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Edited by P. L. H. McSweeney

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Preface

Although cheese is a very ancient food product which originated close to the dawn of agriculture, it is still not possible to guarantee the production of premium quality cheese. The way in which cheese ripens and its quality are often heavily dependent on very small differences in its compositional characteristics. Most cheeses are also very dynamic products and change substantially during ripening. For these reasons, more scientific knowledge is necessary for the successful manufacture of cheese than for perhaps any other food product.

The objective of this book is to provide practical knowledge about cheese and problems which occur during its manufacture in a unique question-and-answer format which will allow cheesemakers to find information quickly. Because many of the issues dealt with in this book are complex, it is often possible to provide only an overview of the topic and to highlight its main points. In the case of some entries, the objective is to start the reader thinking along the right lines; cheesemakers will require further information before being confident of the solution to a particular problem. Hence, most entries contain a list of Further reading to which the reader is directed for more detailed information on the problem being discussed. In addition, there are relatively few simple cause-and-effect relationships in cheese, and varying one factor often causes changes to numerous other parameters in the cheese. Because of this and to avoid overlap between certain questions, each entry contains cross-references which direct the reader to other entries containing information of relevance to the topic being discussed. This book presupposes the level of knowledge of dairy chemistry and cheese science and technology that would be common among people working in the dairy industry. Hence, there is little discussion of cheesemaking technology or science beyond that essential for the topic under consideration. There follows

a list of texts on cheese science and technology and dairy chemistry to which the reader is directed to learn more about the science and technology underpinning cheese manufacture.

I hope that this book will be of benefit to cheesemakers and will help to solve at least some of their problems. Finally, I would like to thank the 21 contributors to this book for so generously sharing their experience and for making my task as editor a pleasure.

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Milk

1 Introduction

P. L. H. McSweeney

Milk is a fluid secreted by the female of all mammals, the primary function of which is the complete nutrition of the neonate of the species. Since the nutritional requirements of the young of the different mammalian species differ greatly, it is unsurprising that the compositions of milks of different species vary considerably. Typical compositions of the milks of the principal dairy species are shown in Table 1. In addition to interspecies differences, milk from a particular species will also vary with the individuality of the animal, breed, nutritional status, stage of lactation, age, interval between milkings, health (mastitis and other diseases) and stage of lactation [**2**, **3**]. The principal components of milk are water, lactose, protein (caseins and whey proteins), fat and minerals.

Milk is the principal starting material for cheesemaking. Its caseins form the structural matrix of cheese and the fat entrapped contributes to cheese texture and flavour. The minerals of milk (particularly the colloidal calcium phosphate

Table 1 Typical compositions (%) of milks from the major dairying species

Species	Total solids	Fat	Protein	Lactose	Ash
Cow	12.7	3.7	3.4	4.8	0.7
Goat	12.3	4.5	2.9	4.1	0.8
Sheep	19.3	7.4	4.5	4.8	1.0
Buffalo	16.0	3.7	6.9	5.2	0.8

associated with the caseins [**4**]) are factors that affect cheese texture and lactose is the essential fermentation substrate for lactic acid bacteria.

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2 What is the typical composition of cow's milk and what milk constituents favour cheesemaking?

A. L. Kelly

The principal constituents of milk of any species are water, fat, protein (caseins and whey proteins), sugar (lactose) and minerals (salts such as calcium and phosphate; see **4**), with trace quantities of vitamins and enzymes. Milk is a complex system, being both an emulsion (of milk fat in globules protected by the milk fat globule membrane), a colloidal suspension (75–80% of the protein is casein, which is found in aggregates called casein micelles) and a solution containing many dissolved components.

The typical range of composition of cow's milk is as follows:

Lactose	Range 4.0–5.0%	Average 4.8%
Protein	Range 3.0–3.5%	Average 3.3%
Casein	Range 2.2–2.8%	Average 2.6%
Whey protein	Range 0.5–0.8%	Average 0.65%
Fat	Range 3.0–5.0%	Average 3.5%
Salts	Range 0.6–0.9%	Average 0.7%

The levels of fat in milk are much more inherently variable than those of other constituents. Milk composition can vary according to diet of the cows, stage of lactation (e.g. reduced casein and lactose contents in late lactation), mastitis and seasonality; the latter two will be discussed in [**3**].

For the cheesemaker, the most important constituents, with the reason for their importance, are shown in Table 1. The pH of milk is an important characteristic of milk for cheesemaking that depends on its composition, particularly the levels of salts and whether they are ionised. High milk pH (as sometimes occurs in mastitis or late lactation milk) renders conditions less favourable for the action of chymosin, which has an acidic pH optimum.

Whey proteins are normally lost in the whey and are not important unless steps are taken to force their incorporation into curd (e.g. heat treatment to recover denatured whey proteins [**12**] or ultrafiltration [**16**] to recover native whey proteins). Much of the lactose and salts are also lost in the whey. Casein

Table 1 The most important constituents of cow's milk for cheesemaking

Casein	Forms the rennet gel which is primary structural element of cheese; influences texture and flavour through proteolysis during ripening
Fat	Contributes to cheese texture and to flavour, via lipolysis, to an extent dependent on variety
Lactose	Fermentation substrate for starter lactic acid bacteria; products of lactose fermentation also contribute to flavour during ripening
Calcium	Essential for formation of a rennet gel in the initial stages of cheesemaking; level in curd influences texture

and fat have the highest proportional recovery from milk into cheese (ideally > 90% for both).

The composition of milk for cheesemaking can be manipulated by the cheesemaker by:

- standardisation of fat:protein ratio (see [**9**]);
- addition of protein (e.g. sodium caseinate) although rarely practised;
- addition of calcium chloride (see [**33**]).

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3 How do seasonal variations in milk composition affect cheese quality?

A. L. Kelly

The composition of milk produced by mammals changes from the commencement of lactation (i.e. colostrum) to the end of lactation and drying-off. Levels of almost all milk constituents change during lactation, and thus the suitability of milk for cheesemaking can vary throughout the lactation period.

A plot of changing milk composition throughout a typical lactation cycle is shown in Fig. 1. For many countries, year-round calving is practised, and the milk received by cheese factories is generally a mixture of mid-lactation milk, with smaller amounts of early- and late-lactation milk; hence, variations in composition are diluted and quality remains at an average throughout the year.

However, in certain regions (e.g. Ireland, New Zealand and parts of Australia) there are pronounced seasonal calving patterns, with calving being concentrated at certain times of the year. In the case of Ireland, historical preference for economical production of milk from cows fed on summer grass has resulted in a national summer:winter milk volume ratio of around 10:1. As a result, there is only a small amount of milk available in winter, and this is

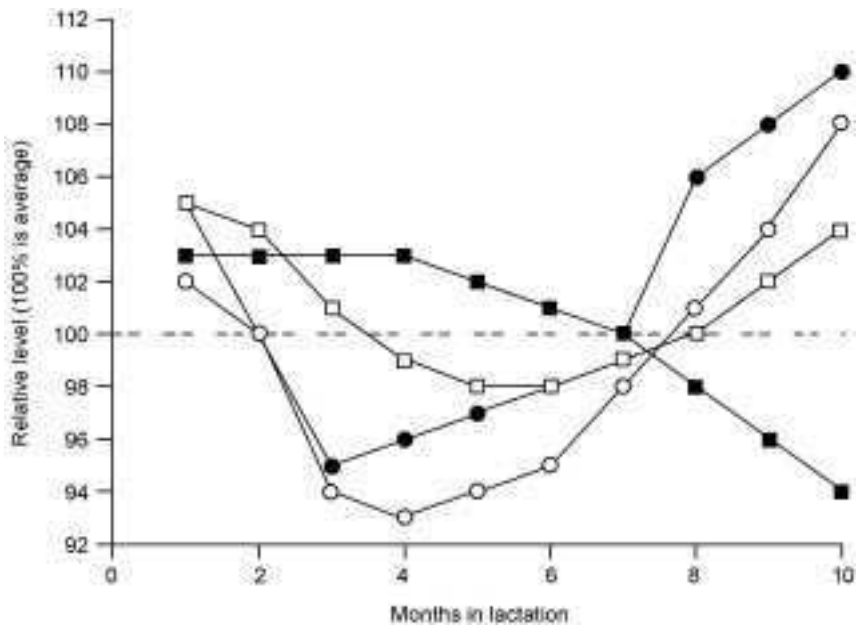

Fig. 1 Schematic diagram illustrating changes in fat (●), protein (○), lactose (■) and calcium (□) contents of milk during lactation, relative to average composition (dotted line). Values are illustrative only to show typical trends (redrawn from Walstra, P., Guerts, T.J., Noomen, A., Jellema, A. and Van Boekel, M.A.J.S. (1999) *Dairy Technology*, Marcel Dekker, New York).

generally utilised for liquid milk products; conversely, during the summer, the relatively large volumes of milk available are used intensively for the production of long shelf-life products, such as milk powder, casein, butter and Cheddar cheese. In Ireland, many cheese factories are closed during winter months due to insufficient availability and poor quality of milk.

In addition, in countries with seasonal milk production, the changes in composition during an lactation cycle of individual cows confound changes in the overall quality of milk collected at different times of the year. For instance, milk collected in Ireland in late autumn/early winter comprises a high proportion of late-lactation milk, which can cause a reduction in the quality of cheese made therefrom. Alternatively, cheesemakers must adapt their cheesemaking protocols and schedules to compensate for any changes in composition (e.g. by addition of calcium chloride [**33**] or changes in standardisation ratio [**9**]).

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4 What are milk salts and how do they affect the properties of cheese?

A. L. Kelly

A minor but highly significant fraction of milk is a mixture of salts, at approximate levels shown in Table 1. Some minerals are present in different phases in milk; of most relevance to cheesemaking, calcium is partitioned between the colloidal phase (i.e. deposited as nanocrystals of calcium phosphate within the casein micelles) and the soluble phase of milk. In addition, minerals in the soluble phase may be ionised or non-ionised. Milk also contains trace levels of zinc, iron, copper, selenium and other minerals.

The partition of salts between the colloidal and soluble phases of milk is affected by the processes applied to milk; cold storage of milk results in solubilisation of colloidal calcium phosphate, which can be reversed by heat treatments such as pasteurisation [**11**] or warming the milk (e.g. to 30 ºC for cheesemaking).

The key mineral constituent for cheesemaking is undoubtedly calcium. Following the first stage of rennet coagulation (hydrolysis of κ-casein by chymosin or another coagulant [**24**]), the formation of the rennet coagulum is dependent on the availability of sufficient soluble calcium, as well as adequate levels of colloidal calcium (without which the micelles would not remain intact). Calcium ions influence coagulation by neutralising negative charges on the casein micelles and probably forming linkages (salt bridges) between negatively charged phosphate groups on the casein micelles. The calcium ion activity of the milk influences the rennet coagulation time and the firmness of the milk coagulum [**28**].

The partition of calcium is strongly influenced by pH, with colloidal calcium becoming progressively more soluble as pH decreases, and solubilisation being

Table 1 Concentrations of some salts from milk

Mineral	Average level (mg per kg milk)
Potassium	1450
Phosphate	950
Chloride	1000
Calcium	
Total	1200
Colloidal	300
Ionic	900
Sodium	500
Sulphate	100
Carbonate	200
Magnesium	130

Adapted from Fox, P.F. and McSweeney, P.L.H. (1998) *Dairy Chemistry and Biochemistry*, London: Blackie Academic and Professional.

complete at pH 4.6; this is particularly important as it influences cheese structure, as the amount of calcium retained in the cheese will depend on the pH at which whey is drained. All else being equal, cheeses containing high calcium levels are more elastic than cheeses with low levels of calcium. Recently, it has been proposed that solubilisation of calcium during the early stages of Cheddar cheese ripening is a key step in the initial softening of its texture. Variations in the level of calcium ions in milk are sometimes compensated for by addition of calcium chloride [**33**], which accelerates rennet coagulation and reduces the pH of milk, facilitating the action of rennet. Overall, mineral salts in milk play a key role in the coagulation of milk, and also play a role in controlling the texture of cheese during ripening.

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5 What are the compositions of other species' milks and how does this affect their cheesemaking properties?

A. L. Kelly

The gross constituents of milk from different mammals are generally ubiquitous (i.e. water, proteins, lactose, fat and salts). However, there are significant quantitative differences in levels of these constituents, and qualitative differences in the nature of the constituents (e.g. the profile and sequence of proteins).

The principal dairy species worldwide are the cow, sheep, goat and buffalo. Different countries rely on different sources of milk, owing to the traditional milk-producing species maintained and farming practices. While the cow has long been the principal dairying species in many regions of the world, buffaloes contribute significantly to milk production in the Indian subcontinent, Egypt and parts of Italy. Sheep and goats are primarily of importance in Mediterranean regions, parts of the Middle East and some regions of Africa.

In 2003, the world production figure of bovine milk was ~505 million tonnes, compared with 73, 8 and 12 million tonnes of buffalo, ovine and caprine milk, respectively. The relative compositions of the milk of these species are shown in Table 1.

The differences in composition of milk from different species can influence either the coagulation properties of the milk or the flavour and texture of the cheese made therefrom. For example, the high levels of short-chain (C_6–C_{10}) fatty acids in goat's milk leads to a characteristic flavour in cheese made therefrom. Also, caprine milk has very low levels of α_{s1}-casein, which results in stronger syneresis, but a more crumbly, or short, texture. Ovine milk contains

Table 1 Composition of milk (%) from different species

Species	Fat	Protein	Lactose	Minerals	Total solids
Antelope	1.3	6.9	4.0	1.3	25.2
Bison	1.7	4.8	5.7	0.96	13.2
Buffalo	10.4	5.9	4.3	0.8	21.5
Camel	4.9	3.7	5.1	0.7	14.4
Cow (Holstein)	3.5	3.1	4.9	0.7	12.2
Cow (Guernsey)	5.0	3.8	4.9	0.7	14.4
Cow (Jersey)	5.5	3.9	4.9	0.7	15.0
Goat	3.5	3.1	4.6	0.79	12.0
Donkey	1.2	1.7	6.9	0.45	10.2
Horse	1.6	2.7	6.1	0.51	11.0
Human	4.5	1.1	6.8	0.2	12.6
Pig	8.2	5.8	4.8	0.63	19.9
Reindeer	22.5	10.3	2.5	1.4	36.7
Seal	53.2	11.2	2.6	0.7	67.7
Sheep	5.3	5.5	4.6	0.9	16.3

Adapted from Huppertz *et al.* (2006).

high levels of fat and protein, the main cheese constituents, and yields a firm coagulum, with rapid syneresis and low final moisture content.

Milks of different species also differ in their enzyme activities; for example, sheep's milk contains very low lipoprotein lipase activity, which influences the flavour of cheese made from that milk. Cheese colour can also depend on the source of milk from which it is made, as sheep, goat or buffalo milk yield very white cheese, because of very low levels of β-carotene in the milk [**14**]; cheese colour can also be influenced by the diet of the milk-producing animal.

Cheesemaking processes used for cow's milk generally have to be modified if milk from different species (especially sheep or buffalo) is used; however, many varieties are only produced from milk of a particular animal (e.g. Roquefort is made from sheep's milk).

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Preparation of cheesemilk

6 Introduction

P. L. H. McSweeney

Although raw, untreated milk is used for the manufacture of certain cheeses, usually on a small scale, most milk for cheesemaking is now subjected to various treatments prior to manufacture [**9**, **10**, **11**, **12**, **13**]. Most cheese is now made from milk that is pasteurised and standardised.

Most milk for cheesemaking is now pasteurised (e.g. 72 ºC × 15 s, although higher temperatures may be used to eliminate *Microbacterium avium* subsp. *paratuberculosis* [**62**]). In addition to improving the hygienic quality of cheesemilk and eliminating pathogens, pasteurisation facilitates making large quantities of cheese of a uniform quality. Raw milk may also be thermised on receipt into the factory [**13**] to prolong its keeping quality at refrigeration temperatures by killing psychrotrophic organisms [**7**]. However, thermised milk is always fully pasteurised prior to cheesemaking. Other pre-treatments that affect the microbial quality of milk include the use of hydrogen peroxide (H_2O_2) or activation of the H_2O_2–lactoperoxidase–thiocyanate system, a very potent indigenous antibacterial system in milk. More commonly, milk may be bactofuged or microfiltered to remove bacterial endospores which can cause problems such as late gas blowing [**91**].

The composition of cheesemilk is usually controlled by standardisation to a defined casein:fat ratio by varying the fat content. Increasingly in large plants protein standardisation is practised where the casein level in the milk is controlled by low concentration-factor ultrafiltration [**16**]. Standardisation of cheesemilk controls the composition and particularly the fat-in-dry-matter ratio of the cheese, maximises cheese yield [**48**] and helps to control cheese quality.

Milk for cheesemaking is not normally homogenised as rennet gels made from homogenised milk synerese poorly and the resulting cheese has a poor texture [**31**]. However, raw cream or milk for the manufacture of Blue cheese [**137**] is sometimes homogenised to activate the indigenous lipoprotein lipase in milk and thus increase lipolysis which is desirable in these varieties. Pre-acidification of the milk promotes rennet activity and leads to demineralisation of the curd [**4**] and may be practised during the manufacture of Camembert-type cheeses. Some cheesemakers control the pH of the milk using an acidogen such as gluconic acid-δ-lactone or by pre-culturing with a starter prior to rennet addition. In addition to its use for protein standardisation, ultrafiltration may also be used to remove much of the moisture from the milk as is sometimes practised for the manufacture of certain varieties (e.g. 'Cast Feta' or fresh cheeses such as Quarg, Ricotta or Cream cheese).

$CaCl_2$ is a common additive used in cheesemaking [**33**] and its addition to cheesemilk improves the rennet coagulation and syneresis properties of the milk. Nitrate is sometimes added to the milk for the manufacture of brine-salted cheeses to prevent the growth of *Clostridium tyrobutyricum*, which can lead to late gas blowing [**91**]. Finally, colouring agents (often annatto) may be added to the milk for cosmetic reasons [**14**]. Low levels of colour may be added to Dutch-type cheeses to give the final product a slight yellow tinge, while much higher levels are added to milk for the manufacture of 'red Cheddar' and similar cheeses which have a distinct orange colour.

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7 What problems are caused by psychrotrophs?

T. Beresford

Psychrotrophic microorganisms are organisms that have an optimum growth temperature in the range 20–30 ºC but are able to grow at refrigeration temperatures, albeit slowly. Psychrotrophs are usually present in raw milk and are of interest to cheese manufacturers as they often produce extracellular enzymes, many of which are heat stable, which can negatively impact on cheese ripening if present at sufficiently high levels.

Milk in the udder of healthy animals is essentially sterile [**8**]; however, opportunities for contamination occur during milking and storage. During this period milk is contaminated with microorganisms that are reflective of the environment. There have been major improvements in milking technology, storage and transport since the 1970s, in particular within the European, American and Australasian industries. A consequence of these advances is that it is now common to harvest milk and store it on farm for 48 h or more and maintain the total microbial load at $<5 \times 10^3$ cfu ml^{-1} on a routine basis. A number of factors have been responsible for this improvement in quality, but central is rapid on-farm cooling of milk followed by refrigerated storage at 4 ºC. The rapidity and degree of milk cooling have a significant impact on its microbial flora. Prior to the introduction of rapid cooling and refrigerated storage, milk was cooled slowly to 15–21 ºC and was dominated by mesophilic microorganisms, particularly *Lactococcus* and *Enterococcus* spp [**18**]. Cooling milk to 4 ºC greatly retards the growth of these mesophilic microorganisms, but psychrotrophic bacteria, such as *Pseudomonas*, Enterobacteriacea, *Flavobacterium* and *Acinetobacter* will continue to grow slowly and dominate the flora.

The majority of psychrotrophic bacteria found in milk are Gram-negative rods. *Pseudomonas* is the most common of these and 'fluorescent' isolates usually form a major portion of this population. The main species, *Pseudomonas fluorescens*, is characterised by the production of a fluorescent pigment when grown on appropriate media. However, some Gram-positive psychrotrophic bacteria are also encountered, usually of the genus *Bacillus*. Many yeasts and mould species are also characterised as being psychrotrophic and may be found in or on cheese.

Pasteurisation is an effective tool to control levels of psychrotrophic microorganisms and the presence of these organisms in cheesemilk or cheese is indicative of post-pasteurisation contamination. In addition, the cheesemaking process involves 'cooking' the curd [**37**] which for many varieties incorporates an additional hurdle to control of psychrotrophic microorganisms. The interior of most cheeses is anaerobic and as the most common psychrotrophic organisms associated with cheese require oxygen for growth, they will not be able to grow within the cheese block. Thus, for most cheese varieties, low levels of contamination is not a major issue, as they will not have an opportunity to grow to significant levels in the cheese curd or in most cheeses during ripening. However, many psychrotrophic microorganisms produce extracellular

proteolytic and lipolytic enzymes, and these can degrade milk fat and protein during storage prior to cheese manufacture. In addition, as many of these enzymes are heat stable they will survive the pasteurisation process [**11**] and may be active during cheese ripening. The presence of such enzymes can result in excessive and uncontrolled lipolysis and proteolysis and the development of undesirable rancid, fruity and bitter flavours [**89**] in the cheese.

The relatively high moisture content of soft cheeses such as Cottage [**170**], Feta and Domiati [**164**] make them susceptible to spoilage by psychrotrophic organisms if the cheeses are contaminated post-manufacture. Spoilage of Cottage cheese, in particular, is well documented since opportunities for contamination occur during the manufacturing process. For example, following whey drainage the curd is washed with chilled water to remove excess lactose and to harden the curd. It is important that this water is of potable quality and free from psychrotrophic organisms. Opportunities for contamination also occur during curd mixing, dressing and filling. The most common species encountered in Cottage cheese are *P. fluorescens*, *P. fragi* and *P. putida.* These bacteria produce very active proteolytic and lypolytic enzymes, which cause bitterness, putrefactive and rancid odours, liquefaction and gelatinisation of curd particles and a slimy appearance on the curd surface. Discoloration, due to the production of fluorescent pigments by *P. fluorescens* or casein hydrolysis, can also occur. Many of these organisms also produce diacetyl reductase. Diacetyl is an important flavour compound in Cottage cheese and its removal through the action of diacetyl reductase leads to 'flat' flavoured cheese. Contamination with psychrotrophic bacilli, yeast and moulds can also occur. Psychrotrophic bacilli can result in bitterness because of excess proteolysis or the appearance of a dark colour through the production of dark pigments. In addition, *Bacillus cereus* strains can cause food poisoning [**58**]. Contamination with yeast and moulds can result in the formation of coloured spots on the cheese surface, the development of fruity, rancid and bitter flavour and aroma, liquefaction of the curd and gas formation.

The most effective means of controlling psychrotrophic microorganisms in cheese is to limit their entry through adherence to strict hygienic milk harvesting, storage, transport and manufacturing practices. Limiting the time period between milk harvesting and product manufacture removes the opportunity for these organisms to grow and produce the range of extracellular enzymes that can damage cheese during ripening. Attention to cleaning within the cheese plant is of the utmost importance to ensure elimination of opportunities for contamination during manufacture. All water used in the process should be of potable quality and wash water should contain 5 ppm available chlorine. The effectiveness of chlorine will be increased if the water is slightly acidic ($\leq$ pH 6.5) thus addition of sufficient food-grade acid, such as phosphoric or citric acids, is recommended. Air quality within the cheese plant should be monitored and sources of dust removed. A number of acceptable preservatives are also available to control psychrotrophic organisms. Addition of propionates and sorbates can control yeasts and moulds. A number of

fermentation-based products, such as MicroGARD^{TM}, are also used. These products are usually made by microbial fermentation and include propionate and acetate in addition to 'bacteriocin-like' substances. As most psychrotrophic organisms associated with cheese are aerobic, adequate packaging is a critical control point in preventing their growth on cheese surfaces during ripening or transport to the consumer. In surface smear [**141**] and mould-ripened cheeses [**128**] this presents a particular problem as oxygen is required to support the growth of desirable secondary flora. Growth of *Penicillium roqueforti* and *Mucor rasmusen* on Camembert cheese are two such examples [**134**]. The approach taken to control this problem is careful attention to the cheese manufacturing and ripening process to ensure that *P. camemberti* growth is favoured over *P. roqueforti* and periodic cleaning and fumigation of the cheese ripening rooms to control *M. rasmusen.*

In summary, psychrotrophic microorganisms can contaminate milk post-harvesting and their growth is promoted by the modern dairy practice of rapid milk cooling and holding at refrigeration temperatures. These microorganisms are controlled by pasteurisation but post-pasteurisation contamination can occur. Psychrotrophs produce extracellular enzymes, many of which are heat stable, which can result in the development of off-flavours and discoloration. Psychrotrophic organisms constitute a particular problem for soft cheeses where they can sometimes grow and result in a range of defects. The most effective control strategy is strict adherence to hygienic milk harvesting and cheese manufacturing practices.

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8 Why do elevated somatic cell counts cause difficulty in cheesemaking?

A. L. Kelly

Milk, even that from healthy animals, contains a low level of white blood cells, or leucocytes, which, in milk, are traditionally referred to as somatic cells; the number of cells is quantified as the somatic cell count (SCC), which is almost universally used as an index of suitability of milk for consumption or processing, including cheesemaking. Limits of acceptability differ between countries, but in the EU are generally set at an SCC of 400 000 cells/ml.

The principal reason for increased SCC of milk is mastitic infection, which may be clinical (severe and obvious) or subclinical (less severe and not obvious). The SCC of milk can also increase in late lactation, perhaps due to dietary or other stresses. The function of somatic cells in milk is protective, and an increase in SCC is generally an indication that the cow is countering a physiological crisis, such as bacterial invasion of the udder.

Mastitis is often restricted to a single quarter of an udder, and the SCC therein can increase rapidly over infection to greater than 1 000 000 cells/ml milk. Clinical mastitis, which results in the highest increases in SCC, is by definition apparent, and usually results in treatment of the animal (by antibiotics [**9**]) and withdrawal of the milk from further use until clearance of the infection. However, inclusion of milk from animals with subclinical mastitis results in increases in farm bulk tank SCC; estimates of the prevalence of subclinical mastitis in herds vary but the problem is generally agreed to be widespread.

Increases in SCC are known to be correlated with significant changes in milk composition, as the changes that allow cells to enter milk from blood also allow increased traffic of substances between the two fluids and are accompanied by changes in the secretory function of the udder. SCC is generally inversely correlated with milk yield and levels of casein and lactose, positively correlated with milk pH and levels of whey proteins and activity of many enzymes, and is also associated with changes in the mineral balance of milk.

Hence, it is not surprising that the SCC of milk is a commonly used index of suitability of milk for cheesemaking. High SCC milk is associated with poor rennet coagulation properties [**30**], reduced cheese yield [**48**], and production of high-moisture cheese, frequently with off-flavours.

However, there remains some disagreement about the exact SCC limit above which milk should be rejected by cheese factories. It is certain that there is not an abrupt change in cheese quality at a particular SCC, but rather that changes are gradual and progressive with SCC. The exact suitability of milk of different SCCs may also depend on the specific variety of cheese being made.

In recent years, there has also been some discussion of the fact that SCC is a cumulative count of the levels of several different cell types, which can vary independently due to physiological factors. Hence, milk samples of similar total SCC can actually have quite different *differential* SCCs, or levels of different

cell types within the total. Whether these differences have significance for milk or cheese quality remains to be determined.

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9 Why must milk be standardised for cheesemaking?

A. L. Kelly

The composition of milk can vary due to a number of factors, including cow to cow variation, diet, season and stage of lactation [**2**]. Generally, the protein content of milk is relatively stable (i.e. it varies within a limited range) but the fat content is much more variable. Assuming high recoveries of both casein and fat into cheese, this may lead to variations in the composition of cheese, in particular the fat content.

Bulking of milk from multiple farms reduces variation in composition; variations in the composition of cheese are generally further reduced by standardisation of cheesemilk, i.e. adjustment of the fat:protein or fat:casein ratio. Standardisation may be achieved in small-scale plants by addition of skimmed milk to whole milk to reduce the fat content or by partially skimming the cheesemilk (or, although less likely and only probably applicable to high-fat cheese varieties, by adding cream to increase the fat content). However, in most large cheese factories, standardisation is achieved using a centrifugal separator (typically of disc-stack design), which separates whole milk into skim milk and cream, which may then be mixed, perhaps in-line in an automated process, in proportions calculated to achieve the desired ratio of constituents. Standardisation ensures that manufacturers deliver levels of fat-in-dry-matter (FDM) required by legal specifications or standards of identity for specific varieties.

Milk may be standardised on a basis of the ratio of total protein:fat, or of casein:fat; the latter is more accurate but requires estimation of the casein content of the raw milk, which requires more advanced analytical capability than the former. For Cheddar cheese, milk is normally standardised to a casein : fat ratio of 0.67–0.72. For low-fat cheese, different ratios may be used; for example casein : fat ratios of 4.79, 1.73 and 1.25 have been used for low-fat, half-fat and reduced-fat Cheddar cheese (Guinee *et al.*, 2000). In addition, ratios recommended differ for different cheese varieties.

While fat can relatively easily be removed or added to milk, it is more difficult to manipulate the protein content of milk. One technological option that is practised in some cases is ultrafiltration to concentrate milk solids [**16**], by a factor of up to two, followed by normal cheesemaking; alternatively, skim milk may be ultrafiltrated to yield a protein-enriched fraction which can be added back to the whole milk before cheesemaking. The advantages of this technology ('protein standardisation') include uniformity of milk composition, increased cheese yield [**48**] and reduced losses of casein in whey.

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10 Why is cheesemilk usually pasteurised?

A. L. Kelly

Pasteurisation is a process originally developed to make milk safe for human consumption by inactivation of the most heat-resistant vegetative pathogenic bacteria that may typically be found in raw milk (i.e. *Mycobacterium tuberculosis* and *Coxiella burnettii*) [**60**]. Pasteurisation, typically involving heating milk to 72–75 ºC for 15–30 s in a continuous-flow plate heat exchanger, also reduces the level of spoilage bacteria in milk, thereby increasing its shelf-life, and inactivates several indigenous enzymes; one of these, alkaline phosphatase, is routinely used as an index of pasteurisation, as its kinetics of thermal inactivation are very similar to those of the pathogens mentioned above. Pasteurisation has little effect on heat-resistant bacterial endospores, the germination of which is generally subsequently controlled by refrigerated storage of milk, or by the conditions in cheese; an exceptional case in the latter regard is that of varieties such as Swiss cheese, which are held for a time during ripening at around 25 ºC, which can facilitate the germination and growth of spores such as those of *Clostridium* spp., if present at high numbers [**91**].

In most developed countries, pasteurisation is accepted as a prerequisite for most dairy products; however, the case for cheese is perhaps unique, in that cases have been made for continuing to produce several varieties from raw milk. The reason for the interest in utilising raw milk lies in the effect of pasteurisation on certain indigenous enzymes (e.g. lipase) and harmless bacteria (e.g. non-starter lactic acid bacteria [**56**]) which can, in theory, contribute positively to the flavour of cheese, and are believed to be responsible for characteristic varietal attributes.

Production of cheese from raw milk obviously implies an increased risk of survival of pathogenic bacteria and their incorporation into cheese. Consequently, other factors should be present which control or eliminate this danger; these include the long ripening time of many cheese varieties, competition from starter and non-starter lactic acid bacteria, low pH and water activity, and high salt content [**59**].

Certain compromise treatments may be applied to retain some of the desirable characteristics of raw milk for cheesemaking; these include sub-pasteurisation heat treatments [**13**] or use of non-thermal methods of inactivating or removing pathogens from milk, such as membrane filtration or, perhaps, novel processing technologies [**53**].

In recent years, there has been some discussion of increasing the severity of pasteurisation processes slightly (i.e. by increasing the temperature or holding time), due to concerns over *Mycobacterium paratuberculosis* subsp. *avium* [**62**], both in terms of its possible causative link to Crohn's disease and its resistance to pasteurisation.

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11 What effects does pasteurisation have on cheesemilk?

P. L. H. McSweeney

Milk for the manufacture of most cheese varieties is now pasteurised. Batch pasteurisation (low-temperature long-time, e.g. 63–65 ºC × 30 min) was used initially but high-temperature short-time (HTST, e.g. 72 ºC × 15 s) is now used more commonly. Thermisation [**13**] (e.g. 63 ºC × 10–15 s) is a sub-pasteurisation heat treatment intended to prolong the keeping quality of raw milk by killing psychrotrophs [**7**].

The effects of pasteurisation on cheesemilk are threefold:

- Killing components of the microflora of the milk, including all vegetative pathogens.
- Inactivation of certain enzymes.
- Partial denaturation of whey proteins.

The most significant change to milk on pasteurisation is to its microflora. Pasteurisation of cheesemilk was introduced as a public health measure to kill the most heat-resistant vegetative pathogen in milk [**60**]. However, pathogenic organisms are usually only a small proportion of the organisms killed by pasteurisation. Most of the bacteria in milk are heat-labile and are thus killed by pasteurisation. Pasteurisation reduces the number of indigenous non-starter lactic acid bacteria (NSLAB) [**56**] and cheese made from raw milk usually contains a higher number of NSLAB and has a more diverse NSLAB flora than cheese of the same variety made from pasteurised milk. The NSLAB in cheese made from pasteurised milk probably survive pasteurisation in a heat-shocked state and recover and grow later during ripening, or they may originate from environmental contamination.

Pasteurisation also inactivates a number of indigenous enzymes in milk. Inactivation of alkaline phosphatase serves as a test in many countries that milk has been adequately pasteurised. Pasteurisation also largely inactivates the indigenous lipoprotein lipase in milk, and cheeses made from raw milk usually have higher levels of lipolysis than cheeses of the same variety made from pasteurised milk [**90**]. Conversely, the activity of plasmin, the principal indigenous proteinase in milk, may be increased on pasteurisation; plasmin is a heat-stable enzyme but its inhibitors are heat labile.

Heating milk to about 65 ºC has a slight beneficial effect on its renneting properties due to heat-induced precipitation of colloidal calcium phosphate and the concomitant slight drop in pH [**4**, **30**]. However, more severe heating of milk leads to the denaturation of whey proteins, particularly β-lactoglobulin, and interaction with κ-casein via disulphide (–S–S–) bonds. While this process occurs to a small extent on HTST pasteurisation (72 ºC × 15 s) with negligible effects on rennet coagulation, higher heat treatment of milk leads to severe impairment of its rennet coagulation properties and there are strategies to offset, at least partially, these negative effects [**12**].

While pasteurisation was introduced initially as a public health measure, it has also had the effect of reducing the risk of producing low-quality cheese resulting from the growth of undesirable bacteria and it is difficult to produce cheese on a very large industrial scale from raw milk.

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12 How does one improve the cheesemaking properties of over-pasteurised milk?

P. L. H. McSweeney

Heating milk at time × temperature combinations greater than those used for high-temperature short-time (HTST) pasteurisation [**11**] results in the denaturation of whey proteins (particularly β-lactoglobulin) and their interaction with micellar casein (particularly κ-casein) via disulphide and other bonds. High heat treatment results in milk with poor rennet coagulation properties including longer rennet coagulation time (RCT) [**30**] and a weak, fine coagulum which has poor syneresis properties [**34**]. Milk may suffer overly high heat treatments by accident or deliberately, in an attempt to increase cheese yield through incorporation of whey proteins into the curd [**48**] or to kill heat-resistant potentially pathogenic organisms such as *Mycobacterium avium* subsp. *paratuberculosis* [**62**].

Strategies to improve the adverse effects of high heat treatments on the rennet coagulation properties of cheesemilk include the following.

Addition of $CaCl_2$

Increasing the Ca^{2+} concentration of milk through the addition of $CaCl_2$ is used frequently to improve its renneting properties [**33**]. Addition of 1–2 mM $CaCl_2$ reduces the gelation time and results in a stronger gel from both heated and unheated milk. Increasing the Ca^{2+} concentration of milk improves the second stage of rennet coagulation (gel assembly) through reducing the electrostatic repulsion between casein micelles which, in turn, promotes their aggregation into a gel [**24**]. Through its effect on the milk salts system, addition of Ca^{2+} also causes a slight decrease in the pH of milk which promotes rennet action [**4**, **30**] and calcium concentrations up to 4 mg ml^{-1} reduce the denaturation of whey proteins.

Reducing the pH of the heated milk to < 6.2

Acidification of heated milk to < pH 6.2 results in a shorter rennet coagulation time [**30**] and increased gel firmness, probably by reducing electrostatic repulsions between casein micelles and increasing the concentration of soluble Ca^{2+} [**4**]. However, this practice may not be compatible with the manufacture of many cheese varieties and will affect the retention of rennet [**28**].

pH cycling

Acidification of heated milk to *ca.* pH 5.5 followed by holding at 20ºC and neutralisation to pH 6.6 reduces the RCT of heated milk and results in slightly firmer gels. Acidification solubilises colloidal calcium phosphate [**4**] which reprecipitates upon neutralisation but in a form closer to that of unheated milk.

Some workers have found that adjusting the pH of milk to 7.3 before pH cycling further improves the rennet coagulation properties of milk. Overall, pH cycling has a greater effect on improving the RCT of heated milk than on gel firmness.

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13 What is thermisation and why is it used?

J. J. Sheehan

Thermisation (also known as thermalisation) is a mild continuous heat treatment, immediately followed by cooling, such that the properties of the raw milk are almost unchanged while its bacterial flora, especially the psychrotrophic flora [**7**], is considerably reduced.

Thermisation is applied to cheesemilk usually to prolong its storage life prior to pasteurisation and/or cheese manufacture, while having a minimum effect on milk constituents and flavour [**11**]. Thermisation involves sub-pasteurisation heat treatments which range from 57 to 68 ºC with holding times of 15–30 s. However, treatments of 63–65 ºC for 15 s are usual. An upper limit of 65 ºC for 20 s for thermisation means that >50% of alkaline phosphatase activity survives. This facilitates the determination of alkaline phosphatase activity as an indicator for subsequent pasteurisation of milk. Cooling and storage of the milk at 4–7 ºC after thermisation is important to maintain microbial quality.

Spoilage of raw milk stored at <7 ºC is predominantly due to its psychrotrophic microflora, e.g. *Pseudomonas* spp., most of which are heat labile but which produce heat stable proteinase, lipase and phospholipase enzymes, which are not inactivated by thermisation or pasteurisation and which cause reduction in yield [**48**] and flavour and texture defects in cheese. Therefore thermisation is used to reduce the bacterial load by minimising growth particularly of psychrotrophic bacteria, and to prolong the keeping quality of raw milk under cold storage for a further 24–72 h after receipt at a processing plant. Extension of storage time without a deleterious effect on milk quality allows greater flexibility to plant processing schedules. The extension of storage time is dependent on the age and microbial quality of the raw milk prior to thermisation, the temperature and time of the thermisation treatment, avoidance of recontamination after treatment, and maintenance at temperatures of 4–7 ºC.

Thermisation inactivates only some pathogenic microorganisms [**60**] and does not fulfil public health requirements as does pasteurisation. In certain countries, cheeses manufactured from raw or thermised milks are required to be stored at 2 ºC for 60 days to allow pathogens to die. Thermisation may result in germination of spores (e.g. *Bacillus cereus*) present in milk during subsequent cold storage, but subsequent pasteurisation of the milk will inactivate these vegetative cells. Thermisation of cheesemilk has little effect on renneting properties during cheese manufacture [**12**, **28**]. Cheeses manufactured with milk that has been thermised rather than pasteurised may develop a more intense flavour profile, possibly because of a lower inactivation of enzymes and non-starter lactic acid bacteria.

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14 Why are colours sometimes added to cheesemilk?

P. L. H. McSweeney

Colour is an important attribute of all foods and may serve as an index of quality. Depending on the nature of the feed, cow's milk fat contains variable levels of carotenoid pigments and thus high-fat dairy products made from cow's milk tend to have a yellow colour. Hence, yellow-orange-red colours have become associated with high levels of fat (or 'richness') in the minds of certain cheese consumers. The most common colourant used during cheesemaking is annatto, a pigment preparation extracted from the pericarp of the seeds of the tropical plant *Bixa orellana* L. Annatto contains two apocarotenoid pigments, nor-bixin, and its methyl ester, bixin. By suitable modification, the pigments in annatto can be made soluble in water for use in cheesemaking. Preparations of β-carotene may also be used to colour cheese.

High levels of colours are added to the milk for the manufacture of 'Red Cheddar' [**100**] and similar variants of British territorial cheeses, and these varieties have a distinct orange colour. Care must be taken when using high levels of colours that the quality of the whey obtained during cheesemaking is not impaired. Lower levels of colour may also be added to the milk for Dutch-type cheeses [**108**] to give the product a desirable slight yellow colour.

Goats, sheep and buffaloes do not transfer carotenoid pigments into their milk [**5**] and hence cheese made from the milks of these species is much whiter than a similar cheese made from cow's milk. Hence, carotenoid pigments are sometimes bleached by treatment with hydrogen peroxide or masked (by chlorophyll or TiO_2) when making cheeses from cow's milk that are usually made from other species' milk. However, these treatments are not permitted in many countries.

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15 What effects does cold storage have on the properties of milk?

T. P. Guinee

In the modern farm, milk is cooled rapidly to less than 8 ºC and may be held for 1–3 days prior to collection. Moreover, cold milk is transported over long distances and is often cold-stored at the cheese plant for 1–3 days depending on the time of year and manufacturing schedules. Hence, milk can be cold-stored for 2–5 days prior to processing. During storage and transportation, the cold milk is subjected to varying degrees of shear due to pumping, flow in pipelines and agitation.

Cold storage (also known as cold ageing) of milk results in a number of changes:

- Chemical changes
  - solubilisation of colloidal calcium phosphate and micellar casein [**2**, **4**],
  - dissociation of the solubilised calcium and phosphate and solubilised caseins (especially β-casein) from the casein micelle into the serum phase of the milk.
- Microbial changes
  - increases in the growth of psychrotrophic bacterial strains such as *Bacillus* and *Pseudomonas* spp. [**7**].
- Enzymatic changes
  - an increase in the level of enzymatic activity in the milk as a result of proteinases by these bacteria and a commensurate increase in the hydrolysis of casein (especially serum casein) and formation of soluble non-protein N.
- Physical changes
  - damage to the native milk fat globule membrane and hydrolysis of free fat by lipases from psychrotrophic bacteria and/or by the native milk lipase, resulting in a lower level of milk fat.

The extent of these changes increases with reduction in storage temperature and with increases in storage time and counts of somatic cells and psychotropic bacteria in the milk prior to cold storage. Cold ageing is generally undesirable as the above changes can increase the rennet coagulation time (RCT, a measure of time for milk to form a gel on addition of rennet [**30**]), reduce the firmness of the resultant gel (curd) and decrease cheese yield. Soft curds are prone to shattering during cutting and early stages of stirring during cheese manufacture. This situation is conducive to the formation of small curd particles, high losses of curd fines and milk fat in the cheese whey, lower cheese yield [**48**] and altered cheese composition (e.g. lower moisture level) and quality.

The increases in the RCT following storage at 4 ºC for 48 h range from 10 to 200% for milks from individual cows and from 9 to 60% for bulked factory milks; variations are due to differences in milk composition, microbiological status and somatic cell count [**8**]. Factors contributing to the increased RCT

include reductions in (i) the level of colloidal calcium phosphate, which may be considered as a cementing agent that helps bind the casein micelles together during rennet coagulation and curd formation, and (ii) the level of micellar casein, which is the major structural component of the gel formed during rennet coagulation. Proteolysis reduces the concentration of gel-forming casein to an extent depending 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 cheese whey. The reduced casein level results in slower gel formation and a soft curd at cutting. The proteolysis of casein associated with cold-ageing can be particularly problematic in large dairy plants where the gel is usually cut at a fixed time after rennet addition rather than at a given firmness. In smaller factories, allowing sufficient time for the gel to attain the desired firmness prior to cutting may offset some of the adverse effects of cold ageing.

The chemical changes and increase in RCT associated with cold storage are almost complete after 24 h in freshly drawn milks and are largely reversed by pasteurisation (72 ºC × 15 s) or milder heat treatments (e.g. 50 ºC × 300 s). In contrast, the enzymatic and physical changes are not reversed by pasteurisation. The microbiological and enzymatic effects of cold ageing of milk may be reduced by thermisation of milk [**13**] (e.g. at 65 ºC for 5 s) prior to cold storage.

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Ultrafiltration of cheesemilk

16 Why is ultrafiltration used for cheesemaking and how is it applied?

J. M. Banks

Ultrafiltration (UF) is a membrane separation process that selectively concentrates milk protein and fat. The technology is used in cheesemaking to increase cheese yield [**48**] by the incorporation of whey proteins. UF can also be used to improve manufacturing efficiency by enhancing the casein content of the milk to optimise gel formation, the recovery of casein and fat during cheesemaking, and to maximise plant throughput. UF is classified into the three categories: low-concentration (LC), medium-concentration (MC) and high-concentration (HC) factor UF, depending on the extent of concentration.

In traditional cheese manufacture, the fat and casein in milk are concentrated by removal of moisture [**34**]. Moisture released during cheesemaking contains water-soluble components which include the whey proteins, lactose and minerals. The whey proteins account for approximately 20% of the total milk protein. The caseinomacropeptide, which is released from κ-casein during renneting [**24**], is also found in the whey and accounts for approximately 4% of milk protein. Membrane separation of milk by UF can be used to incorporate these proteins into cheese. UF of milk produces a permeate (also called ultrafiltrate) containing water, lactose, soluble minerals, non-protein nitrogen and water-soluble vitamins, and a retentate in which the casein, whey protein, fat and colloidal salts are increased in proportion to the amount of permeate removed. As much of the water is removed prior to cheese manufacture, the level of syneresis required is reduced, and whey proteins are entrapped in the curd during cheesemaking.

The most widely used application of ultrafiltration in cheese manufacture is in the production of low concentration factor retentates to facilitate uniformity in milk composition by elimination of seasonal variation [**3**, **9**] ('protein standardisation'). In LC UF the milk is concentrated approximately 1.5-fold. At this level of concentration it is possible to apply conventional cheese manufacturing techniques and the only investment required is UF equipment to concentrate the milk. Milk protein content is standardised to levels ranging from 3.7 to 4.5% prior to cheese manufacture. Low concentration factor retentates are used in the production of a variety of cheeses including Camembert [**128**], Cheddar [**100**] and Mozzarella [**146**]. The advantages of cheesemaking using low-concentration factor retentates are uniformity in milk composition, production of a firm coagulum which encourages lower losses of casein in whey, increased cheese yield (approximately 6% on a protein basis), improved cheesemaking efficiency in terms of a higher throughput per vat, and no requirement for additional cheesemaking equipment with the exception of the ultrafiltration unit.

The increase in cheese yield using a low-concentration retentate is attributable to improved casein and fat retention due to improved curd firmness, and the retention of a small proportion of whey proteins. For Cheddar cheese, concentration of milk 1.6 or 1.7-fold is common. At higher levels of concentration, the rennet coagulum is extremely firm and difficult to handle and as a consequence fat losses in the whey may be high.

In MC UF a concentration factor of 2–6-fold is used to achieve the final solids content of the cheese without the need for whey expulsion. This approach effectively increases cheese yield through incorporation of whey proteins. Additional benefit is derived when the milk is heat treated to denature whey proteins prior to UF and the denatured whey proteins carry additional moisture into the curd.

MC UF has been particularly successful in the production of high-moisture unripened cheeses such as Quarg and Cream cheese [**170**] and of cheeses which are not heavily dependent on proteolysis for flavour development, for example Feta [**164**]. In the commercial production of Feta using UF the milk is concentrated 5-fold. The ultrafiltrated whole milk is homogenised [**31**], blended with lactic starter [**18**], salt and a lipase-rennet mixture [**27**], and poured into 18 kg tins, where the curd, which includes whey proteins, is formed. The curd is then covered with 6% salt or brine and is held for ripening.

Many reports have been published on the use of UF concentration to attain the final dry matter level of soft or semi-hard rennet-curd cheeses, including Camembert, Blue cheese [**137**], Havarti and Mozzarella. However, the use of UF technology for production of these cheeses has been limited owing to problems with flavour, texture and functionality. These problems are partly associated with changes in the buffering capacity of milk [**22**] on concentration by UF which impacts on critical cheesemaking parameters. UF of milk at its normal pH of 6.7 results in an increase in buffering capacity of the retenate. The increase in buffering capacity results from concentration of the colloidal calcium phosphate

[**4**] which is bound to the casein micelles and is concentrated to the same extent as the caseins. Critical factors influencing cheese quality, flavour and texture development are altered owing to the enhanced buffering capacity. These include the rate and extent of acidification by the lactic acid bacteria, the rennet coagulation time [**30**], the rheological properties of the curd, the activity of ripening enzymes [**23**, **28**], the lysis of mesophilic lactic acid bacteria and the water-holding capacity of the cheese.

To avoid undesirable effects on cheese quality due to the increased buffering capacity, e.g. excessive acid taste, crumbly texture or abnormal ripening, the mineral content of UF retentates must be lowered. The extent to which minerals should be reduced is specific to the variety of cheese being produced. Lowering the mineral content of UF retentates can be achieved by solubilisation of the colloidal calcium phosphate by reducing the pH of milk prior to or during ultrafiltration so that soluble minerals pass into the permeate.

The maximum achievable concentration by ultrafiltration is about 7:1 for whole milk, and this is not sufficient to achieve the dry matter levels required for hard cheeses such as Cheddar. Following coagulation of the retentate, whey must be expelled through syneresis to attain the desired solids content in the final cheese, but the high concentration of curd solids prohibits the use of conventional cheese manufacturing equipment. HC UF is therefore used in conjunction with specially designed equipment to produce high-solids curds.

The APV Sirocurd process developed for Cheddar was an example of this technology. The process involved the continuous rennet coagulation of milk ultrafiltered to 40–45% total solids. A small proportion of the ultrafiltered milk was prefermented with lactic acid bacteria and used as bulk starter at the level of 10–12%. The continually formed coagulum was cut with specially designed wire knives and cubed curd pieces were transferred into a rotating drum where syneresis took place during heating to 38 ºC over a 30–40 min period. Automated cheddaring occurred at the optimum pH, followed by milling and salting of the curd. Yield increases [**48**] of 6–8% were claimed with this process. However, after several years of operation this process is no longer in use because of technical difficulties and poor economics.

The most successful commercial applications of UF in cheese manufacture have been in the production of Feta-type cheeses and fresh acid-curd varieties such as Quarg, Ricotta and Cream cheeses, where substantial improvements in yield are attainable.

However, successful manufacture of all cheese varieties by UF technology will require careful consideration of the properties of the protein-enriched concentrates as they determine the quality of the end products, in addition to evaluation of the economics of use of membrane technology.

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Acidification

17 Introduction

P. L. H. McSweeney

Cheeses are fermented dairy products and hence the controlled production of lactic acid from lactose by lactic acid bacteria (LAB) is an essential step during the manufacture of essentially all varieties. Milk for cheesemaking may be acidified by its indigenous LAB or by using a whey culture (i.e. a volume of whey retained from a previous day's cheesemaking) [**18**]. While these traditional techniques continue to be used for certain artisanal varieties, the use of selected cultures of LAB, known as starters as they initiate acidification, is now widespread. Starter cultures are now produced and supplied to the cheese industry by a number of companies and may be mixed-strain starters (containing unknown combinations of unknown strains of LAB) or defined-strain cultures (containing known combinations of known strains of LAB). Mesophilic starters (with an optimum temperature of ~30 ºC) are generally composed of strains of lactococci; sometimes mesophilic starters also contain leuconostocs or citrate-positive strains of lactococci as flavour-producers. Thermophilic starters (optimum temperature ~42 ºC) contain *Streptococcus thermophilus* and a *Lactobacillus* sp. (e.g., *Lb. delbrueckii* subsp. *lactis* or *Lb. helveticus*). In addition to these primary starters that acidify the milk, secondary or adjunct starters may also be added for a function other than acidification (e.g. the moulds in mould-ripened cheeses [**128**, **137**], *Propionibacterium freudenreichii* in Swiss-type cheeses [**117**] and a complex Gram-positive microflora in smear-ripened cheeses [**142**]). Starters may be grown in the cheese plant by propagating in a suitable growth medium (bulk starters) or they may be obtained in a highly concentrated form and added directly to the vat ('direct vat set' or 'direct vat inoculation').

Acidification plays a number of important roles in cheese manufacture and ripening:

- Controls or prevents the growth of spoilage or pathogenic microorganisms [**59**].
- Affects the activity of the coagulant during manufacture and ripening [**30**] and the retention of coagulant activity [**28**] in the cheese curd.
- Solubilises colloidal calcium phosphate [**4**] and thus helps to determine the level of Ca in the cheese curd and the ratio of soluble to colloidal calcium. These factors, in turn, greatly influence cheese texture.
- Promotes syneresis [**34**, **36**] and hence helps to determine cheese composition (particularly the moisture content of the cheese).
- Influences the activity of enzymes during ripening and hence affects cheese flavour and quality.

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18 What are starters and what starter types are used for cheesemaking?

J. J. Sheehan

Starters

Starters are bacterial cultures used in cheese manufacture to produce lactic acid by controlled fermentation of lactose with a consequent reduction in pH. Acidification is central to cheese manufacture, composition, texture and ripening through its effect on rennet activity and retention, syneresis, dissolution of colloidal calcium phosphate and inhibition of adventitious organisms and pathogens. Starter cultures can also contribute to an open texture or eye formation [**57**] through production of CO_2 and to microbial safety by lowering pH, reducing redox potential and through competitive inhibition of pathogens [**58**, **59**]. Starters also influence cheese flavour and aroma through metabolism of citrate or through the activities of peptidases, esterases, lipases and other enzymes released during ripening [**23**].

Starter types

- Mesophilic starters of optimum growth temperatures of ~26–30 ºC are used in cheese manufacture where maximum scald does not exceed ~40 ºC. Examples include O-type cultures (*Leuconostoc lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris*) used in Cheddar manufacture and the citrate-fermenting LD-type cultures (containing *Lc. lactis* subsp. *lactis*, *Lc. lactis* subsp. *cremoris,* citrate-positive strains of Lactococcus and *Leuconostoc mesenteroides* subsp. *cremoris*) used in the manufacture of Dutch-type [**108**] and soft mould-ripened cheeses [**128**, **137**].
- Thermophilic starters with optimum growth temperatures of ~42 ºC are used in the manufacture of Italian [**96**, **146**] and Swiss-type [**117**] cheeses and include *Streptococcus thermophilus* and lactobacilli such as *Lactobacillus helveticus*, *L. delbrueckii* subsp. *bulgaricus* and *L. delbrueckii* subsp. *lactis*. More recently, thermophilic cultures are used as adjunct cultures in Cheddar [**100**] and Gouda-type [**108**] cheeses to enhance acidification (*S. thermophilus*) and flavour profile (lactobacilli) while mesophilic cultures have been used in the manufacture of Mozzarella-type cheeses to metabolise residual sugars to minimise browning [**188**] on subsequent cooking of the cheese.
- Secondary or adjunct cultures such as propionibacteria, bifidobacteria and *Penicillium* moulds may also be inoculated into the cheesemilk but are not considered to be starter cultures as they do not produce lactic acid.

Starters are also subdivided into defined- and mixed-strain cultures. Defined-strain cultures are pure cultures with known physiological characteristics and technological properties. These enable industrial-scale production of cheese of consistent technological quality and consist of ~2–6 phage-unrelated strains used in rotation as paired single strains or as multiple strains. Mixed-strain cultures

contain unknown numbers of strains of the same species and may also contain bacteria from different species or genera of lactic acid bacteria.

Traditional or artisanal starters are used in the manufacture of certain varieties, are of undefined composition and are reproduced daily through the practice of 'back-slopping' (i.e. using some whey from the previous day's manufacture as the starter).

Starter formats

- *Bulk starter*: This involves propagation of a commercial culture in a heat-treated (90–95 ºC for ~30 min) reconstituted skim milk powder or in a milk or whey-based phage inhibitory medium (PIM) which may also contain ingredients to improve culture growth, to control pH and to inhibit phage adsorption [**21**]. Control of pH reduces acid stress and associated loss of activity and helps to achieve higher cell numbers. The cultures are cooled at the beginning of the stationary phase of growth. Bulk cultures offer the advantage of being extremely active and allow flexibility in strain choice; however, they require specialist facilities, skilled labour and constant vigilance against phage infection.
- *Direct vat starters (DVS or DVI)*: Cultures in this format are highly concentrated (10^{10}–10^{12} cfu/g), are produced in freeze-dried powder or frozen pellet formats and are added directly to the vat. Advantages include reduction in risk of phage attack, flexibility of use, mixed strain and species cultures are available and propagation facilities are not required. However, a slightly longer pre-ripening period may be necessary with some DVS cultures due to an initial lag phase on addition to milk and modifications to the make procedures may be necessary to accommodate higher culture activity in the latter stage of cheese manufacture. Such alterations are not always necessary with more recent DVS cultures which have a minimal lag phase. Freeze-dried cultures are usually stored at −18 ºC but frozen cultures require cooling with dry ice during transit and storage at −45 ºC.

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19 What problems are caused by antibiotic residues in milk?

J. J. Sheehan

Antibiotics are used to treat bacterial infections including mastitis (infections of the mammary gland) in lactating cows or as slow release preparations, in dry cow therapy (administration of intramammary antibiotics at the end of lactation to remove any existing infection present at drying off and to prevent new infection during the non-lactating period). Types of antibiotics include cephalosporins, tetracyclines, macrolides, aminoglycosides, quinolones and polymyxins; however, β-lactams (which include penicillins, e.g. cloxacillin) are the most widely used for lactating cows. All antibiotics when administered enter milk to some degree; thus, there is a requirement for a period during which milk must be withheld from supply to creameries. In cases of insufficient withdrawal periods or increased or incorrectly administered dosage, antibiotic residues will occur in milk. Such residues are a public health concern owing to the emergence of antibiotic-resistant strains, their impact on human intestinal flora or their potential for allergic reactions in sensitised individuals. When present in cheesemilk, antibiotic residues pose technological problems due to their partial or total inhibition of the growth of starter cultures [**18**] and thus inhibition of acid production.

The concentration of antibiotics required to inhibit different starters depends on the strain used and on the antibiotic type. In general, lactic acid bacteria are more sensitive to penicillin than to cloxacillin. Lactococci are more sensitive to streptomycin and tetracycline and more resistant to penicillin than *Streptococcus thermophilus* and thermophillic lactobacilli. *Lactobacillus delbrueckii* subsp. *lactis* and *L. helveticus* are less resistant to penicillin than most strains of *L. casei* and *L. delbrueckii* subsp. *bulgaricus* and *Propionibacterium freudenreichii* are less resistant to penicillin than lactobacilli. Antibiotics may also influence the associative growth between two species when growing together.

Prior to cheese manufacture, antibiotic residues present in skim milk reconstituted from powder may inhibit preparation of bulk starter culture. During Cheddar-type cheese manufacture [**100**] lower levels of antibiotic residue result in a reduced rate of acidification particularly in the drain to salting period, which necessitates longer manufacture times, may influence moisture contents and may result in higher cheese pH. Elevated levels of antibiotic residues can result in up to a complete cessation of acidification after renneting and thus an abnormally high cheese pH. Such cheeses may have an uneven texture and pasty body with abnormal flavours described as yeasty, rancid or fermented. Antibiotics also inhibit the growth of non-starter lactic acid bacteria [**56**], which may reduce flavour intensity particularly in raw milk cheeses.

In brine-salted cheeses [**41**], antibiotic residues inhibit starter growth and acidification resulting in poor curd syneresis [**34**], soft curd particles with an excessive whey content and overall in a curd with an elevated moisture content. Coliforms, which are not inhibited by antibiotics like penicillin, can increase in numbers and produce gas which forms numerous holes in the curd [**57**]. Where

pH remains high, growth of putrefying bacteria may occur. In Swiss-type cheeses manufactured with propionic acid bacteria [**117**], abnormal fermentations occur, including the butyric acid fermentation with development of abnormal eyes, slits, cracks, brown spot discoloration and putrefaction. Development of wet, slimy surfaces with a strong off-odour may also occur [**91**]. In white mould cheeses, elevated curd moisture content facilitates growth of adventitious moulds such as *Mucor* and low curd lactate contents impedes the growth of *Penicillium camemberti* on the cheese surface.

Microbial inhibition tests and fast enzymatic and immunological tests are commercially available for detection of antibiotics in milk. Microbial inhibition tests are based on inhibition of growth of bacteria such as *Bacillus stearothermophilus* by antibiotic residues present in a milk test medium which prevents a colour change of a pH indicator. More specific high-performance liquid chromatography (HPLC), gas chromatography (GC) or gas chromatography–mass spectrometry (GC–MS) methodologies are required for identification and quantification of residues. Owing to the widespread availability of these tests, and to better education of farmers, problems with antibiotic residues have declined in many countries.

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20 What are lactenins and how do these natural substances inhibit acid production?

P. L. H. McSweeney

It is important to differentiate between slow acidification during cheesemaking (i.e. inadequate rate of production of lactic acid) and slow reduction in pH. The latter may be a result of inadequate acidification and/or high buffering capacity in the milk [**22**]. Slow acidification during manufacture is a major cause of poor quality cheese. The main causes of slow acidification are:

- presence of antibiotics [**19**];
- bacteriocin production;
- natural inhibitors (often called lactenins);
- bacteriophage [**21**].

Although bacteriophage are by far the most serious cause of slow acid production, the natural systems in milk inhibitory to bacterial growth (often called lactenins) may cause problems under certain circumstances.

Although milk contains a number of natural antibacterial systems (e.g. proteins that bind metal ions or vitamins, antibacterial enzymes and peptides released from the caseins and whey proteins on hydrolysis), those that have been shown to cause trouble during acidification are principally certain immunoglobulins and the lactoperoxidase system. Immunoglobulins can cause susceptible bacteria to aggregate together leading to localised acid production and precipitation, in severe cases, aggregates settle at the bottom of the vat. Although starters continue to grow, the localised production of acid leads to the inhibition of their growth. Fortunately, immunoglobulins are denatured by pasteurisation and hence this problem is associated with raw milk cheeses.

Lactoperoxidase (EC 1.11.1.7) is an indigenous enzyme found in milk, the activity of which survives high-temperature short-time (HTST) pasteurisation. Lactoperoxidase activity produces strongly antibacterial compounds in milk (principally the hypothiocyanate anion, $OSCN^-$, which is the major product of this reaction at neutral pH and hypothiocyanous acid, HOSCN, which may be more bactericidal) in the presence of the thiocyanate anion (SCN^-) and H_2O_2. Thiocyanate anions are found naturally in milk, particularly if the cow's feed contains vegetables such as cabbage, kale, brussel sprouts, cauliflower, turnips or rutabaga. These are particularly rich in glucosinolates, which upon hydrolysis yield thiocyanate in addition to other reaction products. H_2O_2 may be added to milk or produced by the action of catalase-negative bacteria (e.g. the starter [**18**]) or by the activities of xanthine oxidoreductase or glucose oxidase. The lactoperoxidase–H_2O_2–SCN^- system is a very potent natural antibacterial system in milk and has been investigated as an alternative to pasteurisation or to prolong the keeping quality of milk, particularly in regions where mechanical refrigeration is unavailable.

Inhibition of acidifcation by lactenins is now rare in the modern cheese industry because starter strains have been carefully selected so as not to be susceptible to these natural antibacterial systems in milk.

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21 What are bacteriophage and what strategies should be used to avoid phage infection?

T. Beresford

Bacteriophage (or phage) are viruses that attack bacterial cells. They are obligate parasites, unable to replicate outside a host cell. They are composed of a nucleic acid core (DNA in the case of the phage of lactic acid bacteria) and a protein coat. When viewed using an electron microscope, phage are observed to be composed of a 'head' (which contains the DNA) and a 'tail'. The size and shape of the head and the length of the tail varies between different phage; in addition, some phage contain a 'collar' connecting the head and tail sections. Phage classification has received much attention over the years with a view to determining the evolutionary relationships between various phage. These classification schemes originally relied on morphological and serological properties, phage–host relationships and protein content. In more recent studies DNA:DNA hybridisation and DNA sequencing have been used.

The commercial significance of phage for the cheese industry relates to the fact that phage infection of starter cultures [**18**] results in reduced rates or, in extreme cases, complete cessation of acid production [**17**]. This results in disruption of manufacturing schedules, reduction in product quality or, in the case of cessation of acid production, failure to produce any product. In the absence of adequate phage control systems, large-scale commercial cheese manufacture as currently practised would not be sustainable.

Phage can replicate only when they gain entry to a host cell and are often characterised on the basis of the type of life cycle employed, i.e. lytic or temperate. Lytic phage immediately begin to express their genes and replicate their DNA on entry to the bacterial cell and infection leads in a short length of time to lysis of the infected cell. Temperate phage on the other hand can enter either a lytic cycle or their DNA can be integrated into the host chromosome to form a 'prophage' where it is replicated *in situ* by the replication apparatus of the host cell during chromosomal replication and a copy of the phage DNA is passed on to all progeny cells. The prophage may remain inert within the chromosome indefinitely but can be activated by various environmental stresses, which cause it to excise and enter the lytic cycle.

The lytic cycle can be subdivided into a number of individual steps which are important in understanding and controlling the proliferation of phage in industrial settings. The first involves attachment of the phage to the host cell. Attachment is usually in a tail-first orientation and requires the phage to recognise specific receptors on the cell surface much the way that specific antibodies and antigens interact in the human immune system. This requirement for a specific receptor is important in defining the host range of a phage and can be used by the cheesemaker to select starter strains that are phage unrelated (i.e. strains that cannot be successfully attacked by the same phage). Attachment is followed by injection of the phage DNA. Once the phage DNA is injected into the bacterial cell, the host DNA replication and gene expression systems are

hijacked and used to make copies of the phage DNA and to synthesise phage proteins. Phage structural proteins self-assemble, initially into 'pro-heads'. The newly synthesised phage DNA is packaged into the pro-heads and final assembly into mature phage follows. Release of mature phage from the bacterial cell is mediated by a phage-encoded cell wall-degrading enzyme (lysins) that results in rupture and death of the host cell.

