7 References for cheese legislation of 11 selected countries

Country	Title	Source
Austria	Österreichische Lebensmittelbuch. Codexkapitel B 32—Milch und Milchprodukte, 3. Käse	Bundesministerium für Gesundheit. http://www.lebensmittelbuch.at/milch-und-milchprodukte/kaese/
Australia and New Zealand	Standard 2.5.4 Cheese. Australia New Zealand Food Standards Code.	Food Standards Australia and New Zealand. http://www.foodstandards.gov.au/code/Pages/default.aspx
Canada	Food and Drug Regulations C.R.C. c. 870. Part B, Division 8, B.08.030.	Department of Justice (Canada). http://laws.justice.gc.ca/en/showtdm/cr/C.R.C.-c.870
Denmark	Bekendtgørelse om mælkeprodukter. BEK nr 335 af 10/05/2004. Kapitel 3 Ost, 4 Osteprodukter and Bilag 4.	Ministerialtidende Danmark. https://www.retsinformation.dk/Forms/R0710.aspx?id=8011#K1
France	Décret no 2007-628 du 27 avril 2007 relatif aux fromages et spécialités fromagères	Journal Officiel de la République Française. Edition 29 avril 2007—Edition numéro 010. http://www.journal-officiel.gouv.fr/frameset.html
Germany	Käseverordnung	Bundesministeriums der Justiz und für Verbraucherschutz. http://www.gesetze-im-internet.de/Teilliste_K.html
Greece	The Food and Beverage Code Article 83 Cheese Products (Κωδικασ Τροφιμων Ποτων και Αντικειμενων Κοινησ Χρησησ Άρθρο 83 Τυροκομικά προϊόντα)	The State General Laboratory. http://www.gcsl.gr/media/trofima/83-iss3.pdf
Italy	International convention for the use of appellations d'origine and denominations of cheese (English text)	Gazzetta Ufficiale della Repubblica Italiana n.47 del 26-2-1954. http://www.normattiva.it/atto/caricaDettaglioAtto?atto.dataPubblicazioneGazzetta=1954-02-26&atto.codiceRedazionale=053U1099
Spain	Real Decreto 1113/2006, de 29 de septiembre, por el que se aprueban las normas de calidad para quesos y quesos fundidos	Boletín Oficial del Estado (BOE) (BOE num. 239), 34717-34720. http://www.boe.es/diario_boe/txt.php?id=BOE-A-2006-17436
Sweden	Livsmedelsverkets föreskrifter om mjölk och ost LIVSFS 2003:39	Livsmedelsverkets (National Food Agency). http://www.slv.se/sv/grupp1/Lagstiftning/Gallande-lagstiftning/Mjolk-och-ost/

relating to cheese is shown in tabular form for 11 other countries, without any further detail. While the Codex Alimentarius standards for cheese are reflected to a greater or lesser extent throughout the world, at this time it cannot be said that they are acceptable in all, or even in a majority, of countries worldwide. It remains to be seen if the Codex cheese standards, which have been revised extensively in the last few years, get wider acceptance. This may depend on any challenges made at the WTO in the event of disputes between countries.

For the foreseeable future, therefore, in many cases it is still necessary to consult the specific legislation of the individual countries to get a full understanding of the complete requirements. This in itself is a challenge, as outlined earlier, as having access to the legislation is only a start and acquiring a full understanding requires not only what the legislation contains but how it is interpreted in the individual countries and it is also necessary to ensure that one has the latest relevant legislative texts to hand. This is likely to require input or at least advice from experts, whether individuals, legal experts, or organisations in particular interest or concern.

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Chapter 22
Whey and Whey Products

Summary The liquid remaining after removal of the fat and casein from milk by isoelectric or rennet-induced coagulation of the casein is called whey. The whey contains about 90 % of the water of milk, ~98 % of the lactose, ~25 % of the protein and ~50 % of the inorganic salts. Traditionally, whey was an essentially worthless by-product of the cheese industry, to be disposed of as cheaply as possible, e.g., as animal feed. However, lactose and the whey proteins have interesting and unique properties. Advances in protein isolation technology have made it possible to isolate and fractionate the whey proteins in undenatured form. Although of minor importance compared with sucrose, lactose has some important applications, especially in the production of infant formulae; in addition, it can be converted to a number of important derivatives. Human milk contains considerable quantities of unique oligosaccharides (OSs) which are believed to be significant for the development of the neonate. Bovine milk contains only low concentrations of OSs but these can be purified and concentrated from whey and there is considerable interest in developing commercially-viable processes.