Two important parameters of the replication cycle that contribute to the severity of a phage infection are the phage 'generation time' and 'burst size'. Generation time refers to the time it takes from the initiation of infection to the release of mature progeny; for phage of lactic acid bacteria, generation times as short as 20 min have been measured. Burst size refers to the number of mature phage particles released from each infected cell, which for lactic acid bacteria phage can be 150 particles or more. These two factors dictate that the rate of phage replication will far outstrip that of bacterial host replication. A hypothetical situation is presented in Fig. 1 where a starter culture consisting of 10^6 cfu/ml and growing with a generation time of 30 min (conditions typical of an early phase in cheese manufacture) is infected at a level of 1 pfu/ml with phage with the same generation time (30 min) and a burst size of 100. While the starter culture will continue to grow, the replication rate of the phage is 100 times greater with the result that the complete bacterial culture is killed within 150 min and acid production ceases.

With the exception of some cheeses manufactured using traditional methods the majority of cheese, including all the primary varieties such as Cheddar [**100**], Mozzarella [**146**], Swiss-types [**117**], Dutch-types [**108**], white [**128**] and blue mould [**137**], and smear cheeses [**141**], are manufactured in large-scale highly automated plants. The large volumes of starter required to operate such plants creates an environment, which if not properly controlled, is ideal for phage proliferation. In addition, the tolerance for reduced or fluctuating rates of acid production is lower in such manufacturing environments than in more traditional

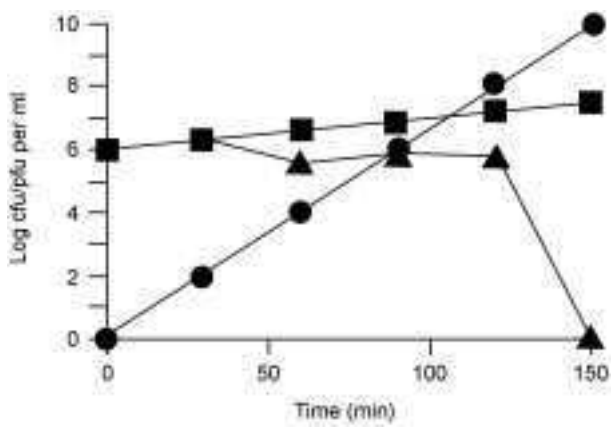

Fig. 1 Hypothetical growth pattern of phage (●) and starter culture in the absence (■) and presence (▲) of phage infection.

processes with fewer time constraints. Thus, the economic consequences of phage infection of starter cultures has resulted in a range of strategies being developed and employed by industry to protect against phage attack. These can be grouped as follows.

Starter selection

Starter cultures can be divided into two primary types, mixed-strain and defined-strain starters [**18**].

Mixed-strain cultures contain an undefined mix of species and strains of bacteria. It is estimated that approximately 90% of the bacteria in the culture contribute to acid production while the remaining 10% are predominantly involved in the production of flavour compounds. Such cultures have a long history of successful use in industry and examples of their use in large-scale manufacture of Dutch- and Swiss-type cheese have been reported. Because mixed strain cultures contain a wide variety of strains, many of which will be phage-unrelated, they have an in-built capacity to survive phage attack; however, owing to the variety of strains present, they also present a breeding ground for phage. Careful handling of the cultures during the preparation of bulk starters is necessary to reduce the number of transfers required and to maintain the strain balance.

Defined-strain cultures were first used in New Zealand in the 1930s and have found widespread use throughout the world in the production of a range of cheese varieties. Defined-strain cultures usually consist of two to six known cultures, selected on the basis of their acid-producing capabilities and that they are phage-unrelated. The fact that they contain a defined strain complement ensures that they have very predictable acid-producing capabilities, which is highly desirable in modern large-scale processing plants; however, as they contain only a limited number of strains, phage infection may have destructive consequences on starter activity. The fact that the strains within the mix are defined means that the cheese manufacturer can accurately monitor phage proliferation within the plant and substitute strains for which phage emerge with phage-unrelated strains before detrimental impacts on acid production are manifest. Indeed, most manufacturers will include a carefully designed rotation scheme based on the use of phage-unrelated strains to help prevent the accumulation of phage within a plant. Defined-strain cultures are also amenable to manipulation using modern techniques for phage hardening cultures such as selection of phage-insensitive mutants or food-grade transfer of phage resistance mechanisms between strains.

Regardless which type of culture is used, the cheesemaker should aim to reduce the number of transfers the culture is subjected to prior to cheese manufacture and starter activity should be monitored constantly within the factory to alert the cheesemaker to emerging problems. Some manufacturers have resorted to the use of concentrated starters produced by commercial culture suppliers either for inoculation of bulk starter tanks (also known as bulk set

starter) or for direct inoculation of the cheese vat (direct vat set – DVS – cultures) [**18**]. There are various advantages and disadvantages of such approaches; however, from the perspective of phage control such cultures offer an advantage as they are produced under stringent conditions by the culture houses and the opportunity for phage contamination within the cheese factory is greatly reduced by eliminating the need for excessive culture transfers.

Starter preparation

Control of environmental conditions within the starter preparation area and application of aseptic methods are probably the most important considerations in the preparation of starter cultures for cheese manufacture. The starter room is a critical control point in ensuring that phage does not cause a problem in the subsequent transformation of milk into cheese.

The starter preparation area should be located far away from cheese manufacture and subsequent whey handling facilities, and movement of personnel, particularly those from the cheese and whey factories, should not be allowed. The air supply to the starter preparation area should be filtered and a slight positive pressure maintained. The construction of the facility should be such that all surfaces are easy to clean and drains should include appropriate traps to reduce the risk of contamination. A regular cleaning regime should be established for the complete facility often including 'fogging' the air with a solution of hypochlorite or alternatively the use of UV light to control phage in the atmosphere.

The utensils and starter tanks used to propagate cultures should be easy to clean and contain no areas which allow standing fluid. A number of mechanically protected systems have been developed which have been widely and successfully used in the dairy industry. Two important aspects of such systems is that heat treatment of the growth medium and growth of the starter culture occur in the same completely enclosed tank and that inoculation of the starter takes place through a barrier that prevents the entry of unclean air. The starter tank should be filled to its maximum capacity; otherwise prolonged heat treatment will be necessary to eliminate phage in the head space. In systems where air escapes from the tank during the heating phase and returns during the subsequent cooling cycle, adequate filtration systems should be fitted to eliminate phage from the returning air. The operators within the starter preparation area should be fully trained, aware of phage and possible sources of contamination and fully competent in the implementation of aseptic handling and inoculation procedures.

A range of growth media are available for the production of starter cultures. The media may be based on whole or skim milk, reconstituted skim milk or whey solids. Many media include internal pH control mechanisms or pH may be controlled externally. The choice is primarily governed by the availability of substrate, cost, tradition and impact on cheese quality. The primary objective of these media is to produce a starter culture with maximum cell numbers and

activity to ensure active fermentation in the cheese vat. This has a subsequent indirect impact as it reduces the time available for phage contamination and growth during the cheese manufacturing phase. In addition, a number of phage inhibitory media (PIM) have been developed. These are based on the observation that attachment of phage to lactic acid bacteria is dependent on the presence of free calcium ions in the growth medium. Most PIM contain phosphates or citrates to sequester free calcium from the growth medium. There are mixed reports in the literature regarding the effectiveness of some of these media in inhibiting phage proliferation; however, most recent literature pertaining to this topic indicates that many of the current media are effective. It should be noted, however, that use of PIM alone in substandard facilities without adherence to the practices outlined above will probably lead to problems with phage.

Manufacturing environment

The typical modern cheese plant processes large quantities of milk, in the region of a million litres or more, on a daily basis. This is fermentation on a large scale and presents a background with significant opportunity for phage contamination and evolution. To help prevent problems with phage, cheese plants should be designed to ensure that effective cleaning is possible. The plant should be audited to ensure that no standing fluids remain following cleaning procedures and that drains are correctly designed and operating. The cheesemaker should endeavour to source quality milk, with low somatic cell counts and free from antibiotic residues.

In summary, the key to effective phage control is continuous monitoring combined with implementation of strict manufacturing practices and aseptic handling of starter cultures.

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22 What factors affect the buffering capacity of cheese?

P. L. H. McSweeney

The pH of cheese is a very important physicochemical parameter which affects the texture of cheese, its flavour and microbiological safety [**17**]. The pH of cheese is determined by the combined effects of acidification by the starter organisms (and deacidification during ripening by secondary organisms in certain varieties) and the ability of the cheese curd to resist changes in pH, i.e. its buffering capacity. The buffering capacity of milk is low near its natural pH (6.7) and increases to a maximum at about pH 5.1. Thus, assuming a steady rate of acid development by the starter, the pH of milk decreases rapidly initially and later slows down. Since the composition of cheese is quite different from that of milk, the pH at which maximum buffering occurs is also different; Cheddar and Emmental cheeses have maximum buffering capacities at ~pH 4.8.

The main components in cheese that buffer against changes in pH are the caseins and their degradation products, inorganic phosphate and organic acids (e.g. lactate, citrate, propionate, acetate and butyrate). The levels of these constituents in cheese vary with the composition of the milk and by the treatments of the curd that affect syneresis [**34**] and moisture levels and pH at whey drainage. The buffering capacity of cheese may also change during ripening owing to the production of CO_2 or organic acids, precipitation of calcium phosphate (as occurs at the surface of Camembert-type cheese), formation of calcium carbonate on the cheese surface (e.g. in hard cheeses), degradation of lactate and proteolysis of the caseins or their dephosphorylation.

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23 What enzymes from starters contribute to cheese ripening?

P. L. H. McSweeney

Lactic acid bacteria (LAB) contain many enzymes that contribute to the development of flavour in cheese during ripening. Of most significance are its proteolytic enzymes (proteinases and peptidases), lipase/esterase systems and amino acid catabolic enzymes. In addition, the enzyme systems of metabolically active cells are of course of great importance as they produce lactic acid (and together with buffering systems in the cheese thus determine pH [**17**, **22**]) and other products that influence flavour.

The proteolytic system of lactococci is generally similar to those of other LAB and consists of a cell envelope-associated proteinase (lactocepin, PrtP) which is loosely attached to the cell surface and requires calcium for stability (Fig. 1). Lactococci also contain transport systems for amino acids, di-/tripeptides and oligopeptides in addition to approximately four intracellular proteinases and a wide range of peptidases. While the cell is growing in milk, the role of the cell envelope-associated proteinase is to hydrolyse the caseins to provide short peptides which are then transported into the cell and degraded further by the intracellular peptidases. However, during cheese ripening, the starter cells die off and lyse at a rate dependent on the strain. Hence, the role of these enzymes during ripening is somewhat different and transport systems are not of significance. In cheese, the cell envelope-associated proteinase generally

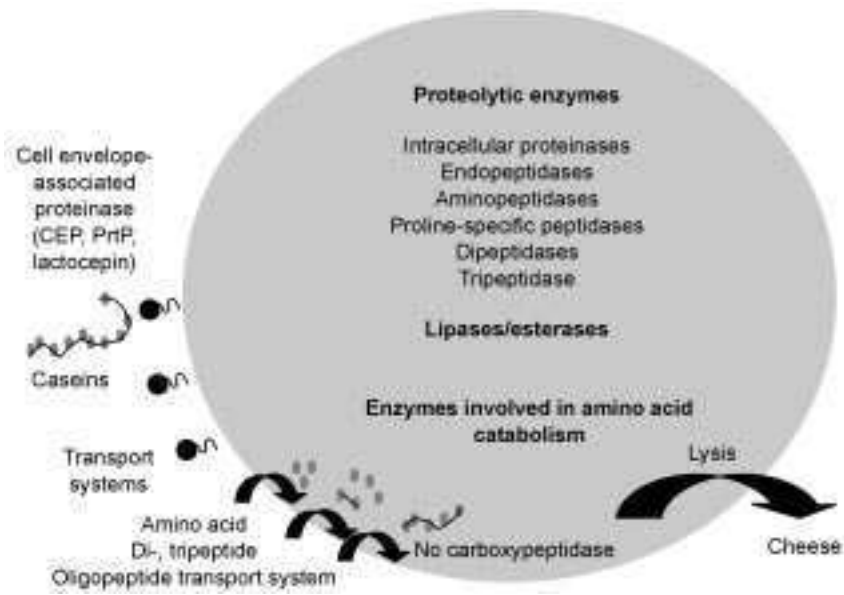

Fig. 1 Enzymes of *Lactococcus* which contribute to cheese ripening. Intracellular enzymes are released into the cheese matrix on lysis (modified from McSweeney, 2004).

acts on intermediate-sized peptides produced by the action of chymosin or plasmin on the caseins and the intracellular peptidases are released into the matrix of the cheese where they hydrolyse short peptides. The role of the intracellular proteinases of LAB in cheese ripening is unclear.

LAB contain intracellular esterases. Compared with other organisms (e.g. psychrotrophs [**7**]), LAB are weakly lipolytic but they are present at high numbers in cheese and for long periods and the lipases/esterase systems of LAB contribute to the low levels of lipolysis characteristic of cheeses made from pasteurised milk and lacking a strongly lipolytic secondary flora and not made using rennet paste (e.g. Cheddar or Gouda). Lipolytic enzymes of LAB are intracellular and a relationship between starter cell lysis and lipolysis has been demonstrated.

An important series of reactions that produce volatile flavour compounds in cheese involves the catabolism of amino acids. The key enzyme in amino acid catabolism appears to be the aminotransferase of LAB which catalyses the transfer of an amino group from an amino acid to an acceptor α-ketoacid (usually α-ketoglutarate), forming a new α-keto acid which can then be degraded further by other enzymes, perhaps also from LAB. The role of enzymes from LAB in the degradation of fatty acids has not been studied in detail but is likely to be important.

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Conversion of milk to curd

24 Introduction: how does rennet coagulate milk?

P. L. H. McSweeney

Caseins (~80% of milk protein [**2**]) occur in milk in the form of large, multi-molecular aggregates called micelles. Casein micelles are approximately spherical aggregates of the caseins (α_{s1}-, α_{s2}-, β- and κ-casein), together with inorganic ions collectively referred to as colloidal calcium phosphate [**4**]. There is an uneven distribution of the different caseins throughout the micelle; in particular, κ-casein is located principally on the surface of the micelle. κ-Casein stabilises the micelles and prevents them from aggregating together in the presence of Ca^{2+}. Were it not for κ-casein, the other caseins would aggregate together in the presence of Ca^{2+} as they are highly phosphorylated.

κ-Casein is divided into two parts. Residues 1–105 (approximately two-thirds of the molecule) are hydrophobic and associate with the other caseins while the C-terminal region of κ-casein (residues 106–169) are hydrophilic (usually containing complex sugar groups esterified to Thr residues) and protrude into the environment, stabilising the micelle (Fig. 1).

Enzymatic coagulation of milk involves modification of the casein micelles via limited proteolysis of κ-casein at or near its Phe_{105}-Met_{106} bond by proteinase(s) in preparations known as 'rennets' [**27**] followed by Ca^{2+}-induced aggregation of the rennet-altered micelles.

κ-Casein is the only casein hydrolysed during rennet coagulation. κ-Casein is hydrolysed to produce *para*-κ-casein (κ-casein fragment 1–105, κ-CN f1-105) and macropeptides (also called glycomacropeptides or caseinomacropeptides; κ-CN f106–169). Macropeptides diffuse into the aqueous phase; *para*-κ-casein remains attached to the micelle core. Macropeptides (~30% κ-casein or 4–5%

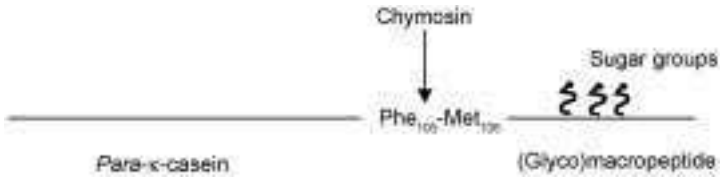

Fig. 1 κ-Casein.

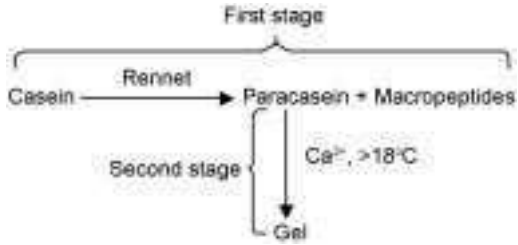

Fig. 2 Summary of the first (enzymatic) and second (aggregation) stages of rennet coagulation.

total casein) are lost. This is an unavoidable loss and a consequence of rennet coagulation but it does have consequences for cheese yield [**48**]. Proteolysis of κ-casein by the proteinase(s) in rennet preparations is referred to as the first stage of rennet action (Fig. 2).

Removal of the macropeptides from micelles reduces zeta (surface) potential of the micelles from -20 to about -10 mV and also removes the steric stabilising layer. When about 85% of total κ-casein is hydrolysed, colloidal stability of the micelles is reduced so much that they coagulate at temperatures above ~18°C in the presence of Ca^{2+}. This event is called the second stage of rennet action.

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25 Why is the Phe-Met bond of κ-casein so susceptible to rennet action?

P. L. H. McSweeney

The Phe_{105}-Met_{106} bond of κ-casein is many times more sensitive to rennet action [**24**] than any other bond in the caseins. However, the Phe-Met residues are not essential; human, porcine and rodent κ-caseins have Ile or Leu at position 106 and, interestingly, the proteinase of *Cryphonectria parasitica* [**29**] cleaves the adjacent bond (Ser_{104}-Phe_{105}). The importance of the Phe-Met bond has been investigated by studying the action of chymosin [**27**] on short peptides with the same amino acid sequence as this region of κ-casein. The smallest peptide hydrolysed by chymosin is κ-casein fragment 104–108 (Ser-Phe-Met-Ala-Ile); extending this peptide out towards the N or C-termini of κ-casein increases its susceptibility to rennet action. κ-Casein fragment 98–111 is hydrolysed as easily as intact κ-casein and certain amino acid residues appear important (e.g. Ser_{104}, Leu_{103}, Ala_{107}, Ile_{108}). The Phe-Met bond is in a very exposed part of the κ-casein molecule in a region of secondary structure composed of a β-strand located between two β-turns which facilitate access to the active site cleft of aspartyl proteinases such as chymosin. Thus, the conformation (shape) of κ-casein in the region of residues 98–111 renders this part of the molecule very susceptible to rennet action.

When ~85% of the κ-casein has been hydrolysed, micelles begin to aggregate progressively into a gel network. There is a rapid increase in viscosity on gelation, following a very slight initial decrease in viscosity after the addition of rennet (caused by the rennet 'shaving' off macropeptides from the micelle, thus reducing its effective volume owing to a large reduction in the hydration of the micelles and a small reduction in their size) (Fig. 1). Coagulation occurs at a lower percentage hydrolysis of κ-casein at higher temperature; however, the temperature of the milk at renneting ('setting temperature') is governed by the starter [**18**] and is usually 30 ºC. Decreasing pH or increasing Ca^{2+} also improves coagulation [**30**].

The actual reactions leading to coagulation are not fully clear but the following points can be made:

- Ca^{2+} is essential but the ability of the caseins to bind Ca^{2+} does not change on renneting.
- Colloidal calcium phosphate is also essential; >20% reduction in CCP prevents coagulation.
- Hydrophobic interactions are important (the rennet coagulum is soluble in urea).
- Electrostatic interactions may be important since moderately high ionic strength has an adverse effect on rennet coagulation.
- pH has little effect on the second stage of rennet action (but it has a major effect on the first stage [**30**]).
- Coagulation is very temperature sensitive; it will not occur <18ºC [**30**].

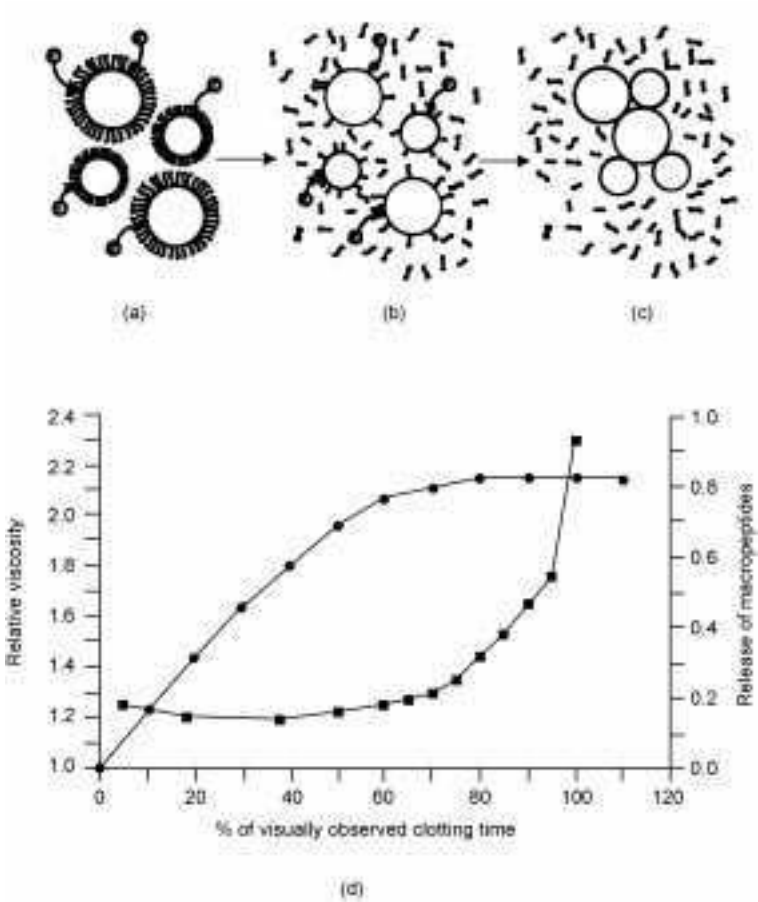

Fig. 1 Schematic representation of the rennet coagulation of milk: (a) casein micelles with intact κ-casein layer being attacked by chymosin (C); (b) micelles partially denuded of κ-casein; (c); extensively denuded micelles in the process of aggregation; (d) release of macropeptides (circles) and changes in relative viscosity (squares) during the course of rennet coagulation.

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26 How can one demonstrate that there are two stages to rennet coagulation?

P. L. H. McSweeney

There are two distinct stages to the rennet coagulation process: the enzymatic cleavage of κ-casein at or near its Phe_{105}-Met_{106} bond and the aggregation of the renneted micelles to form a gel [**24**]. These stages of the rennet coagulation can be separated by exploiting the fact that the second stage (gel assembly) effectively does not occur below *ca*. 18 ºC, while the first stage progresses slowly at low temperatures. To demonstrate the two stages of rennet coagulation, milk is treated with rennet and incubated at 10 ºC, during which period κ-casein is cleaved slowly (Fig. 1). Periodically, aliquots of the renneted milk are removed and warmed to 30 ºC and the time taken for coagulation (rennet coagulation time; RCT) at this temperature is measured [**30**]. As incubation time at 10 ºC increases, the RCT after warming to 30 ºC decreases as part of the rennet coagulation process has been completed at the low temperature. At long incubation times at 10 ºC, all the κ-casein will have been hydrolysed and the milk will coagulate quickly when warmed to 30 ºC. The RCT under these conditions reflects the time taken to complete the second stage of rennet coagulation.

The separation of the first and second stages of rennet coagulation has received some attention as the basis of possible modifications to accelerate or to control better the cheesemaking process. It should be possible to cold rennet milk (e.g. in a silo during storage) and for it then to gel very rapidly when it is warmed in the vat. The principal practical problem associated with cold renneting is the difficulty in uniformly heating quiescently a large volume of milk; non-uniform heating of renneted milk leads to different rates of gel formation and non-quiescent heating would interfere with the gel assembly process. Hence, despite much research, practical cheesemaking systems involving cold renneting of milk have not been used commercially.

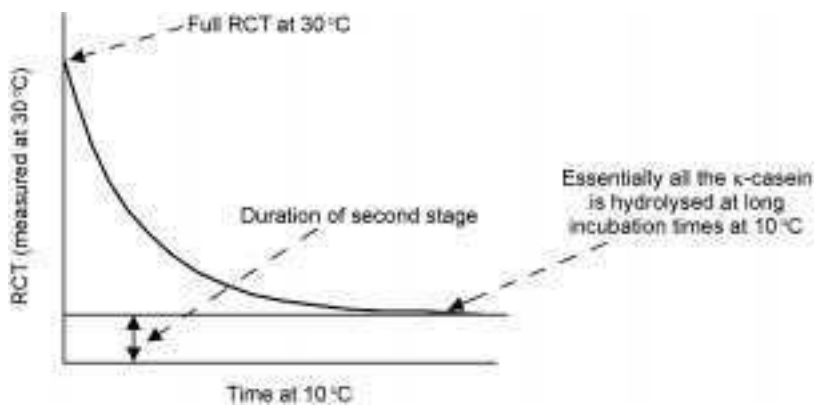

Fig. 1 Rennet coagulation time (RCT) of milk at 30 ºC as a function of pre-incubation at time 10 ºC showing the duration of the second stage of rennet coagulation.

27 What enzymes are in rennet?

P. L. H. McSweeney

'Rennet' is a general term for proteinase preparations used to coagulate milk. Most proteinases will coagulate milk [**24**] under suitable conditions of pH and temperature. However, all but a few enzymes are too proteolytic relative to their milk-clotting activity and thus hydrolyse the coagulum too quickly, leading to losses of short peptides in the whey (and thus reduced cheese yields) and the production of bitter peptides.

Rennets are obtained from a number of sources and contain one or more proteinases. Traditional calf rennet is a brine (~15% NaCl) extract from the stomachs ('vells') of milk-fed calves. The principal proteinase in calf rennet is chymosin but ~10% of the milk clotting activity of rennet is due to pepsin. As the animal ages, the percentage of chymosin decreases and the percentage of pepsin increases.

Bovine chymosin is a monomeric aspartyl proteinase of about 320 amino acid residues and a molecular mass of 35.6 kDa. Like other aspartyl proteinases, the chymosin molecule exists as two domains separated by a cleft which contains the active site of the enzyme. Three genetic variants of chymosin have been identified; calf rennet contains principally chymosins A and B with lesser amounts of C, with specific activities of 120, 100 and 50 RU/mg, respectively. Chymosins A and B differ by a single amino acid substitution. Chymosin generally tends to cleave peptide bonds containing bulky, hydrophobic amino acids. The physiological role of chymosin appears to be to coagulate milk in the stomach of the young mammal, increasing the effeciency of digestion by delaying discharge into the intestine. An important feature of chymosin is that its general proteolytic activity (i.e. action on bonds other than the Phe-Met bond) is low relative to its milk clotting activity (i.e. action on the Phe-Met bond). In countries where sheep or goats are the main dairy animal, lamb rennet and kid rennet, respectively, are used. These products are similar to calf rennet.

Rennet paste is a traditional rennet preparation used to coagulate milk for certain hard Italian and Greek cheeses (e.g. Provolone, the various Pecorino varieties and traditional Feta). This is a paste-like product that is produced by grinding up the entire calf stomach (but not extracting it with brine like calf rennet). In addition to chymosin, rennet paste contains a lipase, pregastric esterase, which is very important for lipolysis in these cheeses. Rennet pastes (particularly those made locally) have been the subject of public health concerns. Most rennet pastes are now produced commercially and are pasteurised. There has been active research interest in producing alternatives to rennet paste by blending commercial lipases into normal rennet extracts.

Plant rennets are used to coagulate milk for some cheese (e.g. the Portuguese variety Serra da Estrella). The most successful plant rennets are from the flowers of the Cardoon thistle (*Cynara cardunculus*) which grows wild in Spain and Portugal.

In addition to these traditional rennets, rennet substitutes are also used commonly [**29**]. Microbial acid proteinases (e.g. those produced naturally by the organisms *Rhizomucor meihei, Rhizomucor pusillus* and *Cryphonectria parasitica*) are available on the market and used widely, as is pure calf chymosin produced by fermentation of organisms modified genetically to produce this enzyme.

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28 What factors affect the retention of rennet in cheese curd?

P. L. H. McSweeney

The amount of active coagulant retained in the cheese curd is of major significance for proteolysis during ripening and the development of cheese texture, flavour and functionality. A range of factors affect retention of coagulant activity including moisture content of the cheese, cooking temperature, pH at whey drainage, ionic strength, amount of coagulant added to the milk, casein content of the milk, casein micelle size and pH in the cheese during ripening.

Since chymosin is dispersed in the aqueous phase of cheese, the more moisture is in the cheese [**34**, **35**, **36**], the more activity chymosin is retained. A major factor that influences retention of active coagulant is the temperature regimes encountered during manufacture; conditions used during the manufacture of high-cook varieties (e.g. Swiss [**117**], Italian Grana-type [**96**]) and during the cooking-stretching step of *pasta-filata* varieties [**146**] denature much chymosin and thus these cheeses start ripening with considerably lower levels of residual rennet than most varieties. Low pH at whey drainage results in higher levels of retention of chymosin in cheese curd (Fig. 1a). The level of rennet added to the milk has relatively little effect on the amount of chymosin retained in curd (Fig. 1b) which is unsurprising since the caseins are effectively saturated with respect to coagulant and most chymosin is lost in the whey. Increasing the ionic strength of milk increases the amount of chymosin retained in the curd (Fig. 1b). Increasing the casein level of milk increases the total amount of chymosin retained as the curd yield is increased, but the level of chymosin per mg dry matter is unchanged (Fig. 1c). Finally, casein micelle size appears to have little effect on the amount of chymosin retained in the curd unless the micelle size is very large.

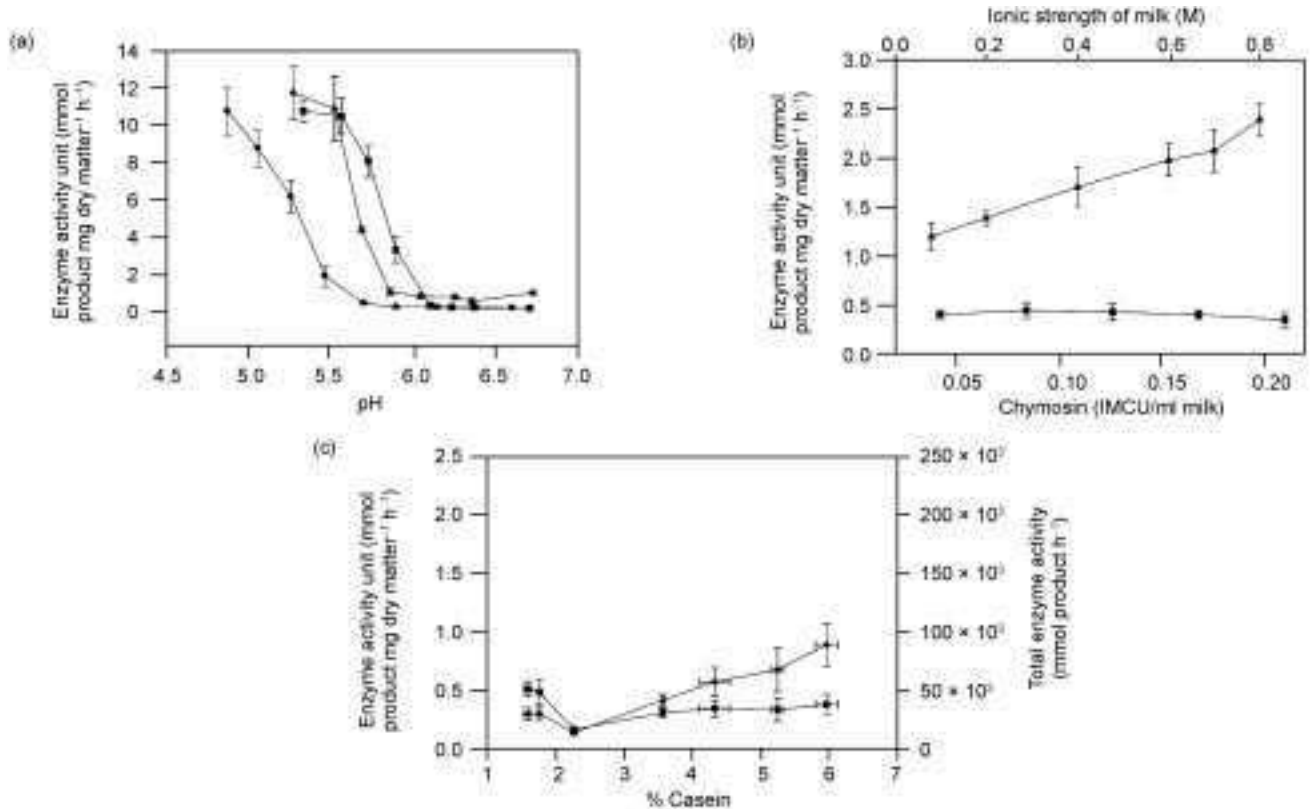

Fig. 1 Residual chymosin activity expressed as enzyme activity units (mmol product mg dry matter^{-1} h^{-1}) in curds as a function of (a) pH of milk at rennet addition ■, pH at whey drainage ● and in curds made using *Cryphonectria parasitica* proteinase as a coagulant as a function of pH of milk at rennet addition ▲, (b) as a function of quantity of rennet added (IMCU/ml) to milk (■) and ionic strength of the milk (▲) and (c) as a function of % casein in milk. Values are means from replicates ($n = 3$); error bars indicate ± standard deviation (Bansal, Fox and McSweeney, unpublished).

29 What rennet substitutes are suitable for cheesemaking?

P. L. H. McSweeney

World cheese production increased during the 20th century, but the supply of calf vells decreased, thus leading to an active search for substitutes to traditional calf rennet [**27**]; all successful rennet substitutes are aspartyl proteinases. Many proteinases coagulate milk but most are unsuitable for use as rennets. Rennet substitutes should have the following characteristics:

- high clotting to general proteolysis ratio;
- proper specificity on κ-casein;
- good activity in milk;
- easily denatured during whey processing (so that products made from whey do not contain active coagulant).

Rennet substitutes include pepsins, microbial aspartyl proteinases and fermentation-produced chymosin.

Pepsins

Pepsins are quite effective, particularly in blends with chymosin. Bovine pepsin is used mainly. Porcine pepsin is very unstable at pH values above about 6. Chicken pepsin has also been used (although it is very proteolytic).

Microbial aspartyl proteinases

Some yeasts and moulds naturally produce proteinases that are suitable for cheesemaking. Enzymes from *Rhizomucor meihei* (formerly *Mucor meihei*) are most widely used. Enzymes from *Rhizomucor pusillus* (formerly *Mucor pusillus*) are also available. Proteinases from *Cryphonectria parasitica* (formerly *Endothia parasitica*) are most suitable for high-cook cheeses (e.g. Swiss [**117**]) in which the coagulant is extensively denatured during manufacture [**28**].

Fermentation-produced chymosin (also called recombinant chymosin, genetically engineered chymosin)

The gene for calf chymosin has been cloned into host microorganisms (*Kluyveromyces lactis*, *Aspergillus niger*, *Escherichia coli*) and a protein identical to calf chymosin is produced by fermentation. These rennets have given excellent results in many cheese varieties and have vegetarian status but their use is subject to regulation.

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30 What factors affect rennet coagulation time?

P. L. H. McSweeney

The time from rennet addition to the onset of gelation (rennet coagulation time, RCT) is an important practical consideration in cheesemaking. The determination of RCT involves measurement of the time elapsed between the addition of a known amount of rennet (diluted) to a known volume of milk at a given temperature (usually 30 ºC) and the onset of gelation (usually assessed visually). To standardise results, low-heat skim milk powder reconstituted in 0.01% calcium chloride and perhaps adjusted to a given pH (e.g. 6.5) is often used as a substrate. An aliquot of the milk is placed in a bottle or tube which rotates in a water bath. When milk is fluid, it forms a film on the inside of the tube or bottle; on coagulation, visible flocs form.

The factors that affect RCT include temperature, pH, Ca^{2+} concentration, pre-heating of the milk (e.g. pasteurisation temperature) and concentrations of enzyme and casein. The effect of these factors on rennet coagulation time (RCT) and their relative influence on the first and second stages of coagulation are shown in Fig. 1.

Temperature

The optimum temperature for the rennet coagulation of milk is ~40 ºC. However during cheesemaking, milk is usually set at 30 ºC because of the starter [**18**]. Coagulation does not occur at an appreciable rate <18 ºC (owing to the effect of temperature on second stage of rennet coagulation [**26**]), while at higher temperatures, rennet is thermally denatured (55–60 ºC, depending on rennet type and pH), thus preventing the first stage of rennet coagulation from occurring.

pH

The rate of coagulation increases as pH decreases as chymosin comes closer to its pH optimum. The effect of pH is thus on first stage of rennet action. Milk may not coagulate if pH is too high.

Ca^{2+} concentration

A high Ca^{2+} level in milk speeds up coagulation (reduces RCT). The effect of Ca^{2+} is mainly on the second stage of rennet coagulation, although it has a slight (indirect) effect on the first stage because addition of calcium to milk alters its salts balance [**4**] by causing a precipitation of colloidal calcium phosphate and the production of H^+, which reduces the pH. Calcium chloride ($CaCl_2$) is often added to cheesemilk to improve its coagulation properties [**33**].

Preheating (e.g. pasteurisation, before it is cooled to its setting temperature) [**11**] Preheating at low temperatures gives a slight decrease in rennet coagulation time as precipitation of soluble to colloidal calcium phosphate occurs. This liberates

Factor	First phase	Second phase	Overall effect, see graphs below
Temperature	+	++	a
pH	+++	–	b
Ca	–	+++	c
Pre-heating	++	++++	d
Rennet concentration	++++	–	e
Protein concentration	+	++++	f

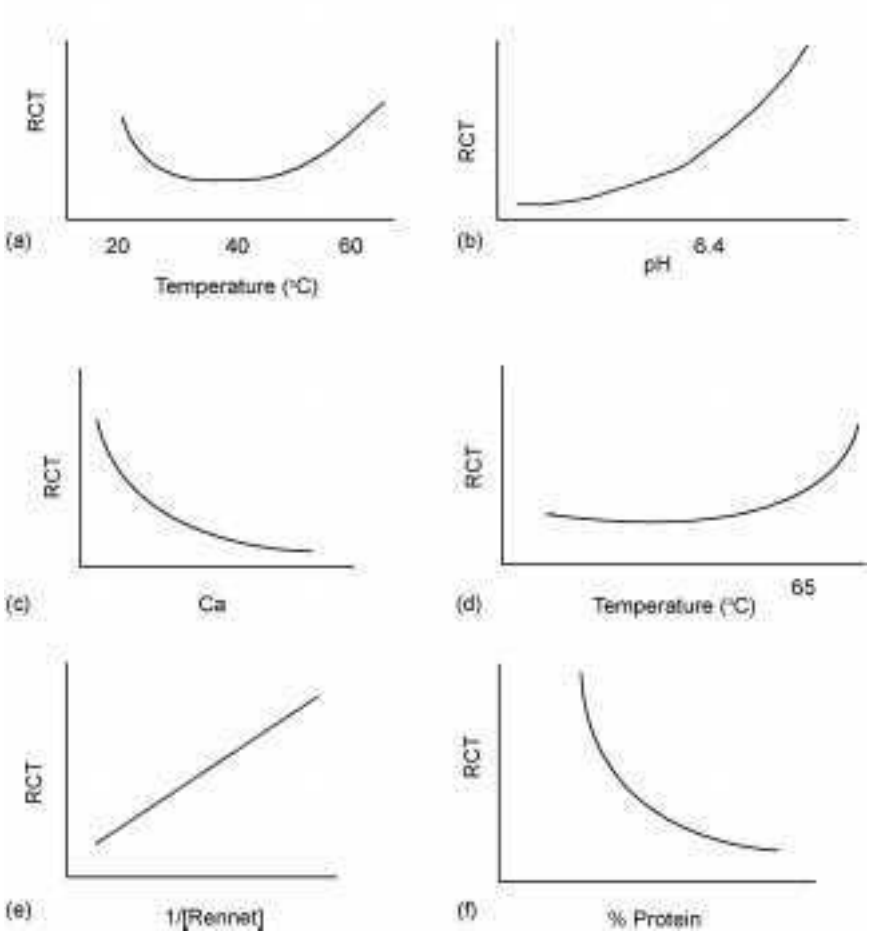

Fig. 1 Principal factors affecting the rennet coagulation time (RCT) of milk (from Fox and McSweeney, 1998).

H^+, which, in turn, decreases the pH, favouring rennet coagulation. However, preheating >70°C (depending on exposure time) causes denaturation of whey proteins (especially β-lactoglobulin). The free –SH group of denatured β-lactoglobulin interacts with κ-casein via a disulphide linkage. This has a

major adverse effect on the first and second stages of rennet action. Indeed, it is very difficult to make good quality cheese from over-pasteurised milk. This fact has a major consequence; it is not possible to sterilise milk for cheesemaking (and thus kill bacteriophage [**21**]). Thus, cheese starter systems must be more complex than those of many other fermentations. Cheesemilk is sometimes exposed to time × temperature combinations greater than high-temperature short-time (HTST) pasteurisation in order to increase yield [**48**] by inclusion of denatured whey proteins or to kill *Microbacterium avium* subsp. *paratuberculosis* [**62**].

Rennet concentration

Coagulation speeds up if more rennet is present; the effect is on the first stage of rennet action.

Protein concentration

Since casein forms the structural matrix of the gel, coagulation speeds up (i.e. RCT decreases) as protein level in the milk increases [**3**, **9**].

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31 What effects has homogenisation of milk on the manufacture and quality of cheese?

T. P. Guinee

Homogenisation of milk is a process whereby the native fat globules are disrupted by passing the milk through small orifices (valves) in series at 45–50 ºC and at pressures typically in the range 15–25 MPa. Homogenisation reduces fat globule size and increases the surface area of the fat by a factor of 5–6. The native protein–phospholipid membrane of the fat globules is sheared off in the process and replaced by a protein layer consisting of casein micelles and whey proteins [**2**]; this layer around the newly formed fat globules is frequently denoted the recombined fat globule membrane (RFGM). The RFGM enables the newly formed fat globules to behave as pseudo-protein particles that can interact with the casein micelles and become an integral part of the gel matrix formed during acid or rennet gelation of milk. Consequently, homogenisation generally leads to shorter gelation times and higher gel firmness, with the effect being more pronounced for acid-induced milk gels [**170**] than with rennet-induced milk gels [**24**].

Nevertheless, homogenisation of milk or cream is not widely practised in the manufacture of rennet curd cheeses because of the increased likelihood of the following effects:

1 poorer ability of the curd particles to knit during manufacture;
2 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);
3 increased moisture content (e.g. 1 to 2% at total pressures of $\geq$20 MPa);
4 altered curd rheology and texture, with the cheese being more easily fractured (lower facture strain), less elastic, 'shorter' and 'bitty';
5 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 or to burn.
6 increased propensity to the development of rancid flavours in the cheese, owing to an increased access of indigenous or microbial lipases in the cheese to the milk fat and the resultant production of free fatty acids.

The extent of these effects, which are normally considered undesirable for most rennet curd cheeses, depends on milk composition (e.g. protein-to-fat ratio, fat level) and homogenisation temperature and pressure. Some of the above effects (1, 2, 4, 5) are due to the higher stability of the RFGM to shearing and heating compared with the native fat globule membrane in non-homogenised milk. This leads to a much lower degree of free fat, which may be considered as a lubricating agent that facilitates the flowing together and knitting of curd particles during cheese manufacture. Similarly, the reduced level of free fat during heating in cheeses made from homogenised milk predisposes the cheese

to dehydration and crusting during baking. Free fat lubricates the relative displacement (movement) of contiguous protein layers in the heated cheese mass and also forms a coat on the surface of melting cheese, thereby reducing moisture evaporation and drying out.

In contrast to the above, homogenisation is desirable in the manufacture of some cheeses. Homogenisation of the cream or part of the milk is often practised in the manufacture of Blue-type cheese [**137**] as the RFGM allows access of mould lipases to the milk fat during cheese maturation and thereby enhances the formation of free fatty acids, which are later metabolised to methyl ketones, the latter being an essential component of the desired cheese flavour. Homogenisation of the raw cream may further enhance lipolysis in Blue cheese due to the action of the native milk lipoprotein lipase on the milk fat, resulting in higher levels of free fatty acids in the standardised cheesemilk (containing added cream) prior to pasteurisation and cheese manufacture. Homogenisation of milk may be exploited as a means of reducing firmness and improving the texture of reduced-fat cheeses, which tend to be excessively firm and elastic as a consequence of their relatively high protein-to-fat ratio. The whiter colour of homogenised milk cheeses is an attribute that may be desirable, e.g. Blue cheese or Mozzarella, or undesirable, e.g. Swiss-type cheese.

Homogenisation of milk is essential in the manufacture of:

- cheeses made from recombined milk (formed by homogenising oils (butter oil and/or vegetable oils) in aqueous dispersions of milk protein (e.g. reconstituted skim milks)) in countries where the demand for milk exceeds the local supply of fresh milk;
- some fresh acid-curd cheeses (e.g. Cream cheese) [**170**], especially where the fat content of the milk is high (e.g. 10% w/w). Here it prevents creaming of fat globules during the relatively long gelation period (~ 12 h) and thereby contributes to product homogeneity; moreover, it contributes to the short and brittle texture of Cream cheese.

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32 How does homogenisation affect the functionality of cheese?

T. P. Guinee

The functionality of cheese may be defined simply as a composite of properties of the unheated and/or heated cheese that affect its eating quality and/or its behaviour when used as an ingredient in assembled (e.g. pizza) or formulated foods (e.g. cheese sauce) [**187**]. The term functionality is usually retained for physical- and rheological-type properties that are dependent *inter alia* on cheese composition, microstructure, degrees of protein degradation and hydration. While cheese flavour and aroma of the unheated and heated cheeses are major quality determinants in most applications of cheese, they are not normally described as functional properties, and for this reason are also not included here. The various functional attributes of the unheated and heated cheese are listed in Table 1. Depending on the application in which the cheese is used, one or more are required.

Homogenisation of milk [**31**] reduces the fat-globule size and replaces the native phospolipid-protein membrane with a membrane consisting of mainly of

Table 1 Functional requirements of unheated cheeses

Functional requirements	Description	Application	Required rheological characteristics*
Shreddability	Ability to cut cleanly into long thin strips with low susceptibility to fracture sticking/matting or clumping	Shredded cheese for retail or catering, pizza	ϵ_f – high σ_{max} – medium–high
Sliceability	Ability to cut cleanly into thin slices, with low tendency to fracture and ability to bend before breaking	Slices for retail and food service	ϵ_f – high σ_{max} – medium–high
Grateability	Ability to fracture easily into small hard particles that resist matting during shearing, crushing or vibrating	Dried cheese for sprinkling	σ_f – high ϵ_f – low σ_{max} – high
Spreadability	Ability to spread easily when subjected to shear stress	Cheese for spreading, e.g. on crackers and bread	ϵ_f – high σ_f – low σ_{max} – low
Crumbliness	Ability to fracture easily into small irregularly shaped pieces when rubbed	Tossed salads, *crêpes au fromage*, soup garnishes	ϵ_f – low σ_f – medium–low σ_{max} – medium–low

* Rheological terms relating to large strain deformation using uniaxial compression tests: ϵ_f, fracture strain; σ_f, fracture stress; σ_{max}, firmness.

Table 2 Functional properties of heated cheese

Types of properties	Description	Level of property required	Cheese types with property	Applications where property is required
Meltability	Ability to soften	High	Most cheeses, apart from low-fat and skim milk cheese	Application: most, if not all, applications
Flowability	Ability to flow or spread	High	Many mature full-fat cheeses such as Cheddar, Gouda, Raclette, Cheshire, Blue	Gratins, cordon bleu products
		Moderate (plastic-type consistency)	Most young full-fat hard/semi-hard cheeses, Mozzarella, half-fat Cheddar	Many culinary dishes, such as toasted sandwiches and pizza
Flow resistance	Ability of cheese to resist flow and retain original dimensions on heating	Medium–high	Acid-heat coagulated cheese such as Paneer; rennet-curd cheeses made from high-heat treated milk or homogenised milk, some processed and imitation cheeses	Fried cheese, deep-fried cheese sticks, cheese for kebabs, cheese insets in burgers, cheese pieces in casseroles
Stretchability	Ability to form strings and/or sheets when extended	Medium–high	Mozzarella, Halloumi, Provolone, Kashkaval	Pizza
		Low	Most cheeses apart from Mozzarella and related stretched curd cheeses	Most applications, especially gratins, cordon bleu applications
Oiling-off	Ability to exude some free oil and create surface sheen on melted cheese	Low–moderate (with surface sheen)	Most rennet curd varieties	Most applications, ranging from moderate for gratins to low for omelettes and pizza
		Low–very low	Some processed and imitation cheeses, low-fat cheeses, cheeses made from homogenised milks	Flow-resistant applications such as fried cheese

casein. It affects the functionality of both the heated and unheated cheeses. It generally reduces the stress required to fracture (σ_f), firmness or hardness (σ_{max} force required to compress the cheese to a percentage of the original thickness) and springiness (recovery in height of a cheese sample following compression) of the unheated cheese. These changes usually coincide with a higher moisture level and lower protein content of homogenised-milk cheeses. However, the magnitude of the changes depends on homogenisation pressure, milk composition (e.g. protein-to-fat ratio), cheese type and composition (moisture, pH, calcium level, fat content). Consequently, inter-study discrepancy *vis-à-vis* the effect of milk homogenisation on the functionality of unheated cheese is evident in the published literature.

The effects of homogenisation of cheesemilk on the functionality of the heated cheese include reductions in free oil, flowability and stretchability; the cheese becomes more flow-resistant, which may be advantageous in certain applications such as fried cheese (Table 2). These effects are due to the concomitant reductions in the degree of fat coalescence in the unheated and heated cheese and consequently the decrease in the level of free oil released on baking. The casein membrane of the newly formed fat globule in homogenised milk cheese is much more stable to shear and heat than the native fat globule membrane in cheeses made from non-homogenised milk, and thereby reduces the level of free oil formed on heating/baking the cheese. Normally, free oil released during heating of cheeses from non-homogenised milk lubricates casein surfaces and facilitates the movement of adjoining casein layers of the cheese matrix. Consequently, functional properties of heated cheese, such as flow and stretch, that rely on displacement of adjacent layers of the casein matrix are markedly impaired by homogenisation of the cheesemilk.

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33 Why is $CaCl_2$ often added to cheesemilk?

T. P. Guinee

The addition of $CaCl_2$ at levels of ~0.2 g/l, i.e. ~1.8 mM Ca, to milk is common commercial practice, especially if the cheesemilk displays poor rennet coagulation and curd forming characteristics. Poor rennet coagulability of milk [**30**] can be the result of a variety of factors such as low protein level in milk, late lactation milk [**3**], high pH (e.g. >6.7), prolonged holding of milk at low temperature prior to cheese manufacture, high somatic cell count [**8**], high enzymatic activity or elevated pasteurisation temperature. Some of these factors are associated with a reduction in the levels of ionic and/or micellar calcium, an increase in the dissociation of casein from the casein micelle to the serum, and/or hydrolysis of the casein to proteose peptones and other soluble peptides by plasmin and/or proteinases from somatic cells. Soluble peptides do not contribute to gel formation and are largely not recoverable in the cheese. Deterioration of the coagulation properties is undesirable in cheese manufacture, especially in large modern dairy plants where the rennet gel tends to be cut on the basis of time rather than on gel firmness or gel firming rate.

Addition of $CaCl_2$ generally improves the rennet coagulation properties of cheesemilk, as reflected by a reduction in rennet gelation time (RGT) and increases in curd firming rate ($1/K_{20}$) and curd firmness (A_{30}), as measured using the Formagraph method (Fig. 1). Depending on the cheese manufacturing protocol (e.g. firmness of gel at cutting, cut programme), the addition of $CaCl_2$ may also increase the level of milk fat recovered to the cheese and the cheese yield. The positive effects of $CaCl_2$ on rennet coagulation properties are due to the following effects on the cheesemilk:

- increase in the concentrations of ionic (Ca^{2+}) and colloidal calcium phosphate [**4**];
- the concomitant decrease in pH (Fig. 1), an effect thought to be due to the reaction of some of the added Ca^{2+} ions with sodium phosphate salts, resulting in an increase in the hydrogen ion activity.

However, the curd firming rates and curd firmness plateau at addition rates of 2 to 9 mM $CaCl_2$ and decrease again at levels $\geq$9 mM $CaCl_2$ (~1 g/l). The decrease in curd firmness at the higher Ca levels may be due to the interaction of the excess Ca^{2+} with the negatively charged carboxyl groups on casein molecules, an effect that would increase the net positive charge on the casein and reduce its susceptibility to aggregation. As expected, the addition of calcium chelating agents such as ethylenediaminetetraacetic acid (EDTA), sodium citrate or sodium phosphate salts reduce firmness of rennet-induced milk gels.

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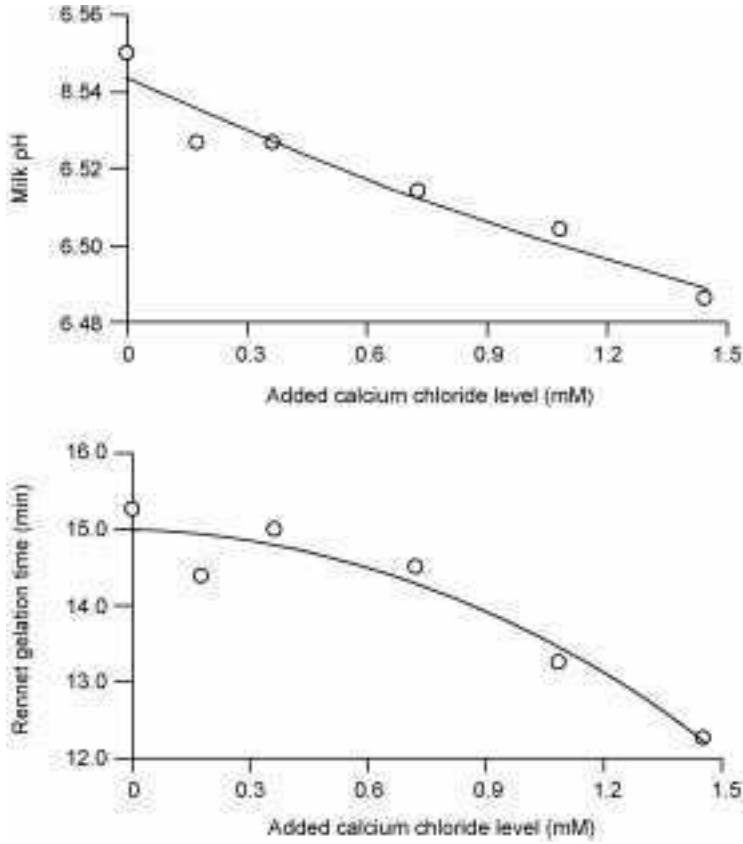

Fig. 1 Effect of adding calcium chloride on the pH and rennet coagulation properties of milk. The calcium chloride was a commercial preparation (33%, w/w); the following rennet coagulation properties were measured at 31 ºC using a Formagraph (Type 11700, Foss Electric, Hillerød, Denmark). The following parameters were obtained from the bifurcated displacement/time output signal: rennet gelation time (RGT, min) – a measure of time to the onset of gelation; curd firming rate index ($1/K_{20}$, min^{-1}) – inverse of time for signal to reach width of 20 mm; and curd firmness index (A_{30}, mm) – width of the signal at 30 min. (Guinee, T.P., unpublished results),

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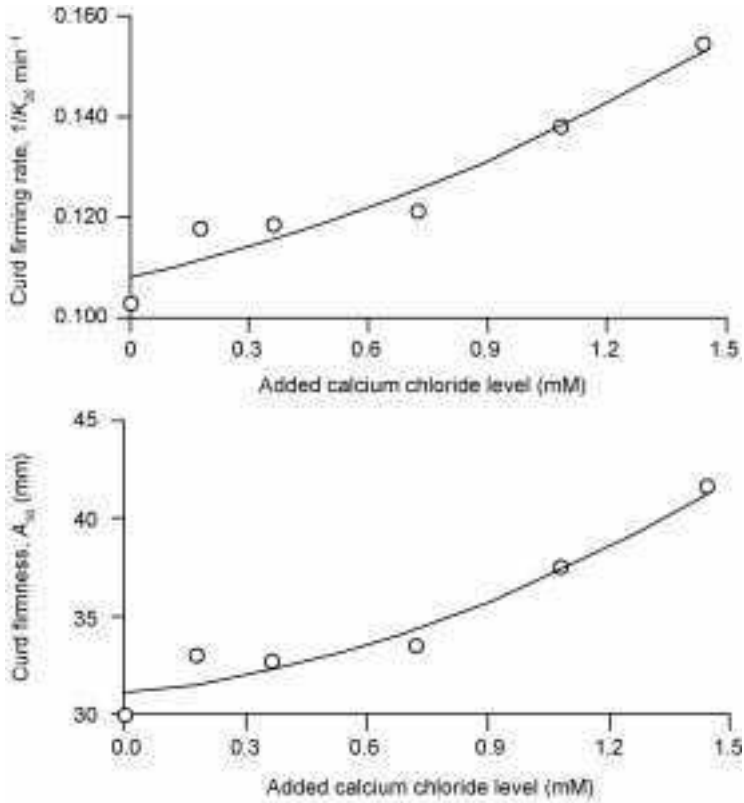

Fig. 1 (continued)

Syneresis

34 Introduction: what is syneresis?

P. L. H. McSweeney

After gelation of the milk during the manufacture of acid- or rennet-curd cheeses, the coagulum is subjected to various treatments with the objective of expressing whey. Rennet- or acid-induced milk gels are quite stable if left undisturbed but if they are subjected to external pressure or are cut and broken, the paracasein matrix rearranges and contracts, owing to various protein–protein interactions, expressing the aqueous phase of the gel (known as whey). This process is essentially a continuation of the gel assembly process and is known as syneresis. Control of syneresis [**35**, **36**, **37**] is essential as it allows the cheesemaker to control the moisture content of the cheese; moisture level, in turn, has many effects on cheese quality, texture and flavour. In general, the higher the moisture content of the cheese, the faster it will ripen and the less stable it will be.

Syneresis is promoted by cutting or breaking the curd, acidification through starter action, heating ('cooking') and stirring the curds–whey mixture, and pressing and salting the curds. The composition of the milk also affects syneresis; decreasing the fat content decreases the rate of syneresis and increasing the casein content of milk may increase the rate of syneresis. Methods used experimentally to measure syneresis include measuring the shrinkage of curd, determining the amount of whey expelled (either directly or by following the dilution of an added tracer dye), determination of the dry matter level or the density of the curd. However, all these methods have certain inherent drawbacks and experimental conditions must be standardised carefully to obtain reproducible results. Some workers have attempted to mimic the cheesemaking

process (e.g. by the addition of starter, cooking and stirring) when determining the effects of various factors on syneresis.

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35 How does the composition of milk affect syneresis?

P. L. H. McSweeney

The composition of milk affects syneresis but the effect is usually not large. Increasing the fat content of the milk slows syneresis somewhat and increases the water-holding capacity of cheese curd. Fat globules act as obstacles for the outward movement of moisture from the curd and for this reason increasing the fat content of milk increases cheese yield [**48**] (absolute yield, Y_a) by about 1.2 times the mass of added fat. The effect of the casein level in milk on syneresis is not entirely clear, as studies in which this factor was varied were often confounded with other factors. However, it appears that syneresis tends to be directly related to casein content in the milk (i.e. syneresis is better in gels made from milk with high casein levels). Since fat and casein levels in milk change in parallel during lactation, their effects on syneresis tend to be offset. Also, cheesemilk is often standardised [**9**] to a given composition or casein : fat ratio before manufacture which further minimises the effects of these factors on syneresis. Concentration of milk (e.g. by ultrafiltration [**16**]) reduces the rate of syneresis.

Increasing concentrations of calcium ions in milk generally promote syneresis although the Ca^{2+} concentration in cheesemilk may be increased routinely by the addition of $CaCl_2$ [**33**], which also increases syneresis. However, the effect of added $CaCl_2$ on syneresis may be negative at certain pH values and at high levels of addition, particularly if the gel is held for a long period prior to cutting. This negative effect has been attributed to the interaction of Ca^{2+} with negatively charged glutamate and aspartate residues on the caseins, leading to an increased net positive charge, swelling of the proteins and less syneresis. Addition of low levels of NaCl to the cheesemilk (as is practised during the manufacture of certain cheeses, e.g. Egyptian Domiati [**164**]) improves syneresis but higher levels of added NaCl retard syneresis.

The minor variations in the rate of syneresis that have been observed with stage of lactation [**2**] may be related to changes in Ca^{2+} concentration. Milk from cows suffering from mastitis [**8**] clots poorly and has somewhat diminished syneresis. Proteolysis by enzymes from psychrotrophs [**7**] reduces syneresis markedly but considerable proteolysis by plasmin appears hardly to affect whey expulsion.

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36 What processing variables affect syneresis?

P. L. H. McSweeney

Since cheesemaking is essentially a dehydration process, it is unsurprising that many operations during cheese manufacture are intended to promote syneresis [**34**]. The principal processing variables that affect syneresis are: cut size, cooking temperature [**37**], rate of acid development [**17**], stirring the curds–whey mixture, pressing and salting [**39**].

Pretreatment of milk can affect syneresis. Excessive heating of milk (e.g. over-pasteurisation) causes denaturation of whey proteins, poor rennet coagulation and reduced syneresis [**11**, **12**]. This decrease in syneresis is related almost linearly with the denaturation of β-lactoglobulin. Homogenisation of whole cheesemilk greatly reduces syneresis [**31**]. Homogenisation reduces the size of fat globules and largely replaces the natural milk fat globule membrane with casein. Casein-coated fat globules become incorporated into the paracasein gel network and hinder its shrinkage during syneresis. Most authors agree that rennet concentration has little effect on syneresis, although some workers claim a slight increase in syneresis when more rennet is added to the milk.

Whey is lost from the surface of the curd particles during syneresis. Hence, all else being equal, the smaller the cut size, the greater will be the surface area available for whey loss and the shorter the distance in the curd grain through which moisture must move and thus the faster will be the rate of syneresis. In contrast, curds for high-moisture cheeses (e.g. Camembert [**128**]) in which syneresis must be limited, are traditionally not cut but rather large gel pieces are scooped into the moulds. Increasing cooking temperature increases syneresis greatly; the cooking temperature used is characteristic of each variety and must be suitable for the starter used to acidify the milk during cheesemaking [**18**]. Acid production by some strains of lactococci is greatly reduced at cooking temperatures between 35 and 42 ºC and hence excessive cooking of curds for certain varieties can have a negative effect on syneresis by reducing acidification. Likewise, too severe a rate of cooking early during cooking can lead to case hardening [**38**] and reduced whey loss. Acidification during cheesemaking promotes syneresis as the net negative charge on the casein is reduced as its isoelectric point is approached, thus facilitating protein–protein interactions.

Stirring the curds–whey mixture during cooking promotes syneresis to an extent proportional to the intensity of stirring. Stirring facilitates heat transfer throughout the cheese vat, prevents the curd pieces from matting together and causes collisions between curd pieces and with the vat wall, all of which improve syneresis. However, initial stirring should be gentle; vigorous agitation of soft curd causes excessive losses of fat and casein in the whey and thus reduction in cheese yield [**48**]. Dry stirring of the curds after whey drainage (e.g. as practised during the manufacture of stirred-curd Cheddar or Colby cheeses) promotes syneresis [**41**]. Pressing the curd after the drainage of the majority of whey promotes syneresis as do cheddaring operations [**100**]. The addition of

NaCl causes considerable loss of moisture from the curd [**43**] but varying salting levels is inadvisable as a method to control moisture levels in cheese owing to the many effects of salt on cheese quality.

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37 Why are certain cook temperatures used for certain cheeses?

J. J. Sheehan

Certain cook temperatures or maximum scalds are used in the manufacture of certain cheeses because of their influence on starter growth and acidification profiles and on curd syneresis [**34**], both of which influence cheese composition and the development of texture and flavour during ripening:

- Cook temperature is used to control the rate of curd syneresis and thus cheese moisture content. Increased temperature during cooking directly increases the rate of syneresis. However, cooking rate should not be excessive as case hardening may occur, thus reducing syneresis [**38**]. Cook temperatures for high-moisture varieties such as Brie/Camembert [**128**] are low (~31–33 ºC), medium moisture cheeses such as Cheddar [**100**] and Gouda [**108**] are intermediate (36–40 ºC) and low-moisture cheeses such as Grana types [**96**] have high cooking temperatures (50–55 ºC).
- Cook temperatures are related to the starter cultures used. Mesophilic cultures [**18**] have temperature optima of 26–30 ºC and will acidify and survive up to ~40 ºC, dependent on strain. Thermophilic cultures have temperature optima of ~42–44 ºC and will survive up to ~55 ºC, dependent on strain. Syneresis is also directly related to acid production. Acidification under optimal temperatures for each starter strain promotes syneresis, but excessive maximum scalds that induce thermal stress and inhibit acidification will have a negative effect on syneresis [**34**].
- Cook temperatures also affect the buffering capacity of cheese [**22**]. Increasing cook temperature increases syneresis, reduces curd moisture and thus its lactose and lactate contents, which result in a lower lactate to protein ratio and thus a higher buffering capacity in the cheese. This results in higher pH values in the cheese.
- The effect of cook temperature on acid production during manufacture determines the extent of solubilisation of colloidal calcium phosphate [**4**]. Greater acidification while in the vat results in greater solubility of colloidal calcium phosphate and thus a greater proportion is removed in the whey during drainage. In general, curds with a low pH and low Ca content tend to have a crumbly, friable texture while those with a higher pH and higher Ca content tend to have a rubbery elastic texture.
- Cook temperature affects proteolysis due to rennet activity, both directly and indirectly [**88**]. Use of high cook temperatures such as in Swiss or hard Italian-type cheeses partially or nearly totally inactivates the activity of residual rennet in the curd. Rennet is responsible for primary proteolysis during ripening which has been associated with softening of cheese texture. Proteolysis by rennet also produces large peptides which undergo further degradation by starter proteinases and peptidases [**23**] during ripening to produce smaller peptides and amino acids. The effect of cook temperature on

acidification and cheese pH influences rennet retention as lower pH at drain leads to greater retention of rennet [**28**] in the curd and a greater proteolytic activity by rennet (optimum pH ~5.0) during ripening.

- High cook temperatures help to inhibit spoilage and potentially pathogenic bacteria [**59**]. The hygienic safety of Emmental cheese made from raw milk was concluded to be comparable to that produced from pasteurised milk due to its high cook temperature and other hurdles including ripening for at least 4 months (Fröhlich-Wyder and Bachmann, 2004).
- Cook temperature also affects the activity of indigenous milk enzymes and those of the starter cultures. Elevated cook temperatures increase plasmin activity in cheese through inactivation of inhibitors, leading to increased activation of plasminogen. Elevated cook temperatures can also have an attenuating affect on starter cells, resulting in a greater leakage of cytoplasmic enzymes due to lysis after death. These enzymes produce and degrade amino acids producing flavour and aroma compounds.

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38 What is case hardening and what problems does it cause?

P. L. H. McSweeney

Case hardening is caused by an excessive increase in the rate of cooking of the curds/whey mixture during cheesemaking. Although the rate of increase of temperature and maximum cooking temperature reached are characteristic of each variety, it is normal to increase the temperature of the curds/whey mixture slowly soon after cutting. If the initial rate of increase in temperature is too great, it can lead to excessive syneresis [**34**] at the outside of the curd piece, leading to the development of a dehydrated protein layer which inhibits further movement of moisture out of the curd piece and thus acts to reduce whey loss (Fig. 1). Case hardening can be avoided by reducing the rate of increase in temperature during the early stages of cooking and/or by introducing (or extending) a healing time between cutting and the start of cooking. Case hardening leads to an increase in the moisture level of the cheese which, in turn, leads to a range of problems and to generally poorer cheese quality.

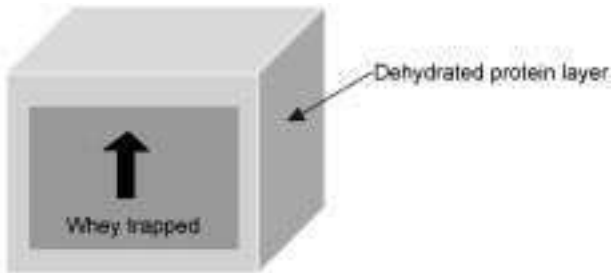

Fig. 1 Schematic representation of case hardening. Excessive rate of increase in cooking temperature during the early stages of cooking can lead to a dehydrated protein layer at the surface of the curd piece which traps moisture within.

Salt in cheese

39 Introduction: what are the functions of NaCl in cheese?

T. P. Guinee

Salt in cheese serves two major roles, namely it acts as a preservative [**46**] and contributes directly to flavour and quality (Fig. 1). The preservative action of NaCl is due to its depressing effect on the water activity (a_w) of the cheese: $a_w = p/p_o$, where p and p_o are the vapour pressure of the water in a cheese and of pure water, respectively. Water not contributing to the vapour pressure of cheese may be considered as being held by the cheese matrix and not available for microbial growth. Moreover, salt increases the osmotic pressure of the aqueous phase of foods, causing dehydration of bacterial cells, killing them or, at least, preventing their growth. The concentration of NaCl in cheese moisture is a major determinant of the a_w of young cheese and $a_w \approx 1 - 0.005\,65$ [NaCl], where [NaCl] is the concentration of NaCl as g/100 g cheese moisture. The a_w of cheeses range from ~0.99 in Quarg [**170**] to 0.92 in Parmesan [**97**], and the minimum a_w required for growth ranges from ~0.8 for most yeasts and moulds to ~0.95–0.96 for pathogenic bacteria such as *Escherichia coli* and *Yersinia enterocolitica* [**58**].

NaCl contributes directly to 'saltiness' in cheese, a flavour that is generally highly appreciated. The flavour of salt-free cheese is insipid and 'watery', with a minimum concentration of 0.8%, w/w, NaCl being required to overcome the insipid taste. NaCl contributes indirectly to flavour of cheese by its controlling influence on microbial and enzymatic activities which, in turn, influence lactose metabolism, cheese pH, degradation of fats and casein, and the formation of flavour compounds such as peptides, free amino acids and free fatty acids.

In addition to these functions, salt exerts a number of important effects on cheese (Fig. 1). Salt, together with pH and calcium level, has a large effect on

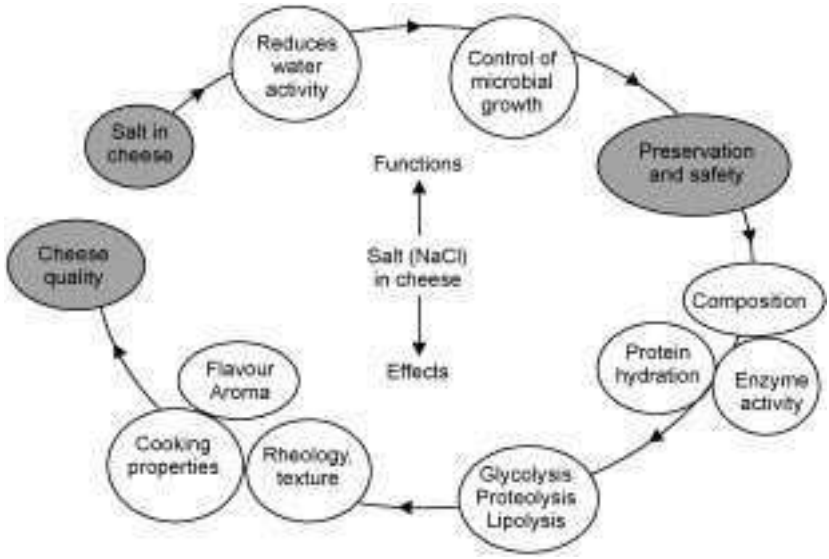

Fig. 1 Functions and effects of salt on cheese. The shaded ovals summarise the function and effect of salt in cheese, while the non-shaded ovals indicate how salt contributes to these.

the extent of paracasein hydration, or aggregation, which in turn affects the water binding capacity of the casein matrix, its tendency to synerese, its rheological and textural characteristics, and its cooking properties.

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40 What are the typical NaCl levels in different cheeses?

T. P. Guinee

The salt content of salted cheese varieties differs markedly (Table 1), typically ranging from about 0.5–0.7% (w/w) in acid curd varieties [**170**], such as Cottage cheese, and Emmental-type cheese [**117**] to ~4 to 6% (w/w) in pickled cheeses such as Domiati and Feta [**164**].

Most natural cheese varieties, apart from fresh, short shelf-life, acid-curd types with a low pH (4.5–4.8) such as Fromage frais, Quarg (and related types), contain added salt (NaCl). In Quarg, which is not salted, the presence of salt at a level of ~0.15% (w/w), is due to the presence of the indigenous Na^+ and Cl^- from milk (~50 and 95 mg/100 ml, respectively) in the moisture phase of the cheese. NaCl may also be added indirectly to cheese by way of dressings or condiments, such as cream dressing in Cottage cheese.

Intra-variety differences in salt content can be quite large, e.g. ranging from 1.6 to 2.4% (w/w) NaCl in commercial full-fat Cheddar cheeses and from 1.4 to 2.1% (w/w) in commercial Brie cheeses [**128**]. Factors contributing to such intra-variety differences include *inter alia*:

- inter-factory differences in make procedure, curd handling and salting technologies (e.g. equipment for metering, applying and/or mixing salt with curd or preparing cheese brines);
- variations in curd dimensions (e.g. of curd chips during dry salting of Cheddar curd or of wheel size and surface area-to-volume ratio during brine salting) and salting time,
- variations in milk composition and curd composition at salting (e.g. moisture level and pH).

Table 1 Approximate NaCl and moisture levels in different cheese varieties*

	NaCl (%, w/w)	Na (%, w/w)	Salt-in-moisture (%, w/w)
Quarg	0.15	0.06	0.19
Emmental	0.7	0.27	1.8
Appenzeller	1.3	0.51	3.6
Low-moisture Mozzarella	1.4	0.55	3.1
Cheddar	1.9	0.75	5.1
Limburger	2.0	0.79	4.4
Gouda	2.3	0.90	5.7
Danish Blue	3.3	1.29	7.7
Roquefort	4.1	1.61	10.1
Romano-type	4.1	1.61	13.8
Feta	4.5	1.76	7.1
Domiati	6.0	2.35	10.9
Pasteurised processed cheese products	0.7–1.62	1–1.5	–

* Data compiled from various sources.

However, intra-variety variation in salt can be minimised (e.g. 1.72 ± 0.12% w/w in commercial Cheddar cheese) by standardisation of cheesemaking procedure and manufacturing practices.

The NaCl content of retail pasteurised processed cheese products (PCPs) [**189**] varies from ~ 0.7 to 1.62% (w/w), as determined by measuring the level of chloride ion. However, compared with natural cheeses, PCPs contain a relatively high level of Na^+ because of the addition of NaCl at levels of ~ 0.4 to 1.05% (w/w), emulsifying salts (sodium phosphates or sodium citrates) at levels up to 3% (w/w), and optional ingredients, condiments and preservatives/ingredients (e.g. sodium propionate) to the formulation. The sodium level in retail PCPs available on the Irish market ranged from 1 to 1.5% (w/w); comparable values for Cheddar cheeses were 0.55 to 0.78% (w/w).

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41 What are the differences between dry-salting and brine-salting?

T. P. Guinee

There are two principal methods of salting cheese curd:

- *Dry-salting* (DS) – direct addition and mixing of dry salt crystals to broken or milled curd pieces at the end of manufacture, e.g. Cheddar [**100**] and Cottage cheeses [**170**];
- *Brine-salting (BS)* or *brining* – immersion of moulded cheese in brine, e.g. most varieties, including Edam, Gouda, Saint Paulin, Provolone;

Another method which is used less frequently is *surface dry salting* which involves the rubbing of dry salt, or salt slurry, to the surface of the moulded curds, e.g. some Blue-type cheeses [**137**]. Sometimes, a combination of the above methods is used.

There are several differences between DS and BS (Table 1). Most notable are the differences in the salting medium (dry salt or brine), application/mechanism of salt uptake, duration of the salting process, and the effect on whey composition. When cheese is placed in brine, salt uptake (absorption) occurs immediately and continues as long as there is gradient between the NaCl concentration in the brine and in the moisture phase of the cheese. In response to the concentration gradient, there is a net movement of Na^+ and Cl^- ions by diffusion from the brine (high concentration) into the cheese (low

Table 1 Differences between brine-salting and dry-salting

Characteristics of salt process	Brine-salting	Dry-salting
Form of salt used	Brine: aqueous solution of NaCl (typically ~20%, w/w) containing added calcium (typically 0.5%, w/w $CaCl_2$) and pH-adjusted to ~5.1 using food grade acid. Temperature maintained at typically 12 °C.	Dried crystalline salt.
Curd dimensions or weight at salting	Differs with variety and market: typically 2–10 kg, but ranging from ~0.2 kg for Camembert-type cheese to 100 kg for Emmental-type cheese blocks in modern manufacture.	Relatively small curd pieces: e.g. curd particles (Cottage cheese), rectangular-shaped curd chips ($1.5 \times 1.5 \times 10\,cm^3$) for Cheddar or irregularly shaped broken pieces (~30 g) for Stilton.
Time of salt application	On completion of moulding and pressing of cheese curd to final size/shape of cheese.	Prior to moulding of cheese curd.

Table 1 (continued)

Characteristics of salt process	Brine-salting	Dry-salting
Method of salt application	Immersion of moulded curd in brine.	Automated weighing, mechanical distribution and mixing of dry salt with curd pieces in modern production.
Mechanism of salt uptake	Pseudo-diffusion process: (i) diffusion of NaCl molecules from moisture phase of brine (high concentration) into the moisture phase of cheese (low concentration) in response to concentration gradient; (ii) simultaneous movement of water molecules in opposite direction.	(i) Dissolution of some added dry salt in the surface moisture of curd pieces creating saturated brine around curd pieces. (ii) Pseudo-diffusion process: salt uptake then occurs as for brine-salting; the consequent loss of water from the cheese dissolves more of the added dry salt.
Duration of salting process	Few hours to several days, dependent on size and shape of cheese, NaCl content of brine and temperature, and required salt content in finished cheese.	15–30 min.
Speed of salt uptake	Fast or slow depending on cheese shape/dimensions and NaCl level of brine.	Rapid because of relatively large surface area of curd pieces.
Effect on whey	No effect on cheese whey composition or quality.	Results in the generation of a 'salty whey' owing to loss in ~20–30% of applied salt during salting process and during subsequent pressing of salted curd. Salty whey (~7% w/w NaCl) accounts for 2% (v/v) of total whey and is subject to centrifugal separation to reduce fat content from ~ 4 to 0.12% (w/w). In modern dairy plants, the separated whey is de-salted using nanofiltration and mixed in with the bulk 'sweet' whey and further processed into ingredients such as whey powders, whey protein concentrates/isolates. In smaller plants the separated whey is either discarded to drain or collected and sold for pig feed.

concentration). Simultaneously, water molecules diffuse out through the cheese matrix to the brine to restore osmotic pressure equilibrium. When dry salt is distributed over the surface of milled curd or curd granules, some NaCl dissolves in the surface moisture and diffuses slowly inwards a short distance. This causes a counterflow of moisture from the curd to the surface, which dissolves the remaining salt crystals and, in effect, creates a supersaturated brine solution around each particle, provided mixing of curd and salt is adequate. Salt uptake then occurs as for brine salting.

The duration of BS varies from <12 h for cheeses such as Camembert [**128**] to ~15 days for Parmesan [**97**], with exact times depending on factors that affect the rate of salt uptake. In contrast, the times for DS curd in the form of milled curd chips (10–20 min) are much shorter because of the relatively large surface area to volume ratio of the curd mass as a whole, which results in salt uptake from many surfaces simultaneously. During DS, some of the saturated 'brine' on the surface of curd particles drains away through the curd mass while more is physically expelled from the curd particles during pressing and is lost in the 'press whey'. This results in the generation of salty whey, which for Cheddar cheese accounts for ~2% (v/v) of the total whey.

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42 What factors affect salt uptake in cheese curd?

T. P. Guinee

The factors affecting salt absorption/uptake by brine-salted and dry-salted cheeses [**41**] are summarised in Table 1. While the level of salt absorbed by brine-salted cheeses increases with brining time, the rate of salt uptake decreases owing to the decrease in the concentration gradient of salt between the cheese moisture and the brine; consequently the level of salt uptake has been found to be proportional to the square root of brining time. Similarly, where cheeses have been partly dry-salted prior to brining, the magnitude of the increase in salt-in-moisture decreases with the level of pre-salting owing to the decrease in salt gradient between cheese moisture and the brine. During dry-salting, the level of salt absorbed increases less than proportionally with level of salt added to the curd, an effect due to the increase in whey released from the curd and the concomitant higher salt losses at increased salting levels.

Increasing brine temperature (5 to 25 ºC) increases the quantity of salt absorbed in brine-salt cheeses, an effect due partly to an increase in the rate of diffusion of NaCl molecules into the cheese matrix. In contrast, an increase in curd temperature from 24 to 41 ºC during dry salting has the opposite effect; this is thought to result from the higher loss of fat at the surfaces of curd chips, which forms a barrier to the salt molecules entering the cheese.

The increase in salt uptake in brine-salted cheese as the level of cheese moisture increases is due to the simultaneous decrease in the level of cheese protein which reduces the frictional effects of the protein matrix of the cheese on the inward diffusion of Na^+ and Cl^- salt ions. In dry-salting milled Cheddar curd, the reverse situation occurs: as the initial moisture level increases, the rate of salt absorption decreases, giving lower salt levels in the cheese for a fixed salting level. The lower salt uptake is due to higher loss of whey from the curd: more of the added dry salt is dissolved to make concentrated brine, which percolates through the spaces between the chips, drains away, loses contact with the chips and thereby lowers the effective amount of salt available for uptake.

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Table 1 Factors affecting salt uptake in brine-salted and dry-salted cheeses*

	Brine-salted cheeses		Dry-salted cheeses
Factor	Effect on salt uptake	Factor	Effect on salt uptake
Brine concentration (BC)	Increases as BC increases in range 5–25% (w/w) NaCl	Salting level (SL): quantity of salt added to curd	Increases as SL increases
Salting time (ST)	Increases at a diminishing rate with ST	Mixing time (MT) of salt with curd	Increases as MT is increased from 20 s to 6 min
		Holding time between salt addition/mixing and pressing (TSP)	Increases as TSP is increased from 15 to 30 min for Cheddar
Brine temperature (BT)	Increases as BT is increased from 5 to 20 ºC	Curd temperature (CT)	Decreases as curd temperature is increased from 24 to 41 ºC
Surface area-to-volume ratio of curd (SAV)	Increases as SAV increases	Curd chip size/surface area (SA)	Increases as SA is increased by reducing chip size
Shape: surface curvature (SC)	Decreases with increase in SC		
Curd moisture (CM)	Increases as CM increases	Curd moisture (CM)	Decreases as CM increases
Curd pH	Decreases as pH is increased from 4.7 to 5.7	Curd acidity (CA) at salting	Decreases as CA increases
*Salt-in-moisture (S/M) level of curd before brining	Decreases as S/M of curd increases		

* Combined dry-salting and brine-salting is sometimes used (e.g. for low-moisture Mozzarella, the objective being the reduction in brining capacity and time, which can be expensive).

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43 How does NaCl affect cheese composition?

T. P. Guinee

It is generally accepted that there is an inverse relationship between the levels of salt and moisture in both brine- and dry-salted cheeses [**41**] (Figs 1 and 2). This is most readily observed in brine and/or dry-salted moulded cheeses during, or immediately after, salting, where a decreasing salt gradient from surface to the centre is accompanied by a decreasing moisture gradient in the opposite direction (Fig. 1). Owing to the slow diffusion of salt from the rind inwards, these gradients disappear slowly and equilibrium of S/M is eventually reached at some stage of ripening.

Salt and moisture content have a major effect on water activity, and thereby exert control over microbial growth [**46**], enzyme activity and biochemical changes during cheese ripening. Additionally, salt content influences the degree of casein hydration, and hence degree of casein aggregation, in cheese which affects its susceptibility to enzymatic degradation. Consequently, salt content also influences aspects of cheese composition other than moisture content. For most varieties, the salt content of cheese is positively correlated with levels of unfermented lactose and pH (Fig. 2) and inversely correlated with the levels of primary proteolysis (as measured by the extent of degradation of both α_{s1}- and

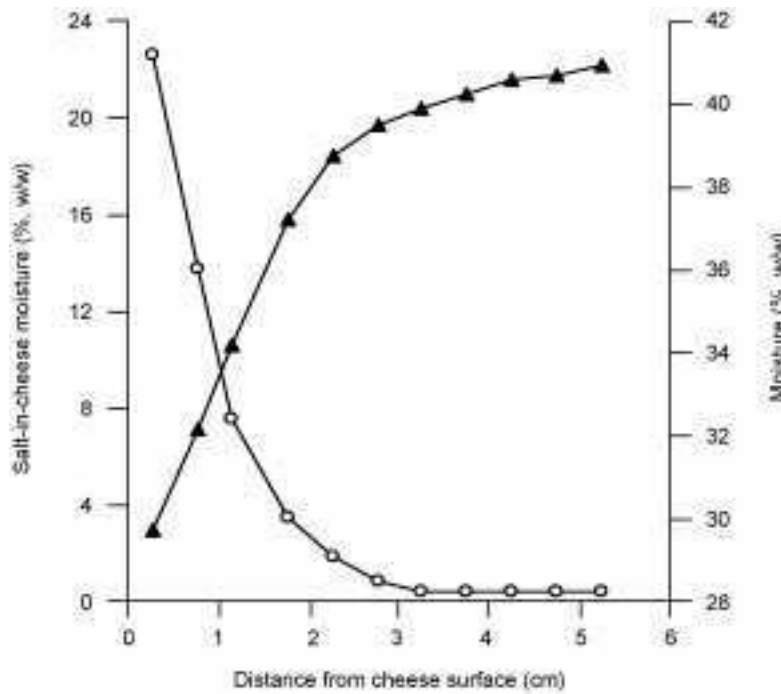

Fig. 1 Moisture (○) and salt-in-moisture (▲) in Gouda cheese as a function of distance from the salting surface after brine-salting for 4 days at 20 ºC in 24 (%, w/w) NaCl brine.

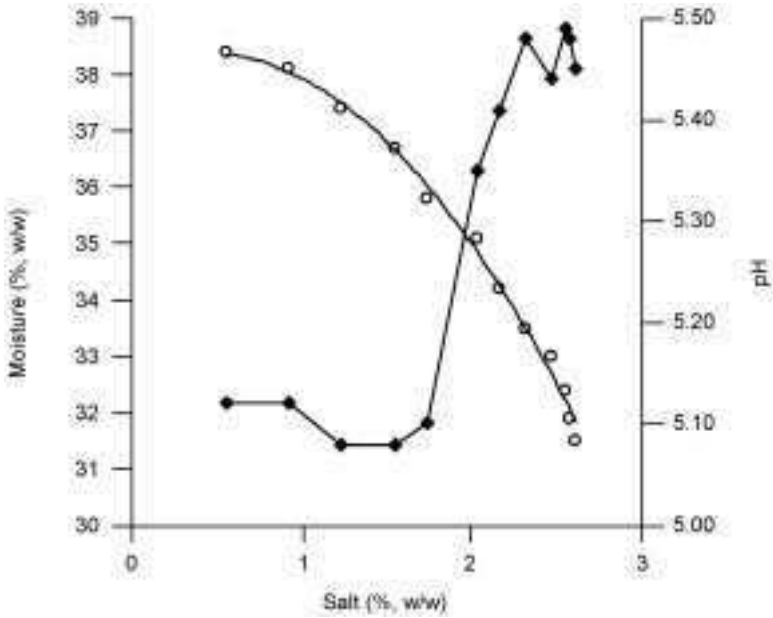

Fig. 2 Moisture (○) and pH (♦) in Cheddar cheese as a function of salt content (Drawn from data of O'Connor, 1974).

β-caseins, or levels of pH 4.6 soluble N) and lipolysis, as measured by concentrations of free fatty acids. The inhibitory effect of salt on the proteolysis of β-casein in Cheddar cheese is particularly important in reducing the incidence of bitterness [**89**], the occurrence of which is greatly increased at salt-in-moisture levels <4.9% (w/w).

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44 What causes the outside of brine-salted cheese to become slimy and sticky?

T. P. Guinee

The root of this defect is an increase in protein hydration, and water uptake, in the surface layer (outer 3–4 mm) of the cheese; this layer is normally denoted the rind. This problem is often referred to as *soft-rind* or *rind rot*. The symptoms of the problem depend very much on the cheese type, being more pronounced for low-calcium, high-moisture and surface mould-ripened or smear-ripened cheeses. Immediately after brining, the obvious symptoms include: a surface that is damp, soft and 'velvety', slimy and sticky. With surface mould-ripened cheese [**128**], the defect, which may be scarcely noticeable after brining, may become acute with progressive ripening and mould growth, as manifested by the easy displacement or tearing away of the outside mould layer from the main body of the cheese when removing the cheese from the shelf or when handling.

Factors that induce the defect are:

- using fresh brines (with NaCl levels of ~≤20%, w/w) without calcium;
- using low concentration brines (e.g. ≤18%, w/w, NaCl), with the effect becoming more pronounced and extending deeper into the cheese as the NaCl concentration is lowered;
- not adjusting the pH of the brine to ~5.0;
- combinations of above.

Other factors that promote a greater degree of hydration of the cheese protein, such as low calcium content, low brine temperature (≤4 ºC) and proteolysis, accentuate the defect. An explanation of the defect is given below.

On salting in brines of typical composition (e.g. 18–23%, w/w, NaCl and 0.5%, w/w, $CaCl_2$), cheese loses water during brining, resulting in a net weight loss of ~2.0% (w/w) [**43**]. This is because the inward diffusion of NaCl from the brine (region of high NaCl concentration) into the cheese (region of low NaCl concentration) is accompanied by the mutual outward diffusion of water molecules from the cheese to the brine, so as to restore osmotic pressure equilibrium between the cheese moisture and the brine. Hence, the inward diffusion of NaCl continues as long as a gradient between the NaCl concentration in the brine and in the moisture phase of the cheese exists. The quantity of water lost is about twice the quantity of salt gained because of the smaller size of the H^+OH^- ion pair compared with that of Na^+Cl^- and the different interactive effects of the ions with the cheese matrix in which the diffusion medium (water) is enclosed.

In contrast, salting in freshly prepared dilute brine (e.g. ≤18%, w/w, NaCl) without added calcium leads to an increase in the moisture content in the outer rind region. In the absence of brine calcium, calcium in the moisture phase of the cheese (which, at a cheese pH of 5.2–5.3, amounts to ~35% of the total calcium) diffuses out of the cheese into the brine. Consequently, the casein-bound calcium in the cheese solubilises so as to restore chemical equilibrium between soluble and colloidal forms of calcium. The reduction in the casein-bound

calcium enables the cheese protein (paracasein) to bind water to a degree dependent on the pH of the cheese. In cheese, protein hydration is maximum at pH ~5.2 to 5.4, decreases rapidly as pH is reduced below 5.2, and is minimum at the isoelectric pH of the casein (~4.6). Reducing the level of NaCl in brine to levels lower than 18–20%, w/w, accentuates the defect; this is because high brine levels dehydrate the protein (*salting-out* effect) while low levels promote hydration (*salting-in* effect).

The problem rarely occurs in 'old', well-used brine because of the accumulation of soluble calcium and lactic acid which migrate from the cheese with the moisture into the brine; the calcium level and pH of mature brines are typically $\geq$0.25% w/w, and ~5.0, respectively.

When preparing fresh brines, the defect can be avoided by ensuring:

- an adequate level of NaCl (18–25%, w/w) in the brine – higher levels are undesirable as they lead to dry, whitish rinds;
- an adequate calcium level (~ 0.25–0.5%, w/w) in the brine – high levels (e.g. $\geq$1%, w/w) can adversely affect taste of the cheese and lead to dry rind;
- adjustment of the pH of the brine to ~5.0, using food grade acid – lower pH values can cause a dry rind and a whitening of the surface, while higher pH values favour hydration and swelling of protein;
- a brine temperature of >10 ºC (lower temperatures favour soubilisation of protein-bound calcium in the cheese).

The exact composition and temperature of brine can be optimised to suit the particular cheese type being salted, e.g. when preparing fresh brine the level of calcium chloride added should be such that the calcium level in the brine is similar to that in the cheese.

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45 How should cheese brine be prepared and maintained?

T. P. Guinee

Brine should be prepared by dissolving at room temperature the appropriate quantities of sodium chloride and calcium chloride in reverse-osmosis treated water that has ideally been pasteurised or sterilised by UV radiation. The final levels of NaCl and Ca in the brine should typically be ~22% (w/w) and ~0.3 to 0.5% (w/w), respectively, even though the exact calcium level will depend on the level in the cheese. Dissolution of the NaCl is aided by constant recirculation of the brine. The pH of the brine is then adjusted with a food grade acid (e.g. lactic acid) to a pH value of ~5.0–5.1, and then cooled to ~12 ºC. At the target values for concentrations of NaCl and Ca, pH and temperature, the risk of surface defects such as rind rot, or soft rind are minimised [**44**].

Despite the fact that cheese brine is an unfavourable environment, some halotolerant microorganisms such as salt-resistant lactobacilli, yeasts and moulds can survive and grow. These microorganisms are undesirable as they can lead to surface patches of mould growth, pigment spots and softening of the cheese surface due to excessive proteolysis. Several systems are employed to maintain a satisfactory microbiological quality in the brine: low pH, high level of plant hygiene, addition of preservatives (subject to legislation) and/or application of bacteriostatic treatments such as periodic pasteurisation (e.g. 75–80 ºC for 15–25 s), microfiltration, filtration using diatomaceous earth, and use of ultraviolet radiation or ozonation. Added preservatives include chlorine, potassium sorbate, hydroperoxide, ozone and/or natamycin may also be added (subject to legislation). In the case of ultraviolet radiation and ozonation, which are perhaps the brine treatments most commonly used in practice, the brine is continually recirculated though cells/reactors where it exposed to the treatment. Ozonation involves subjecting oxygen molecules to high electrical voltage, and the resultant formation of O^{3-} which is sparged into the brine where it, *via* its oxidative effect, kills any microorganisms (i.e. yeasts, moulds and bacteria) present.

Cheese brines are maintained for long periods, sometimes for many years, so as to avoid potential problems associated with incorrectly prepared fresh brines (e.g. with low calcium levels, high pH) such as rind rot. However, even with incorrectly prepared brines, these problems disappear with ageing and use of the brine as the levels of lactic acid and calcium levels in the brine approach equilibrium with those of the moisture phase of the cheese type being salted. Maintenance of the brine requires:

- regular replenishment of the sodium chloride content;
- pH and calcium levels to be kept at desired values;
- maintenance of the desired microbiological status (essentially free of microorganisms);
- continuous filtration to remove insoluble cheese material.

The frequency of salt replenishment depends on the level of salt in the cheese type being brined, the weight of cheese being brined, and the weight ratio of

brine-to-cheese. Obviously, the higher the throughput and salt content of the cheese, the more frequently salt should be added. While calcium levels and pH should remain more or less constant in mature brine owing to the equilibrium established between the concentrations of calcium and lactate in the brine and in the cheese moisture, these parameters should be frequently tested to ensure that they are within the desired range.

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46 How does NaCl affect the microbiology of cheese?

J. J. Sheehan

Salt affects the microbiology of cheese by increasing the osmotic pressure of its aqueous phase, causing dehydration of bacterial cells, either inactivating them or preventing their growth. The preservation effect of NaCl is due to its effect on water activity, a_w. The a_w of most cheese varieties is not low enough to prevent the growth of yeasts, moulds and many bacteria. However, NaCl in combination with low redox potential, low pH and low ripening temperature, is sufficient to control microbial growth and growth of pathogens.

Dry-salted cheeses

In Cheddar [**100**] and territorial-type cheeses (hard pressed cheese-types with make procedures similar to Cheddar, e.g. Cheshire, Double Gloucester and Wensleydale), dry salt is added to milled curd chips to arrest acidification by inhibition of the starter bacteria and, along with the buffering components in the cheese [**22**], to maintain pH at a desired level. However, dependent on curd chip size, starter growth and acidification through lactose metabolism may continue for a short period directly after salt addition as salt requires time to diffuse from the surface to the centre of the curd chips. Growth of lactococci in Cheddar curd is generally not inhibited by $\leq$4% salt-in-moisture (S/M), but acidification is significantly inhibited at S/M levels >5%; S/M levels of 4.7–5.7% are desirable to produce high-grade Cheddar cheese. The sensitivity of starter cultures to NaCl varies depending on the strains used but *Lactococcus lactis* subsp. *cremoris* is generally more NaCl sensitive than *Lactococcus lactis* subsp. *lactis*. Low S/M levels in Cheddar-type cheeses may lead to high numbers of active starter cells and increased rennet activity on β-casein which may lead to bitterness.

Where lactose metabolism by starter bacteria is inhibited by excessive S/M levels during manufacture, residual lactose may be metabolised during ripening by non-starter lactic acid bacteria (NSLAB) [**56**]. NSLAB are more salt-tolerant than starter lactococci and most strains are capable of growth at 6% S/M and some at 8% S/M.

Brine-salted cheeses

In brine-salted or surface-salted cheeses [**41**], salt diffuses inwards from the surface of the cheese towards its centre. Acidification and growth of starters ceases due to lactose depletion, high acidity or low temperature before an inhibitory concentration of salt is attained in the cheese centre. It is therefore of little practical significance that thermophilic starters *Streptococcus thermophilus* and *Lactobacillus* spp. such as *Lb. helveticus* and *Lb. delbrueckii* subsp. *lactis* are all less NaCl tolerant than *Lactococcus* spp.

Cheeses with propionic acid bacteria

Growth of propionic acid bacteria (PAB) is inhibited even by low salt levels [**119**]. Inhibition of growth at salt levels of 0.5 to 3% have been reported but inhibition is affected by varying pH and S/M levels to an extent dependent on strain. Increasing S/M levels from 1 to 3% reduces CO_2 production in Swiss cheese and its volatile fatty acid content by 4 to 5-fold. Cheeses with PAB consequently have low salt levels, such as 0.4 to 0.7% NaCl in Emmental cheese.

Blue mould cheeses

In Blue mould cheeses [**137**], germination of spores of *Penicillium roqueforti* is stimulated by 1% NaCl but inhibited by 3–6% NaCl. However, growth of germinated spores can occur in cheeses containing up to 10% NaCl. Blue cheeses are often salted by the surface application of dry salt, and a salt gradient exists from the surface to the cheese centre. High initial levels of salt near the surface may inhibit spore germination resulting in areas within the cheese without mould growth.

Surface-ripened cheeses

Microflora of commercial brines include halotolerant lactobacilli and yeasts such as *Debaromyces hansenii*. Immersion of cheeses in brine leads to halotolerant microflora developing on the surface of the cheese. In white mould-ripened cheeses [**128**], growth of *Penicillium camemberti* is promoted by low levels of NaCl and is unaffected by levels up to 10% NaCl. Growth of *P. camemberti* is poor at levels less than 0.8% NaCl. If brining is delayed, *Geotrichum candidum* grows well but it is inhibited by relatively low salt levels and is totally inhibited by ~6% NaCl. Smear-ripened cheeses [**141**] are brined and usually also have smear liquid (dilute brine) applied to their surface during ripening. This promotes the growth of a halotolerant surface microflora including coryneforms, brevibacteria, micrococci and staphylococci [**142**] which are capable of growth at >10% NaCl.

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47 How can one make low-sodium cheese?

T. P. Guinee

Although sodium is an essential component in the human diet, excessive intakes have undesirable physiological effects, the most significant of which are hypertension and increased calcium excretion (which can lead to osteoporosis). The recommended daily requirement of sodium for the adult human is ~2.4 g Na^+, which is equivalent to ~6 g NaCl, per day. Sodium intake in the modern western diet is 2–3-fold higher than recommended. This has given rise to recommendations for reduced dietary intake of Na^+ and an increased demand for reduced-sodium foods, including cheese. However, owing to the important role of salt in cheese [**39**], reduction in salt level must be such that the quality and safety of the cheese are not compromised. Probably the most effective approaches to date for reducing sodium are:

- reducing added NaCl to the minimum level required for optimum quality;
- partial substitution of NaCl with KCl.

Maintaining the salt content at the minimum level required for optimal quality of any given variety requires large databases showing the relationships, if any, between the salt content and grading scores/cheese quality [**80**]. Published information of this type is readily available for Cheddar cheese [**100**], but less so for other varieties. Studies investigating relationships between composition and quality/grading scores of Cheddar have identified four *key compositional parameters* that have a major influence on quality. These include levels of salt-in-moisture (S/M), moisture-in-non-fat-substances, pH and fat-in-dry-matter. S/M level has a critical effect on quality, with grade deteriorating rapidly at S/M levels <3.0 and >6% (w/w). The recommended ranges for S/M are 4.7–5.7% S/M for first-grade Cheddar, and 4–4.7% and 5.7 to 6% S/M for second-grade Cheddar. Reducing the S/M to the lower end (4.7–5.0% S/M) of the range prescribed for first-grade quality cheese enables a 12% reduction in sodium content while maintaining excellent cheese quality. The implementation of this approach would necessitate a high degree of process control to ensure that the mean salt concentration is consistently kept within a narrow window of tolerance. For cheeses other than Cheddar further studies relating quality (grading scores, consumer acceptability) to salt level, are required so as to establish the minimum level to which the NaCl can be reduced without compromising quality.

Owing to the varying effects of different anions and cations on saltiness, the partial substitution of NaCl by an alternative salt with a non-sodium cation offers potential as a means of reducing sodium in cheese. Consequently, KCl, $MgCl_2$ and $CaCl_2$ have been extensively investigated as potential substitutes for NaCl in the production of low-sodium cheeses. These salts on their own or in 1:1 mixtures with NaCl are unsuitable because of associated sensory defects such as crumbly, soft greasy texture, and metallic and bitter off-flavours in the cheese. In contrast, the partial substitution of NaCl with NaCl : KCl mixtures with

weight ratios $\geq$70:30 does not markedly alter biochemical, textural and microbiological characteristics of cheeses, and offers significant potential for reducing sodium level (by $\leq$30%) in cheese.

Other approaches to salt reduction include (i) protein-enrichment of cheesemilk by supplementation of cheesemilk with reverse osmosis/ultrafiltered milk retentate [**16**], and (ii) the addition of flavour-enhancing substances to natural cheese. The potential of protein-enrichment of cheesemilk as a means of salt reduction has been ascribed to the higher levels of calcium and phosphate in the resultant cheese, which contribute directly to its 'saltiness'. They also increase the buffering capacity of the cheese and thereby prevent the likelihood of low pH and associated defects such as excessive proteolysis and bitterness, which are otherwise likely in low-salt cheese. However, major differences have been found in the effectiveness of this approach between the limited number of studies undertaken. Flavour-enhancing substances added to compensate for the reduced saltiness include autolysed yeast extract, gluconic acid-δ-lactone, glycinamide hydrochloride, monosodium glutamate and/or 5′-ribonucleotides. While such substances may enhance the perception of saltiness, they have often been associated with the development of off-flavours described as metallic, bitter, burnt, scorched, meaty and brothy.

For cheese and other food products, our long-term ability to reduce sodium will be further enhanced by developments in sensory research investigating product factors (e.g. structure, rheology, texture) affecting the release of salty flavour during mastication and how the perception of saltiness during mastication is affected by the presence of other taste and/or odour compounds.

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Cheese yield

48 Introduction: why is cheese yield important?

J. M. Banks

Production yields are an important determinant of profitability and economic success in all sectors of the dairy industry. More than 35% of milk produced worldwide is currently utilised in cheese manufacture and therefore maximising cheese yield is a critical and constant challenge to the dairy industry. A difference in cheese yield of only 0.1% over a year's production impacts greatly on the economic success of a cheese manufacturing plant. Cheese yield measurements are used to determine multiple component pricing systems for milk, to assess the effectiveness of processing modifications, and to evaluate effectiveness of new ingredients for use in cheese manufacture.

Cheese yield is influenced by many factors, including the composition and quality of the raw milk [**2**], milk handling, the cold storage of milk, pretreatments such as standardisation [**9**] and pasteurisation [**10**], cheesemaking parameters, equipment and technology. Maximising cheese yield requires extensive knowledge of factors influencing milk composition and curd formation. Measurement of cheesemaking efficiency is essential so that inefficiencies are identified and procedures put in place to eliminate them. Indices of cheesemaking efficiency include cheese yield, and/or the recovery of milk constituents in curd or their loss in the whey, in particular the casein and fat.

While there are many potential routes to improving cheese yield, maximisation of yield is profitable only if the cost of implementing new technologies and procedures is economically viable and savings are significant. Introduction of equipment for the recovery of fines from whey may improve cheese yield, but

the capital cost of new equipment may be too high to improve profitability in the short term.

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49 How is cheese yield defined?

J. M. Banks

Cheese yield [**48**] can be defined in several ways. The simplest definition of cheese yield is the weight of cheese in kg produced from 100 kg of milk. This is also termed the 'percentage yield'. Cheese yield is also sometimes expressed as the volume of milk in litres required to manufacture one tonne of cheese; in Cheddar production this is approximately 10 000 litres. These basic definitions are of limited value for comparisons of manufacturing efficiency unless they are adjusted to take into account the variability of the moisture content of the cheese [**94**]. Cheese yield may be more accurately expressed as the quantity of cheese of a given dry matter content produced from a stated quantity of milk of a defined protein and fat content (kg cheese/100 kg milk).

The determination of actual yield requires the measurement of the weight of all inputs and outputs in cheesemaking. The inputs include the milk, starter and salt. The outputs include the cheese and whey.

The actual cheese yield may be calculated using the equation:

$$\text{Actual yield}(Y_a) = \frac{\text{weight of cheese}}{\text{weight of milk} + \text{starter culture} + \text{salt}} \times 100$$

The actual yield does not take into account the moisture content of the cheese, and variations in moisture content for a given variety of cheese are common. Comparison of the actual yield between batches of a given variety may therefore reflect differences in both moisture content and/or the efficiency of recovery of the milk constituents in curd. It is important to differentiate between these two parameters. A comparison of cheese yields between batches of cheese made from milk of the same composition that show differences in moisture content is best considered as a moisture-adjusted cheese yield.

Adjusting the moisture content of the different batches of cheese to a reference or desired value eliminates the effects of variations in yield due to moisture content and therefore allows comparisons of yield on the basis of efficiency of fat and protein recovery.

The moisture-adjusted cheese yield (MACY) is calculated as follows:

$$\text{MACY (kg/100 kg)} = Y_a \times \frac{100 - \text{actual cheese moisture content}}{100 - \text{reference cheese moisture content}}$$

The recovery of milk components, fat, protein or casein, can be determined when their concentration in the inputs (milk and starter) and outputs (cheese and whey) are known. Thus the recovery of fat, casein, non-fat solids or protein can be calculated as follows:

$$\%\text{ of fat recovered in cheese} = \left[\frac{\text{wt of cheese} \times \text{fat content}}{(\text{wt of milk} \times \text{fat content}) + (\text{wt of starter} \times \text{fat content})}\right] \times 100$$

The composition of milk and the yield of cheese derived from a given quantity of milk is determined by a multiplicity of factors, which include the animal species (e.g. cow, goat or sheep) [**5**], the breed of animal, the stage of lactation [**3**], nutrition, the lactation number and animal health. The two most important constituents with respect to yield are the fat and casein in milk. The casein forms the paracasein network from which the cheese structure is formed, and fat and moisture are held within this structure. The fat level can be adjusted by standardisation of the milk [**9**] and the moisture levels in the curd are controlled by adjusting the rates of heating and acidification of the curds in whey, and the salting of the curd [**36**]. While the theoretical yield of cheese is limited by the fat and casein content of the milk used, the effectiveness of the manufacturing protocols in recovery of fat and casein, together with the target moisture and salt contents of the cheese, are also critical in determining yield.

Optimisation of yield potential of milk for cheesemaking can be achieved by technological intervention. In addition to standardising the casein to fat ratio of milk for cheesemaking, the casein content of milk can be increased and maintained at a constant level throughout the year by low concentration ultrafiltration (LCUF) [**16**], or fortification with extra low heat treated skim milk powder. LCUF is widely practised, especially in regions where large variations in milk composition occur throughout the manufacturing season [**3**]. By producing milk of a more uniform composition, consistency of cheese composition in manufacture is assured, and gel formation properties are improved, which reduces curd fines, casein and fat losses.

The heat treatment or pasteurisation treatment applied to milk can influence the amount of protein that can be recovered in cheese curd. Approximately 5% of the total whey protein is denatured using the standard pasteurisation treatment of 72 °C for 15 s [**11**, **12**]; the whey proteins interact with κ-casein and are retained in the cheese curd.

The composition of most cheese varieties falls within certain specifications prescribed by national or international standards of identity. However, intravarietal differences in composition are usual. The moisture content of Cheddar cheese [**100**] typically varies from 34 to 38%. Moisture content must be maximised to optimise yield, but the composition of the cheese must remain within legal specifications and quality must not be impaired by the inclusion of too much moisture in the curd.

The percentage recovery of milk fat and casein influences the efficiency of the cheesemaking process and therefore impacts on cheese yield. Information on the recovery of fat and casein is useful in establishing the cause of reduced fat recovery, e.g. inadequate curd firmness at cutting. The level of curd fines and fat lost in whey may also be used as a measure of cheesemaking efficiency and provides information relevant to yield. Curd fines are fragments of curd that are broken off the curd particles during cutting and in the initial stages of stirring.

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50 How can cheese yield be predicted?

J. M. Banks

Cheese yield can be predicted using yield formulae based on milk composition. Predictive yield formulae are specific to a particular cheese variety and milk from a particular species. The formulae are developed using data derived from trials in which yield measurements are related to milk compositional parameters and from theoretical estimations of the partitioning of milk components during cheese manufacture. While the theoretical yield of cheese is limited by the fat and casein content of the milk used for manufacture, the effectiveness of manufacturing protocols in recovery of fat and casein, together with the target moisture and salt contents of the cheese, are also critical in determining yield. Predictive yield equations must take these factors into consideration.

The comparison of actual yields with predicted yields can provide a useful efficiency index for the industry providing that historical data used in development of predictive yield formulae has been obtained when plant is running efficiently. Prediction of cheese yield allows companies to anticipate labour requirements, equipment and materials required for the production process, enabling them to calculate profitability in advance. As in the calculation of actual yields, the results derived from the use of predictive equations will be dependent on the cheese variety, the processing conditions, the recovery of casein and fat, the level of moisture in the cheese and the accuracy of the analytical methods used.

Predictive yield formulae for a particular variety can be developed on the basis of information obtained from both cheese yield experiments in which the yield and component recovery are related to milk composition, and theoretical considerations of the influence of the cheesemaking processes on the partition of various components of milk (e.g. milk salts, the caseinomacropeptide and fat) between the curd and whey.

Predictive yield formulae are generally of the following types:

$$Y = aF + bC$$

or

$$Y = aF + bC + k$$

where Y is the yield, F and C are the fat and casein content of the milk + starter, and k is a constant dependent on the loss of casein and the levels of non-fat, non-casein solids in the cheese; a and b are coefficients, the magnitude of which depends on the contributions of fat and casein to yield.

One of the simplest and most widely used formulae for predicting cheese yields was published by van Slyke in 1936. The van Slyke formulae for actual (Y_a) and moisture-adjusted yields (Y_{MACY}) are:

$$Y_a = \frac{[F \times (\%FR/100) - C - a] \times b}{1 - (\text{actual moisture}/100)}$$

$$Y_{\text{MACY}} = \frac{[F \times (\%\text{FR}/100) - C - a] \times b}{1 - (\text{reference moisture}/100)}$$

where F and C are the fat and casein content of the cheesemilk (with added starter culture), %FR is the fat recovery, a is the coefficient for casein loss, and b is the coefficient to account for cheese solids non-fat, non-protein.

The values for %FR/100, a and b for Cheddar cheese [**100**] as predicted by van Slyke are 0.93, 0.1 and 1.09 respectively. This formula can be rewritten in the form $Y = aF + Bc$, where the values of the coefficients are 1.66 and 1.78 respectively for Cheddar cheese containing 39% moisture. The van Slyke formula has been modified for other types of cheeses, such as low-moisture Mozzarella [**146**], where the reported values for %FR/100, a and b are typically 0.86, 0.36 and 1.09 respectively.

There is considerable intravarietal variation in the reported values of %FR/100, a and b. Values for %FR/100 for Cheddar cheese have been found to range from about 83 to 93%. Discrepancies between studies are indicative of differences in milk composition, milk quality and storage conditions, milk heat treatment, cheese manufacturing parameters and cheesemaking equipment. Variations in the coefficients also occur between cheese factories due to the above factors. Hence the application of a generic cheese yield formula for a given cheese variety may not accurately predict cheese yield in all plants. Plant-specific formulae must therefore be developed based on milk composition, milk quality, fat and protein recovery and cheese salt and moisture content.

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51 What factors associated with the milk affect cheese yield?

J. M. Banks

Many factors associated with milk have important implications for cheese yield [**48**]. The main components of the milk contributing to cheese yield are the casein and fat as together these account for 94% of the dry matter in Cheddar cheese. Damage to casein or milk fat prior to or during cheesemaking, through enzymic activity or physicochemical effects, will reduce recovery of these components during cheese manufacture. Properties inherent in the milk supply can also impart advantage for yield potential in cheese manufacture, e.g. the BB genetic variants of κ-casein and β-lactoglobulin are associated with higher yields.

The casein has a great impact on cheese yield since a higher proportion is retained in curd as compared with fat. It also forms the structural network in which fat and moisture are entrapped [**34**, **35**]. The formation and properties of the coagulum derived from the casein network determines the efficiency of retention of milk constituents in cheese.

Fat acts as a relatively inert filler in the coagulum, but its inclusion in the curd physically inhibits syneresis [**34**, **35**], thereby influencing moisture retention in curd. Moisture held in curd contributes directly to cheese yield and also contributes indirectly as it carries with it soluble components of whey such as whey proteins, the caseinomacropeptide, lactate and soluble milk salts. Casein also carries micellar calcium phosphate [**4**] into curd.

There are distinct seasonal trends in the level of fat and casein in milk [**3**], which reflect lactational changes superimposed by nutritional, mastitic and environmental effects. Seasonal changes in the fat and casein levels in milk result in changes to the casein to fat ratio. This ratio has important implications for cheese yield, cheese quality and manufacturing efficiency. As a consequence, milk for cheesemaking is standardised [**9**]. The optimum casein to fat ratio for manufacture of Cheddar cheese is 0.7:1. Increasing the casein to fat ratio, by reducing the level of fat in milk, results in a higher moisture content and inclusion of a higher level of soluble whey solids in curd. Reducing the casein to fat ratio increases the level of fat and decreases the level of moisture in cheese.

Genetic variants of milk caseins and whey proteins can impact on cheese yield. Bovine milk caseins consists of four types of milk protein. The α_{s1}-, α_{s2}-, β- and κ-caseins are present at approximately 38%, 10%, 34% and 15% of the whole casein. Each of the caseins exhibits genetic polymorphisms in which one or two amino acids are substituted in the protein chain. These substitutions have important implications in terms of cheesemaking properties. The BB genotypes of β-lactoglobulin and κ-casein are generally associated with higher concentrations of casein and superior renneting properties. These variants are associated with superior cheesemaking properties, and result in higher recoveries of fat, lower levels of curd fines in cheese whey, higher actual and moisture-adjusted yields. These effects have been validated in Cheddar [**100**], Edam

[**108**], Gouda, low-moisture Mozzarella [**146**] and other cheese varieties. Reported increases in moisture-adjusted yield with the κ-casein BB variant range from about 3 to 8%, depending on milk composition and cheese variety.

Damage to casein or milk fat prior to cheese manufacture has important implications for cheese yield. Casein degradation due to high levels of somatic cell proteinases [**8**], or psychrotrophic proteinases [**7**] associated with excessive cold storage of milk reduces the yield potential of milk. β-Casein can also dissociate from micellar casein on extended cold storage of milk. High somatic cell counts (SCC) result from mastitic infections of the mammary gland. Cellular damage at the site of infection initiates chemical signals which attract white blood cells to the area. Some of these white blood cells are transferred to the milk and therefore the SCC of milk increases during mastitis. Mastitic infections can be classified as either clinical or subclinical. Subclinical mastitis is defined as inflammation that is not visibly apparent, and requires a diagnostic test for detection. Cows with clinical mastitis may exhibit swelling of the udder and apparent pain or discomfort, while their milk may contain flakes or clots.

The SCC is an important parameter of milk quality since it reflects the health status of the cow, is an index of quality and processing properties of milk, and is widely used as a payment parameter by dairy companies. Milk produced by cows with either clinical or subclinical mastitis will have cheesemaking properties different from cows without mastitis.

With increases in the SCC of milk there are changes in the quantity and type of proteolytic enzymes in milk [**8**]. These proteinases degrade casein and result in a loss of soluble casein-derived peptides in whey, which results in a lower cheese yield. Two main sources of proteinases have been defined: those from within the somatic cells, and the native alkaline milk proteinase plasmin. Casein degradation products from these two sources of enzyme activities differ. In milk with somatic cell counts $<2 \times 10^6$ cells ml^{-1}, plasmin is responsible for most of the proteolysis. As the somatic cell count in milk exceeds 2×10^6 cfu ml^{-1}, other proteinases from within the somatic cells are present in sufficient quantity to contribute more significantly to proteolysis. Proteolysis of casein occurs in the udder prior to milking when somatic cell count is high. The average decrease in cheese yield efficiency for milk from an individual cow with a milk SCC > 100 000 cells ml^{-1} is 4%. Cooling raw milk quickly, maintaining a cold temperature on storage (<4 ºC) and limiting storage time of the raw milk will minimise further degradation of casein by plasmin.

Extended cold storage of milk impairs the rennet coagulation properties of milk [**24**, **30**], reduces the recovery of fat and protein and reduces cheese yield. The extent of yield reduction is influenced by a number of factors and published studies report differing levels of yield reduction. This is not surprising since variations in experimental conditions such as the temperature history of the milk [**11**], milk pH, somatic cell count [**8**], bacterial count, species/strains of psychrotrophic bacteria [**7**] and their potential to produce extracellular proteinases and lipases will independently impact on cheese yield. However,

it is generally agreed that at levels of $<10^6$ cfu ml^{-1} the psychrotrophs have little effect on cheese yield.

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52 What factors under the control of the cheesemaker affect yield?

J. M. Banks

Factors within the control of the cheesemaker that affect cheese yield [**48**] are the handling and storage of the milk prior to cheese manufacture, the selection of appropriate ingredients such as the starter culture [**18**] and coagulant [**27**], and the milk and curd handling techniques during cheese manufacture.

Milk handling and cold storage

A cheese factory operating to capacity may have to store milk cold for a period of 1–3 days prior to manufacture. Prior to arrival at the creamery, the milk may have been cold stored on the farm for up to 3 days and then transported substantial distances from farm to creamery. During storage and transport the temperature will be below 6 ºC, and the milk will be subjected to shear through pumping and agitation. Cold storage and shearing encourage a number of physicochemical changes in the milk which potentially impact on cheese yield. These include the solubilisation of micellar caseins, particularly β-casein, and of colloidal calcium phosphate [**4**], leading to an increase in serum casein and soluble Ca. There is an increased susceptibility of serum casein to hydrolysis by plasmin and proteinases from psychrotrophic bacteria [**7**] or somatic cells [**8**] and plasmin. The milk fat globule membrane may be damaged by shearing, and free fat may be hydrolysed by lipases from psychrotrophic bacteria or the indigenous lipoprotein lipase in milk, resulting in a decrease in the level of fat. Excessive cold storage of milk can impair its rennet coagulation properties [**30**], leading to reduced recovery of protein and fat and a reduction in cheese yield.

It is generally agreed that a level of $<10^6$ cfu psychrotroph ml^{-1} will have little effect on the cheesemaking properties of milk, and that pasteurisation effectively reverses the physicochemical effects of cold storage. Provided milk is not excessively cold stored, the psychrotroph counts do not exceed 10^6 cfu ml^{-1} and coagulum cutting times are suitably adjusted, the effects of cold storage of milk for several days will be minimal.

However, to ensure psychrotroph levels do not become excessive, milk may be deep cooled ($\leq$2 ºC), or thermised [**13**] at a subpasteurisation temperature (e.g. 57–68 ºC) for 10 to 15 s, prior to cold storage. These treatments reduce the psychrotroph load during storage so that casein and fat degradation are minimised.

Standardisation and fortification of milk

Fat and protein levels in milk show seasonal variations. Standardisation of the casein to fat ratio [**9**] is undertaken to produce cheese of the required composition consistently through the year. The casein to fat ratio used is specific to a particular variety and is influenced by efficiency of fat and protein recovery

in the manufacturing plant. Further advantage may be gained in terms of cheese yield and vat throughput by fortification of the protein content of milk. Spray dried milk protein concentrate or low concentration-factor ultrafiltration retentate [**16**] can be used to increase the protein content of cheesemilk to achieve increased yield in terms of both milk solids retention and plant throughput. Recent studies suggest an optimum level of fortification between 4.56% and 6.48% for Cheddar [**100**], 5.42% for Feta [**164**] and 5.38% for Mozzarella [**146**]. Above these values yield benefits may be lost owing to physical damage of curd in a traditional cheesemaking process.

Calcium chloride may be added to improve the rennet coagulation properties of late lactation milk [**33**]. Improved curd formation on addition of calcium is thought to be associated with a reduction in pH and an increase in the concentration of Ca^{2+}. Addition of calcium chloride at a level of 0.02 g/l is commonly used in late lactation milk [**3**]. Yield studies suggest an improvement in the efficiency of recovery of milk fat and protein in cheese which leads to a significant improvement in cheese yield. Beneficial effects are thought to relate to enhancement of casein aggregation, which reduces the susceptibility of the curd to fracturing during cutting and the initial phase of stirring.

Starter cultures

Starter cultures will hydrolyse casein to various degrees dependent on their proteolytic activity [**23**]. Casein degradation can occur either during preparation of a bulk starter culture or during curd manufacture and can have consequences for cheese yield. Model studies have shown that starter cultures can produce significant losses of casein (0.7–6.6%) as compared with direct acidification. The extent of casein loss is dependent on the proteolytic activity of the starter strain. Proteinase-negative single-strain starter cultures generally give higher dry matter yields of Cheddar cheese than the corresponding proteinase-positive starters, with the yield advantages ranging from 1.4 to 2.4% dependent on starter strain. However, proteinase-negative strains rely on indigenous amino acids and peptides in milk for growth, reproduce very slowly, and therefore reduce the pH too slowly for cheese manufacture. Their use may also lead to slow proteolysis and flavour development during maturation. Proteinase-negative strains are therefore not used independently but rather in blends with proteinase-positive strains. Such blends are commonly used as commercial cheese cultures.

Coagulants

The various rennets available to the cheesemaker differ in their milk clotting : proteolytic activity ratio [**29**]. Different rennets hydrolyse 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 drainage. Some breakdown products of casein are soluble in whey and are lost in whey at whey drainage.

The principal role of the coagulant in cheese manufacture is the specific hydrolysis of the Phe_{105}-Met_{106} bond of κ-casein and this initiates the formation

of the coagulum in the presence of calcium at a suitable concentration [**24**]. The ideal rennet should hydrolyse only the Phe_{105}-Met_{106} bond of κ-casein during milk coagulation, with further cleavage of caseins, essential for flavour and texture formation, occurring only after complete removal of whey. Under these circumstances the recovery of casein is maximised and cheese yield is increased.

Calf chymosin or fermentation-derived chymosin has the lowest level of non-specific proteolytic activity compared with bovine pepsin or microbial rennets [**29**]. The clotting to proteolytic ratio of chymosin is over 25 times higher than that of pepsin. Fungal rennets are also more proteolytic than calf chymosin, with proteolytic activity being in the following order: *Cryphonectria parasitica* proteinase >> *Rhizomucor miehei* proteinase > *R. pusillus* proteinase > calf chymosin. In some cases the fungal rennets have a higher thermostability, which can cause more degradation of casein to peptides during manufacture, leading to a reduction in cheese yield.

The extent of casein hydrolysis during manufacture of cheese curd is lowest with calf rennet and recombinant or fermentation produced chymosins, intermediate with 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 depends largely on the pH at whey drainage. Rennets with a high level of proteolytic activity compared with calf rennet probably have little effect on whey drainage when the pH is high (e.g. $\geq$6.15), as in the case of Cheddar, Gouda and Emmental, but reduce yield when the pH at drainage is below 6.0, as in the case of Blue cheese [**137**] and Camembert [**128**]. The thermostability of the different rennets at the cook temperatures for a given variety probably also determines how differences in proteolytic activity impact on yield.

Pasteurisation of milk

Pasteurisation of milk (e.g. 72 ºC for 15 s [**10**, **11**]) results in a low level of denaturation of whey proteins ($\leq$5% of total). These denatured whey proteins complex with κ-casein and are retained in cheese curd where they contribute to a yield increase of 0.1–0.4%. However most of the whey proteins, which account for 20% of the total milk protein, are lost in the whey. Theoretically, if all whey proteins were retained in curd, a yield increase of 12% would be achievable. However, inclusion of high levels of whey protein (>35% of total whey protein) in denatured form impedes rennet coagulation and impacts negatively on the functionality and quality of most rennet cheeses.

Curd firmness and cutting

Cutting the coagulum formed in cheesemaking is a critical control point in cheese manufacture with respect to yield. Cutting the gel initiates the dehydration process in which the colloidal constituents of milk (fat, casein and micellar salts) are concentrated to form cheese curd [**34**]. In large mechanised plants the coagulum is cut after a specified set-to-cut time to conform to factory schedules.

However, many factors that affect gel firmness are not consistent throughout the cheesemaking season. Hence firmness at cutting can vary and result in variability in yield potential of milk. The factors that can influence curd firmness include milk composition, stage of lactation, somatic cell count, milk heat treatment, culture type and pH.

During gel formation, firmness increases progressively from the onset of gelation as a consequence of the aggregation of paracasein micelles. The gel eventually becomes firm enough to withstand cutting without shattering. Traditionally in commercial cheese manufacture, the curd particles are allowed to sit quiescently in the whey after cutting.

During this period, referred to as healing, syneresis proceeds rapidly, and the curd particles become firmer and develop a surface film. The combined effects of this film and the cushioning effects of the expressed whey limit the damage inflicted on the curd particles by impact with the agitators and vat surfaces during the initial phases of stirring. Healing reduces the tendency of the curd particles to shatter (i.e. fracture along their weakest points into smaller particles with jagged edges). The surface layer becomes progressively stronger as a consequence of the dehydrating effects of heat, acid and stirring, and it seals and protects the fat and casein within the curd particles.

In large mechanised factories, the curds are not given a defined period of healing following cutting. Instead the curds heal during the cutting programme which determines the number of alternate cutting and rest cycles. Insufficient healing may result in curd shattering, which results in an increase in surface area through which fat globules can escape from the surface of the curd particle. It also results in the formation of cheese fines (curd particles less than 1 mm), which may contribute to yield loss and necessitate further downstream processing to recover and minimise losses.

The curd particle size distribution during the initial stages of cutting influences yield efficiency since it determines the surface area through which fat can escape into whey. The curd particle size and fat losses in whey are influenced by a combination of the speed and duration of cutting and the subsequent speed of stirring prior to cooking.

For a given vat design, proper maintenance of knives (i.e. edge sharpness and knife angle) is essential to enable clean cutting and thereby reduce the risk of tearing the curds which results in high fat losses in whey. For individual vat designs to operate to maximum efficiency, in-plant studies are required to optimise the interactive effects of coagulum firmness, cutting programme and stirring speed so as to achieve the best curd particle size and fat retention results. In the future, the use of in-vat curd firmness sensors will undoubtedly lead to the consistent realisation of maximum yields.

Yield losses post-vat

Most of the losses in cheese manufacture occur in the cheese vat. About 6.5% of milk fat and 4–5% of the casein are lost during commercial Cheddar

manufacture. The losses that occur post-vat are comparatively small (approximately 2.0% of milk fat) but are important determinants of cheesemaking efficiency. Following whey drainage the curd is subjected to a variety of handling procedures such as stirring, cheddaring [**101**], milling, salting [**39**] and prepressing. During these operations, moisture and fat are lost to varying degrees, thereby influencing cheese yield.

Milling of cheddared curd exposes fresh surfaces from which fat is lost in whey during salting. Losses are increased with elevation of temperature, reduction in chip size and increased severity of mechanical squeezing either in the mill or in worn conveyors. Fat losses in whey released from blockformers may also be influenced by strength of vacuum, but there is little published information available.

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New technologies

53 What potential uses do high hydrostatic pressures and high-pressure homogenisation have in cheesemaking?

A. L. Kelly

In recent years, there has been a significant interest in a number of novel, emerging or non-traditional methods for food processing. Generally, these have been driven by consumer demand for minimally-processed and 'fresh-like' products, which have desirable safety and shelf-life characteristics but are apparently not overly processed, i.e. do not suffer the loss of sensory or nutritional quality that can sometimes arise from thermal processing.

Perhaps the foremost of this family of novel processes is high-pressure (HP) processing, in which food products are exposed to pressures many thousands of times greater than atmospheric pressure (i.e. 100–1000 MPa), for short periods of time. Treatment is generally applied in a batch-wise manner, with packaged product being held in a large thick-walled steel vessel and subjected indirectly to high pressures via a surrounding pressure-transmitting medium which is brought to very high pressures, usually using a pump or piston. For most food products, high pressures kill vegetative cells (unfortunately HP treatment has little effect on bacterial endospores) but result in little change in sensory or nutritional quality.

In the case of cheese, HP processing may be applied in two different ways:

1. *Treatment of milk for cheesemaking*. The effects of HP on milk are complex, and will not be reviewed here. However, their net effect, depending on the pressure applied, may be a change in rennet coagulation time [**30**], incorporation of whey proteins into cheese, with a concomitant increase in

yield [**48**], and increases in rennet gel strength. The effects of HP treatment of milk on the quality and ripening of cheese made therefrom are less well studied.

2. *Treatment of cheese*. Reports from Japan in the early 1990s that HP treatment of Cheddar cheese curd could greatly accelerate cheese ripening have largely been disproved by later scientific studies, although some, generally relatively minor, acceleration of proteolysis and inactivation of contaminating microorganisms certainly occurs. However, the effects of HP treatment on Mozzarella cheese [**146**] are potentially far more interesting, with very significant effects of even short HP treatments on fresh curd, resulting in hydration of protein fibres and rapid attainment of desirable functional properties.

Overall, HP processing is an extremely expensive, and still relatively small-scale, technology, and benefits for cheesemakers to date probably do not warrant the investment that would be required for a reasonably sized cheese factory. However, there remain several applications where research suggests HP treatment may be of benefit, in particular in the context of the ripening characteristics of cheese made from HP-treated milk. Applications of HP processing for attenuation of starter bacteria for use as adjuncts [**18**] have also been investigated in a preliminary way, and may prove of future interest.