The processing of whey into various products is described in this chapter.

Keywords Whey powders • Lactose • Lactose derivatives • Whey proteins • Whey cheese

22.1 Introduction

The liquid remaining after removal of the fat and casein from milk by isoelectric or rennet-induced coagulation of the casein is called whey. The term "milk serum" is used for the supernatant obtained on ultracentrifuging skimmed

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(fat-free) milk; 95 % of the casein micelles are sedimented by ultracentrifugation at 100,000 g for 1 h. Milk serum represents the aqueous phase of milk, unchanged by the process of separation, although it does contain small casein micelles.

The whey prepared by isoelectric precipitation or rennet-induced coagulation is called acid whey and sweet (rennet) whey, respectively. They differ from each other in composition (Table 22.1) and from milk serum owing to changes that occur during their preparation, e.g., acid whey contains a much higher concentration of calcium, magnesium, phosphate and citrate than sweet whey or milk serum owing to the solution of the colloidal milk salts on acidification; if properly prepared, acid whey should be free of casein. Commercially, acid whey is usually prepared from efficiently skimmed milk in the manufacture of acid-coagulated cheeses (see Chap. 16) or acid (isoelectric) casein and is therefore essentially free of fat, although it does contain some phospholipids. Sweet whey is a by-product of the manufacture of rennet-coagulated cheese or rennet casein and its composition varies depending on its source, e.g., pH 6.2–6.6, depending on the extent of acidification that had occurred prior to whey separation (hence the concentration of some salts varies somewhat). Most rennet-coagulated cheeses are made from full-fat or partially skimmed milk and, typically, ~10 % of the fat in such milk is lost in the whey as a

Table 22.1 Typical composition and pH of whole milk and sweet (rennet casein or Cheddar cheese) and acid (lactic and mineral acid) wheys[a]

	Composition (g/l)				
	Sweet wheys		Acid wheys		
Component	Rennet casein	Cheddar[b] cheese	Lactic acid casein	Mineral acid casein	Whole milk
Total solids	66.0	67.0	64.0	63.0	122.5
Total protein (N×6.38)	6.6	6.5	6.2	6.1	33.0
Non-protein nitrogen (NPN)	0.37	0.27	0.40	0.30	–
Lactose	52.0	52.0	44.0	47.0	47.0
Milk fat	0.20	3.0	0.30	0.30	35.0
Minerals (ash)	5.0	5.2	7.5	7.9	7.5
Calcium	0.50	0.40	1.6	1.4	1.2
Phosphate	1.0	0.50	2.0	2.0	2.0
Sodium	0.53	0.50	0.51	0.50	0.5
Lactate	–	2.0	6.4	–	–
pH	6.4	5.9	4.6	4.7	6.7

[a]Modified from Mulvihill (1992)
[b]Unseparated whey

result of the formation of free (non-globular) fat during pasteurization and pumping of the milk and the loss of fat globules from the curd pieces during cutting and cooking. Rennet casein is produced from skimmed milk and, therefore, the resulting whey is essentially fat-free. As discussed in Chap. 7, rennet coagulation involves cleavage of κ-casein and the resulting macropeptides are present in rennet whey. Other casein-derived peptides may be present in whey if an excessively proteolytic rennet (substitute) is used. If the rennet coagulation process is incomplete when the gel is cut, the whey may contain some uncoagulated casein as well as small particles of curd. The compositional data for acid and rennet wheys shown in Table 22.1 are typical values which vary considerably.

It will be apparent from Table 22.1 that whey contains ~50 % of the total solids of milk viz essentially all of the lactose and whey proteins (provided that the whey proteins were not denatured by heat treatment prior to coagulation), 50–100 % of the milk salts (depending on whether it was produced by acid or rennet coagulation) and some fat (depending on whether skimmed or whole milk was used). Thus, whey is a valuable source of food constituents from which numerous food products are produced. In this chapter, the principal products produced from whey will be described very briefly. The reader is referred to Sienkiewicz and Riedel (1990), Zadow (1992) and Jelen (2002, 2011) for detailed discussions on whey and whey utilization; certain aspects of whey proteins are covered in Fox and McSweeney (2003) and lactose and its derivatives in IDF (1993) and McSweeney and Fox (2009).

Traditionally, whey was regarded as a waste product and was disposed of by the cheapest possible method, e.g., fed to animals (especially pigs), spray irrigated onto land, dumped in waterways or treated as effluent. Some whey is still disposed of by such methods but dumping of whey is unacceptable today for environmental reasons and improved technology makes it possible to recover whey constituents in a cost-effective way. World production of whey is about 2×10^8 tonnes per annum, containing ~9×10^6 tonnes of lactose and 1.4×10^6 tonnes of whey proteins.