A related technology of recent research interest is homogenisation at considerably higher pressures than conventionally used, which results in very fine fat globules, but also inactivates enzymes and bacteria in milk. The potential of this technology for treatment of cheesemilk is the subject of considerable current research effort.

Other processes commonly grouped in the category of novel technologies include use of pulsed electric fields, oscillating magnetic fields and high-intensity light pulses; little is known to date of the potential significance of these technologies for cheese-related applications, as these fields are in their infancy.

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The microbiology of cheese ripening

54 Introduction

P. L. H. McSweeney

Cheeses are ripened for periods ranging from ~2 weeks (e.g. Mozzarella) to >2 years (e.g. Parmigiano Reggiano or extra-mature Cheddar). During ripening, a wide range of biochemical [**88**] and microbiological changes occur. Microbiological changes which occur include the continuation of starter activity [**17**] into the early stages of ripening, until the salt-in-moisture level become inhibitory (which takes longer in brine-salted than in dry-salted varieties [**41**, **46**]). In most varieties, there then develops an adventitious non-starter microflora ('non-starter lactic acid bacteria'; NSLAB [**56**]) usually comprising facultatively heterofermentative lactobacilli. They often originate from the cheesemilk and survive pasteurisation, if used, in a heat-shocked state, but they may also come from the cheesemaking environment. NSLAB may contribute to the flavour of cheese and to variability between factories or days of production. NSLAB grow from very low initial numbers in the cheese (typically $<10^2$ cfu g^{-1}) and tend to reach a maximum of ~10^7 cfu g^{-1}. Growth rate and final numbers of NSLAB are influenced by factors including initial numbers, cooling rate of the cheese block and ripening temperature.

Certain cheeses are characterised by the growth of a secondary microflora during ripening. The fermentation of lactate to propionate, acetate, CO_2 and H_2O by *Propionibacterium freudenreichii* is essential for the development of eyes and flavour in Swiss-type cheeses [**117**] while *Penicillium camemberti* and *P. roqueforti* dominate the ripening of Camembert-type [**128**] and Blue cheeses [**137**], respectively. During the ripening of smear-ripened varieties, the cheese surface is deacidified initially by the growth of yeasts (e.g. *Debaryomyces*

hansenii) and *Geotrichum candidum* before a very complex Gram-positive bacterial flora develops (containing organisms from genera such as *Corynebacterium, Brevibacterium, Arthrobacter, Staphylococcus* and *Micrococcus*), which give these cheeses their distinctive red-orange colour and greatly contribute to their flavour [**142**].

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55 What factors affect microbial growth in cheese?

T. Beresford

From a consumer perspective, cheese is a nutritious food, rich in protein and amino acids, vitamins including folic acid and vitamins B6 and 12, minerals in particular calcium and the fatty acids, including conjugated linoleic acid which has proven health benefits [**69**]. However, while cheese is rich in many nutrients, it presents a challenging environment for growth from the perspective of a microorganism [**61**]. Notwithstanding the environmental challenge, a surprising range of microorganisms have evolved to grow in or on cheese. The main examples of such organisms include starter and secondary flora [**18**], adventitious non-starter lactic acid bacteria (NSLAB) [**56**] and potentially deleterious microorganisms including spoilage and pathogenic organisms [**58**].

The starter flora, which may include lactococci, thermophilic lactobacilli or *Streptococcus thermophilus* depending on the cheese variety, represents the major biomass present in cheese, in particular immediately post-manufacture and early in the ripening process. The starter culture is usually added to the cheesemilk at the beginning of manufacture except in a limited number of traditional raw milk cheeses that rely on organisms indigenous to milk to produce acid during the fermentation process. However, while starters grow during the manufacturing phase, growth for most species stops in the first few days of ripening and thereafter many organisms lose viability and release their cellular contents in a process referred to as autolysis.

Many cheeses contain a secondary flora that play a vital role in the ripening process. These include propionic acid bacteria (PAB) present in Swiss-type cheese [**117**], micrococci, staphylococci, coryneform bacteria and yeasts present in smear-ripened cheese [**142**] and blue and white moulds present in mould-ripened cheese. These secondary flora grow during the ripening process and their metabolism directly influences the key quality attributes of the mature cheese. Secondary flora are often added during the manufacturing or ripening process but can also occur as natural contaminants.

NSLAB [**56**] have been isolated from nearly all cheese varieties studied to date. Mesophilic lactobacilli are the dominant members of this group although pediococci are also encountered occasionally and some authors include enterococci within this category. NSLAB are adventitious microorganisms and gain entry either as contaminants of the milk or from the cheesemaking environment. NSLAB, in particular mesophilic lactobacilli, grow during the ripening process and represent a considerable portion of the biomass of most long-ripened cheeses.

Potential spoilage and pathogenic organisms, which can include coliforms, clostridia, *Staphylococcus aureus, Listeria monocytogenes* and *Salmonella enterica*, all gain entry as contaminants from milk or during the manufacturing process. In general, such organisms find cheese a difficult environment for growth and survival; however, they may attain levels in particular cheese types sufficient to cause spoilage of the product or illness to the consumer.

The manufacturing process plays a key role in defining the microbial flora of cheese as follows:

1. *Limits entry of microorganisms to cheese.* While most cheese, in particular in modern processing plants, is manufactured under highly hygienic conditions the process is not aseptic and offers many opportunities for entry of microorganisms. They can gain entry as part of the starter or secondary flora, as contaminants of the raw milk prior to processing or other ingredients used in cheese manufacture, or from the cheesemaking and ripening environment.
2. *Kills many microorganisms that gain entry.* The initial step in the manufacture of many cheeses involves pasteurisation of the milk [**10**]. During pasteurisation, milk is heated to 72 ºC for 15 s, a treatment that inactivates all but the most heat-tolerant microorganisms in the milk. Manufacture of most cheese varieties involves coagulation at temperatures of 30–37 ºC followed by cooking to 37–54 ºC. Few of the microorganisms present will be killed by such heat treatment; however, the temperatures achieved during cooking have the potential to inhibit growth of some organisms. For example Swiss-type cheese is cooked to 52–54 ºC and is maintained above 50 ºC for up to 5 h. This heat treatment is considered to play an important role in controlling the growth of starters and undesirable microorganisms. Salt [**39**] is added to nearly all cheeses towards the end of the manufacturing process and plays a major role in the control of microflora [**46**] as will be discussed in more detail below.
3. *Defines the environmental conditions that prevail during ripening.* The manufacturing process, in particular the influence it exerts on expulsion of water from the curd [**34**] and rate and degree of acid production, dictates the gross composition of the cheese which is best defined by the four parameters of salt-in-moisture (S/M), moisture in non-fat-substance, fat-in-dry-matter and pH. Of these, S/M and pH are the most significant in the context of microbial growth. Additional environmental factors influencing microbial growth include the presence of organic acids and nitrate, oxidation/reduction potential and ripening temperature.

Salt-in-moisture (S/M)

Water is required for growth of all microorganisms and one of the most effective ways of controlling their growth is to reduce the available water either through dehydration or addition of some water-soluble component such as sugar or salt. Both dehydration and salt addition are used during the manufacture of cheese. It is the availability of water rather than the absolute amount present that is critical for microbial growth. The concept of 'available water' or 'water activity' (a_w) developed during the 1950s has provided a basis for an increasing understanding of microbe/water relations in food. a_w is a thermodynamic concept defined as the ratio between the vapour pressure of the water present in a system (p) and that of pure water (p_0) at the same temperature:

$$a_w = p/p_0$$

Thus, potential values for a_w range from 0 to 1. It clearly follows that a relationship exists between salt concentration and a_w. This relationship is almost linear (correlation coefficient, $r^2 = 0.997$) and is defined as:

$$a_w = -0.0007x + 1.0042$$

where x is the salt concentration in cheese ($g\,kg^{-1}$). The salt concentration in cheese ranges from 7 to 70 $g\,kg^{-1}$, and this corresponds to an a_w of 0.99 to 0.95, respectively. A depression in a_w occurs during cheese ripening due to (i) continued water loss by evaporation from cheeses that are not packed in wax or plastic, or stored in an environment with controlled humidity and (ii) as a consequence of hydrolysis of proteins to peptides and amino acids as hydrolysis of each peptide bond requires one water molecule.

In general, bacteria have higher minimum a_w requirements than yeasts, which, in turn, have higher requirements than moulds. At the initial stages of cheese manufacture, its a_w is ~0.99, which supports the growth and activity of most microorganisms found in cheese. After whey drainage, salting and during ripening, a_w (0.917–0.988) is significantly lower than the optimal requirements of starter bacteria; however, many secondary organisms and NSLAB can continue to grow under such conditions.

pH and organic acids

Most bacteria grow optimally at pH around neutrality and growth is often poor at pH values <5.0. Notable exceptions are lactobacilli, yeasts and mould, which grow well at pH 4.5. Owing to the fermentation process, organic acids accumulate in the cheese curd post-manufacture and the pH is decreased to between 4.5 and 5.3; these low pH values will not allow the survival of acid-sensitive organisms. The real inhibitor, however, is the undissociated form of the organic acid. The principal organic acids found in cheese are lactic, acetic and propionic acids which have dissociation constants (pK_a) of 3.08, 4.75 and 4.87 respectively. Hence, lactic acid is the least and propionic acid the most effective inhibitor at the same concentration. However, lactate in cheese curd is invariably present at a much greater concentration than either acetic or propionic acid, except in the case of Swiss cheese where the concentration of propionic acid may be higher than that of lactic acid.

Nitrate (NO_3^-)

Nitrate is not always added during the cheesemaking process, but in some brine-salted cheeses it is used to control the growth of *Clostridium tyrobutyricum* a potential spoilage organism. *C. tyrobutyricum* can metabolise lactate to butyrate which imparts a rancid off-flavour to cheese and in the process produces CO_2 and H_2 gas, which cause the cheeses to 'blow' [**91**]. Growth of *C. tyrobutyricum* is possible in brine-salted cheeses as it may take some weeks (depending on the

size of the cheese) for salt to migrate through the curd and attain levels that will be inhibitory to growth of this spoilage organism. Nitrate is often used for this purpose in the production of Dutch-type cheeses, such as Edam and Gouda where it is added to the cheesemilk as KNO_3 or $NaNO_3$. Lactic acid bacteria are not affected by nitrate but PAB, essential for eye formation in Swiss-type cheeses, are and thus it cannot be used in such cheeses. Nitrates are reduced to nitrites (the actual growth inhibitors) during ripening by an indigenous enzyme in milk, xanthine oxidoreductase, and levels of nitrite should be below the permissible level of 50 mg kg^{-1} by the time the cheese is ready for consumption. Nitrite can react with aromatic amino acids in cheese to produce nitrosamines, many of which are carcinogenic. The reaction is pH dependent, occurring preferentially in the pH range of 2 to 4.5. As cheese has a higher pH, the reaction leading to the formation of nitrosamines is thus slowed.

Oxidation–reduction potential (E_h)

The oxidation–reduction (redox) potential (E_h) is a measure of the ability of a chemical/biochemical system to lose (oxidation) or gain (reduction) electrons. An oxidised or reduced state is indicated by a positive or negative redox potential respectively. The E_h of milk is about +150 mV, while that of cheese is about −250 mV. While the mechanism of E_h reduction in cheese is not fully established, it is most probably related to fermentation of lactic acid by the starter during growth, and the reduction of O_2 in the milk to H_2O. As a consequence of these reactions, the cheese interior is essentially an anaerobic system, which can only support the growth of obligatory or facultatively anaerobic microbes and thus E_h is a major factor in determining what organisms will grow in cheese. In some cheese types, such as Danish Blue and Roquefort, the cheesemaker deliberately alters the cheese E_h to facilitate growth of *Penicillium roquforti* in the cheese interior.

Ripening temperature

Temperature is one of the few parameters the cheesemaker can manipulate to control microbial development during the ripening stage of cheese production. The microorganisms involved in cheese manufacture and ripening are either mesophilic or thermophilic, having temperature optima between 30 and 42 ºC. The temperature at which cheese is ripened is a compromise between the need to promote ripening reactions and control growth of the desirable secondary flora, and the need to prevent the propagation of potential spoilage and pathogenic bacteria. Most cheeses are ripened at temperatures in the range 6–15 ºC; exceptions are Swiss-type cheeses which undergo a period at 22–25 ºC to promote the growth of PAB. Higher temperatures promote accelerated ripening, but the changes to body and flavour are often detrimental.

The final factor to consider with regard to the growth of microorganisms in cheese is availability of nutrients. Cheese has a relative abundance of protein,

amino acids and fat and these are not limiting factors for the growth of most microorganisms. However, the carbohydrate content of cheese is relatively low. Residual lactose is present in cheese following manufacture but this is rapidly depleted in the early days of ripening and is often totally absent during the time in which many of the secondary and NSLAB flora are actively growing. Citrate is present in small amounts (~8 mmol kg^{-1}) but is not considered to be a significant energy source for microbial growth in cheese. Lactate, which is the major end product resulting from lactate fermentation by the starter bacteria, is present in cheese and is metabolised by some microbial flora including PAB, mould and yeasts. The growth of NSLAB [**56**] has received particular attention as significant growth continues following depletion of lactose. Recent research has demonstrated that many possess glycoside hydrolases and can utilise sugars derived from the glycomacropeptide of casein and the glycoproteins of the milk fat globule membrane.

In summary, while a wide diversity of microorganisms have the capacity to grow in cheese, the manufacturing process greatly restricts most either directly, by preventing entry or inactivation on entry, or indirectly through the creation of unfavourable environmental conditions in the cheese.

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56 What are non-starter lactic acid bacteria and how do they affect cheese quality?

T. Beresford

Introduction

The microflora of cheese can conveniently be divided into two main groups consisting of (i) starter [**18**] and (ii) non-starter organisms. The primary distinguishing feature between these two groups is that starters produce acid during the manufacturing process while secondary flora contribute little to this activity. The secondary flora are a diverse group of organisms that include (i) propionic acid bacteria [**117**], (ii) moulds, (iii) smear flora [**142**] and (iv) non-starter lactic acid bacteria (NSLAB). The NSLAB complex, in turn, can be considered to consist of facultatively heterofermentative mesophilic lactobacilli, pediococci, enterococci and leuconostocs. The mesophilic lactobacilli are the best studied members of this flora, but inclusion of the other members is merited on the basis that they are lactic acid bacteria, are found in cheese and do not contribute significantly to acid production during the manufacturing phase. NSLAB, in particular mesophilic lactobacilli, have been isolated from all ripened cheeses. With the exception of leuconostocs, which are deliberately added to some cheese varieties, NSLAB are adventitious microorganisms that gain entry from the milk, ingredients or utensils used in cheese manufacture. Their contribution to the ripening process has been the subject of much investigation, and with the exception of leuconostocs, which are responsible for 'eye' formation ensuing from CO_2 production and flavour development resulting from production of diacetyl and acetate in some cheese types [**108**], the contribution of NSLAB to cheese quality is not clearly defined.

Bacteria of the NSLAB complex

Mesophilic lactobacilli

The genus *Lactobacillus* consists of Gram-positive, catalase-negative, generally non-motile, rod-shaped bacteria with complex nutritional requirements. Cell length varies considerably between species and strains from long slender rods to short or coccobacilli-shaped cells. Rod may be straight or curved and chain formation is common. Lactobacilli are generally aciduric with an optimal pH usually in the range 5.5–6.2 and grow at temperatures from 2 to 53 ºC. The genus can be divided into three groups on the basis of being either (i) obligatory homofermentative, (ii) facultatively heterofermentative or (iii) obligatory heterofermentative. Mesophilic lactobacilli most frequently encountered in cheese as part of the NSLAB complex are members of the facultatively heterofermentative group of lactobacilli and are thus sometimes referred to as facultatively heterofermentative lactobacilli (FHL). While a wide variety of species have been isolated from cheese, the organisms most frequently encountered include *Lactobacillus casei*/*Lactobacillus paracasei*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus* and *Lactobacillus curvatus.*

Pediococci
The genus *Pediococcus* is composed of Gram-positive, catalase-negative, non-motile, spherical cells found in pairs and tetrads. Single cells are rarely found and pediococci do not form chains. They are generally facultatively anaerobic and grow in the temperature range 25–50 ºC. They are salt tolerant, with some strains able to grow in media containing 6.5% NaCl, and grow over a wide pH range (4.5 to 8.2). *Pediococcus pentosaceus* and *Pediococcus acidilactici* are the dominant species isolated from dairy products.

Enterococci
The genus *Enterococcus* consists of Gram-positive, catalase-negative, spherical or ovoid, non-motile cells, which are typically arranged in pairs or chains. They are facultative anaerobes and most species within the genus will grow in the temperature range 10–45 ºC. Most are capable of growth in media containing 6.5% NaCl, at pH 9.6 and can hydrolyse aesculin in the presence of 40% bile salts. The dominant species isolated from cheese include *Enterococcus faecium, Enterococcus faecalis* and *Enterococcus durans*.

Leuconostoc
The genus *Leuconostoc* consists of Gram-positive, catalase-negative, non-motile cells with irregular coccoid morphology. Their optimum growth temperature is in the range 20–30 ºC. They share many features in common with lactococci but can be distinguished on the basis of that they (a) ferment sugars hetero-fermentatively rather than homofermentatively, (b) produce the D rather than the L isomer of lactate and (c) do not, with the exception of *Leuconostoc lactis*, grow well in litmus milk unless it is supplemented with yeast extract (0.3 g/100 ml). The species most frequently encountered in cheese include *Leuconostoc mesenteroides* subsp. *cremoris* and *Leuconostoc lactis.*

Source of NSLAB in cheese
NSLAB are adventitious bacteria which gain access to the cheese either from the ingredients used in its manufacture or from the environment. However, an exception are leuconostocs which are sometimes added as part of the starter culture. In addition, as a consequence of the accumulating evidence that some strains of mesophilic lactobacilli have the capacity to enhance cheese flavour, selected strains of lactobacilli are now sometimes added with the starter.

Mesophilic lactobacilli can be isolated from raw milk and thus milk is a likely source, in particular, for cheeses manufactured from raw milk [**11**]. The heat sensitivity of mesophilic lactobacilli has been extensively studied but the findings are equivocal. In general the studies agree that significant inactivation occurs on heating for the majority of strains tested; however, survival of low numbers of some strains, possibly in a stressed state, is likely to occur. Such strains are likely to recover during cheese manufacture and ripening and then grow in the cheese. Lactobacilli that enter the cheese plant either in the milk or

in other ingredients, equipment or personnel may survive within the plant in the form of biofilms. Such biofilms can be resistant to the cleaning procedures used and could potentially survive over long periods of time within a plant. No data have been published on the source of pediococci in cheese but are likely to gain entry in a similar manner to lactobacilli.

As with lactobacilli, the source of enterococci in cheese is also not clearly defined. However, it is generally assumed that milk is a source as it may be contaminated easily during the milking process. Enterococci can be readily isolated from bovine faeces; however, other organisms present in faeces are not always found in high numbers in milk, suggesting the sources other than bovine faeces need to be considered.

Growth of NSLAB in cheese

The factors that control the growth of microorganisms in cheese are discussed in [**55**] and include pH, level of salt, water activity and temperature. While these parameters combine to inhibit the growth of most microorganisms, many NSLAB, in particular non-starter lactobacilli and enterococci, are tolerant of these environmental conditions and will survive and/or grow during ripening. Many studies have demonstrated the capacity of non-starter lactobacilli to grow during ripening attaining populations of up to 10^7 cfu g^{-1} within the first 2–3 months of ripening. While enterococci have been isolated from a wide range of cheese types, most of the data relating to population changes during ripening would suggest that in general their population remains stable during ripening. A similar situation pertains to leuconostocs. Little data are available regarding the growth of pediococci in cheese; however, the fact that they are sometimes isolated from mature cheese from which they were not detected early in ripening would suggest that they have the capacity to grow.

The growth rate and final population density of non-starter lactobacilli are not affected significantly over the pH range, salt and moisture levels that normally occur in the curd during cheese manufacture and ripening. Non-starter lactobacilli have a generation time of approximately 8.5 days in cheese ripened at 6 ºC and viable cells can be recovered from cheese stored at 10 ºC for 3 years. While growth rate of non-starter lactobacilli is temperature dependent, temperature modification has little impact on the final numbers in cheese.

Cheese is a rich source of many nutrients required for microbial growth; however, carbohydrates required as a source of energy would appear to be limiting. Lactose, which is present in abundance in milk [**2**], is present in cheese at relatively low levels and then only in young curd. Lactose is probably used by residual starter activity or non-starter lactobacilli in the first days of ripening; however, as significant growth occurs following lactose depletion, alternative carbohydrate source(s) must be available in the cheese. Recent studies demonstrated that non-starter lactobacilli possess glycoside hydrolase enzymes and can utilise sugars derived from the glycomacropeptide of casein and the glycoproteins of the milk fat globule membrane. Release of sugars following

starter cell autolysis has also been proposed as a source of carbohydrate. Peptides and amino acids are catabolised by lactobacilli provided that a keto acid acceptor is present as a co-factor for aminotransferase activity and it was recently proposed that arginine could act as one of the main energy sources for non-starter lactobacilli in cheese.

Impact of NSLAB on cheese quality

The impact of NSLAB on cheese quality has been the subject of much investigation. The approach taken in this research has (i) endeavoured to control the entry and growth of NSLAB in cheese and (ii) added a selected strain in an effort to ensure that these strains dominate cheese during ripening and can thus be associated with any subsequent impact on quality. This research is complicated by the fact that NSLAB are adventitious microorganisms and few studies have succeeded in gaining full control of the population during extended ripening.

Many studies have used stringent hygienic approaches to control NSLAB entry to cheese, in particular in laboratory and pilot-scale trials. Such approaches are successful for controlling leuconostocs and enterococci. However; control of non-starter lactobacilli has proved more difficult. The fact that non-starter lactobacilli are ubiquitous microorganisms and can readily grow in cheese presents particular hurdles to this type of research. The approaches involved sterilisation of all cheesemaking utensils prior to cheese manufacture, more stringent milk pasteurisation procedures and attempts to avoid contamination from the environment during manufacture. Interpretation of the outcome of this research is complicated by the fact that full control of wild non-starter lactobacilli was rarely achieved; however, many of the studies concluded that non-starter lactobacilli have a role to play in cheese quality.

Control of non-starter flora through the addition of antibiotics to the cheese curd at the end of the manufacturing process or through the use of bacteriocin-producing starter cultures has also been attempted. In studies with antibiotics, growth of non-starter lactobacilli was greatly reduced, though not totally inhibited. While the overall patterns of proteolysis, considered an important quality indicator, in the cheese was not impacted upon by the antibiotic treatment, lower levels of amino acids were reported in cheese containing antibiotics. Bacteriocins produced by the starter culture within the cheese, are an alternative to antibiotics to control growth of secondary flora. The broad spectrum bacteriocin, lacticin 3147, can be produced by starter cultures in cheese during manufacture and has the capacity to inhibit growth of non-starter lactobacilli. As with the use of antibiotics, non-starter lactobacilli grew in cheese containing lacticin 3147 albeit at reduced rates and final population size. In these studies little impact of reduced non-starter *Lactobacillus* populations was noted.

Control of NSLAB through manipulation of the ripening temperature has also been attempted. In all such studies to date while reducing the ripening temperature inhibits growth of non-starter lactobacilli, no cheese was

maintained free of lactobacilli using this technology, even when the temperature was reduced to 1 ºC.

The effect of adding adjunct cultures [**18**] of non-starter lactobacilli to milk for cheesemaking has been studied for several decades. The results of these studies are equivocal, with some studies showing positive effects while others report negative effects on flavour development. The reason for the equivocal nature of these findings probably results from the potential of the isolates selected to generate flavour combined with growth of adventitious strains during ripening. However, most of the more recent studies on this topic have indicated that non-starter *Lactobacillus* adjuncts exert a positive effect on flavour and may help to control some defects such as gas production by heterofermentative lactobacilli during ripening.

The potential impact of pediococci on cheese quality has not been well studied, due primarily to the infrequency with which they have been encountered. Enzymes, including protease, peptidase and lipase activities [**23**], with potential to promote cheese ripening have been identified in various strains. Further investigation regarding the prevalence of pediococci and their growth and survival during cheese ripening is required prior to defining their role, if any, in flavour development.

The impact of enterococci on cheese quality is also unclear; indeed, their presence in cheese may be considered an indicator of insufficient sanitary conditions during the production and processing of milk. While most strains studied have relatively low extracellular proteolytic activity, many have significant lipolytic activities in addition to the capacity to metabolise citrate, which can result in the production of cheese flavour compounds.

The potential of leuconostocs to impact on cheese ripening is well documented. They produce acetate, CO_2, diacetyl, acetoin and 2,3-butanediol resulting from citrate metabolism. The CO_2 produced is responsible for small eye formation in Dutch cheeses such as Edam and Gouda, while diacetyl and acetate contribute to the flavour of products such as Quarg, Fromage Frais and Cottage cheese. They also contain intercellular proteolytic and esterase activities which are likely to contribute to the ripening process.

NSLAB adjuncts for quality improvement of cheese

The increasing evidence that NSLAB have the potential to improve cheese quality has led to much research into the identification of suitable adjunct strains, as such adjuncts would offer considerable financial benefit to cheese manufacturers. An important characteristic of NSLAB, and non-starter lactobacilli in particular, is their ability to grow in the cheese during ripening, thus enabling the cheesemaker to add them at very low levels to the cheesemilk at the beginning of manufacture and still obtain a positive impact in the cheese. The key to this approach is selection of suitable strains, as much research has demonstrated individual strains that may have positive or negative impacts on quality.

One approach has been to develop 'reference flora' either by isolating mixed NSLAB cultures from raw milk or good quality cheese. Such cultures have been developed either by selective plating or, in the case of milk, by ultrafiltration. The outcome has been mixed, with some authors suggesting improvements while others report deleterious effects.

An alternative approach is to select individual strains for addition to the cheesemilk. There have been numerous reports in the literature that have used this approach. Most involve the selection of strains from good quality cheese followed by their inoculation into cheesemilk. Again the results have been ambiguous. With regard to non-starter lactobacilli, recent studies have indicated that their population is dynamic and that few cheeses are dominated by single strains or groups of strains throughout ripening. Thus, selection of strains at a single time point during ripening may not include all the strains necessary to bring about an improvement in quality. The criteria for strain selection, in particular in the early studies in this area, were limited to a minimal range of biochemical characteristics. More recent studies have applied molecular techniques to characterise strains and have included a broader range of biochemical traits. The outcome of such studies is the identification of strain blends that have a demonstrated capacity to enhance cheese quality. This demonstrates that a systematic approach to strain selection based on the available knowledge of microbial physiology, cheese microbiology and biochemistry of flavour development can lead to the selection of strains of non-starter lactobacilli with the potential to improve cheese quality.

Studies on the selection of other NSLAB for use as starter adjuncts are limited but in general similar approaches to those for selection of adjuncts of non-starter lactobacilli were used. Pediococci have been selected from raw milk and cheese, while enterococci have been isolated from natural whey cultures and cheese.

Summary

The NSLAB complex of cheese is composed of non-starter lactobacilli, pediococci, enterococci and leuconostocs. NSLAB are differentiated from starter bacteria on the basis that they do not produce significant acid during the manufacturing phase but can grow and/or survive in cheese over an extended ripening period. The population is complex, composed of a number of species and strains, which are in a dynamic state during ripening. Control of NSLAB, in particular non-starter lactobacilli, is difficult and while application of stringent hygienic practices can limit the initial number they will ultimately grow, even in cheeses held at low temperature, and attain populations of $\leq 10^7$ cfu g^{-1}. There is mounting evidence to support the hypothesis that NSLAB have the potential to influence cheese quality and selection of suitable strains for use as starter adjunct is crucial if the economic potential of these bacteria is to be realised.

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57 What causes the development of gas during ripening?

J. J. Sheehan

Development of gas in cheese during ripening is evident by the occurrence of eyes, cracks, slits, fissures, holes or gas within the packaging. Gas produced during cheese ripening may occur within the first few days of ripening (early gas) or towards the latter stages of ripening (late gas).

Early gas

Gas creating numerous small holes and produced in cheeses shortly after manufacture is usually caused by the growth of coliform bacteria or yeasts. This defect is more common in soft and semi-soft cheeses because of their higher a_w than in other cheese types. Gas caused by coliforms is mainly H_2, produced from formate, a product of lactate metabolism. Gas caused by yeasts is CO_2 produced from metabolism of lactate or lactose. Sources of contamination include:

- poor hygiene of plant and equipment;
- post-pasteurisation contamination of cheesemilk;
- contaminated starter, or starter with too high or too low acidity which may permit undesirable microorganisms to grow.

Other microorganisms causing gas defects may include:

- citrate-positive lactococci or *Leuconostoc* spp. [**18**] which can produce CO_2;.
- heat-resistant streptococci growing in plate heat exchangers, which may be responsible for CO_2 production in cheese [**116**];
- salt-tolerant lactobacilli have also been associated with CO_2 production and unclean flavours and have been reported in under-strength brine solutions.

Problems due to H_2 gas may be controlled, where permissible, by the addition of KNO_3 or $NaNO_3$ at low levels (0.2%) to the cheesemilk. NO_3^- promotes breakdown of lactose to CO_2 and H_2O rather than to the fermentation of formate to H_2 by suppressing the formation of the hydrogen lyase system and inducing the formation of a formate dehydrogenase/nitrate reductase system.

Late gas in Cheddar-type cheese

Gas production during ripening and openness in Cheddar-type cheeses [**100**] may be due to contamination of milk by heterofermentative organisms such as lactobacilli. Non-starter lactic acid bacteria (NSLAB) [**56**] grow during ripening and heterofermentative lactobacilli such as *Lactobacillus brevis* and *Lactobacillus fermentum* are able to produce gas in Cheddar cheese. Gas production occurs from the fermentation of residual lactose and galactose to CO_2. Salt-in-moisture (S/M) levels influence starter activity in Cheddar-type cheeses. Slow starter activity can lead to high levels of residual lactose. S/M level also affects lactose utilisation and lactate production by NSLAB.

Contamination by heterofermentative starters may also result in CO_2 production by metabolism of citrate in such cheese types. Growth of *Clostridium tyrobutyricum* may also result in late gas blowing [**91**] but this is rare in Cheddar-type cheese due to the rapid increase in S/M levels.

Late gas in brine-salted cheeses

Gas produced in brine-salted cheeses during ripening may be due to the germination of spores of *Clostridium tyrobutyricum* or *Cl. butyricum* [**91**, **115**]. These organisms ferment lactate to acetate, butyrate, CO_2 and H_2. Late gas blowing is a particular problem with Swiss-type cheeses where germination of spores and growth of clostridia can occur during hot room ripening. Factors that may influence this include:

- use of milk from herds fed with silage;
- poor quality milk;
- poor hygiene of plant and equipment;
- starters with low activity;
- inadequate pressing of the curd, resulting in weak areas in the curd mass or unexpelled whey pockets which could establish conditions for the formation of gas holes.

Such problems are prevented by not using milk produced from cows fed with silage, by the bactofugation of milk or by the addition of NO_3^- or lysozyme to cheesemilk.

Furthermore, during ripening or storage of Swiss-type cheeses the propionic acid bacteria (PAB) may produce excessive CO_2. Factors which may also influence gas development include:

- inappropriate storage temperature;
- temperature fluctuations during cold storage;
- additional CO_2 production by decarboxylation of amino acids during ripening;
- production of lactate and substances stimulatory for PAB by other microorganisms, e.g. enterococci or *Lactobacillus lactis*;
- PAB with high aspartase activity.

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Pathogens and food poisoning bacteria

58 Introduction

P. L. H. McSweeney

Milk is a highly nutritious medium for bacteria with a pH close to neutrality and can support the growth of many pathogenic organisms. Milk has been responsible for many outbreaks of food poisoning. In contrast, cheese, despite also being very nutritious, has been responsible for relatively few outbreaks and hence is a relatively safe food product. However, there has been a number of food poisoning outbreaks associated with cheese, particularly with cheese made from raw milk and/or soft varieties [**81**, **83**], often with a surface smear [**141**], in which the pH increases during ripening. The principal organisms associated with food poisoning outbreaks caused by cheese are *Salmonella* spp., *Staphylococcus aureus, Listeria monocytogenes* and enteropathogenic strains of *Escherichia coli*. The primary reasons for cheese-related outbreaks of food poisoning include poor starter activity (due to bacteriophage [**21**] or the presence of antibiotics [**19**]), poor hygiene, gross environmental contamination or faulty pasteurisation of the cheesemilk.

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59 What cheeses are most liable to pathogens?

C. W. Donnelly

The characteristics of the specific cheese variety dictate the potential for growth and survival of microbial pathogens. In general, ripened soft cheeses present a higher risk for growth and survival of pathogens in comparison with aged hard cheeses [**83**] where a combination of factors including pH, salt content and water activity (a_w) interact to render cheeses microbiologically safe. Many hard cheeses aged for 60 days or more are made from raw milk (e.g. Parmigiano Reggiano, Grana Padano [**96**], traditional Swiss cheese varieties [**117**] and some Cheddar cheeses [**100**]) and these products enjoy an excellent food safety record. Soft cheese varieties such as Camembert [**128**], Brie and Hispanic-style cheeses such as Queso Fresco [**170**] present a higher risk from the perspective of microbiological safety. These cheeses have a higher pH and higher moisture content which can promote the growth of microbial pathogens. In the United States, the Code of Federal Regulations (21CFR, sections 133.182 and 133.187) permits manufacture of semi-soft and soft ripened cheeses from raw milk provided that these cheeses are aged for 60 days at $\geq$1.67 ºC (35 ºF) (Fig. 1). Pathogens such as *Listeria monocytogenes* can grow to high levels during 60 days of ageing, so the safety of cheeses in this category must be achieved through use of control strategies other than a 60-day holding period. High risk cheeses are those for which there is a low curd cooking temperature, neutral pH and high moisture in the finished product. Cheeses where there is a high curd cooking temperature, low moisture and long ageing have a low risk. The 60-day ageing rule currently applies to high risk soft cheeses. The arrow suggests the need for changes so that the 60-day ageing rule does not apply to these cheeses.

The pathogens *Salmonella* spp., *L. monocytogenes*, *Staphylococcus aureus* and enteropathogenic *Escherichia coli* pose the greatest risk to the safety of cheese. If active lactic acid starter cultures [**18**] are used, *S. aureus* is considered to be a low-risk pathogen. However, in traditional cheeses where active starter cultures are not utilised, *S. aureus* may pose a significant risk for toxin production in cheese if numbers are sufficiently high. The factors that contribute to the safety of cheese with respect to pathogenic bacteria include milk quality, starter

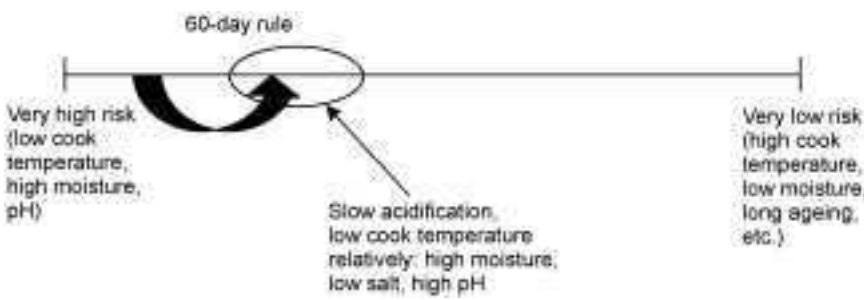

Fig. 1 The risk continuum associated with raw milk cheeses (Kindstedt, 2004).

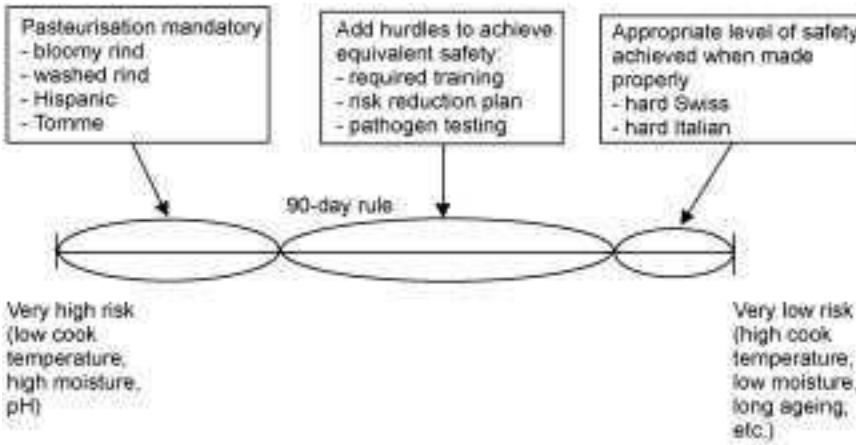

Fig. 2 Interventions in the risk continuum to improve cheese safety (Kindstedt, 2004).

culture or native lactic acid bacteria growth during cheesemaking, pH control, salt, control of ageing conditions, and chemical changes that occur in cheese during ageing. Other technologies (e.g. use of starter cultures that produce substances inhibitory to pathogens) may provide opportunities to add additional barriers to the growth of bacterial pathogens. It is particularly important for the producers of raw milk cheeses to have a documented and systematic approach to ensure product safety.

For high-risk cheeses such as bloomy rind cheeses, washed rind cheeses, certain Hispanic-style cheeses and Tomme-style cheeses pasteurisation should be mandatory to ensure pathogen inactivation. For raw milk cheeses, a minimum of 90 days of ageing coupled with mandatory technical training for cheesemakers, a mandatory risk reduction plan and mandatory pathogen testing could achieve safety equivalent to pasteurisation. For certain low-risk cheeses (hard Swiss and Italian varieties), the appropriate level of safety is achieved by high curd cooking temperatures used in manufacture, the low moisture achieved by these cheeses during ageing, and the long ageing time. Circles on the risk continuum in Fig. 2 indicate proposed interventions by risk category.

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60 Which pathogens survive pasteurisation and which are killed?

C. W. Donnelly

Pasteurisation [**11**] is a heat treatment designed to inactivate the most heat-resistant vegetative pathogen of public health concern. In milk, this pathogen has been determined to be *Coxiella burnetii*, the causative agent of Q fever. Pasteurisation of milk intended for cheesemaking is accomplished using high-temperature short-time (72 ºC for 15 s) or vat (holding) pasteurisation at 63 ºC for 30 min. If correctly pasteurised, this process will eliminate pathogens from cheesemilk. Efficacy of pasteurisation is dependent upon the use of properly designed and operated equipment as well as raw milk quality. Once pasteurised, milk and cheese must be protected from post-process recontamination. Extremely low levels of *Listeria monocytogenes* (typically 0.5 to 1.0 cells/ml) exist in commercial bulk tank raw milk. *Listeria* is inactivated by pasteurisation and contamination of processed dairy products is therefore probably a function of post-pasteurisation contamination from the dairy plant environment.

Pasteurisation alone will not assure the microbiological safety of cheese. Pathogens can still be present in cheese made from pasteurised milk if cheese is not protected from post-process recontamination. The production environment and contamination from humans who handle cheese can be sources. In fact, post-process recontamination of cheeses rather than pathogens surviving pasteurisation has been documented as the causative factor in several outbreak investigations.

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61 Do pathogens grow during cheese ripening?

C. W. Donnelly

Whether pathogens grow or decline during ripening depends largely on the chemical and compositional properties of the cheese variety in question. In general, cheeses with high moisture contents, or those with a neutral pH due to bloomy rind or smear development, will support the survival or growth of pathogens during ripening. Conversely, in hard, low-moisture cheeses with a low pH, pathogens die during ripening. Pathogens can be present in cheeses either as a result of surviving pasteurisation or through recontamination from the ageing environment after manufacture. In studies of Swiss hard and semi-hard cheeses [**117**] where high levels of pathogens, including *Aeromonas hydrophila*, *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Staphylococcus aureus* and *Yersinia enterocolitica* were inoculated to raw milk, no detection of pathogens beyond 1 day was recorded. The high curd cooking temperatures used for Swiss hard (Emmental-type) and semi-hard cheeses (Tislit-type), coupled with acidity development during ageing, facilitate control of pathogens in these cheese types. In general, pathogens such as *Listeria*, which can contaminate Swiss-type cheeses after manufacture, decline in numbers during ripening due to acidity, water activity and other chemical constituents.

Cheeses that depend on surface ripening (smear [**141**] and surface mould-ripened [**128**]) in general support growth of pathogens due to the pH increases (pH 6.0 and above) during ripening. Growth of several pathogens including *L. monocytogenes*, *Enterobacter aerogenes* and *E. coli* O157:H7 has been reported in Camembert cheese. *E. coli* O157:H7 was shown to decline during ripening of Cheddar cheese, but grew in Camembert cheese once the cheese pH had reverted from acidic to basic owing to development of bloomy rind. Death of *E. coli* O157:H7 was observed in a smear-ripened cheese, and death was correlated with growth of the smear microflora which may have produced antimicrobial substances. However, in general, the increase in the pH of smear-ripened cheese during ripening facilitates growth of bacterial pathogens. However, studies have shown that manufacture of smear-ripened cheese from raw milk containing as few as 40 *E. coli* O157:H7/ml would result in cheese hazardous to public health.

Studies by Ryser and Marth (1987a,b) examined the fate of *L. monocytogenes* during the manufacture of Cheddar and Camembert cheese. Rapid growth of *Listeria* to high populations was observed in Camembert cheese (see Fig. 1a). The high moisture content and neutral pH of Camembert cheese facilitates growth and survival of *Listeria*, and growth parallels the increase in cheese pH during ripening, creating a favourable growth environment for *Listeria*. In contrast, populations of *Listeria* decline during ripening of Cheddar cheese (see Fig. 1b). Of interest is the fact that Camembert and Feta [**164**] have nearly identical composition in terms of moisture content, water activity, percentage salt-in-moisture and ripening temperature, but fully

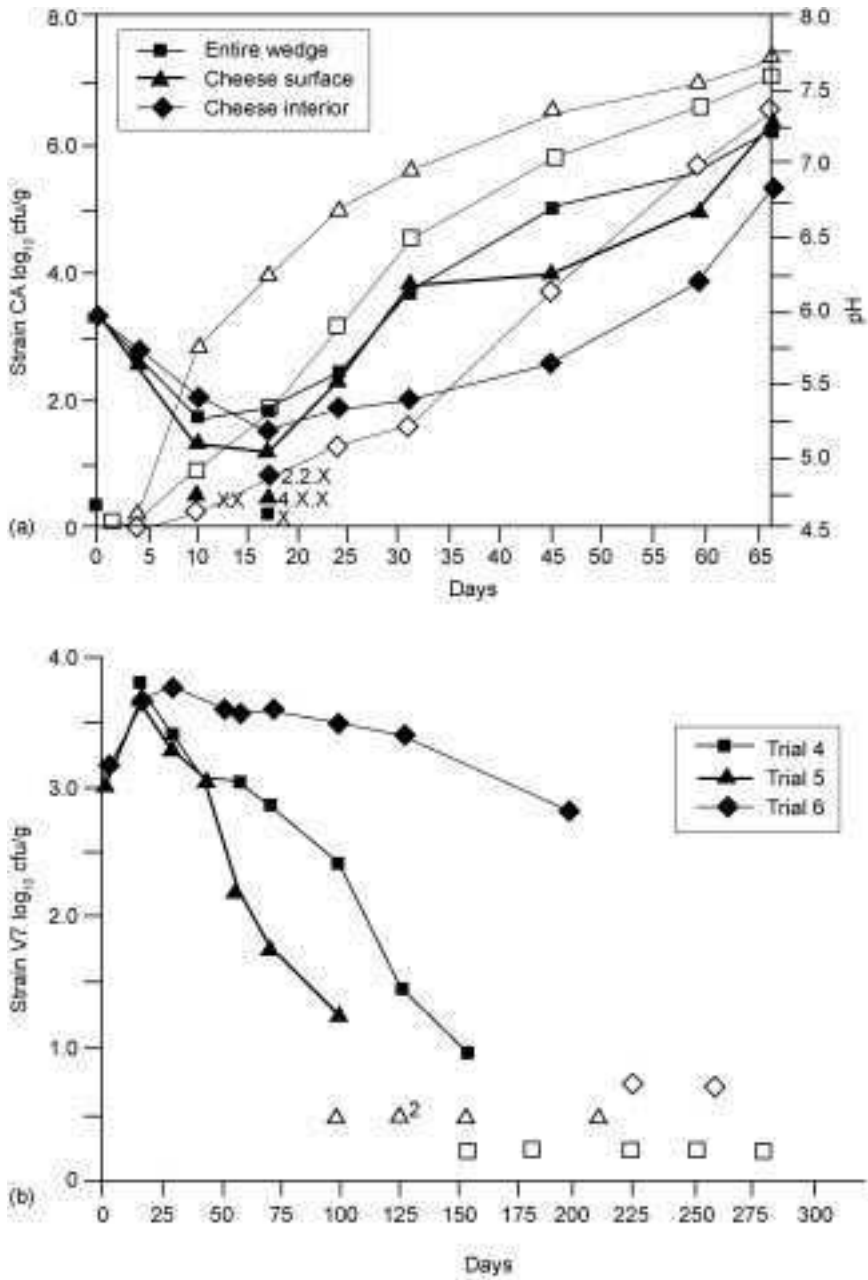

Fig. 1 Comparison of the survival of *L. monocytogenes* in Camembert (a) and Cheddar (b) during ripening. Open symbols indicate pH values; closed symbols depict *Listeria* counts (Ryser and Marth, 1987a,b).

ripened Camembert has a pH of 7.5, unlike Feta, which has a pH of 4.4 that prevents growth of *Listeria*.

The safety of the Italian hard cheeses (Grana Padano and Parmigiano Reggiano; [**96**]) is associated with several factors including (a) cooking of cheese curd to temperatures between 53 and 56 ºC for 15–20 min, with a total holding time of up to 85 min at these temperatures; molding of the cheese, whereby it is held at temperatures of 52 and 56 ºC for at least 10 h at pH 5.0; brine-salting of the cheese which lowers the water activity (a_w) to 0.9; and extended ripening for periods of 9 months (Grana Padano) up to 24 months (Parmigiano Reggiano) which promotes a further decrease in the a_w to levels inhibitory for growth of bacterial pathogens.

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62 What is *Mycobacterium avium* subsp. *paratuberculosis* and how is it controlled?

C. W. Donnelly

Mycobacterium avium subsp. *paratuberculosis* (MAP) causes paratuberculosis, or Johne's disease, an inflammatory bowel disease affecting ruminants. This disease is chronic and contagious and eventually results in death of affected animals. In the US, approximately 22% of the dairy herds are affected with Johne's disease. Infected dairy cattle are able to shed MAP in milk. This pathogen has been epidemiologically implicated in association with Crohn's disease in humans. While pasteurisation is thought to provide public health protection from this organism, several studies have revealed the presence of MAP in retail fluid pasteurised milk.

Many studies have been conducted to explore the potential for survival of MAP during cheesemaking. Swiss studies examined the fate of MAP in raw milk during the manufacture of hard and semi-hard Swiss cheeses [**117**]. Counts of the pathogen decreased slowly but steadily during ripening, with a 120-day ageing period achieving a 3–4 log inactivation of MAP. Analysis of retail cheeses from Greece and the Czech Republic for MAP found presence of this pathogen at low levels. Some 31.7% of cheeses tested positive by DNA-based methods compared with 3.6% which tested positive by cultural methods. Cheese pH, salt concentration and temperature during ripening and manufacture were all shown to be factors which prevented multiplication of MAP in cheese. Sung and Collins (2000) demonstrated that heat treatment of raw milk coupled with 60 days of ageing achieved inactivation of 10^3 MAP cells/ml during manufacture of soft Hispanic-style cheese [**170**].

Cheeses can be best safeguarded by using milk from animals testing negative for MAP. Prevention of MAP is difficult within dairy herds, but can be achieved through good biosecurity, along with testing and knowledge of livestock origins when introducing new animals to dairy herds.

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63 Is *Escherichia coli* O157:H7 of concern to cheesemakers?

C. W. Donnelly

Yes. *Escherichia coli* O157:H7, a pathogen first characterised in 1982, is now a leading cause of foodborne illness. *E. coli* O157:H7 is a very dangerous human pathogen, particularly for young children. Just a few cells of this bacterium can permanently inactivate kidney function in young children. Bloody diarrhoea, haemolytic uremic syndrome (HUS) and kidney failure are associated with *E. coli* O157:H7 illness.

A major reservoir of *E. coli* O157:H7 in nature is the gastrointestinal tract of healthy dairy cattle, deer, goats and sheep. Cattle are the principal reservoir for this important human pathogen and carry *E. coli* O157:H7 in their intestines. Cattle manure is therefore an important source of *E. coli* O157:H7. Contaminated drinking water is the most probable vehicle for infection of animals and a potential intervention point for on-farm control of this pathogen. The shedding of *E. coli* O157:H7 by infected animals is intermittent, suggesting that animals are re-inoculated from an environmental source rather than being colonised by this pathogen. *E. coli* O157:H7 can readily contaminate raw milk on the farm, and cases of *E. coli* infection have been traced to the consumption of raw milk, with a few additional cases in England linked to yogurt. A number of outbreaks of *E. coli* O157:H7 infections have been traced to county fairs in the US, where infections have been linked to petting zoos and contact between children and infected animals.

Cheeses are an infrequent source of *E. coli* O157:H7 infection. An outbreak linked to consumption of fresh cheese curd from a dairy plant in Wisconsin has been reported. Case patients had purchased cheese curds from an unrefrigerated display at a cheese plant. To be legal in the US, cheese curds must be manufactured from pasteurised milk. Vats of raw milk Cheddar cheese were inadvertently used to make fresh curds, which were incorrectly labelled as 'pasteurized' Cheddar cheese curds.

Haemorrhagic colitis caused by *E. coli* O157:H7, which affected 13 patients in 2002 in Edmonton, Alberta, was linked to Gouda cheese made from unpasteurised milk (Honish *et al.*, 2005). Consumption of unpasteurised Gouda cheese produced at a local dairy farm was reported in 12 out of 13 outbreak cases, with illness onset 2 to 8 days following consumption. *E. coli* O157:H7 was isolated from 2 of 26 cheese samples manufactured by the producer implicated in the outbreak. The cheese isolates had indistinguishable genetic profiles as compared with outbreak case isolates. Gouda cheese [**108**] obtained 104 days after production was found to be contaminated with *E. coli* O157:H7, even though the cheese had met microbiological and ageing criteria. This is the first outbreak confirmation of *E. coli* O157:H7 infection in Canada associated with a raw milk hard cheese.

Numerous studies have examined the survival of *E. coli* O157:H7 during the manufacture and ripening of Cheddar cheese. In general, when *E. coli* O157:H7 levels in milk are low (1 cfu g^{-1}), survival is not observed. Even when numbers

are high (1000 cfu ml^{-1}), a 2 log cfu/g reduction after 60 days of ageing has been noted in Cheddar cheese. Schlesser *et al.* (2006) recently reported survival of *E. coli* O157:H7 during Cheddar cheese manufacture when raw milk was inoculated with as few as 10^1 cfu ml^{-1}. These investigators warned of the inadequacy of 60 days of ageing to control this pathogen.

Cheesemakers should be alert to the dangers posed by *E. coli* O157:H7 contamination of cheese and take appropriate measures to screen milk intended for cheesemaking for this pathogen.

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64 What factors should be considered to reduce coliform counts?

C. W. Donnelly

Coliforms refer to a broad group of aerobic and facultatively anaerobic, Gram-negative, non-sporeforming, rod-shaped bacteria that ferment lactose. Coliform bacteria of significance in cheeses include the non-enteric genera *Serratia* and *Aeromonas*, along with *Citrobacter*, *Klebsiella, Escherichia* and *Enterobacter*. Some coliforms are indicators of faecal contamination and if counts are high, they can indicate the potential presence of bacterial pathogens [**58**]. Methods for detection of coliforms in cheese are easy and rapid to perform, and can provide valuable information. Coliforms are useful to indicate inadequate sanitation of equipment as they are highly sensitive to chemical sanitisers, so their presence in high numbers indicates inadequate cleaning and sanitation. Because they are heat sensitive, the presence of coliforms in pasteurised products may indicate post-pasteurisation recontamination. Coliform counts are very important as indicators of insanitary milking practices. Raw milk of high microbiological quality should have coliform counts of <10 cfu ml^{-1}. High levels of coliforms can lead to defects in cheese during ageing, which include excessive gas formation [**57**] which leads to structural defects and negative impacts on cheese sensory quality. Coliforms are known to be major contributors to early gas production in raw milk cheeses. In aged Cheddar cheese, coliforms may be a cause of early gas production, particularly when there is a failure of the starter culture. *Enterobacter aerogenes* has been shown to be problematic with respect to early gas production in cheese, and it is found in raw milk. Coliforms are acid sensitive, and sensitivity is further enhanced with increased salt concentration and decreases in water activity.

In a study of raw milk from the Normandy region of France, Desmasures *et al.* (1997) found that 84% of the samples analysed had coliform levels below 100 cfu ml^{-1}. In general, higher levels of coliforms are found in soft cheeses when compared with hard or semi-hard cheeses [**83**]. Melilli *et al.* (2004) found that presalting of curd prior to stretching of a raw milk *pasta-filata* cheese [**147**] was a simple and effective means of reducing coliform counts and preventing early gas production in Ragusano cheese. Coliform levels in cheese are dependent upon the cheese characteristics. Low levels of coliforms were found in white brine cheeses [**164**] (Aleksieva, 1980), with *Citrobacter intermedium, Enterobacter aerogenes* and *Escherichia coli* being the predominant coliforms isolated.

EU Directives (92/46/EEC) do not regulate coliforms in raw milk cheeses. De Reu *et al.* (2002) found higher levels of coliforms in soft cheeses when compared to blue-veined [**137**], semi-hard, hard and fresh cheeses. Soft cheeses tend to have a higher pH, shorter ripening periods, higher water activity and lower salt concentration compared to hard, semi-hard, blue veined and fresh cheeses. Araújo *et al.* (2002) found median values of 10^6 coliforms per gram in raw milk soft cheeses. Because coliform populations increase during

manufacture of soft raw milk cheeses, it is important to control coliform levels in raw milk. EU regulations do not specify coliform standards in raw milk intended for cheesemaking, but in general, levels of <100 coliforms/ml raw milk are recommended target levels.

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65 What are enterococci and are they pathogenic?

C. W. Donnelly

Enterococci are indicators of faecal contamination, and are useful in providing information about sanitary hygiene during food manufacture. The presence of enterococci in cheese is usually indicative of poor microbiological quality and reflects poor hygience during manufacture. Enterococci reside in the gastro-intestinal tracts of warm-blooded mammals. *Enterococcus faecalis* is the dominant species found in human faeces, while *Enterococcus faecium* is the predominant species in dairy cattle. Both *E. faecalis* and *E. faecium* occur in cheese. Enterococci are nosocomial pathogens (hospital acquired), and are gaining importance as human clinical pathogens because of multidrug resistance.

Enterococci are commonly found in artisanal starter cultures used in cheesemaking [**18**]. *E. faecium* has been used as an adjunct starter culture in the manufacture of Greek Feta cheese [**164**] where it improved the taste, aroma, colour and structure of fully ripened cheese. Gelsomino *et al*. (2002) used pulsed-field gel electrophoresis to identify the source of enterococci in farm-house raw milk cheese. Two *Enterococcus casseliflavus* clones could be traced to the bulk tank and milking machines, even following chlorination, suggesting resident niches which serve as reservoirs of contamination.

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66 What factors should be considered when developing a HACCP plan for cheesemaking?

C. W. Donnelly

In addition to prerequisite programmes which include standard sanitation operating procedures, good manufacturing practices and good agricultural practices, a hazard analysis critical control points (HACCP) programme can identify and control potential hazards to ensure food safety. The seven principles of HACCP (hazard analysis, identification of critical control points (CCPs), establishment of critical limits for the CCPs, identification of monitoring procedures for the CCPs, record keeping, corrective actions and verification of the process) must be identified as components of an effective HACCP plan. Raw milk quality is important in producing all cheeses, but particularly for cheeses manufactured from raw milk. Low bacterial counts and low somatic cell counts are the key indicators of milk quality, and as their numbers increase, there is a higher risk for contamination of milk and cheese with pathogens. Monitoring and controlling bacterial and somatic cells counts in milk should be components of a HACCP programme to ensure product safety. As rapid, cost-effective methods become available for detection of bacterial pathogens in raw milk, the use of specific pathogen testing could become part of a HACCP programme. In general, when raw milk bacteria and somatic cell counts are high, there will be other negative impacts on cheese quality that may reduce consumer acceptability and cheese yield [**48**]. In the manufacture of most artisanal cheeses, the time from milking to cheesemaking is very short and in some cases the milk is made into cheese immediately on the farm without cooling. Minimising the time from milk collection to the initiation of cheesemaking reduces the opportunity for the growth of undesirable bacteria in raw milk. Conversely, when milk is cooled and held in transport, the opportunity for pathogen growth, particularly growth of psychrotrophic pathogens, is increased.

The European Community Directives 92/46 and 92/47 contain regulations for the hygienic production and marketing of raw milk, heat-treated milk and milk-based products. These regulations establish hygienic standards for raw milk collection and transport that focus on issues such as temperature, sanitation and microbiological standards, enabling production of raw milk of the highest possible quality. Raw cow's milk must meet quality standards, e.g. a standard plate count at 30 ºC of $<100\,000$ cfu ml^{-1} and somatic cell counts of $\leq 400\,000$ per ml of milk. To meet these and other established standards, countries employ HACCP principles in the production of fluid dairy products. This involves identification of sites to be monitored and evaluated to ensure that products are produced under the correct conditions, as well as the development of critical limits established by valid and verifiable parameters. In the case of fluid milk products, many processors have identified length of shelf-life as a critical limit. Shelf-life is influenced by a number of factors including cleaning and sanitising of pipelines and milking equipment, condition of raw milk used to produce product, and storage temperature. Pasteurisation [**11**] will eliminate some of the

indigenous microflora in the raw milk including pathogenic bacteria; however, thermoduric organisms survive pasteurisation. Post-pasteurisation contamination of milk is problematic if the processing/packaging environment is not maintained. Moreover, many contaminants, including *Listeria*, are able to form biofilms which protect them from cleaning and sanitising agents. Some regulations, such as those of the EU, have established microbiological limits at the sell-by date for products such as cheeses. With respect to regulations which govern the use of raw milk for cheesemaking, limits have been established for *Staphylococcus aureus* in raw milk. Finished cheeses must meet specific hygienic standards, in which case the presence of *S. aureus* and *Escherichia coli* indicate poor hygiene.

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67 What are biogenic amines and how are they produced?

N. M. O'Brien and T. P. O'Connor

Biogenic amines are non-volatile, low molecular mass aliphatic, alicyclic or heterocyclic organic bases. Typically, they originate in foods from the decarboxylation of specific amino acids. Decarboxylation can occur due to indigenous decarboxylases in foods or to decarboxylases produced by micro-organisms in the food. Biogenic amines are found in a variety of foodstuffs, most commonly fish of the families Scombridae and Scombereoscidae, but also in cheese. In cheese, biogenic amines are produced by decarboxylation of amino acids during ripening. Levels produced vary as a function of ripening period and microflora. High levels of biogenic amines are most likely to be detected in cheeses heavily contaminated with spoilage microorganisms. The principal biogenic amines detected in cheese are histamine, tyramine, tryptamine, putrescine, cadaverine and phenylethylamine. The ingestion of biogenic amine-containing foods may cause adverse toxic reactions. Some of the biogenic amines have vasoactive properties (e.g. histamine, tyramine, phenylethylamine, tryptamine) while others act primarily by inhibiting histamine detoxifying enzymes, e.g. the putrefactive amines, putrescine and cadaverine.

Histamine toxicity can result in a wide variety of symptoms such as rash, urticaria, inflammation, nausea, vomiting, diarrhoea, abdominal cramping, hypotension, tingling sensations, flushing, palpitations and headache. In general, toxic symptoms are relatively mild and many patients may not attend a doctor. Thus, the exact prevalence worldwide of histamine toxicity is unclear. The prevalence of cheese-related toxicity is also unclear although several incidences have been reported in the literature. For most individuals, ingestion of even large concentrations of biogenic amines, such as histamine, does not elicit toxicity symptoms since they are rapidly converted to aldehydes by monoamine oxidase (MAO) and diamine oxidase (DAO) and then to carboxylic acids by oxidative deamination. These enzymes, present in the gastrointestinal tract, may prevent/reduce the absorption of unmetabolised histamine into the bloodstream. However, if MAO and DAO are impaired due to a genetic defect or the presence of potentiators such as foodborne putrefactive amines (e.g. putrescine, cadaverine) or pharmacologic agents (e.g. isoniazid), adverse reactions may occur on ingestion of biogenic amines. Putrescine and cadaverine have been reported to inhibit two histamine-detoxifying enzymes, DAO and histamine *N*-methyltransferase (HMT). Many bacteria, especially Enterobacteriaceae [**65**], are capable of producing putrescine and cadaverine as they possess ornithine decarboxylase and lysine decarboxylase. Tyramine, tryptamine and phenylethylamine can also act as potentiators. Tyramine is the only inhibitor present in significant quantities in cheese. The anti-tuberculosis drug isoniazid inhibits histamine-metabolising enzymes and has been reported to result in histamine poisoning in conjunction with cheese consumption. Other drugs administered as antidepressants, antihistamines and antimalarials can sometimes inhibit histamine-metabolising enzymes.

Factors influencing formation of histamine and other biogenic amines include the following.

The presence of histamine-producing bacteria

Enterobacteriaceae have been implicated in histamine production in cheese. However, *Clostridium*, *Lactobacillus* and some strains of *Klebsiella, Morganella* and *Hafnia* have also been reported to possess histidine decarboxylase, and hence are potential histamine producers. Low concentrations of free histidine are present in milk. However, proteolysis during cheese ripening can produce large amounts of histidine. Non-starter lactobacilli [**56**] play a significant role in histamine formation in Gouda and probably other cheeses. Higher levels of biogenic amines are formed in cheese made from unpasteurised milk than in raw milk cheese. It appears that bacteria responsible for biogenic amine formation are present in milk prior to processing rather than as post-processing contaminants. Thus, adherence to high standards of cleanliness during milk production can play a role in reducing biogenic amine formation in cheese.

Storage temperature

Storage temperature also appears to play a role in histamine formation in cheese through its effect on the growth of non-starter bacteria [**56**]. Elevated storage temperature increases the potential for histamine formation in cheese, particularly if significant numbers of bacteria with decarboxylase activity are present.

pH and salt concentration

High pH and high salt concentration influence the ability of certain bacteria, e.g. *Lactobacillus,* to produce histamine in cheese.

Proteolysis

Enhancing proteolysis during cheese ripening by addition of proteolytic enzyme preparations has been reported to increase the concentration of biogenic amines in cheese.

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68 What are mycotoxins, where do they come from and what problems do they cause?

N. M. O'Brien and T. P. O'Connor

Mycotoxins are secondary metabolites of fungi which can cause acute toxic, mutagenic, teratogenic and carcinogenic effects. For example, aflatoxin B_1 (AFB_1) is regarded as the most potent known animal liver carcinogen. Hence, contamination of the human food chain, including dairy products, with mycotoxins is undesirable. The presence of mycotoxins in cheese may result from contamination of the cows' feedstuffs (indirect contamination), production by fungi used in the manufacture of mould-ripened cheeses [**128**, **137**], or direct contamination by mycotoxin-producing fungi.

Indirect contamination

Early work demonstrated that intake of AFB_1-contaminated feedstuff by dairy cows resulted in rapid excretion of a toxic factor in their milk. Subsequently, aflatoxin M_1 (the 4-hydroxy derivative of AFB_1) was identified as the principal toxic metabolite in milk. Normal carry-over is about 0.4–0.6% and a daily intake of $AFB_1 \geq 70\,\mu g$ by cows would result in greater than the regulatory limit ($0.05\,\mu g\,l^{-1}$) of AFM_1 in milk accepted in most countries. While AFM_1 is much less toxic, less mutagenic and less carcinogenic than AFB_1, it is nonetheless classified as a possible human carcinogen and as such its presence in milk-derived products, such as cheese, is a cause for concern. A number of studies have shown that AFM_1 is stable during cheesemaking, and that 40–57% of total AFM_1 in milk is retained in the curd. An examination of different types of cheese showed high stability of AFM_1 during maturation and storage. Therefore, the presence of AFM_1 in cheese and indeed in other casein-containing products is to be expected if contaminated milk is used as the starting material. Results of quantitative surveys of the level of AFM_1 in milk indicate that the incidence of AFM_1-contaminated milk has decreased significantly in recent years. These studies suggest that typically AFM_1 was not detectable or occurred in cheese at concentrations lower than the legal limits. Studies on the indirect contamination of milk with other mycotoxins such as ochratoxin A, zearalenone, T-2 toxin, sterigmatocystin and deoxynivalenone have indicated that contamination of milk with these mycotoxins does not represent a significant public health issue.

Mycotoxin production by fungi used in the manufacture of mould-ripened cheeses

Cultures of *Penicillium roqueforti* [**137**] and *P. camemberti* [**128**] have been used for a long time in the manufacture of various types of blue-veined and white surface-mould cheeses. Some *P. roqueforti* strains can produce mycotoxins such as patulin, mycophenolic acid, penicillic acid, roquefortine, cyclopiazonic acid, isofumigaclavine A and B and festuclavine. *P. camemberti* strains have been shown to produce only cyclopiazonic acid. Evaluation of

toxicological data together with data on the consumption of mould-ripened cheeses, indicate that the levels cause no appreciable risk to human health.

Direct contamination of cheese with mycotoxin-producing fungi

Unintentional mould growth on cheese during ripening and storage [**74**, **134**, **139**] is a potential problem for manufacturers, retailers and consumers; it results in financial loss, diminishes consumer appeal and often necessitates trimming. However, mycotoxin production is a potentially more serious problem. Such cheese has been reported to contain mycotoxins that are nephrotoxic (ochratoxin A, citrinin), teratogenic (ochratoxin A, aflatoxin B_1), neurotoxic (penitrem A, cyclopiazonic acid), carcinogenic (aflatoxins B_1 and G_1, ochratoxin A, patulin, penicillic acid, sterigmatocystin) or toxic antibiotics (patulin, penicillic acid, mycophenolic acid, citrinin). Of the mycotoxigenic fungi isolated from cheese, *Penicillium* spp. are by far the most frequently reported, with *Aspergillus* spp. and others encountered occasionally. Cheese is generally a good substrate for fungal growth, given suitable conditions of temperature, humidity and oxygen availability. Mycotoxin-producing moulds require oxygen and hence packaging of cheese is important; moulds are unlikely to grow on properly vacuum-packed or wax-coated cheese. Good plant sanitation during manufacture and handling is also important in minimising or preventing mould growth on cheese. Mycotoxins are unlikely to be produced during low temperature storage. Additionally, the presence of mould growth does not necessarily imply that mycotoxins are present in cheese.

Some work has been undertaken on the ability of mycotoxins to migrate from the surface of cheese into the interior. Data on this topic are of significance in making objective decisions on whether or not to trim or discard mould-contaminated cheese. While interpretation of much of the data is difficult, it is recommended that if cheese is visually contaminated with mould growth, the contaminated portion of the cheese be removed to a depth of at least 2.5 cm.

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Nutritional aspects of cheese

69 Introduction

P. L. H. McSweeney

Cheese is a highly nutritious and versatile food that can play an important role in a well-balanced diet. Unlike most dairy products, the per capita consumption of cheese is increasing and cheese has a healthy and positive image in many markets around the world. Nevertheless, since cheese is a high-calorie food, considerable effort has been expended in making low-fat or reduced-fat variants of established varieties [**106**], such as Cheddar [**100**], Gouda [**108**] and Mozzarella [**146**], for health-conscious consumers. The nutritional value of cheese depends largely on its composition which, in turn, is determined during manufacture. Cheese is usually an excellent source of protein, fat-soluble vitamins (water-soluble vitamins in milk largely partition into the whey on cheesemaking) and calcium, and ripened cheeses are essentially lactose-free. However, cheese also contains high levels of fat and NaCl and low levels of iron, and acid-curd cheeses contain significantly lower levels of calcium than rennet-coagulated varieties [**4**, **72**].

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70 What are the typical levels of vitamins in different cheeses?

N. M. O'Brien and T. P. O'Connor

Cheese contains a high concentration of essential nutrients relative to its energy content. Its precise nutrient, including vitamin, content is influenced by the type of milk used (species, stage of lactation, whole fat, low fat, skim), manner of manufacture and to a lesser extent the degree of ripening. Water-insoluble nutrients of milk (coagulated casein, colloidal minerals, fat, fat-soluble vitamins) are retained in the cheese curd, whereas the water-soluble milk constituents (whey proteins, lactose, water-soluble vitamins and minerals) partition into the whey. The concentration of fat-soluble vitamins in cheese is influenced by the same factors that affect its fat content. Most of the vitamin A (80–85%) in milk fat is retained in the cheese fat. The concentration of water-soluble vitamins in cheese is generally lower than in milk due to losses in the whey. The loss of some of the B vitamins is offset, to a certain extent, by microbial synthesis during cheese ripening. In particular, propionic acid bacteria synthesise significant levels of vitamin B_{12} in hard cheeses such as Emmental. In general, most cheeses are good sources of vitamin A, riboflavin, vitamin B_{12} and, to a lesser extent, folate. Cheese contains negligible levels of vitamin C. The vitamin content of a range of cheeses is shown in Table 1.

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Table 1 Vitamin content of selected cheeses, per 100 g (Holland *et al.*, 1989)

Cheese type	Retinol (μg)	Carotene (μg)	Vitamin D (μg)	Vitamin E (mg)	Thiamine (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B_6 (mg)	Vitamin B_{12} (μg)	Folate (μg)	Pantothenate (mg)	Biotin (μg)
Brie	285	210	0.20	0.84	0.04	0.43	0.43	0.15	1.2	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	0.0	2.1	40	0.38	1.8
Emmental	320	140	N	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	0.05	0.02	0.04	0.40	0.13	0.10	1.4	15	N	N
Gouda	245	145	0.24	0.53	0.03	0.30	0.05	0.08	1.7	43	0.32	1.4
Gruyere	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*	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	0.03	0.02	0.19	0.09	0.03	0.3	N	N	N
Roquefort	295	10	N	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

N = The nutrient is present in significant quantities but there is not reliable information on the amount.
Tr = Trace.
* Type not specified.

71 Is cheese good for your teeth?

N. M. O'Brien and T. P. O'Connor

Dental caries involves metabolism of sugars by oral microorganisms including *Streptococcus mutans* to acids that gradually dissolve tooth enamel. It is now recognised that a number of dietary factors and nutrient interactions can modify the expression of dental caries. The cariogenic potential of food is influenced by its composition, texture, solubility, retentiveness and ability to stimulate saliva flow. A considerable body of research has been conducted on the cariostatic effects of cheese.

Early work demonstrated that the incorporation of dairy products into the diet greatly decreased the development of dental caries in rats. Later work indicated that if enamel is treated with milk *in vitro* and subsequently washed, the solubility of the enamel is greatly reduced. This effect was attributed to the high levels of calcium and phosphate in milk or to the protective effects of casein. It has also been reported that both casein and whey proteins significantly reduced the extent of caries, with the former exerting the greater effect. Thus, evidence exists that milk proteins, calcium and phosphate all exert an anticariogenic effect. Additionally, a study in humans found that the consumption of Cheddar cheese after sweetened coffee or a sausage roll increased plaque pH, possibly due to increased salivary output. Rats fed additional meals of cheese while on a high-sucrose diet developed fewer smooth surface carious lesions and exhibited increased salivary output (which buffers acid formed in plaque) and a reduction in numbers of *S. mutans*. Further work on the effect of cheese, with or without sucrose, on dental caries and recovery of inoculated *S. mutans* in rats indicated beneficial cariostatic effects of cheese consumption but little effect on numbers of *S. mutans*. These data suggest that the cariostatic effects of cheese may not be directly related to effects on *S. mutans*.

The effects of Cheddar cheese on experimental caries in humans has been studied using an 'intra-oral cariogenicity test' (ICT). Demineralisation and consequent reduction in the hardness of enamel monitored in this test is assumed to be typical of the early stage of caries development. Enamel slabs were polished and their initial micro-hardness determined using a Knoop indenter. The slabs were then wrapped in Dacron and fastened on a prosthetic applicance made specifically for each subject to replace a missing lower first permanent molar. The subjects chewed 5 g of cheese immediately after rinsing their mouths with 10% (w/v) sucrose. Chewing cheese immediately after sucrose rinses resulted in a 71% reduction in demineralisation of the enamel slabs, raised plaque pH but caused no significant change in the microflora of plaque compared with controls.

A study to investigate the effects of the water-soluble components of cheese on human caries using the ICT procedure reported an average reduction of 55.7% in the cariogenicity of sucrose, indicating the presence of one or more water-soluble anticariogenic components. Evidence that cheese may inhibit dental caries in the absence of saliva was provided by a study with rats that had

their saliva-secreting glands surgically removed and were reported to develop fewer and less severe carious lesions when fed cheese in addition to a cariogenic diet when compared to an appropriate controls.

Trials on human subjects have confirmed that the consumption of hard cheese leads to significant rehardening of softened enamel surfaces. Saliva flow is greatly reduced in individuals who receive head and neck irradiation for malignancies. These individuals are at high risk of developing dental caries. Consumption of hard cheese by these individuals was effective in controlling caries. Concentration of calcium in plaque was significantly higher in human subjects fed cheese-containing meals than in control subjects fed meals without cheese. The beneficial effects of cheese were observed even when it was incorporated into other foods, e.g. pasta with cheese sauce. Epidemiological studies indicate that high intake of cheese is negatively associated with root caries in elderly populations, many of whom are at high risk for such lesions.

In conclusion, while more research is needed to define the precise mechanism(s) involved in the cariostatic effects of cheese, there is ample evidence to support the consumption of cheese to avoid the development of dental caries. The most plausible mechanisms for the protective effect of cheese appear to be related to the mineralisation potential of the casein-calcium phosphate of cheese, to the stimulation of saliva flow induced by its texture and/or flavour, the buffering effects of cheese proteins on acid formation in dental plaque, and inhibition of cariogenic bacteria.

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72 What are typical calcium levels in different cheeses?

P. L. H. McSweeney

Cheese is generally a good source of dietary calcium but calcium levels in cheese vary widely. Levels of calcium in milk (typically *ca*. 1142 mg l^{-1}) are affected by factors including the breed of cow and stage of lactation [**4**]. Calcium levels in cheesemilk may also be increased through the addition of $CaCl_2$ prior to manufacture [**33**]. In addition to its nutritional significance, calcium levels in cheese are of significance to cheese texture.

Calcium in milk is distributed between the colloidal (associated with the casein micelle, *ca*. 66.5%) and soluble (*ca*. 33.5%) phases [**4**]. During cheesemaking, colloidal calcium is incorporated into the curd while calcium in the soluble phase is largely lost on whey drainage. The balance between colloidal and soluble calcium is affected by pH and, as milk is acidified, colloidal calcium phosphate associated with the micelles dissolves and thus levels of soluble calcium increase. Thus, the pH at whey drainage is a critical factor in determining the calcium levels of the cheese. All else being equal (e.g. levels of residual rennet activity and casein and moisture levels), draining the curd at a high pH will result in higher calcium levels in the curd and a more elastic cheese texture than draining at a low pH at which point more colloidal calcium phosphate has dissolved and thus is lost in the whey, leading to low calcium levels in the cheese (and a more crumbly, friable texture).

Calcium levels are generally quite low in acid-curd cheeses [**170**] as whey separation occurs at a low pH. Conversely, the whey is drained from Swiss-type cheeses [**117**, **124**] at a high pH, meaning that more calcium is retained in the cheese, which is a factor contributing to the elastic nature of these cheeses. In cheeses that loose much moisture during ripening such as Italian Grana-type varieties [**97**], calcium together with all other solids in the cheese, become more concentrated. Calcium levels in Camembert-type cheeses [**128**] are relatively low since much calcium is lost during manufacture as syneresis is driven by acidification while the cheeses are being moulded. Also, much of the calcium in these cheeses is at their surface where it precipitates at the high pH caused by lactate metabolism by *Penicillium camemberti.*

Typical calcium levels in cheese are shown in Table 1.

Table 1 Typical calcium levels (mg/100 g) in different varieties of cheeses (modified from O'Brien and O'Connor, 2004)

Brie	540	Cream cheese	98	Gruyère	950
Caerphilly	550	Danish Blue	500	Mozzarella	590
Camembert	350	Edam	770	Parmesan	1200
Cheddar (normal)	720	Emmental	970	Processed cheese	600
Cheddar (reduced-fat)	840	Feta	360	Ricotta	240
Cheshire	560	Fromage frais	89	Roquefort	530
Cottage cheese	73	Gouda	740	Stilton	320

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Packaging

73 Introduction: how may cheese be packaged?

A. L. Kelly

Packaging is increasingly recognised as an important factor in protecting and controlling the quality of cheese. The packaging requirements of cheese varieties can broadly be divided into two categories:

1. For certain varieties, particularly those with an active surface microflora (e.g. bacterial surface-ripened [**141**] or mould-ripened cheeses [**128**, **137**]), and generally a short shelf-life, the packaging plays a critical role in controlling the ripening of the cheese, through its moisture and gas permeability characteristics.
2. For hard varieties [**83**], which generally ripen for long times in an anaerobic environment, a complete barrier package (e.g. a vacuum package) may be preferable.

There are several stages of packaging of many cheese varieties. Immediately after manufacture, many hard cheese types made on a large industrial scale are bulk-packaged in 20–25 kg blocks before ripening. Subsequently, at some point adjudged suitable for sale to consumers, and depending on the grade of cheese being produced (e.g. mild, mature, extra-mature), these packages are opened and the cheese block is subdivided into multiple smaller consumer packs, which often have built-in convenience attributes (e.g. resealability).

Factors that must be considered in selecting a cheese packaging material include permeability to water vapour, oxygen, NH_3, CO_2 and light, potential for migration of compounds from food to packaging or vice versa, and practical

considerations including suitability for labelling and compatibility with conditions during distribution and sale.

Hard varieties are typically packaged in polyethylene/polyamide vacuum-pack bags, which retard growth of aerobic spoilage bacteria and contamination of the cheese from the outside. For certain varieties (e.g. Gouda), paraffin wax was traditionally used as a packaging material, while today a latex emulsion (plastic coat) may be used.

The ripening rate of respiring cheeses, such as surface mould-ripened varieties, may be manipulated by use of modified atmosphere packaging combined with appropriate permeability characteristics of a plastic over-wrap. Care must be taken in packaging very soft cheeses, as mechanical stresses may result in collapse of the cheese structure within the package. High-moisture fresh cheese varieties [**170**] are sensitive to dehydration, and must be packaged in suitable barrier materials, which also provide light and oxygen barriers.

Overall, increasing attention is today being paid to packaging of cheese, and what was once regarded as an inert and passive protectant for cheese is being acknowledged as a potentially significant means of controlling ripening, quality and safety.

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74 Why does mould develop under the packaging?

J. J. Sheehan

Mould defects in vacuum-packed Cheddar-type cheeses

Moulds require oxygen to grow and sporulate but many cheeses produced commercially, particularly Cheddar [**100**], are vacuum packed during ripening. Moulds grow if air pockets exist between the cheese and packaging, perhaps as a result of small pinholes in the packaging, or if the cheese has been improperly sealed. The starter lactic acid bacteria [**18**] are no longer viable and thus cannot competitively inhibit the moulds, and moulds are also capable of growth at cheese storage and ripening temperatures.

Moulds found in Cheddar-type cheeses include *Penicillium* spp., such as *Penicillium commune* and *Penicillium roqueforti*, and black moulds, such as *Cladosporium cladospoiroides*. Moulds causing 'thread-mould' defect in the folds and creases of vacuum-packed cheese blocks include *C. cladospoiroides, P. commune*, *C. herbarum*, *P. glabrum* and *Phoma* spp. 'Thread mould' defects can occur on cheese surfaces but are usually associated with free whey drawn from the cheese blocks during vacuum packing.

Mould species responsible for defects in Cheddar-type cheeses are found in the cheese factory environment, on cheese manufacturing equipment, in air and in curds and whey providing a wide range of sources of contamination. Mould spores have different means of dispersal and this is species dependent. Moulds with moist spores such as *Mucor* are spread in humid atmospheres with wet surfaces, while moulds with dry spores such as *Penicillium* spp. are spread through movement of air.

Mould defects in other cheese types

Moulds such as *Penicillium discolor* and *Aspergillus versicolor* may grow on Dutch-type cheeses [**108**] which have been treated with natamycin to inhibit mould growth. Moulds may grow on cheese surfaces where cheeses are not vacuum packed or gas flushed prior to packaging and where the cheeses are stored in a humid environment and where anti-fungal agents have not been applied.

Mould defects within soft or white mould cheeses [**128**] are often due to the growth of *P. roqueforti* which is capable of growth in the low oxygen environment of mechanical openings created during cheese manufacture. Such mould contamination has been attributed to sources such as the cheese manufacturing environment and air quality and, in the case of raw milk cheeses, from spores contained in milk produced from maize silage.

Black moulds causing defects include *Mucor* spp. and *Rhizopus* spp. and may cause spoilage in cheeses where insufficient acidity has developed, where poor handling practices are employed and where humidity may be excessive in ripening and drying rooms.

Cladosporium herbarum forms dark green spots on cheese surfaces. It is not acid tolerant and grows on the surfaces of certain varieties where the pH has

increased during ripening, and at low ripening temperatures. It can colonise ripening rooms, ceilings, air ducts and temperature control and air conditioning units. *Scopulariopsis fusca* forms brown coloured spots on cheese surfaces and it also is not acid tolerant. This organism can colonise paper and packaging materials stored under unsuitable conditions and thus can spread to cheese surfaces.

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Whey processing

75 What products may be produced from whey?

A. L. Kelly

For perhaps centuries, whey has been regarded as a problematic by-product of cheesemaking, and not an insubstantial one, with 90% of milk volume generally being released as whey. Options such as feeding to animals, dumping in waterways and spreading on land were practised until relatively recently in many countries, and may still be practised in certain regions.

Recognition of problems such as the high biochemical oxygen demand of whey, rendering it a potent pollutant, and interest in recovery of the milk constituents in whey drove a strategic re-evaluation of the potential of whey. This has led today to a point where whey is viewed as a valuable resource, from which many products are produced. Facetiously, it is sometimes quipped that cheese is a low-value by-product of the manufacture of whey!

Whey is classified on the basis of its pH into acid (pH $\leq$ 4.6) or sweet ($\geq$5.0) types; these differ most significantly in their mineral content, acid whey having a far higher level of calcium due to pH-induced solubilisation of colloidal calcium phosphate from the casein micelle [**4**]. Most cheese whey is of the sweet type, with acid whey originating from the production of Quarg or similar varieties [**170**], in addition to acid casein.

Before further processing, cheese whey must generally be clarified or separated centrifugally, to recover fat and curd fines; the former may be churned into whey butter. It is usually also rapidly cooled and often pasteurised, to control or eliminate the starter bacteria present and inactivate the rennet activity. For certain products, very low levels of fat may be achieved by microfiltration of the whey or by addition of calcium followed by a controlled programme of pH

and temperature (i.e. so-called thermocalcic aggregation). The main products which can be produced from whey are listed below.

Whey beverages
Flavoured or unflavoured drinks represent a low-cost relatively simple utilisation option for whey in certain countries.

Whey powder
Whey contains a very high (>92%) level of water, and may be stabilised and made easier to transport by dehydration, either partially (i.e. by evaporation or reverse osmosis) or almost fully (by subsequent spray-drying). The major constituent of whey powder is lactose, and the crystallisation thereof must be carefully controlled to avoid defects such as caking of the powder on storage.

Demineralised whey powder
The relatively high mineral content of whey renders it unsuitable for use in certain applications, particularly infant formulae; demineralisation using ion exchange or electrodialysis can yield deminieralised whey products suitable for these applications.

Whey protein concentrates
Probably the most functionally significant constituents of whey are its proteins. Powders enriched in protein (and consequently with lower levels of lactose and salts) can be produced by ultrafiltration of whey, and protein contents can be increased further by diafiltration, a process by which the protein-containing ultrafiltration retentate is diluted with water and re-ultrafiltered. Powders containing 30–70% whey protein (compared with ~10–15% in whey powder) are referred to as whey protein concentrates (WPC). The by-product of ultrafiltration, an aqueous solution of lactose and salts, is called whey permeate.

Whey protein isolates
Products with higher protein levels than WPCs (e.g. >80%) may be produced, for example by recovering proteins using ion exchange; these are called whey protein isolates (WPIs).

Denatured and fractionated whey protein products
Whey proteins in WPCs and WPIs are generally native, but may also be recovered in denatured form by high-heat treatment and pH adjustment. Fractions enriched in α-lactalbumin or β-lactoglobulin may be produced by specific

processes which exploit the different relative solubilities of these proteins under certain conditions (e.g. pH). Finally, biologically active compounds such as lactoferrin and lactoperoxidase are produced commercially by large-scale ion exchange processes, which exploit the high isoelectric points of these proteins to facilitate their recovery from whey. The value of these proteins justifies the large volumes of whey that must be processed to recover significant quantities.

Lactose-derived products

Whey permeate may be processed in a number of ways to utilise the high level of lactose therein; concentration by evaporation followed by controlled crystallisation and separation in decanter centrifuges yields crystalline lactose, which can be re-dissolved and reacted with activated carbon to produce pharmaceutical-grade lactose, for use in tablets and coatings. Whey may also be fermented with a yeast such as *Kluyveromyces marxianus* to yield ethanol, or a range of chemical reactions utilised to yield products such as lactulose, lactitol and lactobionic acid.

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Analysis of cheese

76 Introduction

P. L. H. McSweeney

Cheese is analysed for a wide range of reasons including to ascertain its composition as part of a quality control system or to generate data for nutritional labelling [**69**, **70**, **72**], to ensure compliance with standards of identity of a particular variety, to assess the efficiency of production and to ensure the microbial safety of the product [**58**, **59**]. Before analysis, cheese must be sampled properly, as the reliability of the results of any analytical procedure is dependent on how representative is the sample taken for analysis. The gross composition and pH of cheese provide some very important data related to quality, and hence measurement of the pH and moisture, fat and salt levels in cheese is common. Derived parameters such as moisture-in-non-fat-substances, salt-in-moisture and fat-in-dry-matter are also calculated commonly. Cheese samples are often sent for microbiological analyses to ensure that the product is free from pathogenic organisms. In the context of scientific research in cheese, compositional data are also of great value as are counts for starter and non-starter organisms. Proteolysis, lipolysis and the products of lactate and citrate metabolism [**88**] can be followed by suitable laboratory techniques and volatile compounds determined, often by gas chromatography–mass spectrometry. A wide range of techniques has also been developed to measure the texture, functionality and sensory properties of cheese.

77 What is the correct way to sample cheese for analysis?

P. L. H. McSweeney

Cheese is analysed for a number of purposes, including determination of composition for nutritional purposes, to ensure compliance with standards of identity, to assess the efficiency of production or as an index of quality, to assess the microbial safety of the product or the influence of microflora on cheese quality [**76**]. Unless an entire cheese is sufficiently small to form the sample, following the correct procedure is essential to ensure that the sample obtained is representative. Methods of sampling dairy products, including cheese, were described in detail by IDF (1995).

In general, samples should be taken by an experienced and responsible person who is familiar with standard methods. Since traceability may be an important consideration in industry, the samples should be sealed and labelled appropriately and accompanied by a sampling report. The apparatus used most commonly for sampling cheese is the cheese trier, although a suitable knife or a cutting wire may also be used. Cheese triers are made from stainless steel and should be sterilised before sampling for microbiological or sensory analysis (Fig. 1).

Cheese samples should be stored in a suitable container (e.g. a plastic container or bag or aluminium foil); containers for microbiological samples

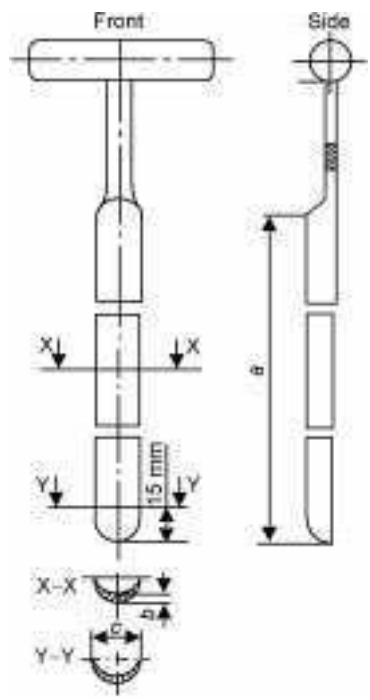

Fig. 1 Cheese trier (from IDF, 1985, with permission).

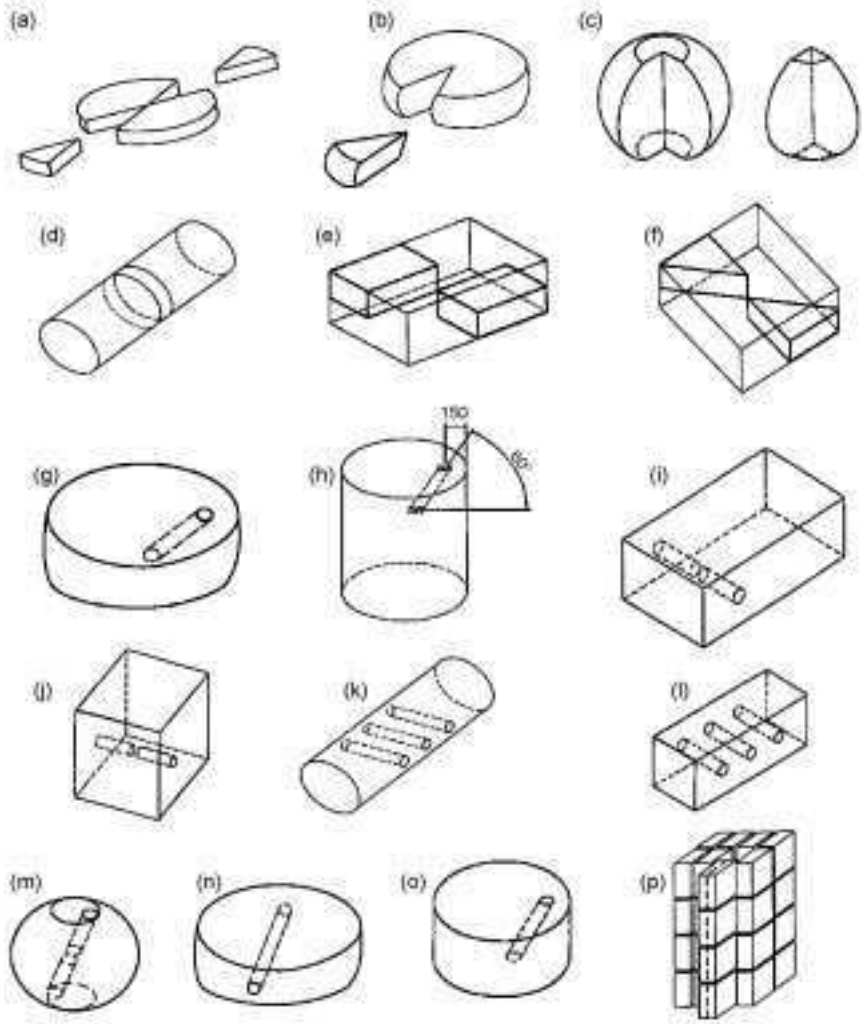

Fig. 2 Suggested sampling techniques for cheese. Sampling cheese by cutting (a) two sectors, (b) one sector, (c) a sector from a spherical cheese, (d) a sector from a cylindrical cheese and by cutting sectors from block-shaped cheese in which the largest face is rectangular (e) or square (f). Sampling cheeses using a trier: (g) a large loaf-shaped cheese, (h) a tall cylindrical cheese, (i) a block-shaped cheese, (j) a cubic cheese, (k) cylindrical cheese, (l) loaf-shaped cheese, (m) a spherical cheese, (n) a loaf-shaped cheese sampled from the side and (o) a large cylindrical cheese sampled from the top, (p) sampling cheeses in brine containers with more than four blocks of cheese (modified from IDF, 1995, with permission).

must be sterile and particular attention should be paid to the risk of moisture loss by evaporation. In general, samples for microbiological, sensory or rheological analyses should be stored at 0–4 ºC until analysed, which should be performed as soon as possible after sampling (preferably within 24 h) although samples for

composition and certain biochemical analyses are routinely stored frozen (−20 °C).

In general, duplicate samples (100–200 g) should be taken and should include any surface layer. However, for research purposes, it is common to analyse separately surface and core samples of certain varieties (e.g. surface-ripened cheeses or those with a salt gradient caused by brining). It is recommended that brine-salted varieties should not be sampled using a trier but rather by a technique that involves cutting the cheese because a trier will not give a representative sample from cheeses with radial salt and moisture gradients. Care should also be taken when sampling fresh cheeses to avoid whey separation. Methods of sampling cheeses of different geometries are shown in Fig. 2.

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78 How are volatile flavour compounds measured in cheese?

P. L. H. McSweeney

The flavour of cheese is a critical quality attribute and sensory analysis is the best method for its determination [**79**]. However, much attention has been focused on instrumental quantification of flavour compounds to learn more about the biochemical pathways which produce specific flavour compounds [**88**] or because instrumental techniques are sometimes more cost effective or convenient than sensory analysis. Attempts have been made to correlate sensory data and cheese flavour chemistry or rheology to link cheese flavour or texture with cheesemaking technology and thus to optimise product quality.

Instrumental techniques have been used primarily to determine the levels of volatile flavour compounds although techniques exist also for measuring levels of non-volatile flavour compounds (e.g. bitter peptides [**89**], NaCl [**39**], lactic acid, lactose, amino acids and other compounds which contribute to the sweet and sour tastes of cheese).

Like other foods, the volatile flavour compounds in cheese are usually hydrophobic, distributed in an heterogeneous manner throughout the cheese and are present at low or even trace levels. Hence, the first step in the quantification of volatile compounds in cheese involves making a preparation or extract suitable for further analysis. Preparation methods include steam distillation, high vacuum distillation and condensing the volatiles in traps cooled by liquid nitrogen, solvent extraction and dialysis techniques based on the ability of molecules of a certain size to pass through a membrane. In addition, headspace methods (static or 'purge and tarp') are very useful. Finally, solid-phase micro-extraction methods have become popular in recent years. In these methods, volatile compounds partition, usually from the cheese headspace, into a solid phase deposited on the surface of a fibre. The advantages and disadvantages of the various sample preparation techniques were discussed by Le Quéré (2004). The actual separation of volatile compounds in cheese extracts nearly always involves various forms of gas chromatography (GC) followed by identification of the separated compounds by mass spectrometry.

Gas chromatography–olfactometry (GC–O), in which the human nose is used as a detector, is a very useful technique for determining the key volatile compounds that contribute to cheese flavour. Specific instrumental techniques have been developed in an attempt to determine the complete flavour of foods. Electronic noses (based on gas sensor technology) or the use of mass spectrometry without prior GC separation of compounds (i.e. directly on cheese headspace or extracts) have been studied as potential means of classifying cheeses.

Instrumental techniques applied to the study of cheese flavour usually generate large data sets and multivariate statistical analysis or other appropriate techniques are useful to produce maps for classification or quality control purposes.

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79 What procedures are available for the sensory analysis of cheese and are they reliable?

J. M. Banks

Sensory evaluation is generally employed to assess cheese quality or to characterise cheeses during product development or to test consumer acceptance of cheese. It is important that a suitable procedure is selected for sensory evaluation and that the test conditions are rigorously controlled to ensure reliability. Sensory analysis may be used to establish if cheese has undesirable characteristics or defects, to identify differences in sensory attributes of two or more cheeses, to quantify differences in specific sensory attributes of a number of cheeses or to study consumer acceptance of cheese. Sensory evaluation is a particularly valuable tool when the sensory data are used as a means of translating consumer preferences into product specifications to facilitate the development of innovative products.

A number of sensory evaluation tools are available. These include grading [**80**], sensory discrimination methods, descriptive profiling and consumer acceptance tests. Cheese grading is a tool for quality control; it is a robust procedure for identifying and defining sensory defects in cheese. However, grading is not suitable for use in research and development or for assessing consumer acceptance. Tools such as sensory discrimination methods, descriptive profiling and consumer acceptance tests are required to characterise and assess the potential of product innovations.

Discrimination tests are used to identify if there are differences in sensory attributes between two or more cheeses. These tests involve direct comparisons of cheeses to establish if there is a perceptible difference in a designated sensory attribute. There are several types of test available including paired comparison, duo–trio, triangular and ranking tests. These tests do not require highly experienced assessors but they do require panellists with the ability to recognise and agree on the meaning of the designated attribute. The paired comparison test involves evaluation of two cheeses and panellists are required to indicate which product has a higher intensity of a designated attribute (e.g. ‘fruity’ or ‘acid’). In a duo–trio test, the assessors are asked which of two cheeses is most similar to a third reference cheese. The triangular test requires the assessors to select which two of three products are alike, or which product is most different from the other two. A number of products are compared with each other for a single designated attribute in the ranking test and the assessors are asked to rank the products in order of increasing intensity of that attribute. Discrimination tests can be used in preliminary investigations to establish if a difference exists between samples which warrants further investigation.

Descriptive sensory techniques are used to discriminate between a range of products based on the full complement of sensory characteristics and to determine a quantitative description of all the sensory differences that can be identified. There are several different methods of descriptive analysis which include the flavour profile method, quantitative descriptive analysis, spectrum,

profiling quantitative flavour profiling and free-choice profiling. For each method, three stages can be identified in implementation. The first step involves the selection of a panel of assessors. A vocabulary must then be established which adequately describes the characteristics of the cheese, and finally those sensory attributes must be quantified. For descriptive sensory analysis the panel of assessors must be capable of recognising many different sensory characteristics, they must agree on how these attributes are perceived and labelled, and they must be capable of individually scoring the intensity of each characteristic on line scales in a consistent way. Much training is required before the panel attains the level of objectivity and reproducibility required. The procedure is expensive and suited to innovative product development.

Consumer acceptance tests are also important tools in product development. Producing a cheese with the desired sensory attributes for a target consumer is the first step towards launch of an effective product in the market. Sensory consumer tests make use of rating scales that measure relative dislike and like, discrimination tests that are based on preference rather than difference, and 'just-right scales' that ask a consumer how they feel about a designated sensory attribute. These tests must be carried out with subjective assessors or with untrained consumers. The assessors should be regular consumers of the product type under test or represent the target market for the product.

All the tests outlined above are reliable if properly executed.

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80 How reliable is cheese grading?

J. M. Banks

Cheese grading is carried out by trained experts and is a reliable means of predicting the potential of a cheese to develop satisfactory flavour and texture during maturation. Grading is used to estimate the optimum point of sale for a cheese, thereby ensuring retail quality is maintained. However, grade scores do not relate to consumer preferences and there is a need for a more informative system of assessment of sensory attributes of a cheese at the point of retail sale [**79**]. Grading schemes used throughout the world generally focus on defects in flavour and texture. To relate more closely to consumer preferences, grading schemes should be based on additional positive attributes which characterise consumer demands. This approach, first introduced in New Zealand, is used increasingly in commercial cheese manufacture.

To maintain consumer loyalty and confidence in a brand of cheese, the quality must be consistent. As consumers become more brand conscious, they expect not only absence of defects, but also consistency in the aroma, flavour and texture of cheese. Cheese grading or quality scoring provides a rapid method to assess the overall sensory quality, but does not adequately take into account the individual aroma, flavour and texture character that give the cheese of individual producers, or regions, their distinctive taste. Brand differentiation of aroma, flavour and texture can be achieved through manipulation of manufacturing protocols and selection of appropriate adjunct cultures. These approaches can be used to impart unique sensory characteristics that are not traditionally considered defects but are important determinants of quality for the discerning consumer.

Grading schemes effectively identify out-of-specification cheese early in the maturation process. The grader will determine whether a batch of cheese is suitable for extended maturation and would continue to mature to produce a premium mature product, or if it should be matured for a short time and sold as a mild category cheese. The grader's assessment will also establish if the cheese is unsuitable for table cheese and should be sent for processing. The chemical composition of cheese and its microbiological analysis are sometimes used in conjunction with the graders assessment as part of the manufacturers' quality assessment scheme. The main role of the cheese grader is to estimate the optimum point of sale for a cheese and to determine the ultimate overall quality of the cheese. Grading and quality scoring are appropriate as quality tools which enable rapid evaluation of large volumes of cheese. However, they are not tools for research or marketing as they do not fully characterise the sensory profile of cheese.

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Principal families of cheese

81 Introduction

P. L. H. McSweeney

A wide range of cheeses and cheese-like products are produced worldwide but from a very limited range of raw materials. Natural cheese is made from cow's, sheep's, goat's or buffalo's milk, lactic acid bacteria, rennet (in the case of rennet-coagulated varieties) and salt, yet it has been said that there is 'a cheese for every taste preference and a taste preference for every cheese'. Cheese has a long history and the collective heritage of certain varieties has been ensured in Europe by Protected Designations of Origin [**82**] for certain varieties. No definitive list of cheese varieties exists but it is estimated that there are about 1000–1500 varieties produced worldwide. However, many of these cheeses are in fact very similar and attempts have thus been made to classify varieties into relatively homogeneous groups. Based on method of coagulation of the milk and various technological parameters, cheeses can be classified into about 12 major families:

- Acid-coagulated varieties (e.g. Cottage cheese, Quarg, Cream cheese) [**170**].
- Varieties coagulated by a combination of heat and acid (e.g. Ricotta) [**170**].
- Rennet-coagulated cheeses (most varieties), which can be subdivided based largely on the technology of their manufacture and ripening into:
  - extra-hard (Grana-type) cheeses (e.g. Grana Padano) [**96**];
  - hard cheeses (e.g. Cheddar) [**100**];
  - semi-hard cheeses (e.g. Monterey Jack);
  - Swiss-type cheeses (e.g. Emmental) [**117**];
  - Dutch-type cheeses (e.g. Gouda) [**108**];

- varieties ripened under brine (e.g. Feta) [**164**];
- *Pasta-filata* varieties (e.g. Mozzarella) [**146**];
- surface (white) mould-ripened cheeses (e.g. Camembert) [**128**];
- Blue cheese (e.g. Roquefort) [**137**];
- surface (smear)-ripened cheeses (e.g. Tilsit) [**141**].

In addition, rennet-coagulated cheeses can be dried or converted to processed cheese [**187**, **189**].

Many of these major families contain varieties that are similar in terms of the technology of their manufacture and microbiology and biochemistry of their ripening but may be made from milk of different species. Imitation cheese products (cheese analogues [**196**]) and enzyme-modified cheese [**197**] (which is used as a flavouring) are also produced. Finally, a minor group of Norwegian whey 'cheeses' (*Brunost*, brown cheese) are produced by concentration and crystallisation of whey to give a product with a smooth, firm body and a caramel-like flavour and long shelf-life.

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82 What is a 'controlled designation of origin'?

M. Gobbetti

The idea of protecting and preserving the traditional diversity of foods, including cheese, commenced at the Paris Convention of 1883 where the term 'Appelation d'Origine Contrôlée' was introduced to recognise the specific heritage of food products from particular regions, while guaranteeing product authenticity. This concept became widespread in Europe and was replaced by the EU scheme (1992), 'Protected Designation of Origin' (PDO), which applies to foodstuffs that are produced, processed and prepared in a given geographical area using recognised technology. Foods with the designation 'Protected Geographical Indication' (PGI) have a geographical link with a particular region during at least one stage of production, processing or preparation, while 'Foods with Traditional Speciality Guaranteed' (TSG) status have a traditional character, either in term of composition or means of production.

As reported in the EU Regulation no. 2081/92 (1992), the 'designation of origin' is attributed to foodstuffs originating from a defined and limited geographical area, the character of the foodstuff is exclusively or mainly determined by human and natural factors dependent on the given area, and the production, processing and preparation of the foodstuff take place in the same area. This policy is mainly intended to protect and increase the market for foodstuffs with typical characters which depend on their origin and to favour the consumers who desire the choice of foodstuffs with a determined geographical origin and protocol of manufacture by supplying clear information.

A large number of cheeses have PDO status in different EU countries; a selection of the main productions is as follows: Belgium (1), Germany (4), Greece (19), Spain (17), France (35), Ireland (1), Italy (30), The Netherlands (4), Austria (6), Portugal (11) and United Kingdom (8) (Table 1). Some of the most important PDO varieties are Roquefort, Stilton, Manchego, Grana Padano, Parmigiano Reggiano and Gruyére de Comté. Unlike commercial trademarks, PDO denomination reflects a collective heritage and may be used by all producers of a particular variety in a defined geographical area.

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Table 1 Cheeses with PDO

Country	Variety
Belgium	Fromage de herve
Germany	Allgäuer Bergkäse
	Allgäuer Emmentaler
	Altenburger Ziegenkäse
	Odenwälder Frühstückskäse
Greece	Anevato
	Batzos
	Feta
	Formella Arachovas
	Parnassou
	Galotyri
	Graviera Agrafon
	Graviera kritis
	Kalathakai Limnou
	Kasseri
	Katiki Domokou
	Kefalograviera
	Kopanisti
	Ladotyri Mytilinis
	Manouri
	Metsovone
	Pichtogalo chanion
	San Michali
	Sfela
	Xynomyzithra Kritis
Spain	Cabrales
	Idiazábal
	Mahón
	Picón Bejes-Tresviso
	Queso de Cantabria
	Queso de l'Alt Urgell y la Cerdanya
	Queso de La Serena
	Queso de Murcia
	Queso de Murcia al vino
	Queso Majorero
	Queso Palmero
	Queso Tetilla
	Queso Zamorano
	Quesucos de Liébana
	Roncal
France	Abondance
	Beaufort
	Bleu d'Auvergne
	Bleu des Causses
	Bleu du Haut-jura, de Gex, de Septmoncel
	Bleu du Vercors
	Brie de Meaux
	Brie de Melun
	Brocciu Corse ou brocciu
	Cantal ou Forme de Cantal ou Cantalet
	Camembert de Normandie
	Chabichou du Poitou
	Chaurce
	Comté
	Crottin de Chavignol
	Epoisses de Bourgogne
	Forme d'Ambert ou forme de montbrison
	Laguiole
	Langres
	Livarot
	Maroilles ou Marolles
	Mont d'or ou vacherin du Haut-Doubs
	Morbier
	Munster ou Munster-Géromé
	Neufchâtel
	Ossau-Iraty
	Pélardon
	Picodon de l'Ardèche ou Picodon de la Drôme
	Pont l'Evêque
	Puligny-Saint Pierre
	Reblochon ou reblochon de Savoie
	Rocamadour
	Roquefort
	Saint-Nectaire
	Sainte-Maure de Touraine
	Salers
	Selles-sur-Cher
Ireland	Imokilly Regato
Italy	Asiago
	Bitto
	Bra
	Caciocavallo Silano
	Canestrato Pugliese
	Casciotta d'Urbino
	Castelmagno
	Fiore Sardo
	Fontina
	Formai de Mut Dell'alta Valle Brembana

Table 1 (continued)

Country	Variety	Country	Variety
	Gorgonzola		Tiroler Bergkäse
	Grana Padano		Tiroler Graugkäse
	Montasio		Vorarlberger Alpkäse
	Monte Veronese		Vorarlberger Bergkäse
	Mozzarella di Bufala Campana	Portugal	Queijo Azeitão
	Murazzano		Queijo de Cabra Transmontano
	Parmigiano Reggiano		Queijo de Évora
	Pecorino Romano		Queijo de Nisa
	Pecorino Sardo		Queijo do Pico
	Pecorino Siciliano		Queijo Rabaçal
	Pecorino Toscano		Queijo São Jorge
	Provolone Valpadana		Queijo Serpa
	Quartirolo Lombardo		Queijo Serra da Estrela
	Ragusano		Queijo Terrincho
	Raschera		Queijos de Beira Baixa
	Robiola di Roccaverano		
	Taleggio		
	Toma Piemontese	United Kingdom	Beacon Fell Traditional Lancashire cheese
	Valle d'Aosta Fromadzo		Bonchester cheese
	Valtellina Casera		Buxton Blue
The Netherlands	Boeren-Leidse met sleutels		Dovedale cheese
	Kanterkaas, Kanternagelkaas, kanterkimijnekaas		Single Gloucester
	Noord-Hollandse Edammer		Swaledale cheese, Swaledale ewe's cheese
	Noord-Hollandse Gouda		West Country Farmhouse Cheddar cheese
Austria	Gailtaler Almkäse		White Stilton cheese, Blue Stilton cheese
	Tiroler Almkäse/Tiroler Graukäse		

83 How are cheese varieties classified?

P. L. H. McSweeney

There exists no definitive list of cheeses and estimates of the number of varieties range from ~400 to ~1400. However, many varieties are in fact rather similar in terms of their composition, flavour, texture and manufacturing technology and so should be considered variants rather than varieties. Attempts have been made to classify cheese varieties but no one classification scheme has met with universal approval and all have certain limitations. Three major approaches have been used as a basis for classification of cheese varieties:

- texture, which is largely determined by moisture and fat contents;
- ripening indices;
- method of coagulation as the primary criterion but coupled with other factors.

Common methods for classifying cheese are based on texture, and cheeses are often described as 'hard', 'semi-hard' or 'soft' (e.g. Schultz, 1952; Davies, 1965; Walter and Hargrove, 1972; Burkhalter, 1981; Scott, 1986). These descriptors are somewhat vague and can result in cheeses with very different characteristics being grouped together. Hence, other factors such as cooking temperature, calcium concentration, type of milk, method of coagulation or characteristic ripening agent have been used to subdivide these categories.

Classification schemes based on ripening indices have also been developed and, at least in principle, it should be possible to classify cheeses based on chemical fingerprints. However, cheese is a dynamic system whose composition often changes considerably during ripening, and varies with rennet type [**27**] or cheese microflora [**54**]. However, a number of physicochemical and biochemical studies have been performed to compare cheese varieties based on their peptide profiles obtained by urea–polyacrylamide gel electrophoresis or reverse-phase high-performance liquid chromatography (HPLC), free amino acid levels, profiles of volatile flavour compounds obtained by gas chromatography–mass spectrometry (GC–MS).

Perhaps the most logical classification scheme for cheese varieties is based primarily on the method of coagulation of milk (rennet, acid coagulation, combination of heat and acid) and these super-families of cheese are further subdivided based on technological parameters (Fox, 1993; Fox *et al*., 2000; McSweeney *et al*., 2004; Fig. 1). In this scheme, rennet coagulated cheeses (*ca*. 75% of total cheese production) are subdivided into 10 relatively homogeneous groups (extra-hard [**96**], hard and semi-hard cheeses, Swiss- [**117**] and Dutch- [**108**] type cheeses, cheeses ripened under brine [**164**], *pasta-filata* varieties [**146**], surface mould-ripened cheeses [**128**], Blue cheese [**137**] and bacterial smear-ripened cheeses [**141**]). Acid curd cheeses [**170**], cheese coagulated by a combination of heat and acid (e.g. Ricotta), and other cheeses and cheese-like products [**189**, **196**, **197**] are grouped separately.

However, this classification scheme is not without inconsistencies as cheeses made from different species' milk are grouped together and there is no clear

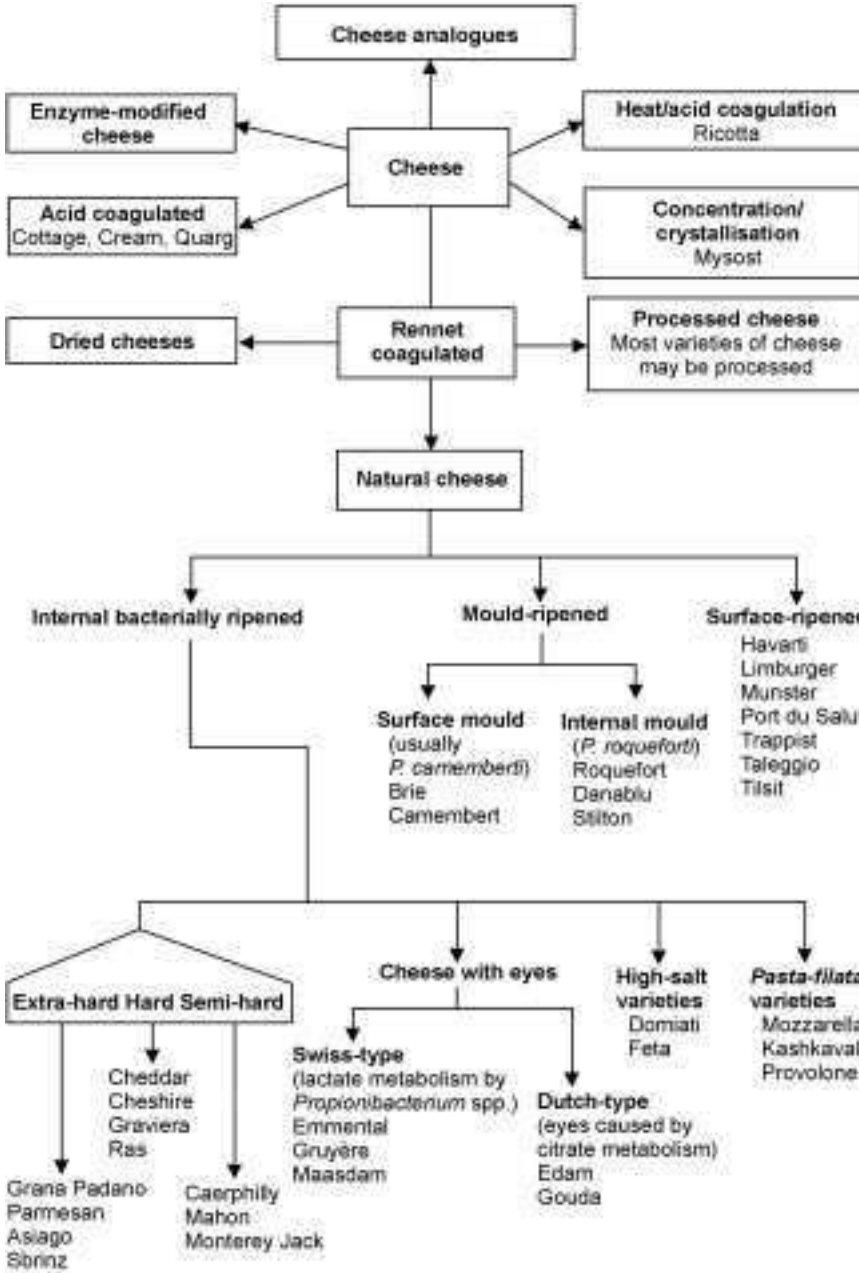

Fig. 1 Classification of cheeses and cheese-like products into super-families based primarily on the method of coagulation of milk and further subdivision of rennet coagulated cheeses based on their characteristic technology or ripening agent (modified from McSweeney *et al.*, 2004).

distinction between hard and semi-hard cheeses. Likewise, Gruyère is classified as an internal bacterially ripened variety with eyes but it is also characterised by the growth of a surface microflora not unlike that of smear-ripened cheeses. Likewise, some varieties classified as bacterial smear-ripened cheeses (e.g. Havarti or Port du Salut) may be produced without a surface smear.

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84 How did cheese originate?

P. L. H. McSweeney

It is commonly believed that cheesemaking originated about 8000 years ago in the 'Fertile Crescent' (i.e. the region surrounding the Tigris and Euphrates Rivers, through what is now southern Turkey around to the Mediterranean coast) as a method of preserving the nutritive value of milk. In the warm climate of these regions, milk would have naturally soured through the action of lactic acid bacteria and the pH may eventually reach the isoelectric point of the caseins, thus leading to the development of fermented milks. When an acid milk gel is broken or cut, it separates into curds and whey and it would have been discovered quickly that whey was a refreshing drink and that the curds could be consumed fresh or stored for future use. No doubt it would have been known that salting or dehydrating the curds extended their shelf-life. It is presumed that acid-curd cheeses evolved in this manner.

The other major group of cheeses, in which the milk is coagulated using rennet, may have originated somewhat differently. It would have been observed that milk in the stomachs of young dairy animals slaughtered for food contained curds. Likewise, curds would have been seen in the vomit of human infants. This observation, or perhaps the use of bags made from animals' stomachs to contain milk, would have led to the use of preparations of gastric enzymes as rennets. In addition, ancient sources contain references to the use of enzymes from plant sources as rennets. Rennet curds have better syneresis properties than acid curds and could be converted to very stable, low moisture, cheeses more easily.

There are many references to cheese and cheesemaking in the Bible, in the tomb art of ancient Egypt and in the literature of ancient Greece. Roman technical writers, particularly Columella (*ca*. AD 50), have left us with detailed descriptions of cheese and cheesemaking. During medieval times in Europe, cheesemaking was fostered on feudal estates and in monasteries, and many important varieties evolved in these self-contained communities. Cheesemaking later spread throughout the world with the migration of European and Middle Eastern settlers to North and South America, Oceania and Africa.

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85 Who are the major cheese consumers and producers in the world?

J. M. Banks

Global cheese production in 2005 was approximately 18.43×10^6 tonnes. This figure, published by the FAO, includes cheeses made using cow's, buffalo's, sheep's and goat's milk [**5**] (Table 1). Cheese made from 'whole cow's milk' accounts for 14.89×10^6 tonnes. The 22 countries listed in Table 2 produced

Table 1 Global cheese production in 2005

Cheese type	Tonnes
Cow's milk (whole)	14 891 465
Cow's milk (skim)	2 155 728
Whey cheese	54 967
Buffalo's milk	264 080
Sheep's milk	643 055
Goat's milk	421 562

Source: www.fao.org

Table 2 Key players in global cheese production in 2005

	Tonnes	World production (%)
United States of America	4 148 300	27.86
France	1 689 000	11.34
Germany	1 259 407	8.46
Italy	1 250 000	8.39
Netherlands	671 000	4.51
Poland	595 000	4.00
United Kingdom	399 000	2.68
Australia	380 000	2.55
Russian Federation	367 000	2.46
Argentina	360 000	2.42
Canada	340 170	2.28
Denmark	336 000	2.26
Egypt	335 000	2.25
New Zealand	293 000	1.97
Belgium	245 348	1.65
Switzerland	166 900	1.12
Austria	152 000	1.02
Ukraine	150 000	1.01
Mexico	132 654	0.89
Sweden	117 800	0.79
Ireland	115 000	0.77
Czech Republic	111 000	0.75
Venezuela	110 000	0.74

Source: www.fao.org

more than 90% of cheese worldwide made from cow's milk. Cheese is primarily a product of Europe and countries populated by European emigrants. There is relatively little production in Asia, Africa or Latin America, but there are some exceptions within these regions, and cheese is produced in some form in most countries globally.

Substantial variations in cheese consumption are evident around the world, even in countries within Europe. The highest consumption levels (approximately 25 kg per head per year) are in Greece, Denmark and France (Table 3). With the exception of Israel and the Dutch Antilles, no Asian, African or South American country is listed among the top 23 cheese-consuming countries. Consumption has grown consistently over the past 20 years in all countries for which data are available and this trend may be expected to continue. With health and nutritional benefits [**69**, **70**, **71**], potential for product innovation by flavour and texture manipulation, cheese is strongly positioned to satisfy the consumer needs of the future.

There is significant international trade in cheese. Europe and Oceania are currently the dominant exporters of cheese (Table 4). Cheese production has increased steadily at a rate of approximately 3% per annum since 1970 and this

Table 3 Consumption of cheese in top 23 countries in 2005

	Cheese supply (kg per capita per annum)
Greece	25.2
Denmark	24.7
France	24.6
Italy	22.2
Switzerland	20.6
Germany	19.7
Netherlands	18.8
Sweden	18.6
Israel	16.4
Belgium	16.3
Finland	15.9
Norway	15.5
United States of America	15.2
Malta	14.5
Czech Republic	13.6
Netherlands Antilles	13.1
Ireland	12.1
Canada	11.8
Slovenia	10.7
Australia	10.4
Poland	10.1
UK	10.0
Portugal	9.3

Source: www.fao.org

Table 4 Key players in international trade in cheese

	Tonnes
Australia	209 359
Belgium	136 086
Denmark	252 488
France	538 813
Germany	664 830
Italy	200 218
Netherlands	510 985
New Zealand	284 933

Source: www.fao.org

trend is expected to continue to increase annually and to account for approximately 40% of milk processed worldwide by 2014. Europe is forecast to continue as the dominant producer. Oceania and the EU are projected to remain the principal exporters of cheese. Japan, the United States, Saudi Arabia and Russia are expected to be among the most significant cheese importing countries of the future.

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86 What are the differences between acid-curd cheese and yoghurt/fermented milks?

A. L. Kelly

There are many similarities between the heat treatment applied to milk, fermentation processes and coagulation principles used for manufacture of yoghurt and for acid cheese varieties [**170**], such as Quarg, Cottage cheese and Cream cheese. However, the key difference is that, for acid-curd cheese varieties, after production of the coagulum, the acid gel is cut and the curd concentrated by expulsion of whey using either filtration or centrifugation, in traditional or more modern processes, respectively.

In addition, fermented milks and yoghurt tend to contain a number of additional ingredients not typically associated with cheese; these may include added milk powder or milk protein powders, hydrocolloid stabilisers, flavours and sweeteners, colours and food particulates (e.g. fruit pieces).

The flavour of these products also differs, owing to differences in the starter culture used [**18**]. The classical fermented milk product, yoghurt, is typically made using a starter culture consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, growing at a temperature around 45 ºC; probiotic fermented milk products may also contain *Lactobacillus acidophilus* or *Bifidobacterium bifidum*, or other bacteria with demonstrated or purported benefits. As a result of the metabolic activities of these cultures, fermented milks derive their flavours from compounds such as acetaldehyde and diacetyl. On the other hand, acid cheese products such as Quarg are typically fermented at lower temperatures (20–25 ºC) using mesophilic cheese starter bacteria such as *Lactococcus* spp. or *Leuconostoc* spp., and diacetyl, lactate and acetate are the most important aroma and flavour compunds.

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Flavour, texture and flavour defects in hard and semi-cheeses

87 Introduction

P. L. H. McSweeney

Hard cheeses are ripened after manufacture for periods ranging from a few months to 2 or more years and it is during this ripening period that the flavour and texture characteristic of the variety develop [**54**, **88**]. Cheese ripening usually involves changes to the microflora of the cheese [**54**, **56**], often death and lysis of starter cells, the development of an adventitious non-starter microflora [**56**] and, in certain cases, the growth of secondary organisms [**117**, **128**, **137**, **142**]. It is often difficult to differentiate between the flavours of freshly made curds of different types of hard cheeses immediately after manufacture. It is during ripening that flavour develops as a consequence of microbial and enzymatic changes to residual lactose and to lactate and citrate, liberation of fatty acids (lipolysis) and their subsequent metabolism to volatile flavour compounds and hydrolysis of the casein matrix of the cheese to a wide range of peptides and free amino acids (proteolysis) followed by catabolism of amino acids to further important volatile flavour compounds. Cheese texture is influenced greatly by manufacture which largely determines the moisture content of the cheese [**34**] and its calcium and fat and fat-in-dry-matter levels. However, texture changes during ripening due to solubilisation of calcium phosphate [**4**], hydrolysis of the casein matrix, changes to water binding within the curd and loss of moisture caused by evaporation from the cheese surface.

While certain changes during the ripening of hard and semi-hard cheeses are always considered defects (e.g. late gas blowing [**91**]), others are considered problems only if they exceed certain limits. For example, very low levels of bitterness are normal in the flavour profile of cheeses such as Cheddar and are

not considered a defect unless levels of bitter peptides exceed certain limits [**89**]. Likewise, lipolysis occurs during the ripening of all hard and semi-hard cheeses but the levels of lipolysis characteristic of an Italian Pecorino variety would be considered a defect in Cheddar [**90**]. Hence, balanced ripening is essential to the quality of hard and semi-hard cheeses.

88 How does flavour develop in cheese during ripening?

P. L. H. McSweeney

It is now generally accepted that there is no one cheese flavour compound and that the flavour of a particular variety stems from the combination of a wide range of volatile and non-volatile compounds present in the correct balance and concentration (the 'component balance theory' of cheese flavour). The biochemistry of cheese ripening is extremely complex and has been a very active area of research in recent years. The reader's attention is drawn to the many reviews of aspects of cheese ripening, and space here permits only the briefest overview of the subject. Conventionally, the biochemistry of cheese ripening is often discussed under three broad headings: (i) metabolism of residual lactose and of lactate and citrate, (ii) lipolysis and metabolism of fatty acids and (iii) proteolysis and amino acid catabolism (Fig. 1).

Most lactose in milk is lost in the whey during cheese manufacture [**34**] and the low levels of lactose trapped in the curd are metabolised quickly by starter activity before salt-in-moisture reaches an inhibitory level [**46**] or by non-starter lactic acid bacteria (NSLAB) [**56**]. Lactate produced by starter activity is an important starting point for a range of pathways that contribute positively or negatively to cheese flavour. L-Lactate may be racemised to DL-lactate by NSLAB activity, which may be of significance to the development of Ca-lactate crystals in cheese [**107**]. Lactate is also the starting point for an anaerobic fermentation by *Clostridium* spp. leading to late gas blowing [**91**]. However, lactate metabolism is of great importance to Swiss cheese where it is metabolised by *Propionibacterium freudenreichii* during the hot-room step of ripening to propionate, acetate, CO_2 and H_2O [**117**]. This secondary fermentation is of great significance to the flavour of Swiss-type cheeses and is essential for eye development. In surface mould-ripened varieties such as Camembert and Brie, lactate metabolism by *Penicillium camemberti* deacidifies the cheese surface with a major impact on cheese texture [**128**, **132**, **133**].

Milk fat contains high levels of short-chain fatty acids which, when liberated by lipolysis [**90**], are highly flavoured. Levels of lipolysis vary widely between varieties and levels expected and desirable in one cheese may be considered a serious defect in another variety [**90**]. Cheeses with the highest levels of lipolysis are those that contain an active source of lipases such as Blue cheese [**137**] (enzymes from *Penicillium roqueforti*) or cheeses (e.g. Italian Pecorino varieties, Provolone or traditional Greek Feta) the milk for which is coagulated using rennet paste which contains pre-gastric esterase [**27**]. Cheeses made from raw milk generally develop higher levels of lipolysis than cheeses of the same variety made from pasteurised milk since the indigenous lipoprotein lipase in milk is largely inactivated by pasteurisation [**11**]. Starter or non-starter lactic acid bacteria are weakly lipolytic but they are present at high numbers for long periods of ripening and their enzymes contribute to the low levels of lipolysis characteristic of varieties such as Cheddar or Gouda. Likewise, *P. freudenreichii* contributes, together with the thermophilic starter, to the low level of lipolysis in Swiss cheese.

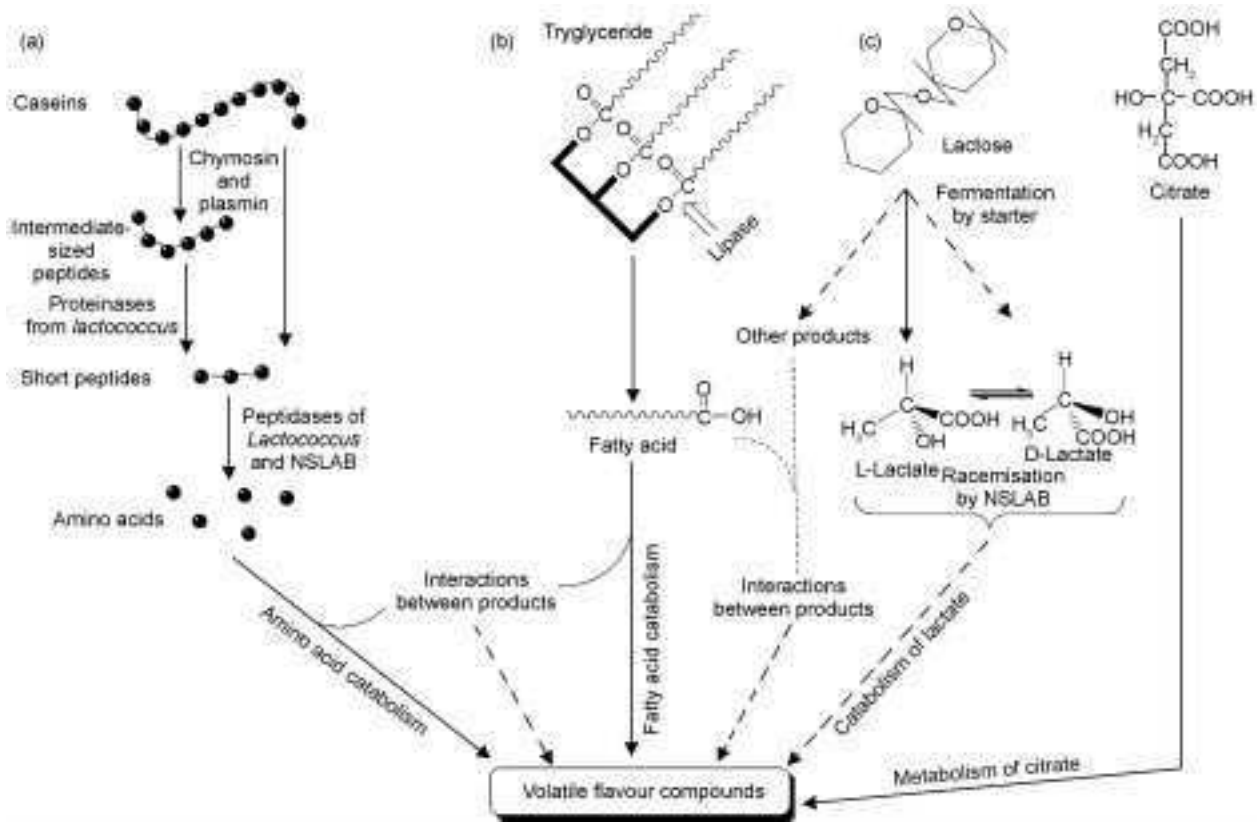

Fig. 1 Schematic representation of the principal biochemical pathways that occur in cheese during ripening (from McSweeney, 2004b).

P. camemberti and the complex Gram-positive bacterial surface microflora of smear-ripened cheese [**142**] also produce lipases that contribute to lipolysis in certain varieties. Fatty acids, particularly short chain acids, have a direct impact on cheese flavour but they also act as starting points for another series of reactions leading to the production of thioesters, ethyl esters, γ- or δ-lactones and, of particular importance to Blue cheese flavour, alkan-2-ones (methyl ketones).

Proteolysis is the most complex and perhaps the most important of the three primary biochemical events that occur during ripening. In most varieties, the caseins are initially hydrolysed by enzymes from the coagulant [**27**, **28**], and to a lesser extent from the milk (plasmin and perhaps somatic cell proteinases [**8**]) forming large and intermediate-sized peptides. The latter peptides are degraded further by the cell envelope-associated proteinase and the wide range of peptidases of lactic acid bacteria, ultimately to free amino acids [**23**]. Proteolysis in hard cheese thus leads to the production of a wide range of peptides (perhaps over 300 in Cheddar) of different sizes and a pool of free amino acids. Peptides may have a direct impact on cheese flavour (some are bitter [**89**]) or may provide a brothy background flavour to cheese. Recent research has suggested that the major role of proteolysis in the development of cheese flavour is the production of free amino acids which are the starting points for a series of pathways ('amino acid catabolism') leading to the production of many important volatile flavour compounds in cheese. Catabolism of most amino acids appears to be initiated by the action of an aminotransferase which transfers the amino group to an acceptor molecule, usually α-ketoglutarate, thus forming glutamic acid and a new α-keto acid corresponding to the amino acid being degraded. The α-keto acids are then degraded by a number of pathways to various flavour compounds. Methionine is the principal sulphur-containing amino acid in cheese and its side chain is the source of many important volatile flavour compounds.