22.2 Clarification of Whey

The curd fines may be removed using a vibrating screen separator but more usually using a centrifugal separator (clarifier); they may be returned to the whey drainage belt and recovered in cheese curd or used in processed cheese or similar products. Removal of fines facilitates further processing of whey, e.g., by ultrafiltration.

Fat, which is typically present at a level of ~0.3 % (w/w) in bulk cheese whey, is recovered from the clarified whey using a centrifugal separator, typically to a level of 0.07 %, w/w. The resultant whey cream (~50 % fat) is normally used for the manufacture of whey butter which is used as a food ingredient, e.g., in processed cheese products.

The phospholipids in whey, which originate from the milk fat globule membrane, exist as lipoprotein particles which block ultrafiltration membranes, reducing the

flux rate of the plant. These lipoprotein particles are not recovered by centrifugation but a number of methods have been developed to aggregate the lipoprotein particles, e.g., by adding $CaCl_2$ and raising the pH to ~7.5. The flocculated calcium phosphate-lipoprotein particles may be removed by sedimentation, centrifugation or, preferably, microfiltration. The lipoproteins have good emulsification properties and may be used in a number of food applications. The clarified whey may be processed into a wide range of products, including the following.

22.3 Whey Beverages

The clarified whey may be used as the base for beverages which have a desirable amino acid profile and are isotonic with blood. They are usually flavoured with natural or concentrated fruit juices or may be fermented to produce whey wine. Such products are available but on a relatively small scale.

22.4 Concentrated and Dried Whey Products

Whey powders have been produced for many years and have several applications in the food industry, e.g., in bakery or meat products and ice cream.

The value of whey powders can be increased and their range of applications extended by one of several process modifications.

22.4.1 Non-hygroscopic Whey Powders

Lactose, which represents about 70 % of the total solids in whey, is difficult to crystallize and if the lactose is not properly crystallized, the whey powder is hygroscopic, making it unstable during storage. Non-hygroscopic whey powder is produced by concentrating the whey to 50–60 % total solids, seeding the concentrate with lactose crystals to induce crystallization and, when complete, drying the concentrate.

22.4.2 Demineralized Whey Powder

One of the important applications of whey solids is in the manufacture of infant formulae. Human milk contains more lactose (~7 %) and less casein (~1 % total protein; whey protein:casein ratio, 60:40, compared with 20:80 for bovine milk) than cows' milk. Most modern baby formulae based on bovine milk are humanized,

i.e., their lactose content and casein:whey protein ratio are adjusted to approximate those in human milk. This adjustment is usually made by blending bovine whey and skim milk. However, the concentration of salts in bovine milk is 3–4 times higher than that in human milk and places an undesirably high renal load on the baby. The problem may be resolved by reducing the concentration of ions in whey by electrodialysis and/or ion exchangers (see Burling 2002; Gernigon et al. 2011).

22.4.3 Delactosed and Delactosed/Demineralized Whey Powders

For many food applications, it is desirable to use a whey product with a higher-than-normal protein content. This may be achieved by the processes described below for the production of whey protein products or alternatively by crystallizing out some of the lactose. This is done by concentrating the whey, seeding with lactose to induce crystallization and removal of the lactose crystals by centrifugation or filtration. The mother liquor may or may not be demineralized (see Sect. 22.4.2) and spray dried to yield a protein-rich whey powder.

22.5 Lactose

Lactose is a sugar unique to milk (see Chap. 4). Among commercially available sugars, lactose has many rather unique properties:

- low solubility
- difficult to crystallize
- a tendency to form super-saturated solutions
- low sweetness
- low hygroscopicity when properly crystallized
- a tendency to adsorb flavours and pigments

These characteristics create problems for the dairy industry but methods have been developed for managing and controlling these problems; in fact, some of these characteristics are exploited in the production of improved dairy products, e.g., crystallization of lactose in the manufacture of instant milk powders or low hydroscopicity icing sugar mixtures (see McSweeney and Fox 2009). However, some of these characteristics are advantageous in some food applications and a substantial market has been developed for lactose although this is very small in comparison with that for sucrose.

Essentially, lactose is produced by concentrating whey to 50–60 % total solids, seeding with lactose crystals and recovering of thew crystals by centrifugation or filtration. If an extra-high purity lactose is required, the first crop of crystals are dissolved and recrystallized (see Muir 2002; Paterson 2009, 2011).

The market for lactose is relatively limited but lactose can be converted, enzymatically, chemically or physically, to a range of useful derivatives (see Playne and Crittenden 2009 and Ganzle 2011a, b):

- *Lactulose* (Fig. 22.1) in which the glucose moiety of lactose is isomerized to fructose by a mild alkaline treatment. Lactulose is not hydrolysed by β-galactosidase in the human intestine and passes to the lower intestine where it may act as a laxative or promote the growth of bifidobacteria which have beneficial effects on the microbial ecology of the lower intestine (Fig. 22.2).
- *Lactitol* (Fig. 22.3): The carbonyl group of lactose may be reduced to an alcohol, lactitol, by chemical or electrolytic methods. Lactitol is not hydrolyzed in the

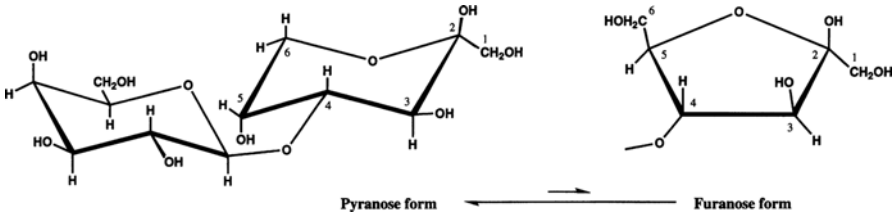


Fig. 22.1 Chemical structure of lactulose

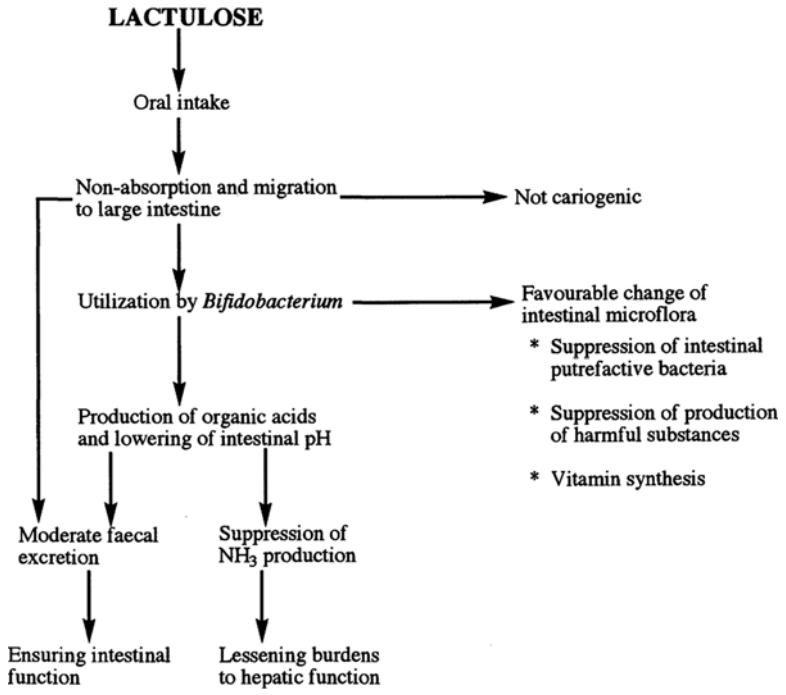


Fig. 22.2 Significance of lactulose in health

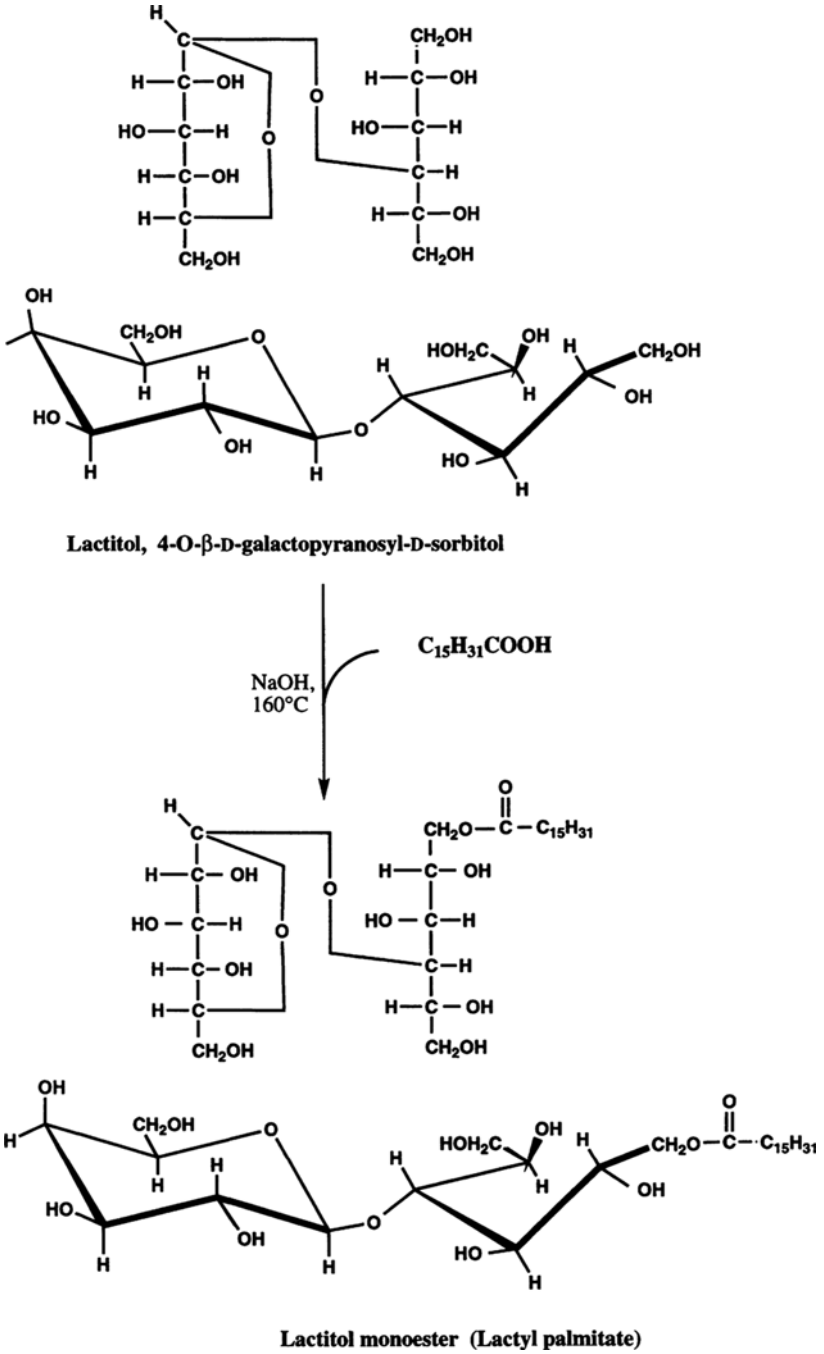


Fig. 22.3 Structure of lactitol and its conversion to lactyl palmitate

human intestine and hence may be used as a non-nutritive sweetener. It is claimed to have anti-cholesterolemic and anti-cariogenic properties. Lactitol may be esterified with one or more fatty acids to produce a range of food-grade emulsifiers, analogous to sorbitans (esters of sorbitol).

- *Lactobionic acid:* Lactose may be oxidized to lactobionic acid which may be converted to lactobionic acid lactone (Fig. 22.4). Both derivatives have a number of food and industrial applications but volumes used are small. Lactobionic acid has a sweet taste, which is a very unusual property for an acid, and therefore may be used as an acidulant without a concomitant acid taste. It prevents the swelling of body organs and is used in the transport and storage of organs for transplant surgery. The lactone may be used as an acidulant for solid foods, with which it can be mixed readily.