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89 How can the problem of bitterness in cheese be solved?

P. L. H. McSweeney

Bitterness is a taste sensation that is perceived at the back of the tongue and should not be confused with astringency or sourness. The bitter defect in cheese nearly always results from the excessive accumulation of hydrophobic peptides derived from the caseins, particularly from the hydrophobic C-terminal region of β-casein. Peptides with a molecular mass < *ca*. 6 kDa and a mean hydrophobicity > 1400 cal per residue are often bitter. Bitterness is a serious problem in low-fat cheeses, probably due to reduced partioning of hydrophobic peptides into the fat phase. Bitterness also develops in cheeses with a low salt level (i.e. low ionic strength) [**40**]. Low ionic strength weakens hydrophobic interactions between the caseins and facilitates the action of enzymes from the coagulant on hydrophobic regions of the caseins, particularly the C-terminal region of β-casein, resulting in the excessive production of hydrophobic peptides (particularly β-CN f193-209).

The development of bitterness in cheese is due to incorrect patterns of proteolysis causing either the excessive production of bitter peptides (usually by enzymes from the coagulant [**27**]) or insufficient peptidase activity to degrade hydrophobic peptides to free amino acids [**23**] (Fig. 1).

The following questions should be considered if bitterness develops unexpectedly:

- Does the milk have an excessive psychrotroph count [**7**]? If so, heat-stable proteinases may be responsible for the production of bitter peptides.

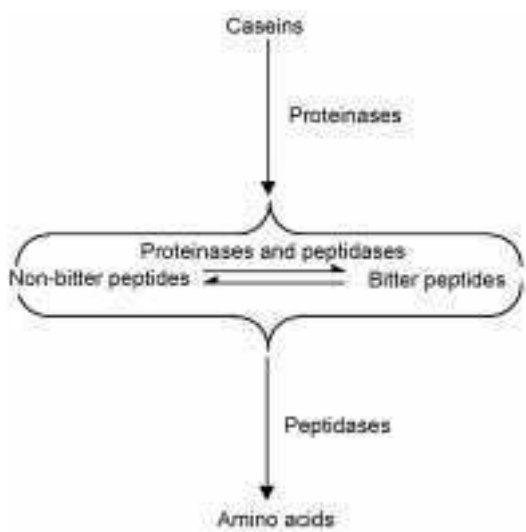

Fig. 1 Schematic representation of the production and degradation of bitter peptides in cheese during ripening.

- Has the rennet preparation been changed recently? Have any other proteolytic enzymes (e.g. in preparations used to accelerate ripening) been added to the milk or cheese?
- Has the starter culture been changed? What is the peptidase activity of the starter and what is the specificity on the caseins of its cell envelope-associated proteinase? Are the numbers of starter cells too high or too low?
- Is the NaCl content of the cheese low?
- Has the fat content of the cheese been reduced?

Strategies to ameliorate bitterness in cheese include changing the rennet preparation used to coagulate the milk to one more suitable for the application, using a starter culture or adjunct with high peptidase activity and ensuring an adequate NaCl level in the cheese (but note the effects of varying NaCl levels [**43**, **46**, **47**]).

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90 What is hydrolytic rancidity and how can it be avoided?

P. L. H. McSweeney

In high-fat foods, lipids may undergo hydrolytic or oxidative degradations. However, the low oxidation–reduction potential of cheese (*ca.* −250 to −350 mV) and the low levels of polyunsaturated fatty acids in milk fat mean that lipid oxidation occurs to a very limited extent in cheese. Hence, the major pathway for degradation of lipids in cheese is hydrolytic and involves the action of lipases on the triacylglyercols of milkfat to produce free fatty acids (FFA) and partial glycerides.

The lipolytic agents in cheese originate from five principal sources:

1. The *milk* contains high levels of lipoprotein lipase (LPL). This indigenous enzyme is largely inactivated during pasteurisation [**11**] and hence is of significance mainly in varieties made from raw milk.
2. The *coagulant* may or may not contain lipolytic enzymes [**27**]. Commercial rennet extracts used to coagulate the milk for the majority of cheese varieties should be free from lipase activity, but rennet pastes used for the manufacture of certain Italian (e.g. the various Pecorino varieties and Provolone) and some traditional Greek cheeses contains a potent lipase, pregastric esterase (PGE). PGE originates from glands underneath the tongue and is washed into the stomach as the animal suckles. Rennet pastes are produced by mascerating the partially dried stomachs of the young of the dairy animal, and their contents, into a paste.
3. The *starter and non-starter lactic acid bacteria* [**18**, **56**] are generally weakly lipolytic but their intracellular lipase/esterases do contribute to the low levels of lipolysis found in varieties such as Cheddar [**100**] and Gouda [**108**].
4. *Secondary organisms* [**128**, **137**, **141**, **142**] (e.g. the moulds in mould-ripened varieties or the smear organisms in smear cheeses) may be very lipolytic. In particular, *Penicillium roqueforti*, which develops within Blue cheeses during ripening, produces potent lipases which lead to extensive lipolysis during ripening.
5. *Exogenous lipases* may be used to accelerate ripening.

Levels of FFA are commonly used as indices of lipolysis and vary considerably between different cheeses. The extent of lipolysis is a characteristic of the ripening of each variety and cheeses with high levels of lipolysis generally have one or more strongly lipolytic agents, are made from raw milk and/or are ripened at elevated temperatures or for long periods of time. Levels of lipolysis typical of Blue cheese (~30 000 mg kg^{-1}) or certain hard Italian cheeses (~15 000 mg kg^{-1}) would be considered as a serious defect in varieties such as Cheddar or Gouda which are characterised by much more limited levels of lipolysis (~1000–4000 and ~400 mg kg^{-1}, respectively).

Factors to consider if an undesirable rancid flavour develops during ripening include:

- damage to the milk fat globule membrane prior to pasteurisation which could allow access of active LPL to its substrate [**31**];
- use of raw milk for cheesemaking;
- high numbers of psychrotrophs [**7**] in the raw milk which can produce heat-stable lipases;
- ripening at elevated temperatures and/or for prolonged durations;
- changes to the starter used or differences in the non-starter microflora [**56**] during ripening;
- possible lipolytic activity in the rennet preparation used [**27**];
- presence of moulds or smear organisms [**142**] during ripening;
- lipolytic activity in any enzyme preparations used to accelerate ripening.

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91 What is late gas blowing and how may this defect be avoided?

P. L. H. McSweeney

Late gas blowing is a serious defect associated with certain hard cheeses [**83**] caused by the anaerobic fermentation of lactate by *Clostridium* spp. (particularly *C. tryobutyricum*) to butyrate, H_2 and CO_2 (Fig. 1). Late gas blowing is principally a defect of brine-salted varieties since diffusion of NaCl through the cheese mass causes a time lag for salt to reach concentrations inhibitory to the growth of *C. tyrobutyricum* [**41**]. Because it is not a brine-salted cheese, Cheddar is not very susceptible to late gas blowing.

Late gas blowing can be avoided by minimising the numbers of spores in the milk by good hygiene and avoiding feeding silage to the cows. Germination of spores and the growth of the vegetative cells may be inhibited usually by the use of nitrate or lysozyme. Spores may also be removed from the milk by bactofugation or microfiltration. In general, bactofugation, an increased level of NaCl in the cheese and a reduced ripening temperature are effective measures for reducing gas production by *Clostridium* spp.

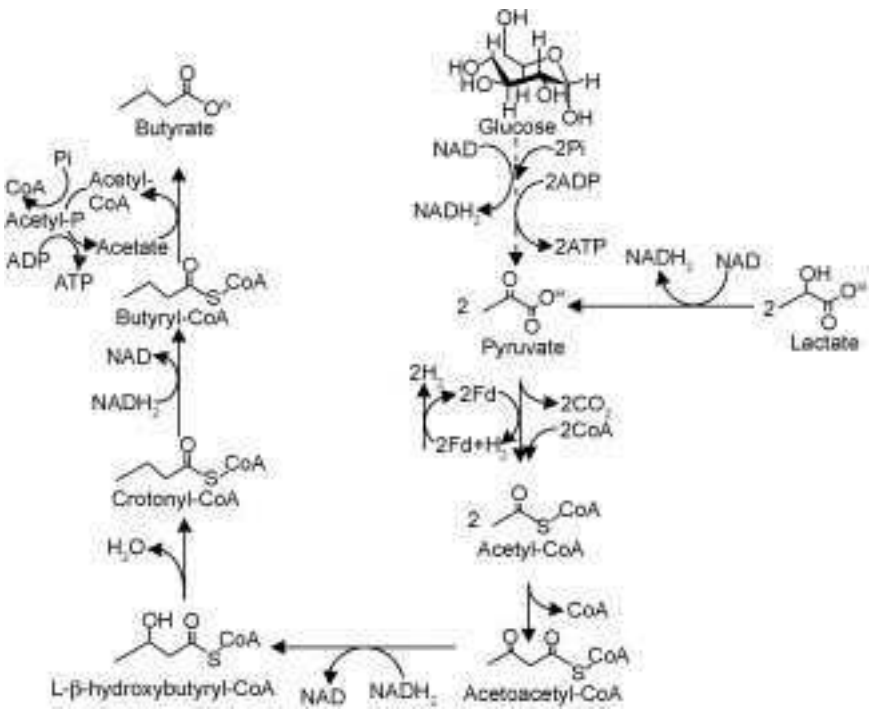

Fig. 1 Pathway for the anaerobic metabolism of lactate to butyrate, CO_2 and H_2 by *Clostridium tyrobutyricum* which causes late gas blowing (from McSweeney and Fox, 2004, with permission).

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92 What general factors affect the texture of hard and semi-hard cheeses?

J. M. Banks

The texture of a ripened hard or semi-hard cheese is determined by a number of factors. The initial composition of the milk [**2**, **3**], the rate and extent of acidification during manufacture [**17**] and the degree of heating and moisture removal during manufacture [**36**] determine the basic curd structure. This basic curd structure comprises a casein network in which fat globules and moisture are entrapped. Water is both bound to the casein and also fills the interstices of the curd matrix. Texture formation is critically influenced by the relative content of protein, fat and water in this structural network. The biochemical and physicochemical changes that occur in the structure during maturation determine the ultimate texture of the ripened cheese.

The first stage in the manufacturing process which influences cheese texture is the preparation of milk by standardisation of the casein to fat ratio [**9**]. This determines the fat-in-dry-matter content of the final cheese. The temperature of the standardised milk is adjusted and the milk is acidified using mesophilic or thermophilic cultures [**18**]. The cultures are carefully selected for the particular cheese variety. This ensures acidification proceeds at the correct rate and solubilisation of colloidal calcium phosphate [**4**] is controlled, thereby ensuring the final cheese has the correct composition. The coagulant is added to the milk and a gel is formed [**24**]. The type and quantity of coagulant are critical: the coagulation temperature, the rate of acid development and the pH of the curd at cutting will determine the coagulant activity and its retention in the curd [**28**] and hence the degree of proteolysis during ripening. Once the gel is formed, the curd is cut. The treatment of the cut curd is crucial to ensuring the desired texture is achieved. The size of the curd particles following cutting, the cook or scald temperature, curd washing in which whey is removed and water is added, the pH of the curd at whey drainage, the temperature of the curd at stretching (for Mozzarella [**146**]), the extent of cheddaring (for Cheddar [**100**]), the method of salting (dry-salting or brining) [**41**] and the amount of salt used, all influence cheese texture. The temperature and humidity at which the cheese is stored during ripening can be used to control the cheese microflora [**55**], enzymatic activity and texture formation. Proteolysis is the most important biochemical event in cheese ripening [**88**] and greatly influences the development of texture. Development of the cheese structure and texture during ripening is primarily achieved by the degradation of the paracasein complex by the proteinases of the coagulant. The duration of the ripening period will determine the extent of proteolysis. However, the softening of texture in the initial stages of ripening results from the solubilisation of colloidal calcium phosphate associated with the paracasein matrix of the cheese [**4**] rather than specific chymosin-mediated proteolysis.

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93 Cheese is weak bodied. What strategies could be adopted to produce a firmer cheese and what are the effects of each treatment?

J. M. Banks

A weak-bodied cheese has a weak casein network structure. The main causes of weak-bodied cheese are high levels of fat and moisture compared with casein levels. A weak network structure can be corrected by increasing the compactness of the curd matrix. This can be achieved primarily by reducing the fat and moisture content of the curd. Standardisation of milk [**9**] to a higher casein to fat ratio and adjustment of the cheesemaking protocol to enhance moisture loss [**34**] (Table 1) will produce substantial improvements in texture. Secondary factors to be considered are increasing the pH at whey drainage to increase calcium levels in curd [**4**, **17**] while decreasing retention of rennet [**28**]

Table 1 Adjustments to the cheesemaking protocol to improve texture of a weak-bodied cheese

Treatment	Effect	Comment
Standardise milk [**9**] to a higher casein to fat ratio	Lowers the FDM in the cheese Creates a more compact casein network structure	Adjust cheesemaking process to maintain MNFS, S/M and pH
Add calcium chloride to milk [**33**]	Improved gel formation and syneresis of curd	Excess use of calcium chloride will give rise to bitterness during ripening
Increase the amount of starter culture [**18**] or prolong the ripening period	Slight improvement in whey expulsion	
Cut the coagulum into smaller cubes	Improved whey expulsion	May increase losses of fat and fines losses
Increase the cooking temperature	Improved whey expulsion	
Increase the stirring time	Improved whey expulsion	
Increase the pH at whey drainage	Reduces the retention of chymosin and plasmin and increases calcium in curd	Reduced casein degradation during ripening
Decrease curd size on milling	Improves whey expulsion	
Increase salt addition at milling	Improves whey expulsion	S/M levels critical controls the activity of residual rennet and plasmin in cheese
Decrease the ripening temperature	Increases the amount of intact casein	Reduced rate of flavour development possible

FDM = fat-in-dry-matter; MNFS = moisture-in-non-fat-solids; S/M = salt-in-moisture.

and plasmin and increasing salt-in-moisture levels and decreasing ripening temperatures. The salt-in-moisture level in the curd and the ripening temperature control the activity of residual rennet and plasmin in cheese. Increasing the salt-in-moisture level and lowering the ripening temperature can reduce proteolysis during maturation [**88**] and result in higher levels of intact casein in cheese which will produce a firmer cheese. Table 1 describes a number of approaches to improve the texture of a weak-bodied cheese.

94 What strategies should be adopted and what are the effects of each treatment to obtain a less acid Cheddar cheese?

J. M. Banks

Acid flavour is a component of the overall sensory profile of a Cheddar [**79**]. An excess or imbalance of acid taste may be regarded as a quality defect. The formation and sensory perception of acid flavours are complex issues which remain poorly understood. To produce a less acid cheese, the activity of the starter [**17**, **18**] must be reduced during cheesemaking. This is effectively achieved by reducing the level of starter culture added and adjusting temperatures [**37**] and cheesemaking protocol. Cheeses develop acidity during production as lactose is fermented by the starter cultures. When lactose is depleted within 48 h of manufacture, the acidity of Cheddar decreases slightly as the cheese matures. Data from manufacturers indicate that cheeses produced using the same recipes at different manufacturing sites can differ markedly in acidic taste. This implies that additional factors play a role in the acid flavour of cheese. Such factors might include the isomeric forms of lactic acid (D or L) [**107**], the presence of other acids (acetic, citric, fatty acids, amino acids), pH (dissociation of acids), buffering capacity [**22**], salt level, fat content, degree of proteolysis [**88**] and cheese texture.

Development of effective strategies to control acid flavour in cheese requires understanding of not only the technological parameters that contribute to development of acidity but also other factors that influence the sensory perception of acid flavours. The mechanism of perception of acid flavours in cheese is complex and only partially understood. The cheese matrix contains many components that contribute to the flavour profile and potentially affect the perception of acidity. In a recent survey of the sensory character of retail Cheddars in the UK, interactive effects were noted in perception of acid and creamy flavours (Fig. 1). This effect was consistent in mild, mature, vintage Cheddars and half-fat Cheddars. Acid flavours were lowest in the mild category cheeses and had a tendency to increase with maturity but there was much variability in perception of acid flavours in each category.

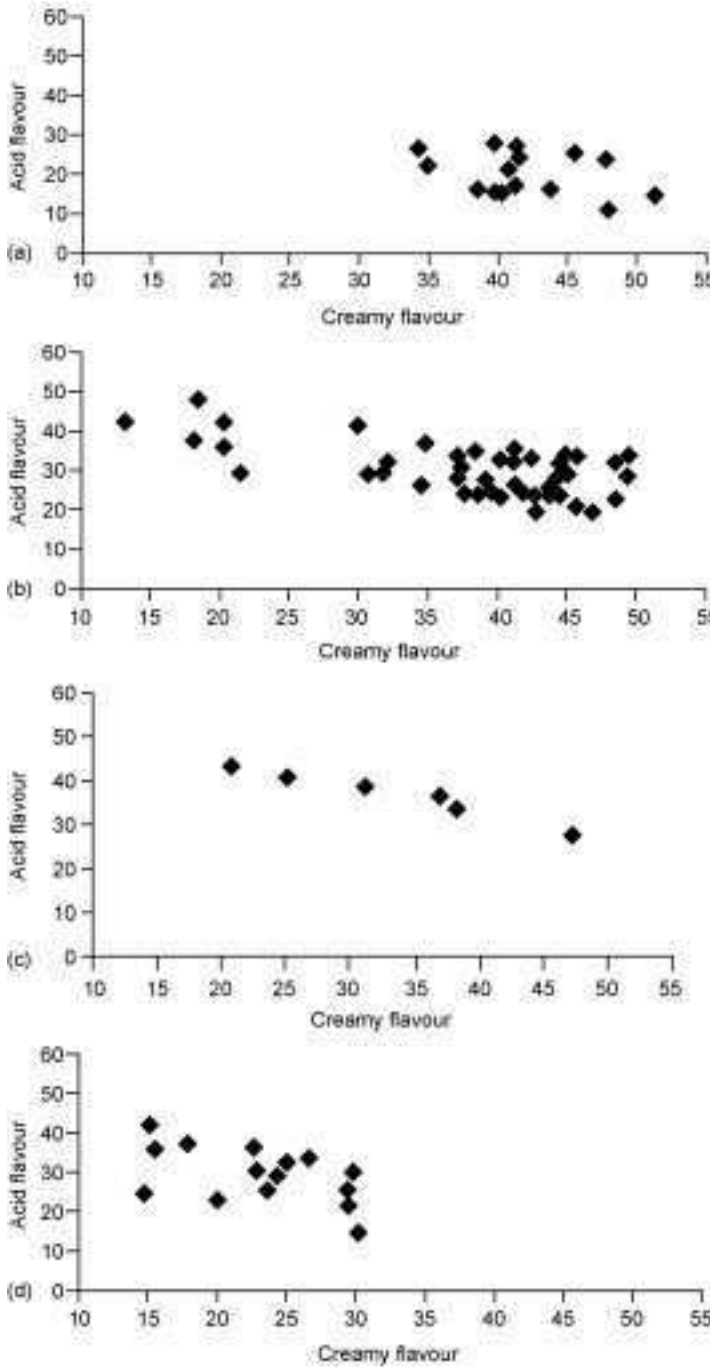

Fig. 1 Perception of acid and creamy flavours in (a) mild Cheddar, (b) mature Cheddar, (c) vintage Cheddar, and (d) half-fat Cheddar cheeses.

95 What strategies can be adopted to soften the texture of a hard cheese?

J. M. Banks

The major structure-forming constituent in cheese is the casein matrix in which fat globules are entrapped. Water or serum is both bound to the casein micelles and fills the interstices of the matrix. The network structure is influenced by the relative content of protein, fat and moisture, as well as the biochemical activities that occur during maturation. An overfirm cheese has an excessively compact casein network structure which is generally associated with reduced fat and moisture levels in curd.

Increasing the fat and water content of the matrix opens up the protein structure and softens the texture. Strategies for adjusting fat and moisture levels are shown in Table 1. Removing fat has a greater effect on texture than removing moisture. In low-fat Cheddar, the casein matrix is extremely compact and the texture is overfirm even when the moisture content is substantially

Table 1 Process modifications to soften the texture of an overfirm cheese

Treatment	Effect	Comment
Standardise milk to a lower casein-to-fat ratio [**9**]	Increases the FDM in the cheese Creates a more open casein network structure	Adjust the cheesemaking process to enhance MNFS
Decrease the amount of starter culture [**18**] or shorten the ripening period	Slight reduction in whey expulsion	
Cut the coagulum into larger cubes	Reduction in whey expulsion	May reduce losses of fat and fines
Decrease the cooking temperature [**37**]	Reduction in whey expulsion	
Decrease the stirring time	Reduction in whey expulsion	
Decrease the pH at whey drainage	Increase the retention of chymosin and plasmin in curd Calcium retention in curd decreased	Increased casein degradation during ripening, giving softer texture
Increase curd size on milling	Reduction in whey expulsion	
Decrease salt addition at milling	Reduction in whey expulsion	S/M levels critical. Controls the activity of residual rennet and plasmin in cheese
Increase the ripening temperature	Enhanced degradation of casein [**88**]	Off-flavours if relative rates of proteinase and peptidase activity unbalanced [**89**]

FDM = fat-in-dry-matter; MNFS = moisture-in-non-fat-solids; S/M = salt-in-moisture.

increased [**106**]. To produce a softer cheese, the casein to fat ratio in milk for manufacture should be decreased to increase the fat-in-dry-matter level in the curd. Cheesemaking parameters should then be adjusted to enhance moisture retention. Increasing the proportion of unsaturated fats in cheese would also result in a softer cheese. The addition of water binders, e.g. denatured whey proteins, generally improves the texture of low-fat cheeses.

To enhance proteolysis during ripening so that casein matrix is broken down and texture weakened, the cheesemaker should decrease the pH at which the whey is drained from the curd. This decreases the calcium levels in cheese and increases the residual levels of chymosin and plasmin [**4**, **28**]. The salt-in-moisture level should be reduced and the maturation temperature increased to accelerate proteolysis.

Grana-type cheeses and Parmesan

96 Introduction

P. L. H. McSweeney

The hardest cheeses are the Italian Grana varieties and their industrial counterpart, Parmesan. The hard grainy texture of Italian Grana-type cheeses (e.g. Parmigiano-Reggiano or Grana Padano) results from the use of raw semi-skimmed milk for their manufacture and a high cooking temperature (~54 ºC) [**36**] and evaporation of moisture during their long ripening period (often 2 years or more). Grana-type cheeses are sometimes consumed as a table cheese when they are young and relatively soft but the mature product is often used grated as a condiment on pasta or other dishes.

In addition to these traditional Italian cheeses with controlled designations of origin [**82**], Parmesan-type cheeses are made worldwide. Parmesan-type cheeses are often smaller than traditional Italian Grana-type varieties and are made from pasteurised milk and cooked to a lower temperature. They are more heavily salted, are ripened for shorter periods and often are made using exogenous lipases, which gives them a strong lipolysed flavour.

97 What causes the traditional grainy texture of Italian Grana-type cheeses?

M. Gobbetti

Parmigiano Reggiano and Grana Padano are known as 'Grana' cheeses because of the grainy texture of the ripened cheese. As indicated in the protocols of manufacture, the cheese structures of Parmigiano Reggiano and Grana Padano are defined as 'fine, brittle granules' and 'fine, grainy and radially fracturing into slivers', respectively.

Especially in the past, a limited role has been attributed to obligately heterofermentative lactic acid bacteria and gas-producing indigenous microorganisms which were considered responsible for the formation of micro-holes, just visible, owing to the synthesis of mainly CO_2. Micro-holes may interfere with the grainy texture. Nevertheless, heterofermentative lactic acid bacteria (e.g. *Lactobacillus fermentum*) are contained in the natural whey starter [**18**] in a ratio of *ca*. 1:10 or higher with obligately homofermentative strains (e.g. *Lactobacillus helveticus*), and the milk currently used for the manufacture of the 'Grana' cheeses has a lower number of indigenous microorganisms than in the past.

The major cause of the grainy texture is associated with the technology of manufacture. Indeed, the grainy texture is associated with the use of semi-skimmed milk and extensive syneresis, which, in turn, is related to the size of the curd after cutting (dimensions of wheat grains, *ca*. 2–4 mm) and to the cooking temperature (54.5–56 ºC), which are typical features of these extra-hard varieties of cheese [**34**, **35**, **36**]. During cooking, curd grains are stirred vigorously in moderately acidic (pH 6.2–6.3) whey. Under these conditions, curd grains undergo a very extensive syneresis. At the end of cooking, they become wrinkled, rough and very poorly cohesive; thus the curd grains tend to retain their individuality both on the bottom of the tank when they were pressed against each other and later in the mature cheese, which effectively determines its grainy texture.

The method of cutting a 'Grana' cheese is also important in maintaining its grainy texture. Considering the way by which a wheel of Parmigiano Reggiano cheese is cut in half before its sale, the most important factor is the particular and original tool used. It is a characteristic knife with a short, pointed and almond-shaped blade. One side is thinner to aid penetration while the other side is thicker because it must act as a wedge. Indeed, a wheel of 'Grana' cheese is not cut, but rather 'opened' so that its internal structure and its grainy texture are not altered.

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98 What common problems are associated with Grana-type cheeses?

M. Gobbetti and R. Di Cagno

The major problem associated with 'Grana' cheeses (Parmigiano Reggiano and Grana Padano) is gas production which is of microbiological origin due to the prolonged ripening (20–24 and 14–16 months, respectively) typical of these cheese varieties. Fermentation by clostridia [**57**, **91**] and, occasionally, by propionic acid bacteria, may be the cause of late blowing.

During ripening spore-forming clostridia are responsible for the butyric acid fermentation usually starting from lactic acid as follows:

$$2CH_3CHOHCOOH \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$$

Three major species of clostridia have been found as agents of the late blowing due to the excessive production of CO_2 and H_2. *Clostridium butyricum* usually grows during the early ripening when lactose is still available as a carbon source and, especially, when the acidifying activity of lactic acid bacteria from the natural whey starter [**18**] is weak. *Clostridium tyrobutyricum* has the capacity to grow in 1-year-old cheese curd, especially when the pH becomes favourable owing to the utilisation of lactic acid and lactate as carbon source. *Clostridium sporogenes* grows after a year of ripening, it is very proteolytic and through the Stickland reaction uses free amino acids as carbon sources. When the butyric fermentation is intense, especially in the presence of *Cl. sporogenes*, off-flavours may be associated with late blowing. An example of late blowing by *Cl. tyrobutyricum* is shown in Fig. 1.

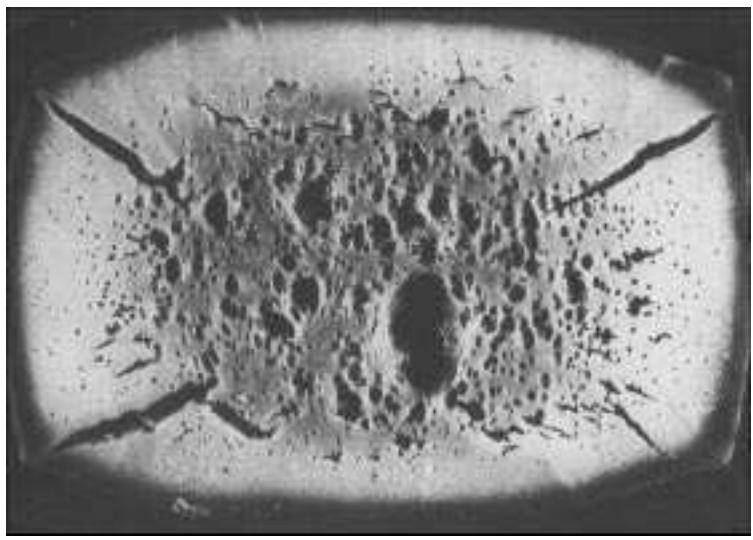

Fig. 1 Section of 'Grana' cheese: 'late blowing' caused by growth of *Clostridium tyrobutyricum* (from Bottazzi, 1993).

Propionic acid bacteria convert lactic acid into propionic acid, acetic acid, CO_2 and H_2O usually as follows:

$$3CH_3CHOHCOOH \rightarrow 2CH_3CH_2COOH + CH_3COOH + CO_2 + H_2O$$

Propionic acid bacteria may grow only occasionally when the curd acidification has been weak and when the cheese has been salted to low levels.

Undesirable fermentations are usually prevented by several practices. The use of silage as fodder for cows is not allowed for the production of Parmigiano Reggiano cheese since it may be a vehicle for spore-forming bacteria, while the addition of lysozyme ($2\,g\,hl^{-1}$) into the cheesemilk is allowed for the manufacture of Grana-Padano cheese as anti-clostridial agent, especially against *Cl. tyrobutyricum*. Overall, high hygiene at milking, the use of natural whey starters with high acidifying capacity and an appropriate, and rapid, salting are considered good practices to inhibit undesirable fermentations.

Other problems that may occur less frequently include: (i) poor gratability of 'Grana' cheeses due to a ratio of fat to caseins of ≥ 1 which is usual when skimming of cheesemilk is not optimal; (ii) off-flavours with a tendency to be bitter or very pronounced when the proteolytic activities of the natural whey starters are not correctly balanced and/or when the enzyme activities of the wild microflora prevail [**91**]; and (iii) excessive concentration of sulphur compounds (e.g. mercaptans), especially when microorganisms such as coryneform bacteria unusually prevail.

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99 How do traditional Italian Grana-type cheeses and industrial 'Parmesan' differ?

M. Gobbetti and R. Di Cagno

Parmigiano Reggiano and Grana Padano are 'Grana'-type extra-hard cheese varieties produced with Protected Designations of Origin (PDO) [**82**]. Unlike commercial trademarks, a PDO reflects a collective heritage and may be used by all producers of a particular variety in a defined geographical area. Protection derives its concept from using cheesemilk from a defined locality and applying technology under strict and traditional conditions which are not easily reproducible under a different geographical area. 'Grana'-type cheeses use cheesemilk produced from farms in a restricted area of the Pianura Padana, northern Italy. Milk from different areas is used for Parmigiano Reggiano and Grana Padano.

Feeding of the cows is carefully regulated. For the manufacture of Parmigiano Reggiano cheese feeding is subjected to the following restrictions: (i) the ratio between forage and other feeds must be $\geq$1; (ii) $\geq$25% of the dry matter (DM) of the forage used must be produced on the same farm where the cheese is manufactured; (iii) $\geq$75% of the DM of the forage used must be produced within the district where the cheese is legally produced; and (iv) the feeding of silage is not allowed, to minimise the number of spore-forming, gas-producing bacteria in the feed [**91**]. Besides, raw milk is used for the manufacture of 'Grana'-type cheeses which are made by unique technology which respects artisanal protocols. The major features are: (i) the use of partially skimmed milk after overnight gravity creaming at *ca*. 20 ºC in special tanks, 'bacinelle' (capacity 10–50 hl), which contain a shallow body of milk; (ii) the use of natural whey starter cultures [**18**] that contain strongly acidifying and synergistic thermophilic strains of lactic acid bacteria which are propagated following a typical procedure; (iii) the coagulation of milk in special copper tanks (*ca*. 10 hl) which have a peculiar shape and contain milk for the manufacture of two cheeses; (iv) the cooking of the curd grains to 54.5–56 ºC which favour the typical grainy texture; and (v) the long time of ripening (12–16 months for Grana Padano and 18–24 months for Parmigiano Reggiano).

As recently stated by the Codex Alimentarius (July 2005), the term 'Parmesan' has just to indicate the English translation of the Italian Parmigiano Reggiano cheese. Indeed, 'Parmesan' cheeses, currently present in the market, are manufactured with: (i) milk produced from any farms which is subjected to skimming by centrifugation and to thermisation or pasteurisation; (ii) selected commercial cultures of lactic acid bacteria; and (iii) shorter time of ripening compared with the 'Grana'-type cheeses. In addition, industrial 'Parmesan'-type cheeses often have higher moisture than Italian Grana-type cheeses and may be manufactured using lipases to accelerate flavour development.

The above technological features are such that 'Grana' cheeses cannot be manufactured successfully under conditions which are different from those regulated by the protocols approved by the Consortia (http://www.parmigiano-

reggiano.it and http://www.granapadano.com) of Parmigiano Reggiano and Grana Padano cheeses.

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Cheddar cheese

100 Introduction

P. L. H. McSweeney

Cheddar is a hard cheese that originated from the village of the same name in the south-west of Britain and is now among the most important cheeses made worldwide, particularly in English-speaking countries. Cheddar cheese is made from pasteurised [**10**, **11**], standardised [**9**] cow's milk (Fig. 1). Mesophilic starter cultures [**18**] are used and, in large cheese factories, defined-strain starter systems are common. The milk is renneted at ~30 ºC and the curds/whey mixture is cooked to 37–39 ºC. After whey drainage, the curds are 'cheddared'. Traditionally, cheddaring involved the repeated piling and repiling of curds which fused together and were cut into blocks. During cheddaring, starter activity continues to reduce the pH, solubilises colloidal calcium phosphate [**4**] and causes physicochemical changes to the curd which changes from being soft and friable to being tough and pliable.

Modern continuous cheddaring systems have dispensed with this traditional piling and repiling of curd blocks and simply provide time for acidification of the curd as it passes through a tower or belt system. When sufficient acidity has developed (~pH 5.4), the curd is milled (cut) into small pieces and dry-salted [**41**]. Dry-salting causes a rapid increase in the salt-in-moisture content of the curd and starter activity stops abruptly. After salting, the curd pieces are pressed into the form of a block before ripening at 6–8 ºC for ~4 months to >2 years depending on the degree of maturity desired.

Cheddar is an internal, bacterially ripened variety [**83**] and its maturation is characterised by the rapid decline in starter activity and the growth of non-starter lactic acid bacteria [**56**]. Metabolism of lactate is usually restricted to

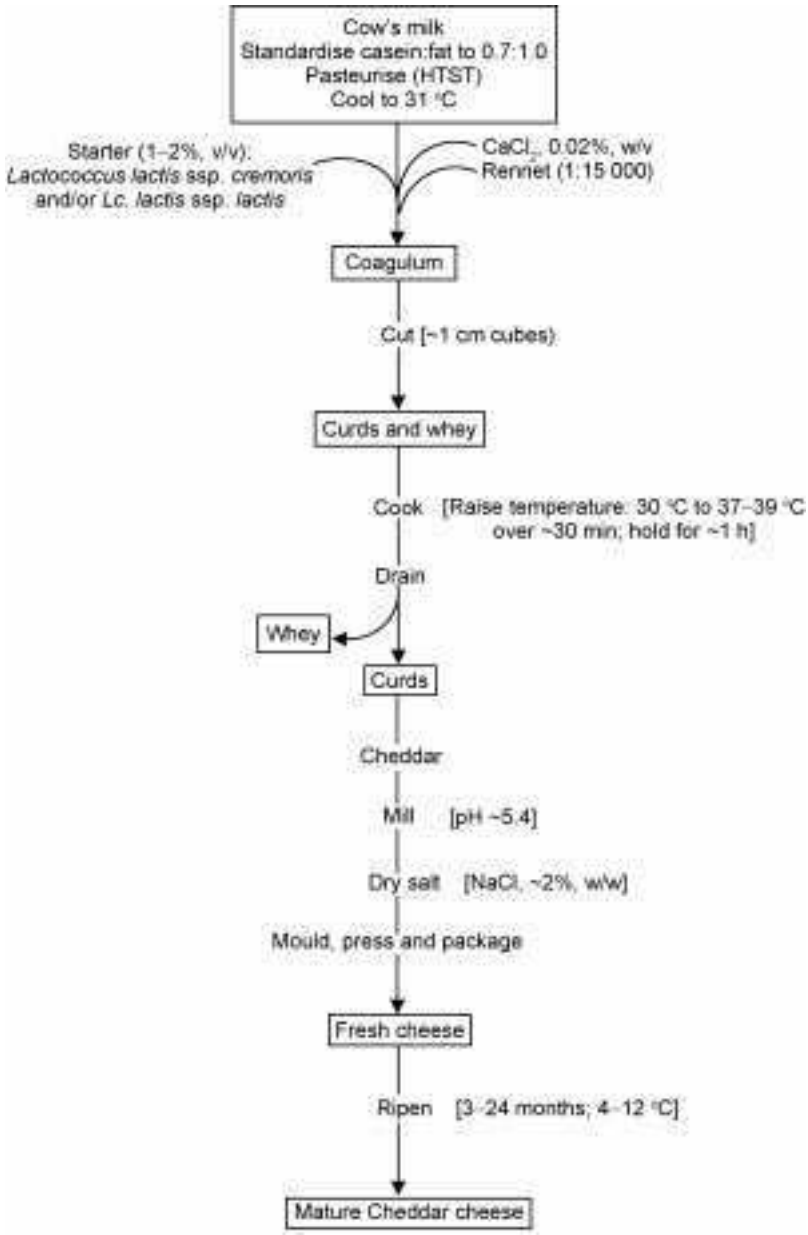

Fig. 1 Flow diagram for the manufacture of Cheddar cheese (modified from Fox and McSweeney, 2004).

racemisation, and citrate metabolism can cause undesirable openness in the cheese. Proteolysis and amino acid catabolism are important to flavour, while lipolysis is usually limited [**88**].

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101 What is cheddaring and what physicochemical changes occur during this process?

J. M. Banks

In traditional Cheddar manufacture [**100**] the curd granules settle at the base of the vat following pitching or whey removal. Under the influence of heat, acid and gravity, the particles matt together and form a solid mass of curd. The cheddaring step in traditional manufacture is characterised by cutting the matted curd into slabs, and piling or stacking the slabs to apply pressure in a controlled and incremental manner over an extended period (60–90 min). During this time the development of acidity is rapid. The combined effects of pressure and acidification lead to the fusion of granular curd particles and the formation of a fibrous structure, not unlike that of cooked chicken breast meat, which is typical of cheddared curd. As the curd is stacked, the pressure on the lower blocks encourages them to flow and develop the requisite fibrous structure. The arrangement of the fibres formed follows the direction of the flow of the curd. The fibrous structure cannot be formed by pressure and deformation unless the pH is less than 5.8. The warmer the curd and the higher its moisture content, the more readily it flows and the fibres become finer, longer and more dense. Curd structure can be influenced by manipulating pH, pressure and temperature, and a direct relationship exists between the structure and the water-holding capacity of the curd.

Cheddaring encourages a number of physicochemical changes which result in curd flow and texturisation. These include solubilisation of micellar calcium which is bound to the casein and acts as a cementing agent between the casein micelles/submicelles. A decrease in the concentration of micellar calcium results in an increase in the ratio of soluble to casein-bound calcium. As the pH decreases from 6.15 to 5.2, the soluble calcium, as a percentage of total calcium in the curd [**4**], increases from approximately 5 to 40%. The hydration of para-casein increases with decreasing pH. The precise mechanisms are unknown, but the loss of calcium phosphate will destabilise the casein micelles, resulting in a change in the conformation of the caseins. As a consequence of the decrease in casein-bound calcium and the increase in casein hydration, the viscoelastic casein matrix, containing liquid fat and moisture phases, has a tendency to flow if unrestricted. The flow effect is enhanced when the curd is piled and pressed under its own weight. The flow of the curd gives the desired planar orientation of the strands of the paracasein network. The physicochemical changes in curd during traditional cheddaring are summarised in Fig. 1. There is, however, little scientific evidence to support the necessity of the traditional cheddaring process for texture development in Cheddar produced commercially in modern mechanised factories.

Mechanised systems have been developed to replace the labour-intensive traditional cheddaring operation. Conveyor belt systems which encourage stretch, flow and inversion of the curd mass, as well as ensuring the required holding time for acid development, mimic the traditional cheddaring process.

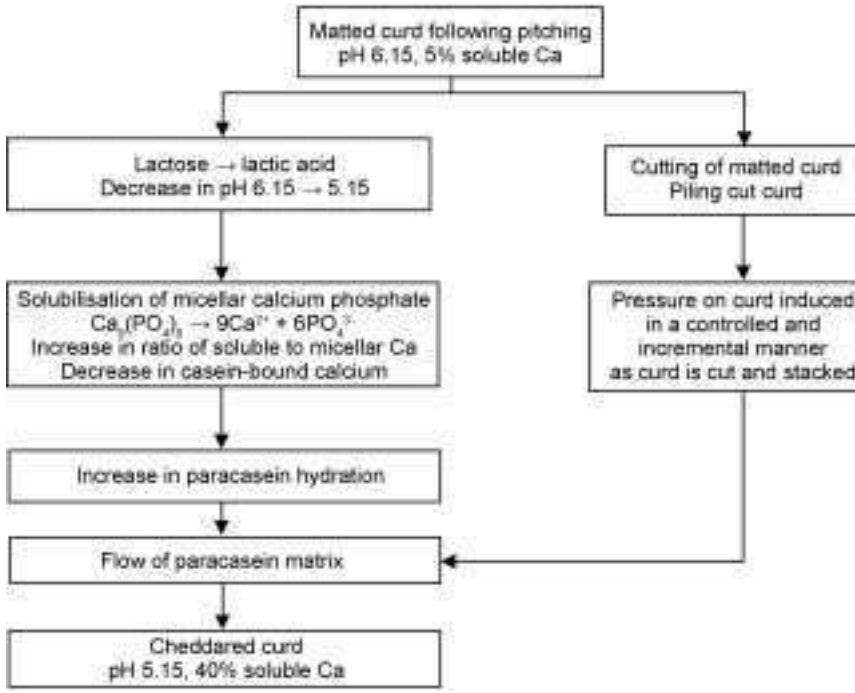

Fig. 1 Physiochemical changes in cheese curd during cheddaring (adapted from Fox *et al.*, 2000).

Curd may also be transferred to a cheddaring tower, approximately 10 m high, and in this system curd flow is restricted. Curd flow in modern mechanised systems is low in comparison with that achieved by traditional methods, yet the texture of the cheese produced is acceptable. In the manufacture of stirred curd Cheddar, the matting and flow of curd is prevented. Since the texture of Cheddar cheese produced by a stirred curd method is adequate, this suggests that curd flow during cheddaring is not essential, and the cheddaring process serves only to ensure the desired level of acid development and syneresis is achieved.

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102 What are the mechanical and slit openings in Cheddar and how may they be avoided?

J. M. Banks

Mechanical and slit openings are texture defects which are associated with traditionally made Cheddar [**100**] rather than cheese manufactured in modern mechanised plants. However, these defects do occasionally occur in cheese produced using modern equipment and, if prevalent, they can cause significant losses in the prepackaging of Cheddar.

Mechanical openness is characterised by irregular shaped holes in the cheese. This defect is not seen in fresh curd manufactured with blockformers but can appear in the early stages of Cheddar ripening. Slit openness is also usually absent in freshly made cheese but develops during maturation. 'Fractured texture' is an extreme manifestation of slit openness and is a defect only of mature cheese. The term 'fracture' is normally used to describe long slits, i.e. longer than 3.5 cm. Excessive fractures can result in the break up of cheese during prepackaging and cause significant losses. It is interesting to note that mechanically open cheese is almost always free from fractures and, conversely, badly fractured cheese usually has few mechanical openings.

The basic mechanism for the formation of these defects in Cheddar cheese is thought to be dependent on either entrapped air spaces in the cheese structure or gas production by adventitious microorganisms. The incidence of these texture defects in modern cheese manufacture is low owing to the use of high pressures during pressing, the introduction of vacuum pressing, and the use of defined single strain cultures [**18**] from which gas-producing strains have been omitted. Production of carbon dioxide by citrate-fermenting heterofermentative non-starter lactic acid bacteria [**56**] during ripening has been associated with the formation of slit openness. Rapid cooling of the curd after moulding and pressing is the most effective way of retarding the growth of adventitious gas-forming non-starter lactic acid bacteria.

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103 Why do salted Cheddar curd pieces not fuse together properly?

J. M. Banks

Inadequate fusion of curd particles during pressing is generally associated with changes in protein and mineral balance on curd surfaces following dry salting [**41**]. Dry salting of milled Cheddar curd particles results in the development of discrete boundaries between individual curd particles. Addition of dry salt causes shrinkage of curd and rapid release of whey containing calcium and phosphate [**43**]. Calcium and phosphate become concentrated at the surface of the curd particles. In extreme cases, the deposition of calcium phosphate crystals results in development of seaminess in Cheddar cheese, a condition in which the junctions of the milled curd are visible after pressing. Seaminess is most frequent and most obvious in cheese that has a low moisture and high salt content, and in some cases persists after the cheese has matured. The presence of seaminess weakens the binding between curd particles owing to incomplete fusion. This can lead to crumbling of cheese when the cheese is sliced or cut into small blocks for packing.

Seaminess in Cheddar results from the formation of crystals of calcium orthophosphate dihydrate, which become concentrated on the surface of milled curd particles to which salt has been applied. Severe dehydration at the surface of these particles occurs on dry salting. Seaminess can be reduced by washing the curd following milling and prior to salting. This removes calcium and phosphate from the surface layer, and consequently the dehydration effect of salt on the surface layer is lessened. Seaminess and poor fusion of curds occur together and washing with warm water as described corrects both defects.

Poor fusion of the curd as a consequence of heavy salting results from changes in the proteins on the surface, from poor contact between the hardened surfaces, from the physical separation brought about by the presence of salt crystals, and when these have dissolved and diffused into the curd matrix, from the growth of calcium orthophosphate crystals. Fusion of curd particles can be improved by increasing the pH, temperature and moisture content of the curd.

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104 Why is it important to control the composition of Cheddar cheese to ensure high quality?

J. M. Banks

The relationship between the composition of cheese and its quality is well established. Cheeses that are manufactured within defined specifications of moisture, moisture-in-non-fat-solids (MNFS: a measure of the ratio of moisture to casein), salt-in-moisture (S/M) and pH have the potential to develop into premium quality Cheddars. These compositional parameters form the basis of compositional grading schemes used in the Cheddar industry. For premium quality Cheddar the MNFS should be 52–54%, the S/M 4.2–5.2%, and the pH 4.95–5.15. Cheeses falling outside these specifications will probably be of poorer quality and will be downgraded; however, producing cheese within these specifications does not always ensure that quality cheese is produced. If the initial milk quality is poor, manufacturing to defined compositional parameters will not guarantee production of premium Cheddar.

A critical factor in the control of Cheddar cheese quality is consistency in the rate and extent of acid production in the vat by the starter culture [**17**, **18**]. The pH at which the whey is drained from the curd determines the mineral content and the amount of chymosin retained in the curd [**28**] and also impacts on the final pH and moisture content of the curd. Approximately 6% of chymosin added to Cheddar cheesemilk is retained in the curd, but if the pH of whey at drainage decreases, chymosin retention increases. Chymosin plays a major role in the degradation of the caseins during ripening [**88**] and in the consequent development of characteristic cheese flavour and texture.

The extent of degradation of casein and the ratio of moisture to casein in the curd is critical to optimising quality. Since most of the laboratories situated in cheese factories are not suitably equipped for estimation of casein in cheese, it is common practice to measure the fat and moisture level in the cheese and from this calculate the solids-not-fat. Since approximately 85% of the solids-not-fat comprises casein, the moisture to casein ratio is related to the MNFS and this parameter provides a better index of cheese quality than does moisture content alone.

The MNFS is greatly influenced by the level of FDM in the cheese. The FDM is subject to substantial variation due to seasonal changes in the milk composition [**3**]. This variability is overcome by standardisation of the casein to fat ratio of milk throughout the year, and this also provides an effective means of controlling the MNFS.

The level of MNFS determines the rate at which a cheese will ripen, and in producing cheese intended to mature rapidly or cheese which will undergo an extended maturation, the cheeesemaker will adjust the MNFS to produce optimum quality at maturity. For a Cheddar cheese that will be matured for 6–7 months, the MNFS will be about 53%. For a Cheddar that is to be matured for only 3–4 months, the MNFS would be increased to about 56%. The higher the MNFS, the greater is the rate of casein degradation and the more rapidly the quality of the cheese will deteriorate after it reaches optimum maturity.

S/M influences the quality of Cheddar by controlling the final pH of the cheese, the growth of microorganisms, specifically the starter bacteria and undesirable species such as coliforms, staphylococci [**58**] and clostridia [**91**], and the overall flavour and texture of the cheese.

The level of S/M controls the rate of proteolysis of the caseins by rennet. The rate of proteolysis decreases with an increase in salt concentration, and as the S/M concentration increases the development of bitterness decreases. At S/M levels greater than 5%, bitter flavours are rarely encountered, but below this threshold value there is an inverse linear relationship between S/M. The incidence of bitterness due to an excess of coagulant activity on β-casein in relation to peptidase activity [**23**]. At S/M values less than 4% Cheddar tends to develop gas and sulphide flavours after it has reached maturity. The final pH of cheese is critical in determining the rate and extent of proteolytic activity during ripening.

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105 Why does cheese develop a pink discoloration?

P. L. H. McSweeney

Pink discoloration in cheese may result from a number of factors and it is important to differentiate between the defect observed in cheeses made using annatto as a colourant [**14**] and cheeses made without annatto.

Pink discoloration of cheeses coloured with annatto may occur during exposure to high-intensity fluorescent lighting in retail display cabinets. This defect is caused by photo-oxidation of lipids and is affected by intensity of the light, storage temperature, exposure time, type of colouring agent and cheese pH (Hong *et al.*, 1995a,b). Pelaez and Northolt (1988) described the development of pink discoloration at the surface of Gouda cheese [**108**] made with nitrate to prevent late gas blowing and coated with a plastic emulsion containing annatto. These authors thought that the problem was caused by the presence of bacteria with a high nitrate-reducing capacity leading to a high concentration of NO_2^- in the rind, which together with annatto and an increase in pH lead to pink discoloration. Pink discoloration in processed cheese [**189**] appears to be favoured by the use of annatto containing high levels of norbixin, high heat during processing, the use of aged cheese which has undergone extensive proteolysis as the base and the use of certain emulsifying salts, particularly phosphates.

Pink discoloration in natural cheeses not containing annatto appears to be mainly due to their microflora. Pink discoloration which occurred in patches on and near (1–2 cm) the surface of New Zealand Cheddar cheese made without the use of annatto was described by Martley and Michel (2001); this pink colour faded quickly (12–24 h) when the cheese was exposed to air. These authors ascribed the development of the pink colour to products of the Maillard reaction caused by the presence in the cheese of galactose which accumulated due to the metabolism of *Streptococcus thermophilus* used as a component of the starter, together with levels of low molecular mass nitrogenous compounds formed by proteolysis [**88**] (peptides, free amino acids) and the establishment of a critical oxygen concentration reflecting the oxygen permeability of the packaging material. This problem appears to be similar to the brownish-pink or 'pink ring' defects sometimes seen in Swiss and hard Italian cheeses, respectively. These cheeses are made using a thermophilic starter containing *S. thermophilus* and in the absence of a galactose-positive *Lactobacillus*, galactose may accumulate and a pink ring may form a certain distance from the rind reflecting a critical oxygen concentration. In a study on pink discoloration of Romano cheese, Shannon *et al.* (1969) found the defect to be associated with certain strains of *Lactobacillus* used as components of the starter and cheeses with the defect appeared to have a higher oxidation–reduction potential than controls. Pink discoloration has also been associated with the presence of yeasts or enterococci in cheese (Forge *et al.*, 1977; Dincheva, 1979; Carini *et al*., 1979).

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106 What factors lead to texture defects in low-fat/reduced-fat Cheddar cheese?

T. P. Guinee

Texture may be defined as a composite sensory attribute resulting from a combination of physical properties or mechanical properties that are perceived through the senses of sight, hearing and touch (including kinaesthesis and mouthfeel). For many cheeses, including Cheddar [**100**], the mechanical properties that are perceived by touch and sensed as forces on the teeth, tongue and mouth during biting, chewing and mastication are the major determinants of texture. Because sensory analysis of cheese is expensive and time consuming, the texture of cheese is often measured indirectly using rheological analysis. This analysis frequently involves subjecting a piece of cheese to compression between two parallel plates under defined conditions designed to simulate the compression of cheese between the molar teeth during mastication. Several rheological parameters may be obtained from the resultant stress/strain curve, such as fracture stress (a measure of the force required to break the cheese), fracture strain (the distance to which the cheese must be compressed or deformed to break), firmness (the force required to compress the cheese by a fixed amount), and others (gumminess, cohesiveness, chewiness).

Reducing fat content has marked effects on the texture of Cheddar and other cheeses (Fig. 1). Generally, the cheese becomes increasingly harder, tougher, chewier and more rubbery as the fat content is reduced, with the extent of these increases being influenced by alterations of the cheesemaking procedure from that used for the standard full-fat Cheddar. These increases generally coincide with decreases in the sensory acceptance of the cheese by the consumer and may be therefore considered as defects.

The adverse effects of fat reduction on texture and rheology are largely due to the increase in the concentration of protein and the concomitant increase in the volume fraction and strength of the protein matrix that forms the structural framework of the cheese. On deforming a cheese between the molar teeth or between the plates of a texture analyser, an increase in the volume fraction of the protein matrix coincides with an increase in the number of stress-bearing strands of the matrix and therefore in the force required to deform or facture the matrix. Moreover, the reduction in fat content per se probably decreases the lubricating effect that liquid fat normally imparts to the surfaces of contiguous layers/planes of protein matrix during displacement.

Approaches used to improve the quality of reduced-fat cheese include:

- alterations to the cheesemaking procedure to reduce the calcium-to-casein ratio [**4**], increase the moisture-to-protein ratio [**34**] and reduce the extent of paracasein aggregation, e.g. by high pasteurisation temperature [**11**, **12**], high-pressure treatment of milk [**53**], reducing the pH at setting or whey drainage, and/or increasing gel firmness at cutting;

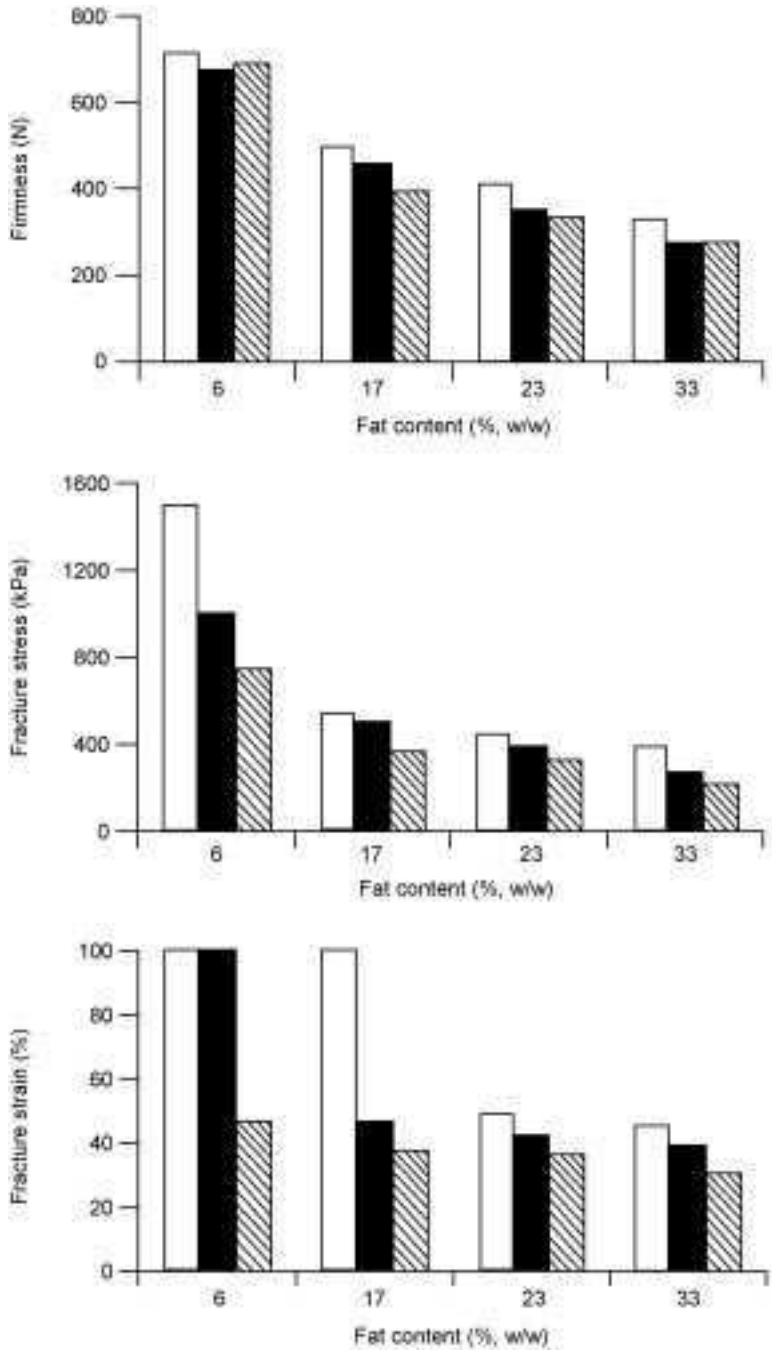

Fig. 1 Effect of fat content on the rheological properties of Cheddar cheese after ripening at 8 ºC for 60 days (□), 120 days (■) and 225 days (▨).

- the use of specialised starter cultures and starter culture adjuncts [**18**], and/or exogenous enzymes;
- and/or the addition of fat mimetics to the milk.

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107 What factors favour the development of calcium lactate crystals in cheese?

P. L. H. McSweeney

The amino acid tyrosine has low solubility and can crystallise in mature cheese, and development of crystals is sometimes a problem during the manufacture of processed cheese [**195**]. However, small calcium lactate crystals are commonly found in mature Cheddar [**100**] and similar varieties.

Ca-lactate crystals are visible on cut surfaces of cheese as small white specks and are often appreciated by connoisseurs of Cheddar as being indicative of a strong-flavoured cheese. Although Ca-lactate crystals are harmless, many consumers mistake them for foreign bodies or reject cheeses containing them as being mouldy; hence, they are usually considered a problem. Crystals form when Ca^{2+} and lactate ions exceed their solubility, supersaturate the serum phase of the cheese and then crystallise at nucleation sites. Nucleation centres include bacterial cells, microcrystals of calcium phosphate or undissolved $CaCO_3$ and a continuous migration of Ca^{2+} and lactate ions to the nucleation site causes the crystals to grow:

$$Ca^{2+} + 2\ \text{lactate}^{-} \leftrightarrow \underset{\text{(soluble)}}{\text{Ca lactate}_2} \leftrightarrow \underset{\text{(nucleated crystals)}}{\text{Ca lactate}_2} \leftrightarrow \underset{\text{(macroscopic crystals)}}{\text{Ca lactate}_2}$$

Most cheese starter bacteria ferment lactose to L(+)-lactate [**18**]. However, considerable amounts of D(−)-lactate are formed during the ripening of Cheddar cheese by non-starter lactic acid bacteria (NSLAB) [**56**] either by direct fermentation of lactose early in ripening, or more likely, by racemisation of L-lactate to DL-lactate during ripening. The solubility of Ca-DL-lactate is lower than that of pure Ca-L-lactate, thus racemisation of lactate favours the formation of crystals. Hence, factors that increase the growth of NSLAB (e.g. slow cooling of the cheese block or high ripening temperature) will favour the development of Ca-lactate crystals. However, crystals have also been observed in cheeses with little or no lactate racemisation and thus NSLAB are not always essential for crystal development. NSLAB can also produce lactate from galactose and citrate and if galactose accumulates in the cheese (e.g. through the use of a Gal^{-} starter culture such as *Streptococcus thermophilus*), this may predispose the cheese to the development of crystals.

Factors which increase the amount of soluble Ca^{2+} in the cheese will also favour formation of crystals. Low pH solubilises more casein-bound Ca [**4**] and thus increases the level of soluble Ca^{2+} as does high levels of salt (due to ion-exchange between Na^{+} and Ca^{2+} ions). Modified atmosphere packaging is another factor that should be considered; cheeses stored in an atmosphere containing CO_2 may develop crystals due to CO_2 dissolving in the aqueous phase of cheese and reducing the pH.

Factors which reduce the solubility of Ca-lactate, particularly low ripening temperatures may also favour crystallisation although this may also slow the

growth of NSLAB. Although there is no guarantee of crystal development, high levels of residual lactose in the cheese which are fermented during ripening to high levels of lactate, may also favour crystal formation depending on the NSLAB flora present. Naturally smoked Cheddar cheeses are also more prone to the development of Ca-lactate crystals, presumably because of dehydration of the cheese surface increasing solute concentrations. Cheese made with certain starters is also prone to the development of crystals.

Remedies suggested for Ca-lactate crystals often include reducing the level of lactate in the cheese, reducing or eliminating undesirable NSLAB, controlling the storage temperature and vacuum sealing the cheese to minimise the airspace around the cheese in which crystallisation occurs.

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Dutch-type cheeses

108 Introduction

E.-M. Düsterhöft and G. van den Berg

Gouda and related cheeses (e.g. Edam) are the most common Dutch-type cheeses. They belong to the group of semi-hard cheeses and have undergone a maturation for at least 4 weeks when brought to the market.

The majority of Dutch-type cheeses have a fat-in-dry-matter (FDM) content of at least 40% and a moisture-in-non-fat-solids (MNFS) content below 63%. Gouda-type cheeses are usually made from pasteurised, partly skimmed milk, milk clotting is by calf or microbial rennet and mesophilic mixed-strain starters [**18**] comprising lactococci and *Leuconostoc* are used. The curd ondergoes only a mild scalding (<36 ºC) to control the moisture content [**36**] and is washed to control the extent of acidification. The cheeses are pressed and brine-salted. Acidification occurs during pressing until the first hours of brining. Gouda-type cheeses, typically in the form of 12–15 kg wheels or blocks, are characterised by a limited number of rather small round eyes, they have a smooth texture when young to medium matured, are easily sliced and have good melting properties. A 4-week-old 12 kg cheese should show 10–20 eyes per cross-section that are regularly distributed and have a diameter between 2 and 10 mm. About 75% of the Dutch production is naturally ripened at around 13 ºC, and 25% is foil ripened at a lower temperature.

Within this general description, a large variation in ripening times (4 weeks to >1 year) and fat contents (50% FDM to < 20% FDM) exists. The use of adjunct cultures [**18**] has brought a relatively large variation in flavours and has been beneficial for the production of low-fat varieties. The volume of low-fat cheeses produced in the Netherlands (34% FDM and below) is around 1%, but is

increasing steeply (Productschap Zuivel, 2005). Outside the Netherlands, Germany is the largest producer of Dutch-type cheeses. In 2004, about 46% of all hard and semi-hard cheese produced in Germany was Gouda- or Edam-type.

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109 Why is the surface of Gouda cheese slimy and discoloured?

E.-M. Düsterhöft and G. van den Berg

The surface of Gouda cheese is normally treated with a copolymer dispersion that, after sufficient drying and repeated treatment, provides the cheese with a smooth, somewhat shiny moisture- and gas-permeable coating [**73**]. This coating hinders growth of microorganisms on the cheese to some extent and normally contains natamycin to inhibit the growth of moulds and yeasts. However, this measure is effective only when certain ripening conditions and proper coating and turning schemes are maintained, in order to ensure that both sides of the cheese are able to dry. The frequency of these treatments becomes gradually less during maturation. It is important to control the yeast load of the brine because high numbers may destroy natamycin to a large extent. A relative humidity (RH) of 85–88% (at 12–15 ºC) is usual in Gouda cheese stores, while, certainly for the younger cheese, sufficient air velocity is a prerequisite; under these conditions the cheeses dry sufficiently to avoid these defects. During the first two weeks a weight loss of 2–3%, depending on the size of the cheese, is usual [**48**]. Thereafter, weight loss diminishes gradually. Too dry conditions, on the other hand, are also undesirable because the coating will be too brittle. Cracks can easily occur and then give rise to mould growth under the coating layer.

At higher RH (>93% and/or insufficient turning of the cheese), however, the cheese surface is more humid and, in spite of the presence of natamycin, yeasts from the brine start to grow. These may degrade remaining lactose and lactic acid and even natamycin. After a short time bacteria, such as coryneforms, also start to grow [**142**]. Firstly *Arthrobacter* spp. and still later, at high humidity, even *Brevibacterium linens* may develop, to give a flora comparable with that of smear-ripened cheese. When humidity is slightly lower (e.g. 90–93%) and particularly in the absence of natamycin, it is easier for moulds to develop in competition with these bacteria. So the microclimate around the cheese is very important in this respect.

Growth of microorganisms at the surface strongly affects the appearance of the cheese. The surface does not dry and does not become yellow, but is dull and soon will become slimy. The cheese sticks to the shelf and, when turning, the coating is easily damaged. The moist surface and the products of microbial growth (e.g. peptides and probably also polysaccharides) cause this sliminess, as also occurs in smear-ripened cheese. Moreover, the coryneforms often produce yellow and lavender to light-purple pigments and, certainly when *B. linens* is present, orange-like colours. Improperly cleaned and dried shelves will enhance these effects.

Such changes are detrimental to the appearance of the cheese, even if an attempt is made to wash and dry the cheese when the rind is still intact. New coating layers properly applied often do not prevent grey to violet discolorations of the cheese surface. In general, any growth on the surface of the cheese – also

after the cheese has been coated – will lead to opaque and unclean layers in between new and old coatings. Thus, faults usually cannot be fully rectified.

A particular problem with slight pink discoloration of the coating layer and the outmost of the cheese itself has been found in practice. This may happen when nitrate and annatto colour [**14**] is used for cheesemaking and when the young cheese is not optimally dried, and when the shelves were not properly cleaned and dried. Although no clearly visible growth and only a slightly slimy surface can be observed, nitrate-reducing bacteria (originating from the shelves) may be active on the cheese. The pH may be somewhat higher at the surface of the cheese and under these conditions nitrite and annatto may react and form a pink colour [**105**].

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110 Why is the texture of Gouda cheese tough and the flavour flat?

E.-M. Düsterhöft and G. van den Berg

The texture of a semi-hard cheese such as Gouda is mainly influenced by its moisture content, fat content, pH and age [**92**]. The former parameters are a result of the processing conditions applied during manufacture. Moreover, the rate of acidification during manufacture and amount of curd wash water have an indirect influence on cheese texture by determining the mineral retention and buffering capacity of the caseinate–calcium phosphate complex in the cheese [**22**].

Toughness is associated with a firm and 'long' (elastic) texture. A moisture content that is too low, especially in combination with a high pH of the final cheese, may lead to a tough texture. For Gouda cheese after 4 weeks' ripening, moisture-in-non-fat-solids will be around 58% and pH should be around 5.30. The higher the pH and the lower the moisture content before brining (approximately equivalent to the moisture content in the core for the first month), the more tough and long the cheese will be. Toughness may be reduced by prolonged or enhanced ripening, as proteolysis makes the cheese more smooth.

A moderate acidification rate (pH of the pressed cheese at 4 h after renneting should be about 5.7) and moderate amounts of curd washing water (25–30% of the curd and whey volume) should be used in order to maintain the desired level of buffering substances (Ca phosphate) in the curd and to control pH. These are the main factors besides the moisture content that should be controlled in order to avoid a tough texture in Gouda cheese.

A flat flavour in combination with a tough cheese texture may arise because of ripening that is too slow (particularly too slow proteolysis). The lower the moisture content [**34**] and the lower the ripening temperature, the slower proteolysis proceeds. The type of starter also influences the intensity of aroma. Starters [**18**] containing citrate fermenting strains (*Leuconostoc* and citrate-positive strains of *Lactococcus lactis*) will generally lead to fuller aroma than in cheeses made without these strains. We have experienced that at higher cheese pH, certain flavours (e.g. salt, bitter) are perceived less. Less intense perception of salt flavour may contribute to an overall flattening of cheese aroma. The texture of a cheese itself plays a role in flavour perception as well. Thus, in a firm and tough texture, at equal concentration of flavour compounds, the flavour will be perceived less intensively than in a more smooth matrix.

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111 Why does Gouda cheese have a soapy off-flavour?

E.-M. Düsterhöft and G. van den Berg

The soapy off-flavour is caused by the combination of different free fatty acids (FFA) derived from hydrolytic degradation (lipolysis) of milk fat [**90**]. Lipolysis during ripening in normal Gouda-type cheese (made from pasteurised milk) is rather low (*ca.* 400 mg FFA/kg at 8 weeks). It is principally the short chain fatty acids (C_4–C_6) which – in low levels – contribute positively to the Gouda flavour profile. In contrast to some other (semi) hard cheeses, even a low level of longer chain FFA (e.g. C_{14} to C_{18}) is considered undesirable and will lead to a 'soapy' off-flavour. This level corresponds to an acidity of the milk fat of 1.5 mmol/100 g or higher in young cheese. Depending on the cause of this soapiness, it may continue to increase with prolonged ripening or may remain relatively stable after about 8–12 weeks of ripening. In general, soapiness will be perceived most in young to medium aged Gouda cheeses, as with increasing maturation, the soapy off-flavour may be increasingly hidden by a higher overall flavour intensity.

The hydrolysis of the triglycerides present in cheesemilk into mono-, diglycerides and FFA is catalysed by lipases. Lipases are found in milk (although their activity is greatly reduced by pasteurisation), they are present (at very low activities) in starter cultures used for Gouda-type cheese production [**23**] and may derive from adventituous microbial flora (e.g. from psychrotrophic bacteria, yeasts and moulds). Lipases of different origins have different specificities. As a consequence, they release different mixtures of free fatty acids (short, medium, long chain) and the corresponding off-flavours may vary.

Lipases act rapidly on triglycerides when they are released from the natural fat globule in which they are normally protected by the fat globule membrane. Thus, any treatment of raw milk, in particular destroying (part) of the milk fat globule membrane, may lead to enhanced lipolysis. Homogenisation, membrane filtration processes and pumping through the equipment at the cheese factory lead to different extents of damage to the fat globules, causing the release of triglycerides. Pasteurisation of cheese milk (72 °C × 15 s) largely inactivates the indigenous milk lipase [**11**] and residual activity is considered irrelevant for Gouda-type cheese made from milk of normal quality. Lipases of the adventitious psychrotrophic microflora [**7**] are, however, more heat resistant and pose a significant risk, if these bacteria have been able to grow to high numbers in the cheesemilk prior to heat treatment or if post-pasteurisation contamination occurs. The lipolytic activity of the usual starter cultures used in Gouda-type cheesemaking, however, is normally very low and probably does not contribute to soapiness, if the quality of cheesemilk is normal.

Although mould growth on naturally ripened Gouda-type cheeses is largely controlled and inhibited by good hygienic conditions and by inclusion of natamycin in the coating, the cheese surface cannot be regarded as sterile. Growth of bacteria and moulds on the surface during ripening may contribute to 'soapy' off-flavours, as many have considerable lipolytic activity.

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112 Why does Gouda cheese have irregular eye distribution?

E.-M. Düsterhöft and G. van den Berg

Eyes in Gouda cheese are normally formed by the production of carbon dioxide from citric acid [**18**]. Air dissolved in the milk is a prerequisite (deaerated milk gives a 'blind' cheese [**119**]) in order to obtain sufficient gas pressure in the cheese at some days after production when the curd particles have well fused and eyes start to grow. Critical factors are the speed of gas production and the number and nature of 'nuclei' (locations where the formation of openings easily starts) present in the cheese matrix.

The activity of the starter used concerning metabolism of citrate into carbon dioxide is important. Too high activity during the first days after production (e.g. when only using citrate-positive *Lactococcus lactis*) will result in a more open texture with irregular eyes, as fusion of the curd particles at that very early stage is still insufficient. Low activity in this respect (e.g. when only *Leuconostoc* spp. is used as citrate fermenter) will give only few and small eyes. Starters without citrate-fermenting strains ('O'-starters) will result in a 'blind' cheese.

The role of nuclei in eye formation is more complicated. To a very limited extent, they are needed to start eye formation. The following should be avoided:

- Small air bubbles already present in the milk (e.g. from a leaking milk pump) will serve as nuclei. However, too many bubbles may easily occur and may result in the 'pin-hole' defect in the cheese.
- If air is mixed with the whey and curd flow and entrapped between the curd particles during drainage, it will cause an open texture with mechanical openings. This may also happen locally and lead to a more 'nesty' cheese. When using a prepressing vat, the whey should not be drained off too quickly to avoid air suction into the top of the curd mat.
- Once moulded, the cheese block should not be subjected to force in any way, nor should (colder) curd remains and fines be added.
- Irregular whey drainage, in particular where curd lumps exist at this stage, causes spots with more whey entrapped between the curd particles. This whey hinders normal curd fusion and after resorption of the whey some days later, gas will replace whey, leading to irregular eyes or 'nesty' spots. Sometimes, when the lumps already exist before washing, they are white because the extra whey (undiluted with water) resorbed inside contains surplus lactose, which is converted into lactic acid. Locally the cheese has a lower pH and contains more salt owing to an uneven moisture distribution during brining. Such 'whey nests' can be very pronounced in cheeses produced with a continuous drainage column when the whey passes through the perforated wall too quickly. Then the curd particles located close to the perforated column wall are strongly deformed, fuse quickly and block perforation, and whey drainage is greatly hindered. This may even result in a weak and nesty ring in the final (round) cheese at a few centimetres from the outside.

Thus it is important that the curd particles are brought together and start to fuse under the whey surface and are compacted before curd blocks are cut for moulding. In low-fat cheeses, the curd particles fuse and deform less easily owing to lower processing temperatures. Therefore irregular eye distribution is more often encountered in these cheese types.

The cheese rind just after brining is white, harder and more brittle because of its extremely high salt content. In this zone, final curd fusion is hindered, especially where small curd lumps are present. Entrapped whey in this zone may result in a number of small, irregular eyes, a defect that is often called 'air rim'. The stronger the citrate fermentation by the starter the more severe may be this defect.

Special attention must also be paid to the packaging procedure used for foil-ripened cheese. A strong vacuum should not be applied too rapidly in order to prevent 'air rims'. The gas that is suddenly released from solution in the rind of the cheese disturbs the structure at weak spots in the matrix and thus initiates the formation of small irregular eyes because carbon dioxide will diffuse into these voids.

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113 What problems do *Propionibacterium* spp. cause in Gouda cheese? How are they controlled?

E.-M. Düsterhöft and G. van den Berg

Propionic acid bacteria (PAB) [**117**, **118**] in cheese are able to convert lactate to propionic acid, acetic acid, carbon dioxide and water. If these organisms grow considerably, after at least 1 month, they cause a sweet taste and a very open texture by increasing the size of the pre-existing eyes. Sometimes pink discoloration of parts of the cheese is observed or small reddish spots without much gas production are found. The normal conditions in Gouda cheese are not very favourable for the growth of these bacteria. At the usual ripening temperature of Gouda cheese (12–14 ºC), PAB develop rather slowly since the usual salt content of Gouda suppresses growth [**46**]. However, it takes considerable time for NaCl to diffuse into the core of the cheese and the salt level in the interior remains below a critical inhibiting concentration for several weeks. Because growth of propionibacteria occurs above approximately pH 5.1, the conditions in normal young Gouda cheese are not very suitable. However, pH increases during ripening to some extent, particularly if the young cheese has a high initial pH. Their growth is also hindered by nitrate, an additive often used in the manufacture of Gouda cheese to prevent from 'late blowing', a defect caused by the growth of butyric acid bacteria (mainly *Clostridium tyrobutyricum*) [**91**].

Raw milk is contaminated with *Propionibacterium* spp. but they usually do not cause serious trouble in the production of cheese from pasteurised milk. However, in the manufacture of raw milk cheese one should take sufficient hygienic measures to prevent contamination from the equipment as much as possible. In the absence of nitrate (or when low amounts are used), when cheeses with reduced salt contents are produced and/or when rather high ripening temperatures are used, cheeses are more susceptible to this defect. A higher cheese pH will further increase the susceptibility.

It is worth noting that special care should be taken in factories that manufacture also Maasdam, Jarlsberg or other varieties for which starters containing PAB are used [**117**], as cross-contamination has been observed in practice. This should be taken into account in the design and cleaning of cheesemaking and starter equipment and the related pipeline systems. A general measure is to manufacture these latter cheese types just before an overall cleaning of the cheese factory will be carried out.

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114 Under which conditions do blisters occur under the wax layer of Gouda-type cheese?

E.-M. Düsterhöft and G. van den Berg

A wax coating is normally applied to Gouda-type cheese after ripening [**73**], in the case of young cheese at least 25 days after production. For Gouda cheese normally yellow or white wax is used; for Edam cheese it is red. This treatment has been used for more than a century to protect the cheese during transport against microbial growth and weight loss due to moisture evaporation. It was, formerly more than nowadays (because of better ripening conditions), general practice to wash and dry the often unclean cheese surface before waxing. The waxed cheeses are wrapped in a foil for better presentation and packed in cardboard boxes. Such cheese should be stored below 10 ºC to maintain its shape and avoid bulging of the cheese which may damage the wax layer, in particular for younger cheeses. However, during overseas transport and storage, certainly in warmer countries, it is hard to maintain such conditions.

The quality of the cheese rind before waxing is key for its successful application. Microbial growth on the cheese surface during ripening, resulting in a slimy and often discoloured exterior, increases pH and decreases the salt concentration due to less drying of the rind zone. Slimy products may be washed away but the rind zone is still too moist and is more susceptible to growth of spoilage bacteria. Moreover, in practice such washing does not result in removal of all microorganisms. It should be noted that, although cheese is briefly immersed in or rinsed with hot wax (100–110 ºC) to form a wax coating layer after solidification, a pasteurising effect of the cheese rind may not be expected in practice. Thus a certain microflora will still be present on the cheese surface after waxing. At high ripening temperatures, this flora shows remarkable activity in cheese previously ripened under poor conditions. It may produce gas, leading to blisters between wax and cheese and is often accompanied by putrid flavours. Such blisters are easily damaged and mould growth will occur because sufficient oxygen becomes available. Under these conditions activity of microorganisms, such as propionic acid bacteria [**113**] and lactobacilli that contribute to this defect, was found.

The conclusion must be that, especially when cheese has to be waxed, the cheese in the store should be kept under good drying conditions and good maintenance in order to prevent any visible microbial activity and to develop a clean, shining and closed surface. The pH of the cheese surface should be well below 6.0, its moisture content should be low and the salt concentration should be high enough to offer sufficient preservation before a cheese can be waxed.

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115 How may late blowing be avoided in Gouda-type cheeses?

E.-M. Düsterhöft and G. van den Berg

The 'late blowing' defect, characterised by excessive eye formation and eventually accompanied by an off-flavour of butyric acid, may occur after prolonged ripening (typically after 4–6 weeks) in Gouda-type cheeses [**91**]. Late gas blowing is caused by the anaerobic fermentation of lactate to butyrate, CO_2 and H_2 by *Clostridium tyrobutyricum* (butyric acid fermentation, BAF). The extent of the defect can vary from slight gas formation and a little larger eyes than normal with almost no perceivable off-taste, to very intensive gas production, leading to a higher number of large eyes, cheeses torn open and a clearly perceivable taste of butyric acid. In cheeses with a strong defect, a few small greenish-grey hard spots of about 0.5 mm can be observed, often at the surface of an eye. These are colonies of *Cl. tyrobutyricum.* When contamination is low, it is sometimes not easy to identify unambiguously the cause of the defect. In case of doubt, analysis of the organic acids content of the cheese is necessary and elevated levels of butyric acid (>100 mg kg^{-1} in Gouda cheese) are indicative. Gouda-type cheese (like other brine-salted cheeses) is particularly sensitive to 'late blowing', as it takes about 8 weeks (in a large 10–12 kg cheese block) before the salt has diffused from the exterior to the middle and before a sufficiently high salt concentration is obtained throughout the entire cheese to inhibit germination of *Cl. tyrobutyricum* spores. *Cl. tyrobutyricum* spores will not germinate at salt/dry matter (DM) contents of >3%, (which corresponds to about 4.2% salt-in-moisture for a typical Gouda cheese at 14 days ripening) [**46**]. Thus, in the interior of the brine-salted cheese, the salt content remains too low for several weeks to prevent effectively outgrowth of the spores. Varieties with reduced salt content therefore have a greater risk for butyric acid fermentation, and the risk is also higher with increasing cheese size. Other factors that may enhance the risk for butyric acid fermentation in Gouda-type cheese are a relatively high pH (e.g. pH 5.4) and a high ripening temperature. Cheeses that are foil-ripened (6 ºC) are therefore less susceptible to late gas blowing than naturally ripened cheeses (13 ºC) as long as cooling is maintained.

The type of starter may also influence the sensitivity of Gouda-type cheeses to butyric acid fermentation. Cheeses made using starters with high citrate-fermenting capacity (containing *Leuconostoc* and citrate-positive strains of *Lactococcus lactis*, LD-type starters) [**18**] appear to be more susceptible to late gas blowing than those produced with starters containing fewer (L-starters) or no citrate fermenting strains (O-starters). The high level of acetic acid formed in the former and its stimulating effect on spore germination may cause this effect.

Butyric acid fermentation can effectively be prevented by bactofugation of the cheesemilk. Bactofuges remove spores with an efficiency of 98%; microfiltration may have a similar effect. The residual spore load has to be controlled using alternative methods, as with cheesemilk which is not bactofuged.

The use of nitrate is the most common protective measure. Typically, 15 g $NaNO_3$/100 l cheesemilk is used (which is sufficient to prevent outgrowth in

cheese from milk with contamination of <10 spores/ml). This dosage results in levels of < 50 mg nitrate/kg cheese at 14 days. In singly bactofuged milk, the dosage of nitrate can be reduced to 5 g/100 l (in winter in Europe). To be effective against the germination of the spores, nitrate has to be reduced to nitrite. Xanthine oxidoreductase, an enzyme occurring naturally in the fat globule membrane, catalyses this reduction and has a specific, essential role. Heat treatment of the cheesemilk will inactivate xanthine oxidoreductase increasingly (82 ºC × 20 s completely inactivates this enzyme) and thus the necessary reduction of nitrate cannot take place. For the same reason, a rather low level of xanthine oxidoreductase makes low-fat cheeses more sensitive to butyric acid fermentation, even when sufficient amounts of nitrate are added.

When the use of nitrate is not allowed, lysozyme can be used to control butyric acid fermentation. Lysozyme degrades the cell wall of *Clostridia* and other Gram-positive microorganisms. Being less effective than nitrate, a high dosage of 500 Units/ml milk has to be used to prevent late blowing in Gouda cheeses, when contamination of milk is low (<0.3 spores/ml) and 1000 Units/ml when spore load of milk is high (up to 13 spores/ml). It is recommended to check the sensitivity of the starter culture to lysozyme before its use in cheese production.

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116 How can excessive gas formation by thermophilic streptococci take place in Gouda cheese?

E.-M. Düsterhöft and G. van den Berg

In the 1970s, energy shortages caused an increase in the number of plates in the regeneration sections of heat exchangers in order to achieve >90% energy recovery. This measure increased the plate surface in the section where the heated milk is cooled down. In particular, strains of *Streptococcus thermophilus* appeared to be able to attach and grow on the (fouling) surface of plates in the regeneration sections where the temperature was favourable for their growth (generation times of 15 min have been found). Contamination of pasteurised milk with this microbe increased to the point that cheese quality became compromised. Although these microbes are present in raw milk, their danger with respect to cheese quality had not previously been recognised. However, increases in the surface area of regeneration sections provide an increased surface at a temperature suitable for growth. Growth to high numbers occurred at longer operating times. *S. thermophilus* contaminates cheesemilk during longer operation times so strongly that they are able to induce excessive carbon dioxide production in the cheese. This results, after about 1 month, in an increase in size of pre-existing eyes. These overly large eyes are usually combined with 'unclean' and 'yeasty' off-flavours. The use of an active citrate-fermenting starter [**18**] will enhance these defects.

Nowadays this phenomenon has been investigated and well-formulated operation rules have been established to allow the modern cheese factory to control this problem. Numbers of this organism in Gouda cheese less than 1×10^7 per gram of cheese will not cause defects. However, organisms are concentrated by a factor of 10 during cheese manufacture and grow in cheese by at least the same factor. So the cheese milk should contain $< 1 \times 10^5$ per ml of these microorganisms.

Normal milk contains a limited number of *S. thermophilus* and some of them may survive pasteurisation (e.g. 74 °C × 15 s). During pasteurisation of the milk in a modern cheese factory, a critical number of organisms will often be achieved after 8 h operation. Then pasteurisation should be stopped and the pasteuriser and milk pipeline positioned behind it should be cleaned and sufficiently heated to kill this microorganism. However, when the counts in the milk before pasteurisation are increased, the operation times should be shorter because the counts in the cheesemilk will increase much earlier. To this end, attention must be paid to thermisation before storage of the milk. So thermisation should be strictly supervised in the same way to avoid considerable increase of the milk counts before the pasteuriser. (It is worth noting that this phenomenon is not restricted to heat exchangers. It may also take place on walls of other dairy equipment at temperatures suitable for growth, thus limiting run times.)

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Swiss cheese

117 Introduction

M.T. Fröhlich-Wyder and H.P. Bachmann

Swiss-type cheeses were originally manufactured in the Emmen valley (Emmental) in Switzerland; their precursors were various mountain cheeses. Emmental cheese is probably the best-known Swiss-type cheese and is frequently referred to simply as 'Swiss cheese'. Swiss-type cheeses have round regular cherry-sized eyes which vary in size from medium to large (1–3 cm).

The propionic acid fermentation leads to characteristic eyes [**118**, **123**] and to a nutty flavour [**125**] and can either occur spontaneously or be achieved by a culture of selected propionibacteria. A spontaneous fermentation leads to irregular eye formation, because of great strain diversity of the natural propionibacterial flora in milk. The number and size of eyes vary markedly and cracks or splits are quite common. Comté and Beaufort are typical examples of cheese varieties with a spontaneous propionic acid fermentation. The use of a culture of selected propionibacteria allows a more regular eye formation as a result of controlled propionic acid fermentation. All these cheese varieties are referred to as Swiss-type cheeses. The body and texture of Swiss cheese are typical of hard or semi-hard cheeses. The characteristics of Swiss-manufactured Emmental cheese are:

- hard cheese made with raw milk from cows that have not been fed on silage;
- high pH of ~5.2 at whey drainage;
- cooking to ~53 ºC (inactivation of much chymosin activity);
- maturation in a warm room (23 ºC) to promote propionic acid fermentation followed by a maturation at 13 ºC;

Fig. 1 Sectional view of a Swiss Emmental cheese (source: ALP Agroscope Liebefeld-Posieux, CH-3003 Berne).

- cylindrical shape;
- firm dry rind;
- weight: 60–130 kg;
- 1000–2000 round eyes, diameter 1–4 cm;
- flavour: mild, nutty, slightly sweet, becoming more aromatic with increasing age;
- cheese body – ivory to light-yellow, slightly elastic;
- ripened for 4–8 months (up to 15 months).

Today, Emmental-type cheese (Fig. 1) is produced in many countries and a great variety of other Swiss-type cheeses are also available on the market, including Jarlsberg, Maasdammer, Leerdammer and many other products denoted as 'Swiss-type cheese'. They are manufactured by methods differing from traditional Swiss production in terms of treatment of milk, extent of mechanisation, the starters used and the weight and shape. Descriptions and analytical values presented in the following entries focus mainly on Swiss Emmental cheese.

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118 What factors affect eye development in Swiss cheese?

M. T. Fröhlich-Wyder and H. P. Bachmann

The main characteristic of Swiss-type cheeses is, besides the nutty and sweet taste, their eyes. For eye formation, the following four main factors are needed:

1. A source of gas.
2. A certain gas pressure and a well-balanced solubility of the gas.
3. Nuclei for eye formation.
4. An adequate body texture and rind.

Propionic acid fermentation produces the CO_2 that leads to the formation of the characteristic eyes. For Emmental cheeses, the inoculation size of *Propionibacterium freudenreichii* is very small (a few hundred cfu ml^{-1} milk). Propionic acid fermentation begins about 30 days after the start of manufacture when the cheese is held at about 20–24 ºC for roughly 7 weeks and then continues at a slower rate at 10 to 13 ºC.

Propionibacteria appear under the microscope as short rods (Fig. 1) which grow only at low oxygen concentrations (anaerobic to aerotolerant). They occur naturally in the rumen and intestine of ruminants, in soil and in silage. Strain diversity of the natural propionibacterial flora is still great. They are sensitive to salt [**46**] and grow optimally at a pH between 6 and 7 (growth range pH 4.6–8.5). The optimal growth temperature is 30 ºC, but growth occurs also at 14 ºC.

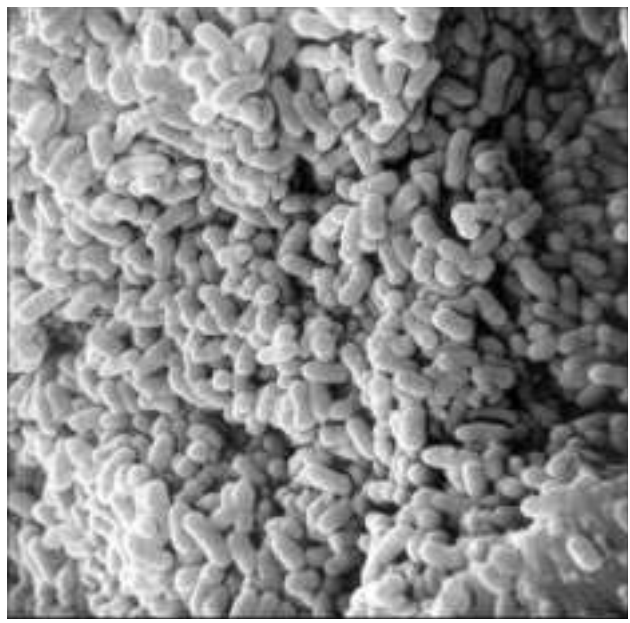

Fig. 1 Scanning electron micrograph of a culture of *Propionibacterium freudenreichii* (source: ALP Agroscope Liebefeld-Posieux, CH-3003 Berne).

Small quantities of CO_2 are also produced during the lactic acid fermentation and through the degradation of citrate. The fermentation of citrate leads to a higher number of eyes in the initial stage of the propionic acid fermentation, but to a lower number of eyes in the mature cheese.

In a cheese loaf of approximately 80 kg, total CO_2 production is about 120 l before the cheese is sufficiently aged for consumption. About 60 l remain dissolved in the cheese body, ~ 20 l are found in the eyes and ~ 40 l are lost from the loaf.

The saturation concentration of carbon dioxide in Emmental is around 34 mmol kg^{-1} and depends on pH and temperature of the cheese body. At 10 ºC, 50% more carbon dioxide is soluble than at 20 ºC; at a pH of 4.8 twice as much CO_2 is soluble than at pH 5.2. The high pH of Swiss-type cheeses and the ripening step in the warm room are therefore two important factors that are responsible for a lower solubility of CO_2 and consequently for better eye development.

Nuclei (i.e. points of development for future eyes), can be achieved by the non-homogeneity of the curd, physical openness and the content of gas in the curd. For eye formation in Emmental cheese, the gas content of the curd is of major importance. Microscopic air bubbles attached to curd particles are the main areas of future eyes. However, proper dip filling of the moulds is imperative since too many air inclusions acting as nuclei for future eye development can lead to undesirable openness as, for example, in the defect of the so-called 'thousand holes'. As the name of the defect suggests, the cheese is littered with thousands of small holes.

A soft and elastic texture is crucial for a regular eye formation. This is why the technology of Emmental cheese production is aimed at the achievement of optimum conditions not only for propionic acid fermentation, but also for the development of a soft and elastic texture. Furthermore, the rind is also essential for eye formation. Brining of the cheeses for 2–3 days and the rather low relative humidity in the ripening room (70–80%) lead to a firm and dry rind [**43**], which reduces the loss of CO_2. The brining and the low relative humidity of the ripening room results in a loss of water from the rind and, consequently, to a compact protein network at the surface of the cheese, which acts as a barrier for gas diffusion. If the rind is too soft and too porous, the brine can be supplemented with calcium, which leads to a stronger protein matrix. On the other hand, if the rind is too rigid, the calcium available in the brine can be eliminated by precipitation. Thus the porosity of the rind can be controlled by adding or removing calcium.

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119 What causes 'blind' Emmental cheese?

M. T. Fröhlich-Wyder and H. P. Bachmann

Since eye formation is an indispensable step in the production of Emmental cheese [**118**], defects related to eye formation are usually unacceptable. Especially in Swiss Emmental cheese production, round, regular cherry-sized eyes which vary in size from medium to large (1–3 cm) are essential. Defects in eye formation include:

- lack of eyes;
- irregular eye formation, slits or cracks.

Propionibacteria are responsible for the formation of eyes and the manufacturing procedure of Swiss cheese must be aimed at the achievement of optimum conditions for propionic acid fermentation.

Propionibacteria are sensitive to low pH and high salt concentration [**46**]. In a cheese with excessive acidification during lactic acid fermentation leading to a low pH at the beginning of ripening, the propionic acid fermentation is reduced markedly. Also at a high salt concentration, the growth of propionibacteria can be slowed down to a great extent. A salt concentration of 5% (w/v) in the aqueous phase can even stop their growth.

Furthermore, propionibacteria are sensitive to copper. In the production of Swiss Emmental cheese, copper vats are used to control propionic acid fermentation. Usually, the copper content in milk is too low to stop propionic acid fermentation completely. Therefore, the lack of eyes is usually not a result of too high copper content.

There might be, of course, additional, technological reasons for the lack of eyes. A cooking temperature that is too high can inactivate large numbers of propionibacteria. Even if the lethal temperature of about 62 °C is not reached during cooking, the temperature remains high during pressing, which can reduce the numbers of propionibacteria considerably. In addition, the conditions in the fermentation room have a great impact on the propionic acid fermentation. Optimal growth temperature of propionibacteria is around 30 °C. Even though growth may also occur at low temperatures (e.g. 14 °C), the temperature of the fermentation room (usually ~23 °C) should not be too far from the optimal temperature, because, as a consequence, the propionic acid fermentation would be greatly slowed down. The length of time cheeses remain in the fermentation room should be adjusted accordingly.

As outlined in [**118**], not only is the liberation of gas by propionibacteria a prerequisite for eye formation, but also the availability of nuclei for future eye development. Elimination of these nuclei, e.g. through milk pretreatments such as microfiltration or centrifugation, prevents the formation of eyes.

During ripening, a high loss of gas might be the result of an overly porous rind or foil. In the case of the former problem, controlling the brining conditions is necessary such as NaCl concentration of the brine, the duration of brining or the use of $CaCl_2$ in the brine in order to get a firmer rind. The choice of the right

foil, if used, with the desired porosity must also be taken into consideration. Furthermore, the size and shape of the cheese also have an impact on gas diffusion. If the volume to surface area ratio is low, the loss of gas might be too high.

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120 What causes irregular eye formation, slits or cracks in Emmental cheese?

M. T. Fröhlich-Wyder and H. P. Bachmann

Irregular eye formation, slits and cracks are often a result of undesirable fermentations and/or an inadequate body texture. The former include excessive propionic acid fermentation or its restart towards the end of ripening (so-called 'late fermentation') and butyric acid fermentation [**91**].

A strong propionic acid fermentation is very often coupled with a strong aspartase activity of the propionibacteria [**121**] used together with a high availability of aspartate. Furthermore, excessive proteolysis leads to a shorter body. This defect becomes particularly evident when a large amount of casein is degraded to low molecular mass peptides [**88**]. The additional carbon dioxide released by decarboxylation of amino acids clearly reduces the keeping quality of the cheese and leads to oversized and irregular eye formation and taller loaves. The cheese body often cannot withstand the pressure of the gas and cracks or splits appear (Fig. 1). Excessive aspartase activity also increases the risk of irregular eye formation with cracks and splits [**121**]. This defect is called late or secondary fermentation.

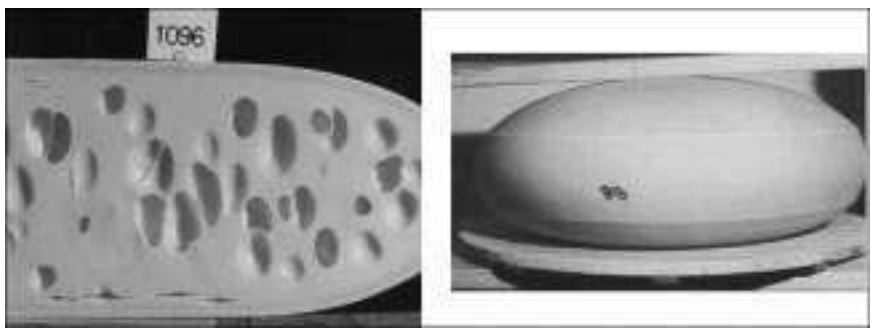

Fig. 1 Swiss cheese with the defect of late fermentation (source: ALP Agroscope Liebefeld-Posieux, CH-3003 Berne).

Butyric acid fermentation is highly undesirable in Swiss cheese, not only because of the production of strong off-flavours, but also because lactate fermentation by *Clostridium tyrobutyricum* into butyric acid, acetic acid, carbon dioxide and hydrogen causes the cheese loaf to blow. The insolubility of H_2 in water is responsible for a very easy and quick cheese blowing. At atmospheric pressure, when 1 g butyric acid (from 2 g lactic acid) is produced, 1000 ml gas (CO_2 and H_2) is liberated. Fig. 2 shows Swiss cheese trials with differing intensity of butyric acid fermentation. The eye formation is irregular, and is accompanied by cracks and splits.

Fig. 2 Swiss cheese trials with differing intensity of butyric acid fermentation (source: ALP Agroscope Liebefeld-Posieux, CH-3003 Berne).

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121 What is aspartase of *Propionibacterium*?

M. T. Fröhlich-Wyder and H. P. Bachmann

Aspartase is an enzyme found in propionibacteria and also other microorganisms that catalyses the deamination of aspartate. For a long time it was known by cheesemakers that different cultures of propionibacteria could lead to very different cheeses. But the cause for this variation was unknown until recently when it was ascribed to the differing aspartase activities of various propionibacterial strains.

The metabolism of propionibacteria in Swiss cheese is rather complex and not yet fully understood. Different metabolic pathways have been described for the utilisation of lactate and aspartate, both of which are available in cheese.

The lactic acid produced by the lactic starters is usually broken down to propionate, acetate and CO_2 as follows (the classic metabolic pathway):

$$3 \text{ lactate} \rightarrow 2 \text{ propionate} + \text{acetate} + CO_2 + \text{ATP}$$

In the presence of aspartate, the fermentation of lactate is coupled to the fermentation of aspartate to produce succinate but no propionate. Consequently, more lactate is fermented to acetate and CO_2 than to propionate:

$$\text{lactate} + 2 \text{ aspartate} \rightarrow \text{acetate} + CO_2 + 2 \text{ succinate} + 2NH_3 + \text{ATP}$$

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122 How does aspartase activity of *Propionibacterium* affect Swiss cheese?

M. T. Fröhlich-Wyder and H. P. Bachmann

Propionibacterial strains can differ strongly in their aspartase activity [**121**]. In the manufacture of Emmental cheese, the use of cultures with differing aspartase activity leads to different products. Strains with high aspartase activity ferment higher amounts of lactate, gain more energy and are thus present in cheese at higher numbers than strains that utilise only a little aspartate. The higher number of propionibacteria is responsible for increased amounts of acetate, propionate, succinate and CO_2 and therefore for more intense flavour and larger eyes. Tables 1 and 2 show the characteristics of Emmental cheeses made with propionibacteria with either strong or weak aspartase activity. Figure 1 shows the outer appearance of Emmental cheeses produced with different propionibacteria. The number and size of eyes and the height of loaves are greater for cheeses made with a culture with strong aspartase activity as a result of increased CO_2 release. The storage time for the cheeses in the warm room may be shortened by up to 10 days. The intensity of aroma is also more pronounced compared with cheeses made with propionibacteria of low aspartase activity. Thus, propionibacteria with strong aspartase activity accelerate ripening by a combined effect of aspartate metabolism and of the increased number of propionibacteria.

The capability of strains to utilise aspartate is a very important factor when selecting new cultures. A very high aspartase activity will increase the amount of CO_2 and therefore the risk of late fermentation. However, moderate aspartase activity may have a positive effect on the quality of Emmental cheese as regards eye formation and flavour intensity.

Table 1 Mean values of metabolites and propionibacterial counts in Emmental cheese (6 and 12 months) made with propionibacteria with weak or strong aspartase activity (adapted from Fröhlich-Wyder and Bachmann, 2004)

Parameter ($mmol\,kg^{-1}$)	Emmental cheeses of 6 months			Emmental cheeses of 12 months		
	Weak ($N = 10$)	Strong ($N = 8$)	*t*-test	Weak ($N = 10$)	Strong ($N = 8$)	*t*-test
Lactate	57.4 ± 10.5	45.3 ± 17.4	ns	47.0 ± 8.5	11.3 ± 6.7	***
Acetate	48.4 ± 1.3	53.1 ± 5.1	*	47.6 ± 0.6	58.7 ± 1.7	***
Propionate	60.1 ± 4.4	67.1 ± 10.2	ns	63.2 ± 4.2	83.6 ± 3.6	***
Succinate	4.0 ± 0.6	11.9 ± 1.7	***	5.1 ± 2.8	17.7 ± 2.5	***
CO_2	27.6 ± 1.6	33.6 ± 2.0	***	nd	nd	
Propionibacteria ($log\ cfu\,g^{-1}$)	nd	nd		6.7 ± 0.9	8.4 ± 0.3	***
Aspartate	2.219 ± 0.861	0 ± 0	***	4.834 ± 0.585	0.588 ± 0.097	***
Asparagine	2.863 ± 1.100	0.125 ± 0.237	***	1.886 ± 0.494	0.054 ± 0.154	***

nd, not determined; ns, not significant; * $p < 0.05$; *** $p < 0.001$.

Table 2 Sensory and quality parameters of Emmental cheese (6 and 12 months) made with propionibacteria with weak or strong aspartase activity (mean values and *t*-test, adapted from Fröhlich-Wyder and Bachmann, 2004)

Parameter (index)	Emmental cheeses of 6 months			Emmental cheeses of 12 months		
	Weak ($N = 10$)	Strong ($N = 8$)	*t*-test	Weak ($N = 10$)	Strong ($N = 8$)	*t*-test
Openness (1–6)	5.3 ± 0.6	4.6 ± 0.6	*	4.6 ± 0.6	4.6 ± 0.8	ns
Number of eyes (0–5)	4.7 ± 0.6	5.3 ± 0.4	*	4.4 ± 0.6	5.4 ± 0.3	***
Size of eyes (1–5)	4.9 ± 0.3	5.8 ± 0.6	**	4.5 ± 0.5	5.8 ± 0.6	***
Maturity (2–8)	4.4 ± 0.8	5.3 ± 0.6	*	6.5 ± 0.5	6.8 ± 0.4	ns
Intensity of aroma (0–7)	3.1 ± 0.2	3.5 ± 0.2	***	3.7 ± 0.4	3.8 ± 0.3	ns
Sweetness (0–7)	2.3 ± 0.2	2.2 ± 0.1	ns	2.5 ± 0.3	2.4 ± 0.2	ns
Height of cheese (cm)	19.1 ± 1.5	21.3 ± 1.7	*	18.1 ± 1.8	20.6 ± 1.0	**

nd, not determined; ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Index indicate the range of appreciation (lowest number = lowest possible score; highest number = highest possible score).

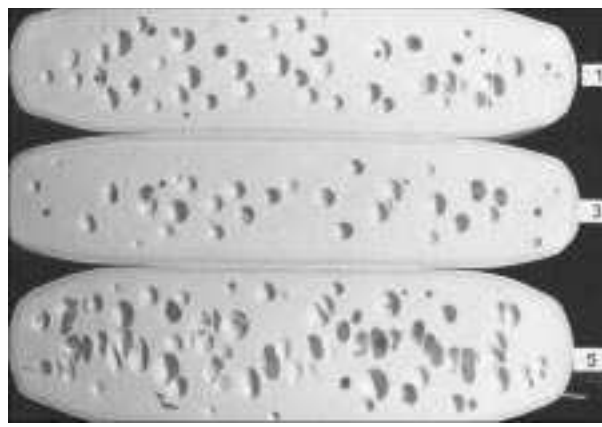

Fig. 1 Emmental cheese (5 months old) made with propionibacteria with strong (no. 5) or weak (nos 1 and 3) aspartase activity (source: ALP Agroscope Liebefeld-Posieux, CH-3003 Berne).

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123 How may the size and quantity of the eyes in Emmental-type cheese be controlled?

M. T. Fröhlich-Wyder and H. P. Bachmann

In order to control the size and quantity of the eyes in Emmental cheese, we need to know the main factors of eye development [**118**]. The size and quantity of eyes can be controlled by controlling the source of gas production, the quantity of nuclei in the cheese matrix and to some extent also the extent of proteolysis.

The main source of gas production is the metabolic activity of the propionibacteria and to some extent also the facultatively heterofermentative lactobacilli [**56**]. In Emmental cheese, interactions between propionibacteria and lactic acid bacteria have a major impact on propionic acid fermentation and, thus, on gas production. Understanding the characteristics of, and the interactions between, these microbial groups results in an easy tool to control eye formation.

As outlined in [**122**], the use of a culture of propionibacteria with high aspartase activity leads to more and larger eyes in Emmental cheese. A culture of propionibacteria with weak aspartase activity, consequently, will produce fewer and smaller eyes. Furthermore it is possible to control stepwise the size and quantity of eyes by mixing these two types of cultures in defined ratios (Fig. 1). Facultatively heterofermentative non-starter lactobacilli (FHL) are used in the Swiss artisanal cheese industry to slow down the propionic acid fermentation, i.e. to control eye formation. As a consequence of inhibition of propionibacteria by FHL, less propionic acid and, thus, less carbon dioxide are produced. The mechanism of inhibition is not yet conclusively clarified, but can be used to control efficiently the size and quantity of eyes in Swiss cheese. Since the

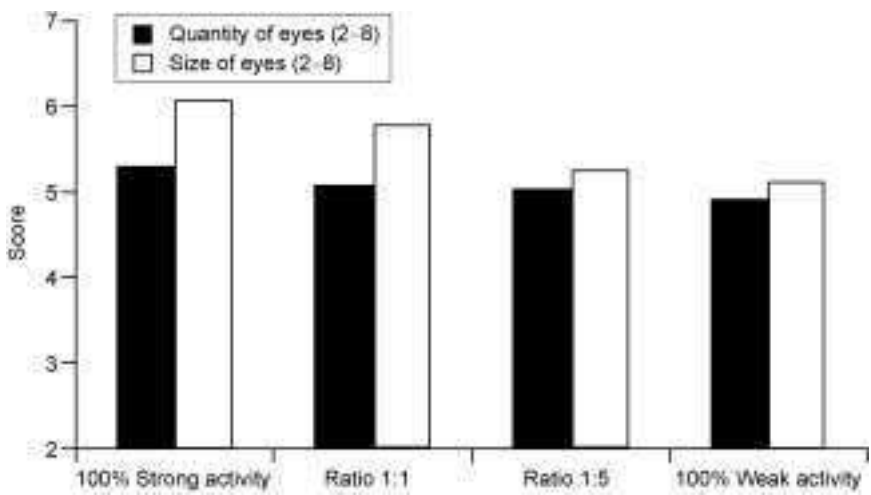

Fig. 1 Influence of different ratios of cultures of propionibacteria with opposing aspartase activity (source: ALP Agroscope Liebefeld-Posieux, CH-3003 Berne).

introduction of cultures of *Lactobacillus casei* in Switzerland in 1989, the defect of late fermentation has decreased considerably.

Propionibacteria with differing aspartase activity are not inhibited equally; propionibacteria with weak aspartase activity are inhibited much more by FHL than are propionibacteria with strong aspartase activity. This is why propionibacteria with strong aspartase activity are generally more prone to causing late fermentation.

Hence, smaller eyes are achievable by the use of a propionibacterial culture with low aspartase activity combined with a culture of citrate-positive FHL; small to medium sized eyes by the use of a propionibacterial culture alone with low aspartase activity; larger eyes by the use of a propionibacterial culture with high aspartase activity.

The quantity of future areas of eye formation, the nuclei, influences fundamentally the quantity and size of eyes [**118**]. At a comparable rate of gas production, fewer nuclei lead to larger eyes and vice versa. The quantity of nuclei can easily be controlled by centrifugation of a certain percentage of the cheese milk (Fig. 2).

Propionibacteria with the ability to ferment aspartate need, as expected, the amino acid aspartate for its metabolism. Increasing levels of proteolysis liberates more amino acids and more aspartate which can be metabolised by propionibacteria. The result is higher gas production, more and larger eyes. A propionibacterial culture with high aspartase activity combined with *Lb. helveticus*, a highly proteolytic *Lactobacillus*, leads therefore to especially large eyes in a Swiss-type cheese.

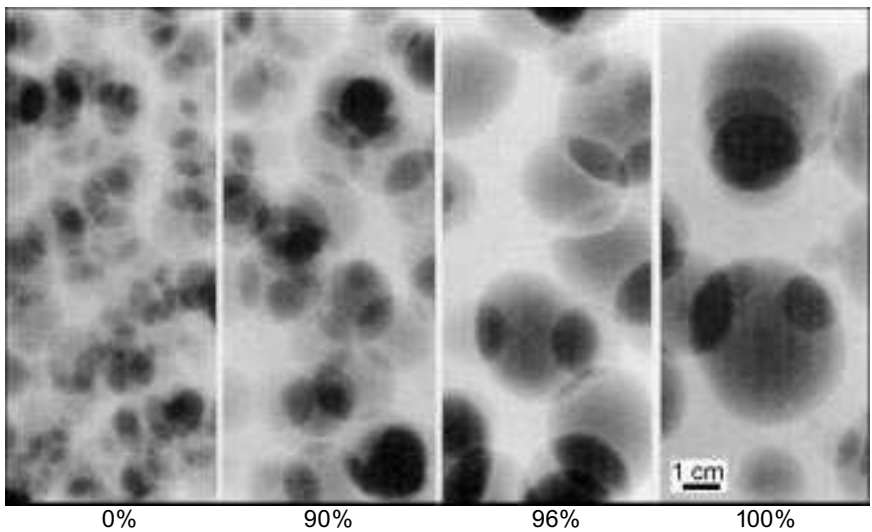

Fig. 2 Eye formation in an Emmental cheese made with uncentrifuged (0%) or partially (90, 96 or 100% v/v) centrifuged vat milk (source: X-ray by ALP Agroscope Liebefeld-Posieux, CH-3003 Berne).

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124 How do I control the elastic texture of Swiss-type cheese?

M. T. Fröhlich-Wyder and H. P. Bachmann

A soft and elastic texture is crucial for a regular eye formation [**118**]. This is why the manufacturing procedure of Swiss-type cheese must be aimed at the achievement of optimum conditions not only for propionic acid fermentation, but also for a soft and especially elastic texture. There are several ways to influence texture. The following will be discussed:

- technology;
- proteolysis;
- composition of milk fat.

A fundamental step during Emmental cheese production is the addition of water (12–18%) to the milk or to the curd in order to dilute the substrate (lactose) for lactic acid bacteria. This leads to a relatively high pH after the lactic fermentation (5.20–5.30), but also at whey drainage, which explains the high calcium content of the cheese and, consequently, the long and elastic texture [**17**]. Calcium plays the key role in the formation of the protein network by building calcium phosphate bridges between the casein micelles.

The role of the water and fat in Swiss cheese should be mentioned: a rather high water and fat content are prerequisites for a soft and elastic texture. Swiss Emmental cheese is a full-fat hard cheese with approximately 50% fat-in-dry-matter and a maximum water content of about 38%. On the other hand, a too high fat content is responsible for a too soft body texture and a lower pH in the cheese. The reason for the latter is the unfavourable protein to fat ratio and a low protein level reduces the buffering capacity [**22**]. This fact leads consequently to a weaker propionic acid fermentation with consequently fewer and smaller holes.

Also, the ripening conditions have an impact on the cheese body. Swiss-type cheeses are kept in the fermentation room for the main part of gas production and eye formation. The temperature of this step (20–24 °C) is rather high to promote growth of propionibacteria and, thus, gas production. In addition to that, the high temperature supports a soft and elastic texture.

The high elasticity is also promoted by a low proteolysis rate during ripening compared to other cheese varieties. Most Swiss-type cheeses are cooked to a high temperature; Emmental cheese is heated to 52–54 °C after cutting. During pressing, the temperature remains at around 50 °C for hours. At this temperature, undesirable microorganisms are eliminated, but also enzymes such as chymosin are largely inactivated. Thus, overly intensive proteolysis is avoided and hence a texture that is too short and too crumbly.

Proteolysis can also be controlled by using an appropriate starter culture: *Lactobacillus helveticus* has higher proteolytic activity than *Lactobacillus delbrueckii* subsp. *lactis* [**18**]. Furthermore, mesophilic lactococci are less proteolytic than are in general thermophilic lactobacilli.

During winter, hay and fodder beet are common components of a basal diet for dairy cows in the lowland regions of Europe. Generally, the concentration of

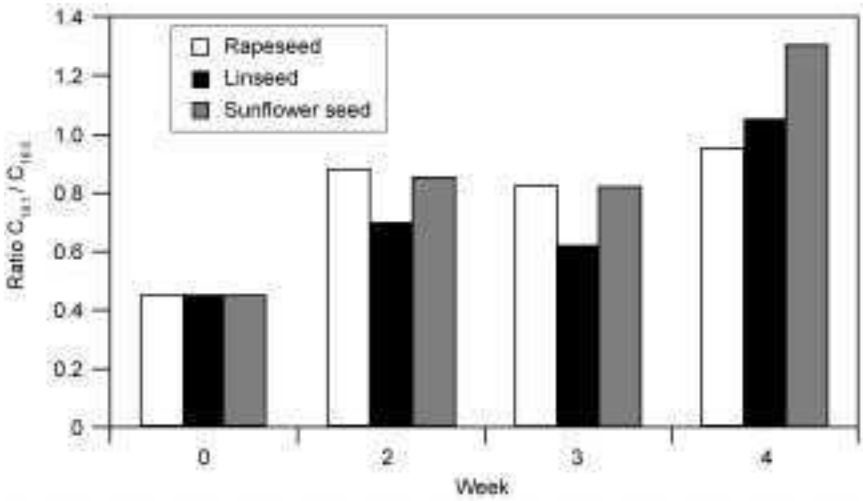

Fig. 1 Influence of oilseed supplementation on the ratio of oleic acid ($C_{18:1}$) to palmate acid ($C_{16:0}$) in the milk fat (blend of milk from 11 cows; Week 0, no supplementation; week 2, 2 weeks feeding with each 1 kg oilseed; Week 3, 3 weeks feeding with each 1 kg oilseed; Week 4, 4 weeks feeding with each 1 kg rapeseed, 1.5 kg linseed or 1.5 kg sunflower seed (source: Agroscope Liebefeld-Posieux; CH-3003 Berne).

saturated fatty acids is very high in milk fat from cows fed such a diet. An elevated level of saturated fatty acids is responsible for a rather hard cheese texture. In order to achieve a softer texture even in winter, it is possible to supplement the cows' diet with oilseeds such as linseed, sunflower seed and rapeseed. Supplementation with oilseeds results in an increase in the proportion of unsaturated fatty acids, and therefore in a 'softer' milk fat, which leads to a softer cheese texture.

The ratio of oleic acid ($C_{18:1}$) to palmate acid ($C_{16:0}$) may be used to describe the hardness of fat. In winter, with a hay and fodder beet diet, the ratio may reach only 0.5. A supplementation with oil seeds can lead to an increase of the ratio to over 1.0 (Fig. 1). A ratio of >0.8 corresponds to a 'soft' milk fat.

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125 Why does Swiss cheese have a sweet flavour?

M. T. Fröhlich-Wyder and H. P. Bachmann

One of the main characteristics of Swiss-type cheeses is its sweet and nutty flavour. The sweet taste of Emmental cheese is considerably higher than that of other hard cheese varieties. The sweet taste of Swiss-type cheeses originates in the main part from the propionic acid fermentation. The following, which are liberated during lactic and propionic acid fermentation, are very potent taste compounds: acetic, propionic, lactic, succinic and glutamic acids, each in free form and/or as their ammonium, sodium, potassium, magnesium or calcium salts, as well as the corresponding chlorides and phosphates of these cations. Magnesium and calcium propionate are the main compounds that cause the sweet taste of Swiss-type cheese.

Other volatile compounds may contribute to the sweetish note of Swiss-type cheeses. These compounds derive from glycolysis, proteolysis and lipolysis during ripening [**88**]. In addition, furanones, which are responsible for caramel-like flavour, may contribute to a sweetish note. Furanones are products of the Maillard reaction which occurs during heat treatment [**188**].

Non-volatile compounds such as free amino acids, which are liberated during proteolysis, may also contribute to the sweet taste of Swiss-type cheeses, but to a lesser extent. Sweet amino acids include proline, serine, glycine, alanine and others.

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126 What are the causes of the most common flavour defects of Swiss cheese?

M. T. Fröhlich-Wyder and H. P. Bachmann

As we already know, Swiss cheese has a particular dominating flavour due to propionic acid fermentation which is described mainly as sweet and nutty [**125**]. However, a flavour defect may occur that becomes evident only with progressing ripening. The most common flavour defects in Swiss cheese are produced by:

- butyric acid fermentation;
- excessive lipolysis;
- excessive proteolysis.

Butyric acid fermentation is totally undesirable, since lactate fermentation by *Clostridium tyrobutyricum* into butyric acid, acetic acid, carbon dioxide and hydrogen causes the cheese loaf to blow [**91**]. Furthermore, even small amounts of butyric acid cause off-flavours. Therefore, in Switzerland, Emmental cheeses have to be manufactured with milk from cows that have not been fed silage. Feeding cows with silage of low microbiological quality is the primary route of contamination of the milk with spores of *Cl. tyrobutyricum*. As few as 50 spores per litre of cheese milk are sufficient to cause a butyric acid fermentation.

Spores can also be eliminated either by physical treatment, i.e. bactofugation or microfiltration prior to processing, or by the use of additives such as nitrate, lysozyme or nisin in order to restrict germination. However, these additives are not permitted in Switzerland for the production of Emmental cheeses. A particularly serious defect results from the presence of *Clostridium sporogenes*. This species leads to a non-specific and very intense proteolysis, leading to putrid spots in the cheese loaf (Fig. 1).

Lipolysis in Emmental cheese is catalysed by bacterial lipases and the indigenous lipoprotein lipase in milk which is, however, thermolabile and

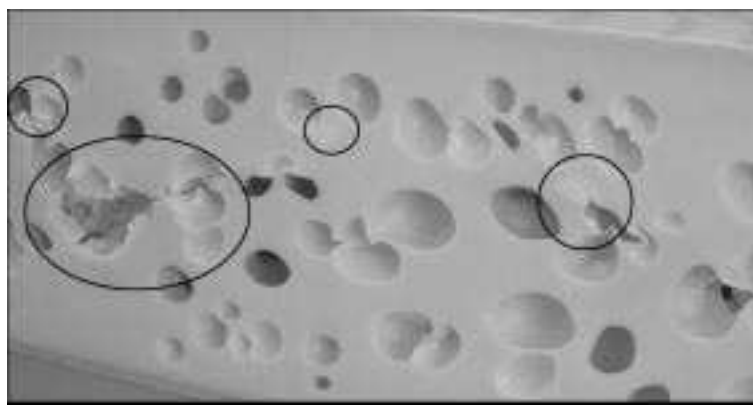

Fig. 1 Intense proteolysis by *Clostridium sporogenes* with putrid spots (source: Agroscope Liebefeld-Posieux; CH-3003 Berne).

therefore its activity is reduced by cooking at temperatures over 50 °C. Lactic acid bacteria have only limited lipolytic activity, with *Streptococcus thermophilus* having the highest. Propionibacteria, in contrast, have lipolytic activity, 10–100 times more than lactic acid bacteria and which is highly strain-dependent. Lipolysis in Swiss-type cheeses is consequently mainly caused by propionibacteria and is generally recognised as necessary to produce typical Swiss cheese flavour. The amount of free fatty acids present varies from 2 to 7 g kg^{-1}. Nevertheless, higher contents give flavour defects such as rancidity (caused mainly by butyric and caproic acids) [**90**]. The release of free fatty acids starts in the warm room simultaneously with the growth of propionibacteria.

Other bacterial, but undesirable, lipases may originate from the raw milk flora. These lipases become especially evident if the raw milk has been stored under unfavourable conditions before processing (too long and at too high temperatures) and these enzymes are usually heat stable.

Excessive proteolysis gives an overripe and sharp taste and a shorter body. This defect becomes particularly evident when a large amount of casein is decomposed into low-molecular compounds and amino acids. The latter are further metabolised to strong flavour compounds, e.g. sulphurous compounds. This is certainly a desirable process in other cheese types, but in Swiss-type cheeses the specific propionic acid flavour should dominate.

Excessive aspartase activity has also a great impact on flavour development [**121**]. Propionibacteria with strong aspartase activity need the amino acid aspartate for this pathway. The more aspartate is available, the stronger is their metabolism. A strong aspartase activity leads also to a stronger propionic acid fermentation and, as a result, to the defect of late fermentation. Consequently, more propionic and acetic acids are liberated, and, if present at excessive concentrations, may also lead to an overripe and sharp taste.

Frequently, the course of proteolysis [**90**] in a cheese loaf varies from one zone to the other, a phenomenon that is due to temperature changes in the cheese loaf during lactic acid fermentation. Since the outer zone cools faster, there often develops a bacterial flora which is proteolytically more active than the microorganisms in the centre of the loaf. This usually leads to cheese defects such as short and firm body, sharp taste, or the development of whitish colour under the rind.

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127 Is Emmental cheese hygienically safe?

M. T. Fröhlich-Wyder and H. P. Bachmann

The original Emmental cheese in Switzerland must be manufactured from raw milk. The contamination of raw milk by pathogenic microorganisms can never completely be excluded, despite intensive efforts at hygiene [**59**, **60**]. Infectious diseases in dairy cattle, contamination of milk during milking, storage, transport or processing present potential hazards. This fact has raised the question of whether Swiss Emmental cheese made from raw milk is hygienically safe. In order to find an answer to that question, the ability of potentially pathogenic bacteria to survive and grow during the manufacture and ripening of Swiss Emmental cheese had been examined. From this research it can be concluded that the hygienic safety of Emmental cheese made from raw milk is comparable to cheese made from pasteurised milk. As it can be seen in Fig. 1, no pathogens can be detected after 1 week of ripening. Already after cooking, there is a remarkable decrease in the number of pathogens.

The number of pathogens decreases in Emmental cheese because of the so-called hurdle technology: each step of the manufacturing procedure of Emmental cheese is a hurdle for the survival and growth of pathogens [**58**, **59**]. The synergistic effect of these steps is responsible for a hygienically safe product. The following technological steps are such hurdles:

- high milk quality;
- short milk storage;

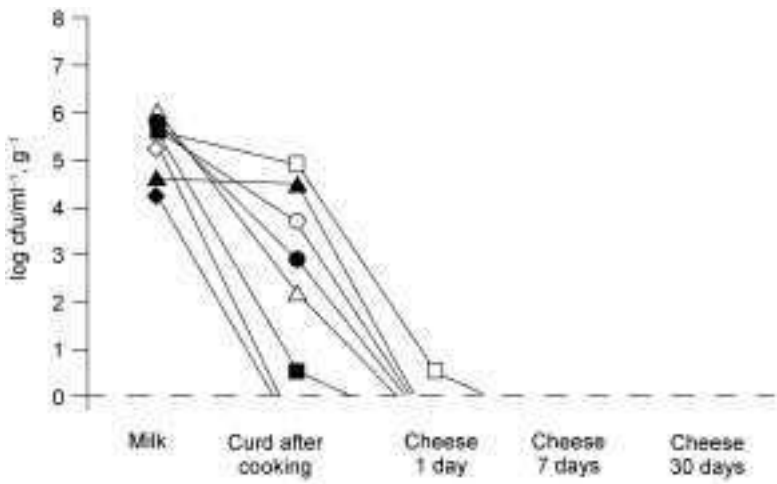

Fig. 1 Survival of *Aeromonas hydrophila* (◇), *Campylobacter jejuni* (◆), *Escherichia coli* (△), *Listeria monocytogenes* (▲), *Pseudomonas aeruginosa* (◯), *Salmonella typhimurium* (●), *Staphylococcus aureus* (□) and *Yersinia enterocolitica* (■) during manufacture and ripening of Swiss Emmental cheese made from raw milk (only data of batches with longest survival are shown). – – – detection limit (Bachmann and Spahr, 1995).

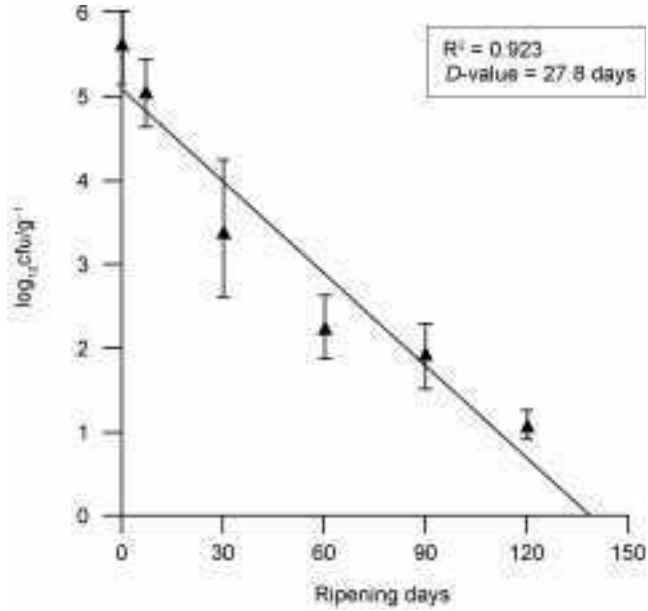

Fig. 2 Inactivation curves for *Mycobacterium avium* subsp. *paratuberculosis* in Swiss Emmental cheese during 120 days of ripening (Spahr and Schafroth, 2001).

- antagonistic starter culture flora;
- rapid acidification;
- antimicrobial effect of lactic acid;
- high cooking temperatures;
- brining;
- long ripening period (more than 120 days).

Another pathogenic agent which could become problematic in cheese production is *Mycobacterium avium* subsp. *paratuberculosis* [**62**]. This bacterium occurs worldwide and is responsible for a chronic enteritis in ruminants, also known as Johne's disease. Crohn's disease, a chronic enteritis in humans, bears considerable similarities to Johne's disease. Studies have shown that a high percentage of people with Crohn's disease are infected with *M. avium* subsp. *paratuberculosis.* Whether the association of this bacterium and the disease is causal or coincidental is not known. But the similarities of these two diseases have raised the question of whether milk, among others, could transfer this bacterium. For that reason, the same investigation as described earlier had been carried out with *M. avium* subsp. *paratuberculosis* only. As Fig. 2 shows, there is a decrease in the numbers of *M. avium* subsp. *paratuberculosis* in Swiss Emmental cheese, but to a much lesser degree than other pathogens. Nevertheless, a Swiss Emmental cheese consumed only after 4 months is hygienically safe, comparable to a cheese made from pasteurised milk.

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White-mould cheese

128 Introduction

H.-E. Spinnler and M.-N. Leclercq-Perlat

Surface mould-ripened soft cheeses are characterised by the presence of a felt-like coating of white mycelia due to the growth of *Penicillium camemberti* on their surface. The presence of this mould gives these cheeses a characteristic appearance, as well as a typical aroma and taste and also leads to a more complex ripening pattern than in other varieties of cheese. These cheeses are becoming increasingly popular with consumers and the demand for them increases.

When the microbial quality of the milk is adequate (microbial population $<10^4$ cfu ml^{-1} is recommended) raw milk can be used even if most of the milk now used for the manufacture of Camembert is thermised [**13**] or pasteurised [**10**, **11**]. After this treatment, the milk is prematurated with lactic acid bacteria in order to get the right mineral equilibrium [**4**] and to acidify the milk before rennetting. Depending on the type of cheese, duration of prematuration may be up to 24 h at 10–12 ºC. When the pH has decreased (6.45–6.5), rennet is added and a soft curd is formed. The temperature of the vat is between 20 and 30 ºC and this temperature stimulates the growth of lactic acid bacteria. The pH continues to decrease quite quickly, leading to demineralisation of the caseins. Owing to the low pH, most of phosphate and calcium will leave the curd to the whey and thus will be removed in drainage. This demineralisation will have two main effects: (i) the buffering capacity of the curd will be low [**22**] and so the pH will change quite easily during ripening and (ii) there are few ionic bonds between the caseins, and the chemical bonds between them are mainly weak. Consequently the curd obtained is very fragile and should be handled with care.

The size of the cheeses made with this type of curd will be small. In some cases, after coagulation, the curd is cut gently with knives or wires. Residual lactose and a part of the lactate can be removed by washing the curd by adding some water to the vat after the cutting of the curd (stabilised curd technology). Traditionally, the curd is then scooped with a ladle to fill the moulds. The curd is very permeable and the whey is easily removed by gravity but the moisture content of the cheese stays quite high (up to 65%). Acidification continues during draining and the final pH after moulding is low (4.4–4.8). Lactic acid bacteria are inhibited by the low pH and the lactose is not completely utilised at the end of the moulding. The residual lactose and the lactate are consumed during the first phase of ripening. At the end of moulding, the cheeses are salted with dry salt or in brine [**41**] in order to have a salt content at the end of ripening of 1.6–1.7%. After salting the cheeses are placed in a room with a low humidity level (about 85% RH) in order to dry the surface of the curd, which is a very important step, permitting the development of the desired microbial flora.

There are two phases in soft cheese ripening. Below pH 5.8, only an acidophilic flora is able to grow and the cheese is deacidified. During this first phase, yeasts (typically *Debaryomyces hansenii*, *Kluyveromyces lactis*), *Geotrichum candidum* and *Penicillium camemberti* raise the pH by consuming the lactate for their growth. When the pH is over 5.8, a second phase of ripening commences; bacteria adapted to the high salt content of the cheese such as *Staphylococcus* or coryneforms will start to grow. This second phase can be considered as a maturation period where the breakdown of proteins and lipids will contribute to some of the typical flavours. The quality of the cheese will depend on the balance between the different species present at the surface and to their enzymatic activities.

Process anomalies or the occurrence of abnormal microorganisms are among the more common origins of problems associated with mould-ripened cheeses. These will render the microorganisms unable to colonise the cheese surface or will bring about diffusion problems from the centre of the cheese to its surface or in the reverse direction.

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129 Why does the surface pH in Camembert cheese not increase adequately?