- *Tagatose:* Tagatose, the keto analogue of galactose, occurs naturally at low levels in some plants, severely heated milk and stored milk powder. It can be produced by treating β-galactosidase-hydrolysed lactose with weak alkali, e.g., $Ca(OH)_2$, which converts galactose to tagatose, which can be purified by demineralization and chromatography. Tagatose is nearly as sweet as sucrose, has a good quality sweet taste and enhances the flavour of other sweeteners. It is absorbed poorly from the small intestine, serves as a prebiotic and has little effect on blood glucose. It is converted in the lower intestine to short-chain fatty acids which are absorbed and provides about 35 % of the energy of sugars metabolized in the normal way. Tagatose, which has GRAS status, was produced in small quantities by SweetGradient (a Arla Foods-Nordzuker joint company) but has been discontinued. However, other companies, e.g., Nutrilabs NV (Belgium), PepsiCo and Yoplait are planning commercialization.
- *Chlorinated derivatives:* The alcohol groups of sugars, including lactose, are very reactive and permit the production of many derivatives. Possible lactose-derived products are discussed by Thelwall (1985). However, such derivatives of lactose are not being produced commercially, probably because similar products can be produced from cheaper sugars. A trichlorinated derivative of sucrose, sucralose, commercialized under the trade name "Splenda" (E 995), is a very successful artificial sweetener (up to 1000 times as sweet as sucrose, twice a sweet as Saccharine and four times as sweet as Aspartame).
- *Glucose-galactose syrups:* Lactose may be hydrolysed by β-galactosidase (lactase), or by free acid or cation exchangers to produce glucose-galactose syrups which are sweeter and more soluble than lactose. Such syrups have several applications in food products but in most cases are not cost-competitive with glucose, glucose/fructose or sucrose.
- *Fermentation products*: Lactose in whey, or, more usually, in UF permeate, can be fermented by lactose-fermenting yeasts or lactic acid bacteria to a range of products (Fig. 22.5), but in many cases it is not cost-competitive with sucrose, or glucose prepared from starch in such applications. The most widespread of these is ethanol, which is being produced on a commercial scale in several factories as

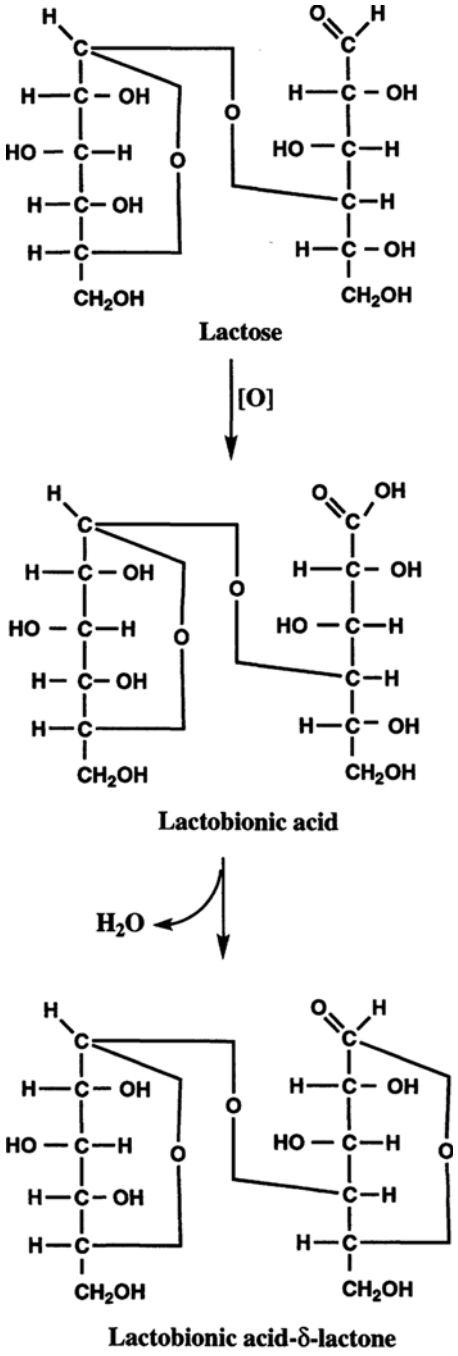


Fig. 22.4 Structure of lactobionic acid and its δ-lactone

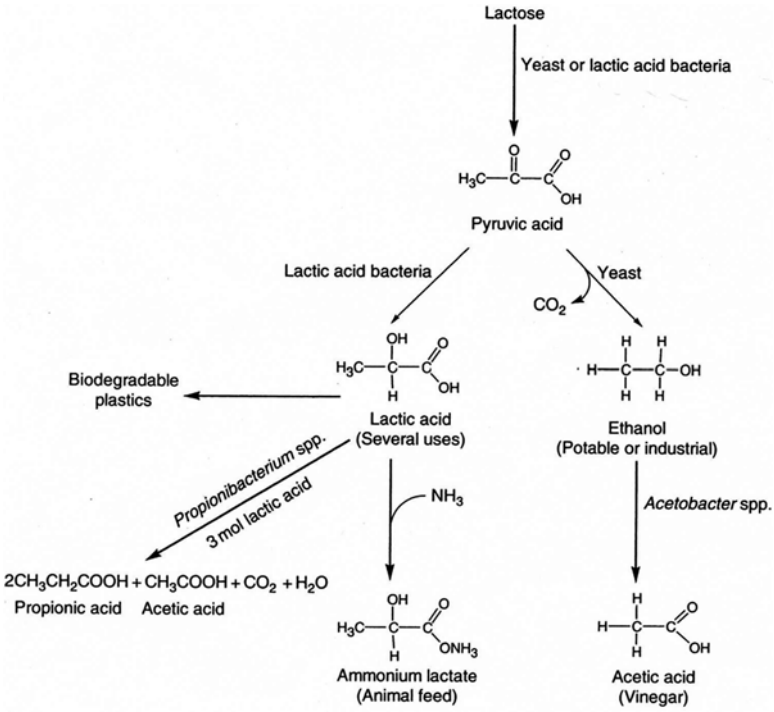


Fig. 22.5 Fermentation product from lactose

a potable product or as a biofuel. The production of yeast biomass by fermentation of whey has been considered but is not economical.

- *Galacto-oligosaccharides*: β-Galactosidase normally functions as a hydrolase but under certain conditions, it may function as a transferase with the production of galacto-oligosaccharides (Fig. 22.6) involving various bond types which are not digested in the human intestine. These oligosaccharides pass into the large intestine where they serve to promote the growth of *Bifidobacterium* spp. These oligosaccharides are quite different from the indigenous oligosaccharides in milk.
- *Indigenous milk oligosaccharides*: As discussed in Chap. 4, the milk of all species that have been investigated contains oligosaccharides (OSs) and human, bear and elephant milk contains very a high concentration and a very complex mixture of OSs (>140 in human milk) which are believed to play significant nutritional and physiological roles (Urashima et al. 2009, 2011).

Although the concentration of indigenous OSs in bovine milk, or that of other commercial dairy animals, is low, they partition into whey and research is ongoing to isolate them on a commercial scale for incorporation into infant formulae and other high-value products (see Merha and Kelly 2006 and Urashima et al. 2013)

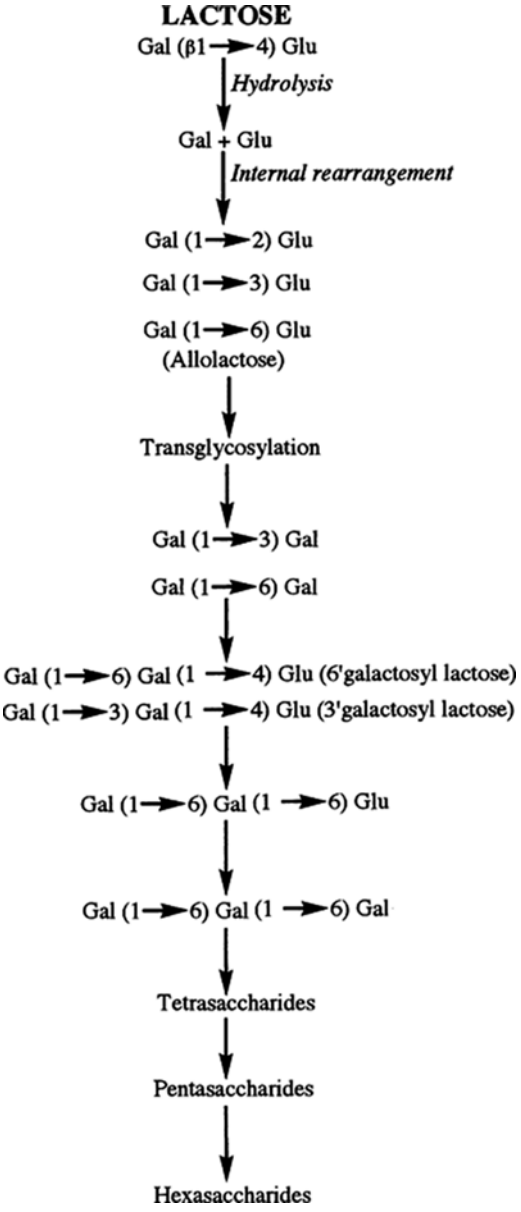


Fig. 22.6 Possible reaction products from the action of β-galastosidase on lactose

22.6 Whey Proteins

Bovine whey contains two main proteins, β-lactoglobulin and α-lactalbumin, with lesser amounts of blood serum albumin and immunoglobulins (mainly IgG_1) and trace amounts of several proteins, especially lactoferrin, and several enzymes (see

Chap. 4). Many of these proteins have desirable nutritional, functional and, in some cases, pharmaceutical properties. Numerous methods are available for the recovery of whey proteins in toto and, more recently, for the isolation of individual proteins.

The first and simplest of these is heat denaturation and recovery of the aggregated protein, known as "lactalbumin". The product is insoluble, has very poor functional properties and is used mainly in nutritional fortification of foods.

Whey protein concentrates (WPC, 30–80 % protein), prepared by ultrafiltration or diafiltration, are widely used as functional ingredients, e.g., for the preparation of gels, foams and emulsions (see Fox and McSweeney 2003).

Products with a higher protein content (up to 95 %; known as whey protein isolates, WPI) have better functionalities and are produced by ion-exchange chromatography. Alternatively, whey defatted by centrifugation is ultrafiltered to 15 % dry matter and microfiltered to remove residual fat; the defatted permeate is further concentrated by ultrafiltration and diafiltration to ~ 20 % dry matter and spray dried. The product typically contains 82 % protein, <0.5 % fat and 96 % dry matter. Since production costs are high, WPIs are usually used as nutritional supplements.

The properties of some whey proteins make them particularly suitable for certain applications, e.g., the gelation properties of β-lactoglobulin are superior to those of α-lactalbumin but it is less suitable for the fortification of infant formulae since it does not occur in human milk and some human infants are allergic to it. Several methods have been developed for the fractionation of whey proteins, some of which are amenable to industrial-scale production, using ion-exchange chromatography, membrane filtration technology or thermal, physical or chemical treatments (see O'Mahony and Fox 2013). Commercially available individual whey proteins include **Bioferrin** (lactoferrin) Glanbia Nutritional, Evanston, IL, USA; **Hilmar 8800** (α-la enriched WPC, from Hilmar Ingredients, Hilmar, CA, USA); **LACTPRODAN –OPN 10**, osteopontin fron Arla Foods, Visby, Denmark.

Some of the minor whey proteins are potentially very valuable as nutraceuticals. Much interest has focussed on lactoferrin which is present at a very much higher concentration in human milk than in bovine milk. Lactoferrin is a non-haem iron-binding protein which has bacteriostatic and other physiological properties and serves as a source of biologically available iron. Because lactoferrin is cationic at the pH of milk (at which most other milk proteins are anionic) it can be easily isolated from milk or whey and is used to supplement infant formulae and other special dietary products (Lonnerdal and Suzuki 2013).

Lactoperoxidase is also cationic at the pH of milk and may be readily isolated. In the presence of H_2O_2 and the thiocyanate anion, lactoperoxidase is a very effective bactericidal agent and has been used as an additive in milk replacers for calves and piglets (Reiter 1985; Fox 2003).

The caseino(glyco)macropeptide (CMP; κ-CN f106-169) produced from κ-casein on the renneting of milk has several interesting biological properties. It contains no aromatic amino acids and therefore is suitable for patients suffering from phenylketone urea. It also inhibits viral and bacterial adhesion, acts as a

bifidogenic factor, suppresses gastric secretions, modulates immune system responses and inhibits the binding of bacterial toxins. The CMP is present at a relatively high level in whey (4 % of total casein, 15–20 % of the protein in whey; an estimated 180×10^3 tonnes are available globally in whey), from which it can be recovered relatively easily. Methods have been developed for the preparation of GMP on a potentially commercial scale (Kawasaki et al. 1993; Maubois 1998).

22.7 Whey Cheese

The whey proteins are recovered as soft cheese by heating a mixture of whey and skim or whole milk, adjusted to pH 6.0, at 90 °C. Ricotta and variants thereof, Anari and Manouri, are examples of this type of cheese; they are discussed in Chaps. 3 and 16.

The whey proteins are also incorporated into some forms of Quesco Blanco which are produced from acidified (pH 5.4) whole milk by heating at 90 °C. They may be incorporated into Quarg by using ultrafiltration technology or the Centri-Whey process (whey is heated at 90 °C to denature the whey proteins which are then recovered by centrifugation, added to milk for the next batch of cheese and become incorporated into the Quarg).

The whey proteins can be incorporated into rennet-coagulated cheese by pre-concentrating the milk to the total solids content of the particular variety by ultrafiltration and coagulating the "pre-cheese" by rennet. This technology has been quite successful for soft cheeses but not to date for semi-hard or hard cheeses.

Finally, whey, usually mixed with whole milk, may be concentrated by thermal evaporation to ~87 % solids to produce a unique family of cheeses, examples of which are Mysost and Gjetost (see Chap. 3). These cheeses are quite different from all other cheeses—they have a sweet taste (owing to the high level of lactose), a brown colour (due to the Maillard reaction between lactose and proteins) and a fudge-like consistency.

22.8 Conclusions

Whey, which contains about 50 % of the total solids of milk and which was regarded as a waste stream until recently, can serve as the raw material for the production of a wide range of food products and food ingredients. Some of these are being produced profitably on a commercial scale. It is very likely that as new technologies are developed, new and improved food ingredients derived from or based on whey will be developed.

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