H.-E. Spinnler and M.-N. Leclercq-Perlat

The main microorganisms able to raise the pH of Camembert type cheeses are yeasts and *Penicillium camemberti.* Normally, owing to the low buffering capacity of the curd [**22**], consumption of lactate changes the pH at the surface very easily, during the first phase of ripening. Yeasts and *G. candidum* develop quickly immediately after moulding, consuming residual lactose and starting to consume the lactate produced by lactic acid bacteria. The main yeasts found in these cheeses are *Debaryomyces hansenii*, *Kluyveromyces lactis* and *Kluyveromyces marxianus*. *G. candidum* grows somewhat later than the yeasts. Other species such as *Saccharomyces cerevisiae*, *Yarrowia lipolytica* and *Candida* spp. are also sometimes present. These organisms may have different metabolisms; for example *Kluyveromyces* spp. will consume residual lactose first and, only after its exhaustion, lactate will be metabolised though *Debaryomyces* will consume both simultaneously. In mould-ripened cheese it is not uncommon that the pH will increase slowly during the first 5 days, but the growth of *P. camemberti* will cause a very fast increase in pH at the surface (Fig. 1). The pH increases from less than 5 to 7.5 in less than 2 days. However, the increase in pH inside the cheese is due to a migration of lactate from the core to the cheese

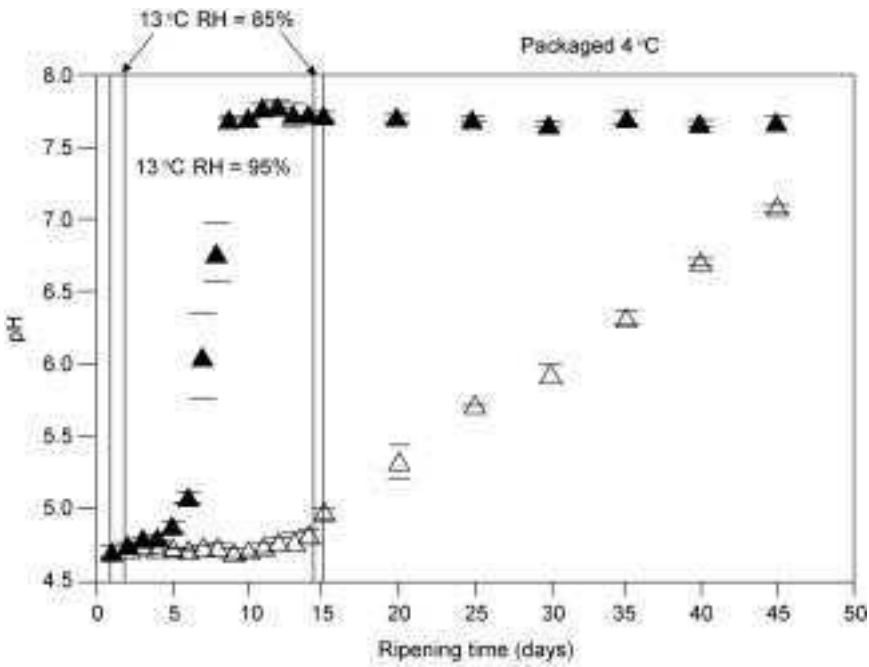

Fig. 1 Change in pH at the surface (▲) and in the core (△) during the ripening of Camembert cheese (Leclercq-Perlat *et al.*, 2004).

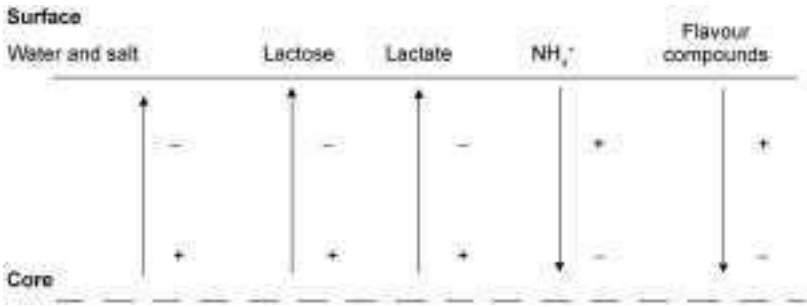

Fig. 2 Transfer of solutes inside Camembert-type cheese from regions of high (+) to low (−) concentration (Leclerq-Perlat *et al.*, 2000).

surface and diffusion of ammonia from the surface to the core (Fig. 2). This mass transfer is quite slow and so, the curd pH rises quite slowly in the core of the cheese (Fig. 1).

Consequently if the pH of a Camembert-like cheese does not increase as quickly as it should, it could be for any of three main reasons:

1. *The buffering capacity of the curd is too high*. This can be due to too high a pH at the end of moulding, leading to an excessive concentration of minerals present in the curd after draining. Thus, even if a part of the lactate is consumed, the pH stays quite low. It is very important to control pH at the different steps of cheesemaking, particularly at renneting, cutting and moulding, at the end of draining (up to 24 h) and just before salting.
2. *Poor development of deacidifying flora*. The acid-tolerant and acid-consuming microflora may not grow well and so the lactate consumption will not be adequate. Spraying *Penicillium* or *Geotrichum* spores onto the surface of the cheese may help to start the deacidification process.
3. *Poor solute transfer*. Poor solute transfer (mainly lactate transfer) is due mainly to limited water transfer. Several reasons can be considered. For example, if there is too much fat or if the curd is too dry, the water mobility is limited. An increase in fat content has a general impact on the solute behaviour inside the cheese.

In conclusion, it is noticeable that an excessively high pH (over 4.8) at the end of moulding will cause a whole series of problems that starts with more difficult drainage of the curd and a more moist cheese. This problem will consequently change the mobility of water and the lactate transfer in the curd. The buffering capacity will also be changed because a high concentration of minerals will still be present, which will cause pH buffering.

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130 Why is mould development on Camembert or Brie inadequate?

H.-E. Spinnler and M.-N. Leclercq-Perlat

Some yeasts, in starting to hydrolyse proteins and fat, will improve the nutrient content of the curd and thus help the growth of *Penicillium camemberti*. After 5–7 days of ripening, the growth of the mycelium of *P. camemberti* is observed and a white felt covers the entire surface of the cheese. The growth of *P. camemberti* is extremely fast compared with that of the other members of the ripening flora. In 2–3 days, its growth is complete and its metabolism changes the surface pH, exhausts lactate at the surface and produces a large amount of CO_2 which may change the gaseous environment of the ripening cellar. It is clear that *P. camemberti* determines many of the principal quality attributes of mould-ripened cheeses and so a mediocre mould growth is a major technological problem in these varieties.

Growth of *P. camemberti* changes the pH and causes the breakdown of lactate, lipids and proteins by its different enzymatic activities. As a consequence of the change in chemical composition of the cheese through its activities, growth of *P. camemberti* gives the appearance, the colour and the texture and contributes significantly to the flavour. Starter companies select the strains of *Penicillium* based on different properties including: growth rate and the capacity to cover the surface, the density and the thickness of the mycelium, the rate of spore germination and the stability of the appearance of the cheese and its colour with time.

Several problems can be suspected when poor growth of *P. camemberti* is observed: (1) the surface of the cheese is too moist, (2) the number or the germination rate of the spores is insufficient, or (3) competition with other organisms does not permit a good growth of *Penicillium*.

Moulds do not like 'to have their feet in water'; this is an important rule among makers of mould-ripened cheese. Consequently, drying cheese surface at the end of moulding is an important step. This is normally done in 1–3 days at less than 85–90% relative humidity at 12–14 ºC. The development of yeasts and *Geotrichum candidum* during the first 5 days of ripening will contribute to drying the surface. However, a very strong development of *Geotrichum* may also hinder *Penicillium* growth. To limit *Geotrichum* growth, we suggest that cheeses be kept at a low temperature (4 ºC) for 24 h. Delaying *Geotrichum* growth will allow time for the spores of *Penicillium* to germinate.

It has also been reported that an atmosphere containing ammonia may slow *Penicillium* growth, although CO_2 in atmosphere at less than 4% will increase its development and activity.

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131 Why does Camembert or Brie have a grey or brown colour?

H.-E. Spinnler and M.-N. Leclercq-Perlat

Initially, two species of white *Penicillium* mould were distinguished, *Penicillium caseicolum* and *Penicillium camemberti*. *P. caseicolum* was considered to be a white mutant of *P. camemberti.* Today consideration of colour is, however, an insufficient criterion to designate a new species and the only name used is *P. camemberti.* However, different forms of *P. camemberti* can be distinguished:

- a form with a fluffy mycelium, white at first and becoming grey-green;
- a form with 'short hair', rapid growth, white close-napped mycelium;
- a form with 'long hair', rapid growth; white, loose, tall mycelium;
- 'Neuchatel form' with vigorous, rapid growth, giving a thick white-yellow mycelium.

Only the white strains of *P. camemberti* are used for cheesemaking. Commercial strains differ mainly in the rapidity of their growth on cheeses and the density of their mycelium. *Penicillium* spores are produced by specialised companies after culture in a fermenter or in 'Roux flasks'. Spores can be added to the cheesemilk or applied to the cheese surface, after salting, by spraying with a dilute suspension of spores. Spores can also be mixed with the salt (when dry salting is used). In these two last cases the level of inoculum is more difficult to control and these methods are often used to complement inoculation of the milk.

The choice of the *P. camemberti* strain has an important impact on the final colour of the cheese. Depending on the technology, especially ripening conditions, the cheese may have different colours. It is important to consider that the mycelium and the spores may have different colours. Today most of the industrial *Penicillium camemberti* strains used have a low ability to sporulate and this is a factor that permits them to have a more constant colour.

Two main reasons may be responsible for the development of *brown* colour in Camembert. Usually, this colour appears late in ripening and is related to lysis of a part of the *Penicillium* mycelia that release enzymes such as polyphenol oxidases, which are very common in fungi. The activity of these enzymes leads to oxidation of phenolic compounds, often tyrosine or compounds derived therefrom, which may polymerise and produce brown pigments. The brown colour appears especially at places where the mycelium can be easily broken, such as at the edge of the cheese. Development of brown colour is significantly increased by Mn^{2+} which is a cofactor of many polyphenol oxidases. Moreover, the proteolytic ability of the *Penicillium* will enhance browning by providing free tyrosine and the use of strains with a low proteolytic activity may help to prevent this problem. If the browning happens quickly, it is often related to a bad physiological status of the *Penicillium* mycelium, which lyses rapidly; this is often associated to a thick rind that may even detach quite easily from the curd. This defect has been attributed to an insufficient drying of the curd when the

Penicillium starts to grow. Another type of yellow/brown colour may appear in the form of small spots and in this case it may be due to the development of coloured bacteria such as *Brevibacterium linens* [**142**]. These spots are often related with flavours typical of traditional Camembert cheese and cannot be considered as a defect.

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132 Why does the texture of Camembert or Brie remain too hard?

H.-E. Spinnler and M.-N. Leclercq-Perlat

Three main factors are involved in texture of the centre of surface mould-ripened cheeses: the pH, proteolysis and lipolysis. The increase in pH is very important for changing cheese texture. The increase in pH causes a resolubilisation of the caseins, which gives a smooth texture to the product. An excessively low pH (pH < 5.5) in the core gives a rough and hard texture.

Two main parameters influence change in pH:

1. The transfer of lactate from the cheese core to the surface; this is determined by the rate of lactate uptake at the surface by the microflora and by the permeability of the curd. Parameters such as pH at drainage, moisture and fat content are probably very important in the lactate transfer from core to the surface but very little is known about the kinetics of this process.
2. The buffering capacity of the curd is determined by components in cheese (e.g. proteins, phosphate and citrate). Mainly due to solubilisation of the phosphate under acidic conditions, the lower the pH at the beginning of moulding and the quicker the acidication rate during moulding, then the lower will be the buffering capacity of the curd [**22**]. A low buffering capacity will favour the deacidification process at the surface, too high a buffering capacity, through a high content of phosphate in the curd will, conversely, hinder deacidification.

A consequence of lactate uptake and the subsequent increase in pH, is resolubilisation of the casein. Caseins become more hydrophilic above their isoelectric pH and so they bind more water. A consequence is a change in texture from a rough and dry texture at low pH to a softer and more creamy texture over a cheese pH of 6.0. This is the reason why the texture usually changes more quickly just under the rind, where the pH increases the quickest, than further into the core of the cheese. Vassal *et al.* (1986) showed a linear relationship between the pH and the firmness of the cheese.

During ripening, the proteolytic and lipolytic activities of the lactic acid bacteria, yeasts and filamentous fungi will change the size of the macromolecules (particularly proteins) and help their solubilisation, but these two parameters are much less important than the pH change.

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133 Why does Camembert-type cheese become too liquid?

H.-E. Spinnler and M.-N. Leclercq-Perlat

Excessively liquid Camembert-type cheese may be due to a high pH. This defect happens mainly just below the surface of the cheese, where the surface microflora raises the pH quickly. Usually this phenomenon is accompanied by strong proteolysis and lipolysis and some ripening microorganisms may help this process, as is the case for *Geotrichum candidum* and *Yarrowia lipolytica*. These two species when present at too high numbers can be responsible for the development of marked defects. In raw milk cheeses, *Geotrichum* is considered the microorganism responsible for many defects such as fragile cheeses or too liquid cheeses. Since thermisation of milk is now common, it is possible to control the level of *Geotrichum* at low numbers (25 spores/ml or less) and so it is possible to prevent these problems and to benefit from the advantages of this species in terms of flavour. The effects of *Y. lypolytica* are less well known but the experimental growth of this species on cheese curd lead to its rapid solubilisation. *Y. lypolytica* is quite often present at the surface of soft cheeses but usually at low numbers.

As has been explained previously, low buffering capacity [**22**] and high humidity (which will favour lactate transfer) may cause a rapid change in pH. Low buffering capacity can be attributed to an excessively low pH at the beginning of moulding or to very quick acidification during moulding [**17**] leading to the loss of a larger amount of minerals in the whey than expected.

A change in the ripening temperature will change the enzyme activities as well as the speed of organic substrates diffusion (e.g. lactate) and may have a strong effect on texture. In particular, an excessively high ripening temperature may cause the cheese to be too liquid, particularly under the rind.

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134 How are spoilage fungi controlled in white-mould cheeses?

H.-E. Spinnler and M.-N. Leclercq-Perlat

The main defects observed due to spoilage fungi are the appearance of blue moulds [**137**, **143**]. These moulds, whose spores are grey, blue or green may belong to several species of the genus *Penicillium* including *P. expansum*, *P. roqueforti, P. janthinellum, P. brevi-compactum* and *P. viridicatum.* All cases described in the literature involve contamination from the environment including wrapping materials. Other moulds may produce colours such as purple blots which have been attributed to *P. funiculosum* or brown blots attributed to *P. bruneoviolaceum. Cladosporium herbarum* may develop dark green or black spots; this organism is sensitive to low pH and it grows only when the pH of the cheese surface is high. *C. herbarum* is very resistant to low temperature; it is very common in cold rooms, on the ceilings of the ripening chambers and in air conditioning ducts. Another mould, *Scorulariopsis brevicaulis*, produces blots with colours from beige to purple. As this organism is cellulolytic, the source of contamination is very often the wrapping papers stored in humid conditions.

The hygiene status of the plant is the first point to check. Hazard analysis critical control point (HACCP) systems [**66**] are now applied in most cheese factories and this approach helps to identify the critical points responsible of the entrance and development of undesirable microorganisms into the plant. With HACCP, monitoring by microbial counts and identification at the appropriate critical control points is necessary. In case of repeated infections, disinfection by an appropriate gas (fumigation) may help restablish a more normal flora. If these measures are not sufficient, increasing the flow of sterile air in the ripening chambers may help to prevent the occurrence of spores of blue fungi.

In some cases in spite of the drastic cleaning methods and the use of HACCP, unexpected fungi may develop in the factory. These problems are very difficult to solve when spores of unexpected fungi, resistant to disinfectant, often present on the surfaces as biofilms, become distributed throughout a factory. A way to limit the germination and growth of these spores is to spray quick-growing yeasts into the rooms to compete with undesirable fungi. The use of *Debaryomyces hansenii* for this specific objective has already been successful in cheese plants.

Keeping the surface of the cheese dry and the use of spores of *Penicillium camemberti* able to germinate quickly can also limit spoilage by unexpected filamentous fungi [**135**].

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135 How may the 'toad-skin' and 'cat-hair' defects of Camembert cheese be solved?

H.-E. Spinnler and M.-N. Leclercq-Perlat

The 'toad-skin' and 'cat-hair' defects originate from uncontrolled development of fungi [**134**]. Specific strains of *Geotrichum candidum* are responsible for 'toad skin', which is characterised by a web of ridges at the cheese surface. This organism is able to produce arthrospores (Fig. 1) which form chains. When these arthrospores are present at high density, the chains join together and are able to create ridges by pushing one against the other, causing the development of 'toad skin'. In some cheeses, 'toad skin' is well appreciated because, usually, it indicates a mature cheese but in some other types of cheeses, when a very white and smooth surface is desired, it is a defect.

Very soon after manufacture, yeasts grow on the surface forming a dense layer about 200 μm thick; *Kluyveromyces lactis, Saccharomyces cerevisiae* and *Debaryomyces hansenii* are the most common yeast species. *G. candidum* appears just after these yeasts but its growth is very sensitive to salt [**46**]. In the past, *G. candidum* caused concern to cheese technologists because of its proteolytic activity which is associated with the 'toad skin'-like surface of the cheese. However, it is now known that through its enzymatic systems, it also plays a major role in the development of flavour. Now, selection of strains of *G. candidum* that do not cause 'toad skin', and use of low inoculum levels (down to 25 spores/ml), have led to a widespread use of this species. In order to improve the organoleptic quality of Camembert made from pasteurised milk, selected strains of *G. candidum*, yeasts and coryneform bacteria are generally added to the cheesemilk, giving a product closer to traditional Camembert, and closer to the expectations of most consumers. The use of dry salting may stop the growth of *G. candidum* for a while. *Geotrichum* likes quite high temperatures (25 °C), and low ripening temperatures may hinder its growth. If *Geotrichum* is added to the milk for its positive effects, the choice of the strain and the level of inoculum have a major impact on the properties of the resulting cheese. The inoculation of

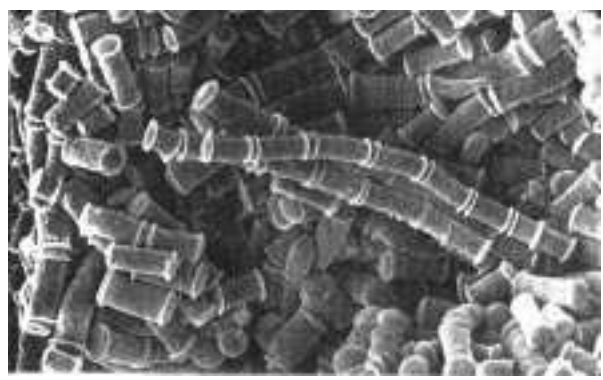

Fig. 1 Arthrospores of *Geotrichum candidum* (5000× magnification).

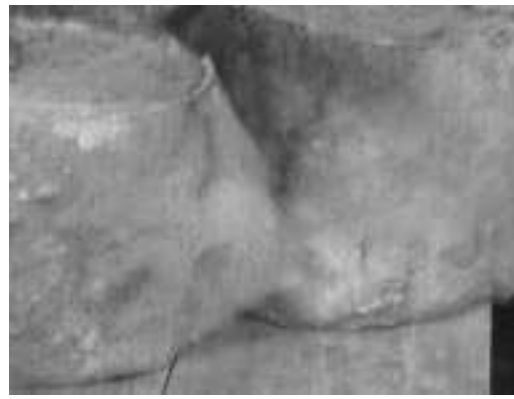

Fig. 2 Development of 'cat hair' on a Blue cheese in an Australian small factory (photo: H.E. Spinnler).

G. candidum should be about 25 spores/ml in the milk, that is to say about 100 times less than the level of inoculation of *Penicillium camemberti*.

'Cat-hair' defect is due to fungi of the genus *Mucor* (*M. racemosus*, *M. sphaerosporus*, *M. circinelloides*, *M. fuscus*, *M. hiemalis*, *M. plumbeus*) and sometimes *M. mucedo* or *Rhizopus stolonifer*. These organisms can produce quite long hyphae; their grey colour (Fig. 2) gives them the appearance of cat's hair and reduces the quality of the cheeses on which they grow. Organisms of the genus *Mucor* are very well adapted to grow on very humid surfaces and so, when the atmosphere is contaminated by *Mucor* spores and when the cheese is not dry enough, the competition between *Mucor* and *Penicillium* favours *Mucor*. Drying the cheese surface is a good method to prevent the growth of these undesirable moulds. Some *P. camemberti* and *G. candidum* strains are able to inhibit the growth of *Mucor*, though the mechanism of inhibition is still unclear; some of these strains are available on the market.

Finally, it has been reported that pH at the end of moulding > 4.8, relative humidity of dry room > 85% and ripening at relative humidity > 91% are factors that favour development of the 'cat-hair' defect. To avoid development of *Mucor,* the cheeses should be kept at least for 1 day at 16–18 ºC and with 75–80% relative humidity to inhibit the germination of *Mucor* spores.

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136 What causes bitterness and other flavour defects in Camembert?

H.-E. Spinnler and M.-N. Leclercq-Perlat

In general, the accumulation of short hydrophobic peptides in cheese is the major source of bitterness [**89**], and mould-ripened varieties are not an exception. The very strong proteolytic activity of *Penicillium camemberti,* especially its acid protease, as compared to its ability to break down peptides, causes the accumulation of bitter peptides. It has been shown using a trained panel [**79**] that the increase in peptide concentration is correlated to the bitter taste descriptor. On the other hand, *Geotrichum candidum* has high peptidase activity (carboxypeptidase and aminopeptidase) and it has been shown that when *Geotrichum* is used in association with *Penicillium*, the cheeses are significantly less bitter. The use of lactic acid bacteria with low or medium proteolytic activity [**23**] may also prevent the formation of bitter peptides.

A few other flavour defects in this Camembert-type cheeses have been reported. Some years ago, in summer, quite often mould-ripened cheeses made using stabilised curd technology had a celluloid taste. It has been shown that *P. camemberti* can be responsible for the production of styrene. When easily usable substrates such as lactose or lactate are exhausted, *Penicillium* attacks proteins and fat. The oxidation of certain amino acids such as phenylalanine is catalysed through action of phenylalanine ammonia lyase. Phenylalanine can be degraded to styrene probably with cinnamic acid as a metabolic intermediate. The addition of phenylalanine labelled with ^{13}C on its benzene ring in a culture medium for *P. camemberti*, together with low concentrations of glucose, leads to the accumulation of styrene with the label on its benzene ring. All conditions resulting in the quick exhaustion of the easily usable substrates (lactose and lactate) lead to the production of styrene. The substrate uptake is more intense at the cheese surface, where *Penicillium* grows, than within the cheese. The concentration gradient causes lactate to migrate from inside the cheese to the surface. If lactate uptake at the surface is quicker than the diffusion of the lactate from within the cheese to the rind, as it is the case for ripening temperatures >15 ºC, the starving *Penicillium* starts to break down the other substrates of the medium such as fat or proteins. This also occurs if curd is washed to remove a part of the lactose and lactate in order to speed up the ripening reactions, or when the enrichment of the curd in fat limits diffusion of lactate. It has been reported that styrene is produced mainly in case of starvation but only by certain strains of *P. camemberti* (Spinnler *et al.*, 1992).

Penicillium camemberti has also been reported to produce geranium-like, musty, potato-like or earthy mushroom flavours. Most of the compounds involved in these defects are related to the catabolism of unsaturated fatty acids. The mushroom-like odour, which can be a desirable note in some mould-ripened cheeses, becomes a defect when the level of this olfactive note is too high. Compounds such as 1-octen-3-ol or 3-octanone are produced by *Penicillium* and have this olfactive property. Geranium odour is related to the

production of 1,5-octadien-3-one and 1,5-octadien-3-ol, while the earthy note is related to 2-methyl isoborneol. Finally, the potato-like odour was attributed to 2-methoxy, 3-isopropyl pyrazine produced by certain strains of *Penicillium*.

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Blue cheese

137 Introduction: what are Blue cheese varieties?

Y. Ardö

Blue cheeses get their typical appearance and flavour from growth of the blue mould *Penicillium roqueforti*. Several different varieties of Blue cheese have been developed over time, each with its own characteristics involving milk of different animals and different manufacturing methods. Worldwide, the best-known blue-veined cheese varieties today are Gorgonzola, Roquefort, Stilton and Danablu (Table 1).

Conidia spores of *P. roqueforti* are added to the cheesemilk, sprayed on the fresh curds before moulding or may occur naturally in the cheesemilk as is often the case for raw milk cheeses. Mesophilic or thermophilic starter bacteria [**18**] are added to the cheesemilk to acidify the curd. The fresh cheeses are produced to contain a relatively high amount of moisture [**34**, **35**, **36**] and thereby the curd contains much lactose that is converted into lactic acid; hence fresh Blue cheeses are more acid than many other cheeses. Blue cheeses are pierced by needles to let in air, because oxygen is essential for development of the mould in the

Table 1 Properties of the four most well-known Blue cheeses

Cheese	Origin	Milk	Moisture (%)	Fat (%)
Gorgonzola	Italy	Cow, pasteurised	42–50	29–31
Roquefort	France	Ewe, raw	42–44	29
Stilton	Great Britain	Cow, pasteurised	37–42	32–35
Danablu	Denmark	Cow, thermised	42–47	29–31

interior of the cheeses. The starter may sometimes contain gas-forming bacteria, the products of whose metabolism open up the cheese structure and facilitate germination and growth of the mould.

After a couple of weeks, the blue mould with its potent lipases and proteases completely dominates the ripening process. Yeasts have been isolated from some varieties of Blue cheese; however, except for a stimulating effect on a secondary bacterial smear microflora [**141**, **142**], their role in the ripening process is not clear. Blue cheeses are salted in brine or by the surface application of dry salt, and their salt content is high compared with other types of cheeses (3–3.5% salt in cheese) [**39**]. The rind is white and quite dry because of the salt, which inhibits growth of the mould in the outer parts of the cheese. Some cheeses are ripened with a microbial surface microflora [**141**, **142**] (e.g. Gorgonzola) while others have dry surfaces during ripening (e.g. Danablu); Blue cheeses are typically ripened for at least 6 weeks.

The body of a blue-veined cheese is white or yellowish with blue-green channels and veins after the growth and sporulation of the mould within the piercing channels and over cavities of the cheese. Blue cheeses made from ewe's milk are whiter than those made from cow's milk [**5**, **14**]; however, homogenisation [**32**] of the cream for cow's milk cheese has been introduced in production of Danablu to make it whiter. An interesting beneficial consequence of homogenisation is increased lipolysis [**90**] and thereby a more intense flavour. The consistency of Blue cheese is at first brittle and crumbly, but becomes softer and spreadable as ripening advances. Proteolysis is more extensive in Blue cheese than in most other varieties. The mould contributes to proteolysis through their highly active and broadly specific proteolytic enzymes, and because pH increases when the lactate is consumed by yeasts and moulds, proteolytic enzymes from milk (plasmin) and starter bacteria (lactocepin) became more active.

The typical flavour of Blue cheese is sharp and piquant as a result of the activities of mould enzymes on milk fat (lipolysis) during ripening producing free short-chained fatty acids ($C_{4:0}$–$C_{12:0}$) and methyl ketones (2-heptanone, 2-nonanone). Also esters and lactones contribute to the large variation in the typical flavour of different Blue cheese varieties.

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138 Why does Blue cheese develop brown spots?

Y. Ardö

During ripening, several biochemical processes [**88**] contribute to the development of typical structure and flavour of each Blue cheese variety. Some of the processes may produce pigment if the enzymes needed are present and the environment in the cheese stimulates its production. Brown spots in a Blue cheese are commonly of microbial origin and may be developed by contaminating yeasts or moulds. It may also be a result of highly active *Penicillium roqueforti* in long-ripened cheese, and thermophilic starter bacteria may also be involved.

Salt-tolerant yeasts may grow to quite high numbers (10^4–10^6 cfu ml^{-1}) in the brine used for some Blue cheese varieties. The complex yeast flora varies considerably between dairies but commonly *Debaryomyces hansenii* (*Candida famata*) is the dominating species [**141**, **142**]. Yeasts in Blue cheese may also originate from the raw milk. Neither of these two sources of yeasts is under the control of the cheesemaker and contamination of species or strains, which produce brown pigments, may occur, which may lead to the development of brown sports. Highly hygienic practices in all steps of milk and cheese production minimise this risk [**66**].

Contaminating moulds [**134**] in Blue cheese that may cause brown spots are mainly of the species *P. commune* or *P. nalgiovense*, but also the newly discovered species *P. caseifulvum*, which has been frequently found in cheese curd, brine and on the cheese surfaces. *P. caseifulvum* is sensitive to CO_2 and therefore it grows mainly on the cheese surfaces where it causes brown spots.

Thermophilic starter bacteria [**18**] and yeasts may have the ability to oxidise tyrosine to dopamine that then may be polymerised into the brown pigment melanin stimulated by changes in the reduction–oxidation potential of the cheese during ripening. This discoloration is typically seen inside the cheese some centimetres from the cheese surfaces, and the reddish-brown area sometimes disappears after being exposed to air for a short time. If it is not possible to omit the strains responsible for the pigment production, the problem must be solved by altering the cheesemaking procedure. The actions taken to prevent the discoloration, however, commonly also influence the flavour development negatively. More research is needed for finding the right balance of the different activities of the complex microflora of Blue cheese.

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139 How may spoilage fungi be controlled in Blue cheese?

Y. Ardö

Blue cheese is a heterogeneous microenvironment comprising different habitats for microorganisms in the core with its fissures and piercing channels and on the surfaces of the cheese. A complex microflora develops during ripening and adapts to the pronounced pH and NaCl gradients and the large variation in content of O_2 and CO_2. Fungi other than the desired *Penicillium roqueforti* may colonise Blue cheeses and grow well, especially on its surfaces. Spoilage of cheese due to growth of contaminating moulds [**134**] causes formation of off-flavours and mycotoxins [**68**] as well as possible discoloration of the cheese.

The most important spoilage fungi of Blue cheeses are *Penicillium* species other than *P. roqueforti*, including *P. commune* and *P. nalgiovense*. Of special interest is the newly discovered species *Penicillium caseifulvum,* which has been frequently found on surfaces of Blue cheeses. *P. caseifulvum* has also been found in various Blue cheese dairies, where it was isolated from cheese curd (100 conidia/g), brine (10–500 conidia/g) and from the surface of Danablu (100–1000 conidia/g). *P. caseifulvum* is sensitive to CO_2, and therefore it grows only on the surface of the cheese, where it may cause discoloration in the form of brown spots. Manufacturing routines must be developed to keep the contaminating *Penicillium* species out, but also more robust and competitive *P. roqueforti* strains should be used as cultures.

Contamination of Blue cheese by *Geotrichum candidum* may cause inhibition of the growth and sporulation of *P. roqueforti*, resulting in white areas without blue veins, which affect the quality of the cheese significantly. This emphasises the importance of good manufacturing practice to prevent contamination by *G. candidum.*

Yeasts are potential adjunct cultures [**18**] that may secure the microenvironment by assimilating residual carbohydrates and organic acids. Very careful selection of strains, however, is crucial to avoid undesirable interactions between the different groups of microorganisms, and to avoid uncontrolled detrimental enzymatic activity leading to the production of pigments or undesirable flavour. *Debaryomyces hansenii* [**129**, **142**] is the yeast species most frequently isolated from Blue cheese, but it is rarely used as an adjunct culture. *D. hansenii* strains will not enhance proteolysis or lipolysis significantly, but may create a stable microenvironment, and protect the Blue cheese against undesired microbial growth during ripening by assimilation of residual carbohydrates and organic acids.

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140 Why does Blue cheese not develop adequate veining?

Y. Ardö

Blue cheese [**137**] develops blue-green veining during ripening as a result of growth and sporulation of *Penicillium roqueforti*. After the piercing that provides *P. roqueforti* with the small amounts of oxygen it needs, the spores easily germinate and grow in the curd, which has been acidified by starter bacteria [**18**] during the first 24 h from the start of production. Strains of *P. roqueforti* have been shown to grow in an atmosphere with an oxygen content as low as 0.5% provided the CO_2 concentration is not higher than 20%. *P. roqueforti* tolerates CO_2 better than many other moulds.

If the cheese is made from pasteurised milk [**11**], conidia of *P. roqueforti* have to be added to the cheesemilk or be sprayed on to the fresh curd before it is placed in moulds. In cheese made from raw milk, *P. roqueforti* may form a natural part of the milk microflora.

Penicillium roqueforti starts growing in the centre of the cheese, because of the salt gradient created from salting in brine [**41**] or by surface application of dry salt. In a fresh cheese, the salt content may be 6% close to the surface while it is ~0% in the centre. The mould grows in fissures and in the piercing channels of the cheese. Its growth rate is strongly influenced by the increasing salt content of the core resulting from equilibration of the salt gradient during ripening [**41**]. Growth and sporulation are influenced by water activity and salt content [**39**], and the sensitivity to these factors is strain dependent. Germination of the conidia is stimulated by 1–3% NaCl but higher salt contents inhibit further development and induce sporulation that is crucial for development of the blue veining. It is only the spores of *P. roqueforti* that have a blue-green colour. The surface layer of a Blue cheese may be too salty or too dry for any mould to develop and the outer part of the cheese is usually without blue-veining.

Contamination of Blue cheese by *Geotrichum candidum* may inhibit growth and sporulation of *P. roqueforti* and create areas without veining ('blind spots'), which affect the quality of the cheese significantly. *G. candidum* is frequently found as a contaminant in Blue cheese and it has similar growth behaviour as *P. roqueforti* provided salt is absent. *G. candidum* competes with *P. roqueforti* in the interior of the cheese during the initial stage of ripening, before a sufficient amount of salt has diffused into the core, which inhibits *G. candidum* but stimulates *P. roqueforti*. *G. candidum* may produce and excrete 2-hydroxy-3-phenylpropanoic acid which has a broad-spectrum antibacterial effect. This emphasises the importance of good manufacturing practice in production of Blue cheese to prevent contamination by *G. candidum.*

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Bacterial surface-ripened cheeses

141 Introduction: what are bacterial surface-ripened (smear) cheeses?

P. L. H. McSweeney

Smear-ripened (bacterial surface-ripened, 'washed rind') cheeses constitute an heterogeneous group of varieties, the distinguishing feature of which is the development of a complex Gram-positive bacterial flora on their surface which is seen as a red-orange smear [**142**]. Most smear cheeses are soft with high moisture but a smear is also encouraged to grow on the surface of Gruyère-type cheeses.

The manufacturing protocols of soft smear cheeses are variable but they are often acidified by a mesophilic lactic starter [**18**], are not cooked to high temperatures and are usually brine-salted [**41**]. Soon after manufacture, a range of halotolerant yeasts (e.g. *Kluyveromyces, Debaryomyces, Saccharomyces, Candida, Pichia, Hansenula* and *Rhodotorula*), together with *Geotrichum candidum*, begin to grow on the cheese surface and, by metabolising lactate, cause an increase in pH. This deacidification favours the growth of a complex Gram-positive bacterial flora comprising various coryneform bacteria (*Corynebacterium, Brevibacterium, Arthrobacter*), micrococci and staphylococci. Some of these organisms are pigmented, which leads to the characteristic red-orange colour of smear cheeses. *Brevibacterium linens* is widely used in smear inocula but recent research has suggested that this organism is usually a minor component of the smear flora.

The number of smear organisms may reach 10^{11} cfu cm^{-2} at the cheese surface and their enzymes and metabolic activities contribute greatly to the flavour of smear cheeses. The smear initially develops as a series of colonies but

the surface of the cheese is regularly washed with a brine solution during ripening, thus distributing the microorganisms evenly over the cheese surface. Although enzymes diffuse very poorly through cheese curd, volatile flavour compounds and other products of smear metabolism do diffuse through the cheese and influence its flavour. Soft smear cheeses are generally very strongly flavoured. Because of the role in ripening played by their surface flora, soft smear cheeses are usually small with a high surface area to volume ratio and thus ripen quickly.

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142 What organisms grow on the surface of smear cheeses?

J. J. Sheehan

After cheese manufacture and salting/brining, smear cheeses [**141**] are ripened under conditions of high humidity and at temperatures of 10–16 ºC. The cheeses may be washed or brushed with a dilute brine solution, which may be inoculated with smear microorganisms. These conditions promote the growth of a surface microflora.

Yeasts

Yeasts are the initial organisms to grow on the surface of smear-ripened cheeses directly after manufacture and salting/brining. The conditions of low pH (~4.9–5.2), relatively low ripening temperature and high salt concentration on the cheese surface favour their growth. Although considerable variation occurs, the most prevalent yeasts reported in many smear cheeses include *Debaryomyces hansenii*, *Candida* spp., *Trichosporon* spp., *Yarrowia lipolytica*, *Kluyveromyces* spp., *Rhodotorula* spp. and *Torulaspora* spp. *Geotrichum candidum* is also prevalent and has the characteristics of both a yeast and a mould. A succession of different species may also occur during ripening.

Yeast growth on the cheese surface serves two functions. Firstly yeasts deacidify the cheese surface by the metabolism of lactate to CO_2 and H_2O and by the deamination of amino acids and the production of NH_3. This leads to an increase in pH of the cheese surface with the development of a pH gradient between the surface and core of the cheese. The increased pH on the cheese surface makes conditions more favourable for the growth of salt-tolerant bacteria. Deacidification is dependent on both the numbers and strains of yeasts present. Secondly, yeasts produce compounds that are stimulatory to the growth of the bacterial smear flora. These compounds include products of proteolysis and vitamins synthesised by the yeasts (e.g. pantothenic acid, niacin, riboflavin).

Staphylococci and micrococci

After growth of yeasts, growth of a progression of bacteria occurs during smear development; staphylococci and micrococci grow early in ripening generally followed by coryneform bacteria. Both staphylococci and micrococci can grow in the presence of 10% salt and they are also acid tolerant and may grow at pH < 6.0. Staphylococci are more important than micrococci and have been reported to account for 5–25% of total counts in certain smear-ripened cheeses. They have been reported at levels of 10^5 cfu ml^{-1} in cheese brines.

Coryneform bacteria

'Coryneform bacteria' is a collective term for *Arthrobacter, Brevibacterium, Corynebacterium* and *Microbacterium* spp. These bacteria are present in high numbers on the surface of smear-ripened cheeses.

Brevibacterium linens has been reported to account for 1–30% of the bacteria on the surface of smear-ripened cheese. Although research has shown that the sensory properties of smear-ripened cheese may not be affected by low numbers of *B. linens*, it remains an important component of the smear microflora due to its proteolytic and lipolytic enzymes, its production of pigments that influence cheese colour and its production of thiol compounds that influences cheese flavour. *B. linens* is halotolerant with optimum growth at 20–30 ºC and at pH 6.5–8.5.

Corynebacterium spp. are also important components of the microflora of smear cheese and have been reported to account for up to 90% of the microflora of Limburger cheese. They influence the flavour profile of smear-ripened cheeses through their esterase and lipase activities and through their ability to produce the flavour compounds such as methanethiol. *Corynebacterium* spp. exist as grey-white or non-pigmented and to a lesser extent as orange-red pigmented microorganisms.

Arthrobacter spp., which are also on the surface of smear-ripened cheese, range in colour from grey-white to yellow and can produce a red colour on the cheese surface, particularly in the presence of *B. linens*.

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143 Why might smear cheese develop excessive mould?

J. J. Sheehan

Moulds are ubiquitous in the environment, including in air and water, and can easily contaminate surfaces and cheeses within manufacture and ripening areas when conditions permit. Moulds grow well on cheese surfaces despite conditions of relatively low temperature, low pH and high salt concentrations.

On certain smear-ripened cheeses [**141**], the appearance of white mould is desirable and is due to *Geotrichum candidum* (an organism that has the characteristics of both a yeast and a mould). *G. candidum* occurs either as a white filamentous growth or as a yellow-grey growth. However, for most smear-ripened cheeses moulds are considered to be spoilage organisms that can cause discoloration of the cheese surface, and can lead to off-flavours and possibly to the formation of mycotoxins [**68**]. The risk of cheese spoilage by mould growth is increased in smear-ripened cheeses matured for long times.

A number of species are particularly associated with mould defects on smear-ripened cheese. Penicillia, particularly *P. commune* and *P. nalgiovense*, are very prevalent but *P. discolor, P. verrucosum*, *P. solitum* and *P. roqueforti* are also often present. *Fusarium* spp. form slimy yellow growths and are associated with mould defects in Tilsit-type cheeses. *Aspergillus versicolor* occurs more frequently in the air of cheese-ripening rooms than on the cheese surfaces but where sufficient contamination occurs, e.g. through smear solutions, it will also grow on the cheese surface.

Excessive mould growth will develop on the surface of smear cheeses due to the following:

- *Poor factory hygiene*. Poor hygiene practices result in heavy mould contamination of the manufacture and ripening environment, which can lead to direct or indirect contamination of milk, cheese or smear solutions.
- *Poor sanitation of ripening areas under use*. In smaller production units where independent ripening areas may not exist, difficulties arise in sanitising the ripening area where mould contamination may have become a problem. A gap in production or a removal of ripening stock may be required.
- *Airborne mould spores*. The concentration of airborne mould spores entering the plant is dependent on the environment external to the cheese factory (proximity to farmyards, decaying vegetation, etc.), the direction of prevailing winds, filtration of air intake and maintenance of positive air pressure within the plant and ripening areas.
- *Movement of personnel*. Mould spores can be carried by personnel unless suitable hygiene measures such as segregation of ripening areas, and limiting personnel movement are implemented.
- *Aerosol generation*. Generation of aerosols and condensates promote development of adventitious moulds and inappropriate use of rotating brushes for smearing may spread mould spores from cheese surfaces to the atmosphere.

- *Manufacture of blue mould cheeses* [**137**]. Manufacture of blue mould cheeses within the same production facility as smear-ripened cheeses is not recommended.
- *Contamination of brine by moulds*. Brines that are not pasteurised regularly develop a salt-tolerant microflora, which consists of yeasts, e.g. *Debaryomyces hansenii*, and bacteria, e.g. *Staphylococcus equorum*, and which develops on the subsequent cheese surface. If the brine is not maintained adequately, it may become contaminated with moulds that will grow on the cheese surface during subsequent ripening.
- *Old–young smearing*. When the microflora from older cheeses is used to promote smear development on young cheeses and where the older cheeses are contaminated with mould, an in-house mould problem is perpetuated.
- *Poor smear development*. Slow deacidification by yeasts and a lag phase prior to the development of a bacterial microflora result in a lack of competitive inhibition of moulds. A brine or smear microflora that deacidifies quickly and promotes bacterial growth is desirable.
- *Inadequate humidity*. Relative humidity of 90–98% is required for normal smear development. Inadequate humidification, excessive air velocity within the ripening area or condensing of steam-generated vapour by temperature control units all result in poor smear development.
- *Inadequate surface treatment*. Surface brushing or smearing aids smear development and physically disrupts mould growth. Mould development is also inhibited due to its slower growth rate in comparison with other microflora.

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144 Why does cheese not develop an adequate smear?

J. J. Sheehan

Development of an inadequate smear on the cheese surface [**141**] may be attributed to compositional, microbial and ripening factors, including the following:

- *Atypical cheese composition*. Cheese composition, most notably moisture, salt and pH, influences the microflora that develops on the cheese surface. Cheese pH and moisture content are influenced by the activity and acidification profile of the starters used [**18**] as well as by manipulation of processing parameters and the temperature profile used during cheese manufacture [**36**]. Cheeses with low pH will require greater deacidification and will have delayed smear development. Moisture content of the cheese [**34**, **36**] influences salt absorption during brining and thus the salt in moisture concentration at the cheese surface.
- *Inadequate humidity*. Relative humidity of 90–98% is required for normal smear development. Inadequate humidification and/or excessive air velocity within the ripening area results in a dry cheese surface and poor smear development.
- *Inadequate temperature control*. Temperatures ranging from 10 to ~16 ºC are usual during ripening of smear cheeses. Temperatures that are too low, undergo fluctuation or are non-uniform between ripening areas may also influence smear development.
- *Inadequate ripening time*. The rate of deacidification by yeasts is strain-dependent and a change in the yeast microflora may lead to slower deacidification of the cheese surface or reduced production of stimulatory compounds. This, in turn, may lead to slower development of the bacterial microflora, necessitating a longer ripening time.
- *Brine microflora*. Brines are a reservoir for yeasts that deacidify the cheese surface and produce compounds stimulatory for the growth of the bacterial microflora. A new or heat-treated brine may result in a change of microflora present in the brine. However, it should also be noted that brine is not the only source of the yeast microflora as they may also be inoculated through smearing or through contamination from the manufacture and ripening environment.
- *Smear microflora*. Depending on whether old–young smearing practices or a smear preparation is used, a change in the composition of the microflora [**142**] may also influence smear development.
- *Commercial smear preparations*. Commercial preparations have a varying complexity of microflora. Some are limited to *Debaryomyces hansenii* and *Brevibacterium linens* and do not provide other necessary organisms including staphylococci, micrococci, *Arthrobacter, Brevibacterium, Corynebacterium* and *Microbacterium* spp., which are then obtained from the environment.
- *Lack of proteolytic bacteria*. The proteolytic ability of certain species, e.g. staphylococci or brevibacteria, appears to promote the growth of other smear

organisms, e.g. corynebacteria. Although, results are inconclusive, production or metabolism of amino acids may also influence colour development.

- *Surface treatment*. Inadequate spraying, brushing or hand smearing of the cheese surface with a brine or smear solution may result in poor dispersal of the surface microflora and/or unfavourable conditions for smear growth.
- *Turning of cheeses*. Where cheeses are ripened on solid or continuous shelving and where the cheeses are not regularly turned, lack of oxygen will limit growth of the aerobic surface microflora on the surface of the cheeses.
- *Shelving*. In the case of wooden shelving, the shelving itself may act as a reservoir for smear microflora. Any change in shelving or in hygiene procedures may influence transfer of microflora to the cheese surface.
- *Smear colour*. A smear may have developed on the cheese surface but the colour of the smear may be atypical owing to a change in the microflora present. Particular microorganisms are associated with different colours, e.g. *Geotrichum candidum* produces a white colour, *Corynebacterium flavescens* produces a yellow colour and a mixture of *Arthrobacter* spp. and *B. linens* produces a red-orange colour.

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145 How may patchy smear development be avoided?

J. J. Sheehan

Similar to the problem of an inadequate smear [**144**], development of a patchy or non-uniform smear on the cheese surface [**141**] may also be attributed to microbial and ripening factors:

- *Inadequate humidity*. Inadequate humidification and/or excessive air velocity within parts of the ripening area may result in a certain surfaces of individual cheeses drying out and thus in patchy smear development.
- *Surface treatment*. Inadequate spraying, brushing or hand smearing of the cheese surface with a brine or smear solution may result in poor dispersal of the surface microflora resulting in patchy smear growth.
- *Turning of cheeses*. Where cheeses are ripened on solid or continuous shelving and where the cheeses are not regularly turned, lack of oxygen may limit growth of the aerobic surface microflora in certain areas of the underside of the cheeses.
- *Residues of cleaning agents*. Such residues, particularly on ripening racks or shelving, may retard or inhibit smear growth or result in patchy growth.
- *Smear colour*. Variable growth of different smear microorganisms [**142**] on the cheese surface may lead to a patchy appearance. Particular micro-organisms are associated with different colours, e.g. *Geotrichum candidum* produces a white colour, *Corynebacterium flavescens* produces a yellow colour, *Arthrobacter globiformis* and *Arthrobacter citreus* produce yellow-green patches, and a mixture of *Arthrobacter* spp. and *Brevibacterium linens* produces a red-orange colour. Pigmented staphylococci may also lead to orange patches.
- *Moulds*. Growth of moulds on the cheese [**143**] may also result in a patchy appearance with grey, green, blue or black colours. Growth of *G. candidum* may provide a white or yellow appearance while cream coloured *Fusarium* moulds may result in a non-visible contamination on the cheese surface, which may lead to a patchy appearance.

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Low-moisture Mozzarella cheese (LMMC)

146 Introduction

P. S. Kindstedt

Low-moisture Mozzarella cheese (LMMC) is a rennet-coagulated semi-hard cheese variety that spans an unusually broad compositional range in terms of moisture (about 45–52%) and fat (about 30–50% fat-in-dry-matter (FDM)). The major use of LMMC is as a pizza topping, thus the term 'pizza cheese' is often used interchangeably with LMMC. Functional properties such as flowability, stretchability, browning [**163**, **188**], free oil formation [**162**] and shreddabilty constitute essential quality attributes of pizza cheese. Considerable amounts of LMMC are also produced in the form of string cheese for snack food products that are either consumed directly or breaded and deep-fried before eating. String cheese is also used as a filling in stuffed-crust pizza and similar products. Important functional characteristics of string cheese include a fibrous peelable texture, long shelf-life and functional stability during refrigerated storage, and resistance to flow during heating in the case of string cheese made for deep-fried and stuffed-crust products.

Commercial manufacturing procedures for LMMC vary considerably, which is not surprising given its wide range of chemical composition and applications. Furthermore, non-conventional manufacturing approaches and ingredients are being used increasingly in pizza cheese production. Conventional LMMC manufacture resembles that of Cheddar cheese as far as the milling stage except that the rate of acidification is much faster, and therefore the manufacturing time from renneting to milling is much shorter. Rapid acidification and shorter make time result in less syneresis throughout cheesemaking, and therefore higher moisture in the final cheese. Rapid acidification also results in greater calcium

losses to the whey, which is necessary to produce a low calcium curd that will plasticise and stretch upon application of heat during the stretching step near the end of cheesemaking. Thermophilic starter cultures [**18**] are commonly used to make LMMC; however, mesophilic starters are also used widely in some countries. Alternatively, LMMC can be produced without starter culture through chemical acidification of the cheesemilk or through a combination of chemical acidification and culturing.

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147 What are *pasta-filata* cheeses and what physicochemical changes occur during cooking/stretching?

P. S. Kindstedt

Pasta-filata is an Italian term, meaning 'stretched curd' or 'spun paste', that refers to the process of cooking and stretching the cheese curd near the end of cheesemaking. Upon completion of stretching, the molten cheese is immediately moulded into its final shape and cooled. In large commercial cheese plants, the curd is plasticised and kneaded in either hot water (if the cheese is brine-salted exclusively) or dilute salt brine (if the curd is dry-salted before stretching, to prevent salt from leaching from the curd) using mechanical mixers with single or twin screws coupled with steam injection for temperature control. It is also possible to perform stretching using an extruder, which appears to be gaining significant commercial acceptance. During stretching, the amorphous paracasein matrix of the cheese curd is rearranged and aligned into roughly parallel paracasein fibres that are interrupted by open columns or channels containing fat globules and free serum. This heterogeneous quasi-laminar structure contributes strongly to the functional characteristics of low-moisture Mozzarella cheese (LMMC) such as peelability and fibrousness in string cheese, and chewiness and stretchability in pizza cheese.

Two conditions are necessary for optimal stretching. First, the curd must be sufficiently acidified [**17**] and demineralised [**4**] during cheesemaking to enable it to plasticise and stretch upon application of heat. Plasticisation and stretching are governed chiefly by the level of casein-associated calcium (or more correctly, colloidal calcium phosphate) in the curd at the time of stretching, which in turn is determined primarily by the total calcium content and the pH of the curd. Acidity development during cheesemaking, therefore, must be controlled so that the correct combination of total calcium content and pH (as well as the desired moisture content) occurs in the curd at the time of stretching. Second, heat transfer during stretching must occur at a sufficient rate to transform the curd to a plastic flowable consistency before it is kneaded and texturised. Premature application of the shearing forces of the screws to incompletely plasticised curd can cause considerable fat and moisture to be squeezed out and results in altered composition and lower cheese yield ([**150**]). Excessive heating, on the other hand, may detrimentally affect functional characteristics (see [**148**]). The temperature of the stretching water (or dilute brine), mixer screw speed and curd feed rate each influence the thermomechanical treatment during stretching; therefore, all three must be balanced and optimised to prevent unnecessary fat and moisture losses and undesirable functional changes.

The thermomechanical treatment during stretching also causes physicochemical changes that strongly influence the functional properties of the final cheese. Heating during stretching induces hydrophobic protein-to-protein interactions, which promote the formation of paracasein fibres. During this process, some of the cheese serum separates from the paracasein matrix and accumulates as free serum (along with liquid fat globules) in the void spaces or channels that

form as the paracasein contracts and becomes aligned into fibres. Heating also causes some of the calcium (more specifically, calcium phosphate) that was dissolved in the serum phase of the curd to interact with the paracasein fibres and become insoluble, which further promotes protein-to-protein interactions and the separation of free serum. The end result is a microstructure characterised by a thick, dense, network of paracasein fibres interrupted by sizeable channels filled with fat globules and loosely held serum. This composite microstructure gives LMMC a resilient elastic texture that retains considerable tensile strength when melted and stretched. When used as a pizza topping, conventional LMMC typically undergoes a brief (e.g. 1–3 week) period of refrigerated ageing to attain optimum functional properties. During this ripening period, protein-to-protein and calcium-to-protein interactions (formed during stretching) undergo partial reversal as calcium dissociates from, and water interacts with, the paracasein fibres. This in turn triggers microstructural changes and the development of a more flowable and stretchable and less chewy melted consistency (see [**148**], [**152**], [**153**]).

The thermal treatment (time–temperature profile) applied to LMMC curd during stretching also affects the microbiological and proteolytic properties during refrigerated storage. The critical temperature range where the effect is greatest falls between about 60 and 66 ºC. At curd temperatures below 60 ºC, thermophilic starter bacteria [**18**] (*Streptococcus thermophilus* and a *Lactobacillus* sp.) and residual coagulant (chymosin; *Rhizomucor miehei* protease; *Cryphonectria parasitica* protease) remain active in LMMC during ripening. Above 60 ºC, starter culture and residual rennet activities decrease progressively with increasing temperature, with little activity remaining when curd temperatures exceed 66 ºC during stretching. Heat inactivation of starter bacteria and rennet may affect the development of functional properties in LMMC during ripening (see [**148**], [**152**], [**153**]).

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148 How can expression of free watery serum be avoided in cooked LMMC?

P. S. Kindstedt

The thick dense paracasein fibres that form during stretching initially have limited ability to bind water. Therefore, the serum that accumulates in the many channels dispersed throughout the fibrous microstructure ([**147**]) is loosely entrapped. When newly made low-moisture Mozzarella cheese (LMMC) melts, this loosely entrapped serum flows between collapsing and sliding layers of protein fibres, and some is expressed as watery serum, which may either pool on the cheese surface or evaporate immediately if intense heating with high-velocity air flow is applied, such as in an impinger-type conveyor oven. Watery serum will continue to separate from melted LMMC during ripening until this loosely held serum is absorbed and immobilised by the paracasein fibres.

Normally, the heat-induced protein-to-protein and calcium-to-protein interactions that render the paracasein fibres hydrophobic (i.e. unable to interact with water) undergo partial reversal during the first week of ripening, which restores the water-binding ability of casein and enables the paracasein fibres to absorb the free serum contained within the adjacent channels. The presence of salt within the cheese structure facilitates this process, which can be observed at the microstructural level (using scanning electron microscopy or confocal laser scanning microscopy) as swelling of the paracasein fibres and the eventual disappearance of the serum channels.

As swelling progresses, the amount of watery serum that separates on melting declines until no separation occurs. Thus, the problem of serum separation usually resolves itself during the first week of ripening. Certain manufacturing factors, however, can cause serum separation to persist much longer, including the following.

High moisture-to-protein ratio

LMMC with high moisture-to-protein ratio may lack sufficient casein for adequate immobilization of the free serum formed within the cheese during stretching. This is usually not a problem in LMMC that contains no more than 50% moisture and 45% FDM. However, very high moisture and fat contents, which may be attractive from the standpoint of cheese yield [**48**], increase the risk of chronic problems with serum separation, as well as other undesirable functional consequences such as soft body and poor shreddability, and excessive flow and lack of chewiness on melting [**150**, **151**, **153**].

High curd temperature during stretching

The critical range where this temperature effect is most pronounced is between about 60 and 66 ºC, the same range in which the activities of thermophilic starter and residual rennet [**28**] are dramatically reduced. Cheese that is stretched at the

low end of this range (curd temperature $\leq$ 60 ºC) will generally display increased water binding and no serum separation after the first week or so of ripening [**147**]. In contrast, cheese that exits the mixer at the high end of this temperature range may continue to produce watery serum on melting for many weeks after manufacture. Higher curd temperature causes more soluble calcium to associate with the paracasein fibres, leaving the serum phase depleted of calcium (Fig. 1a). This has the effect of increasing the hydrophobic nature of the paracasein fibres and impeding the normal reversal of heat-induced protein-to-

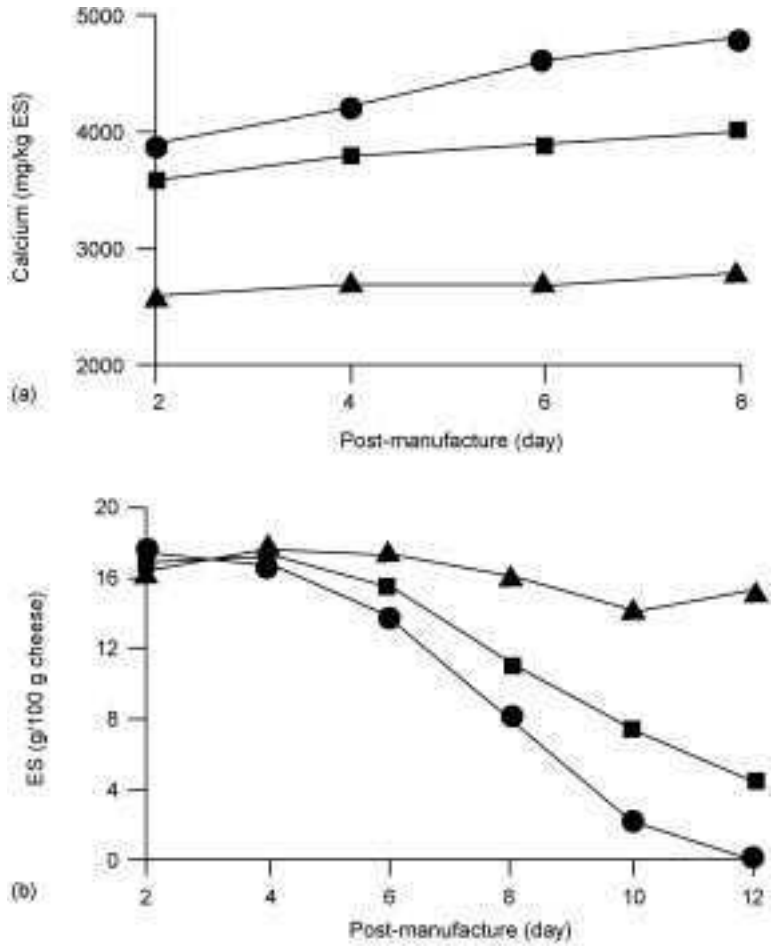

Fig. 1 Impact of stretching temperature on the calcium content (a) and amount (b) of expressible serum (ES) obtained from low-moisture Mozzarella cheese during early ripening. Curd temperatures at the stretcher exit were: ● 62 ºC; ■ 64 ºC; ▲ 66 ºC. Lower concentration of calcium in the ES (a) corresponds to greater calcium-to-protein interactions and more hydrophobic paracasein fibres. Lower amount of ES (b) corresponds to greater hydration and swelling of the paracasein fibres. (redrawn from Kindstedt *et al.*, 1995).

protein and calcium-to-protein interactions during the first week of ripening. Consequently, the paracasein fibres fail to hydrate and swell, and the pooled serum remains loosely entrapped, well beyond the first week of ripening (Fig. 1b). Furthermore, thermal inactivation of residual rennet and starter bacteria mean that proteolysis is dramatically reduced by high stretching temperatures, which also contribute to the slow improvement in water binding. An increase of a few degrees in the curd stretching temperature within this critical range (e.g. an increase from 62 to 66 ºC) can have a surprisingly large effect on water binding and serum separation (as well as on other functional characteristics such as flowability and stretchability). For cheese with high moisture-to-protein ratio, it is particularly important to maintain low stretching temperature ($\leq$60 ºC) to limit serum separation.

Because the water-binding ability of paracasein increases as the level of casein-associated calcium decreases, the proclivity of LMMC to express watery serum can be reduced by lowering the calcium content. LMMC with lower calcium content can be produced by either increasing acid production by the starter culture in the early stages of manufacture (before draining, and especially before renneting) or by combining culturing with partial acidification of the milk before renneting with a food-grade acidulant. Alternatively, serum separation can be avoided by replacing the starter culture completely with direct acidification [**149**]. Be aware, however, that lowering the calcium content may necessitate changes in stretching pH (higher curd pH at stretching will generally be needed) and may require changes in stretching water temperature and screw speed to accommodate the softer and more flowable molten curd. Lowering the calcium content will also produce a softer and gummier cheese that may be more difficult to shred and will flow more easily and be less chewy on melting.

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149 I recently changed from bacterial to direct acidification. Why is my LMMC different?

P. S. Kindstedt

During the manufacture of cultured low-moisture Mozzarella cheese (LMMC) [**146**], acidification occurs gradually through the action of the starter culture [**18**]. In directly acidified LMMC, acidification is accomplished in a single step before renneting by adding food-grade acid to the cold milk before cheesemaking to attain a target pH value, usually around pH 5.6 or 5.7. Directly acidifying milk in this way results in cheese with much lower calcium content and higher pH than would normally be obtained using a make procedure involving a starter culture. Also, it is possible to produce cheese with very high moisture content by direct acidification because directly acidified curd is less prone to syneresis during cheesemaking owing to its lower calcium content and greater water-holding capacity. Furthermore, cooking and cheddaring times and temperatures can be minimised, if so desired, to retain more moisture. The characteristics of directly acidified LMMC differ from those of cultured LMMC in several important respects:

- Lower calcium content in directly acidified curd results in the formation of hydrated and swollen paracasein fibres on stretching, which dominate the microstructure of the newly made cheese. This translates into a drier, softer and gummier texture in the young cheese than would occur in a cultured LMMC of similar composition and age. Consequently, directly acidified LMMC may prove more difficult to shred unless the moisture and fat contents are kept low enough to prevent excessive softness and gumminess. On the other hand, the absence of wetness means that it is not necessary to age directly acidified LMMC before shredding, which may be an advantage.
- Lower calcium content also means that newly made directly acidified LMMC melts to a more flowable and stretchable and less chewy consistency that does not release watery serum, even during the first few days after manufacture. Therefore, ripening is not necessary to attain desirable melting characteristics such as flow and stretch, and changes to functional properties occur very slowly during refrigerated storage. In contrast, cultured LMMC usually must be ripened for 1–3 weeks before comparable flowability and stretchability are attained [**152**], and functional changes during refrigerated storage are larger and less predictable.
- Limited secondary proteolysis occurs during refrigerated storage owing to the absence of starter bacteria. This renders directly acidified LMMC resistant to browning during baking because heat-induced Maillard browning reactions are governed by concentrations of both products of proteolysis (free amino groups in small peptides and free amino acids) and residual carbohydrates (lactose, galactose) present in the cheese [**155**, **163**]. Although directly acidified LMMC contains abundant residual lactose, the scarcity of free amino groups effectively limits browning.

- The flavour will differ owing to both the absence of flavour compounds normally produced by the starter culture and the presence of flavour imparted by the specific acidulant used.
- The cheese yield efficiency will be lower due to elevated losses of minerals (i.e. calcium phosphate) and soluble caseins into the whey.

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150 How may moisture levels in LMMC be controlled and what changes should be expected if moisture changes?

P. S. Kindstedt

Several manufacturing practices can be manipulated to control the moisture content of low-moisture Mozzarella cheese (LMMC) [**146**], including the following.

Acidification schedule/manufacturing time

The total time that elapses from renneting to milling directly influences the amount of syneresis during manufacture. The longer the manufacturing time, the greater the amount of syneresis [**34**] and the lower the moisture content in the final cheese when other conditions are held constant. Make times of 2.5 h or less from renneting to milling are commonly used to produce pizza cheese containing 47–50% moisture. Furthermore, faster acid production in the early stages of manufacture (before draining and especially before renneting) favours curd with lower calcium content [**4**] that retains moisture more readily throughout cheesemaking.

Cooking/cheddaring temperature

Temperature directly influences the rate of syneresis during manufacture [**36**]. The lower the temperature during cooking and cheddaring, the lower the syneresis rate and the higher the moisture content in the final cheese, providing other conditions are held constant. However, changes in cooking and cheddaring temperature will also probably affect the rate of acid production by the starter culture and thus the manufacturing time unless steps are taken to maintain a constant rate of acidification. For example, when a thermophilic starter [**18**] is used to produce LMMC, lowering the cooking/cheddaring temperature from 41 to 38 ºC will favour less syneresis and higher moisture in the final cheese. However, thermophilic starters optimally produce acid at around 42 ºC; therefore acid will be produced more slowly at 38 ºC than at 41 ºC, if the same amount of starter is used, resulting in an increase in the time needed to reach the target pH at stretching. Therefore, the gains in moisture retention due to less syneresis at the lower temperature will be partly offset by additional moisture losses that result from the longer manufacturing time. The greater the increase in manufacturing time in response to a given decrease in temperature, the smaller the gains in moisture retention at the lower temperature. Thus, decreases in cooking/cheddaring temperatures should be combined with measures to keep the rate of acidification and manufacturing time constant (e.g. increases in amount of starter culture added; increases in the ratio of cocci to rods in thermophilic starter culture) in order to maximise moisture gains.

Salting temperature/method

The amount of moisture expelled from the curd during salting [**43**] is strongly influenced by the temperature and method of salting. LMMC was traditionally brine-salted [**41**] but it has become common commercial practice to replace some or all of the brining with dry salting combined with stretching in dilute brine (e.g. 4–6% NaCl) to prevent the salt from leaching from the curd during stretching. In general, less moisture is lost during brine salting than when dry-salting is used to attain the same salt content. For both brine-salting and dry-salting, lower temperature during salting results in less moisture loss. For example, LMMC that is brined at 1 ºC to an average salt content of about 1.4% may decrease by about 1.5–2% moisture from milling to the final cheese (e.g. from 51% moisture before stretching to 49–49.5% moisture after brining). The same cheese brined at 10 ºC may decrease by about 3% moisture, if other conditions are held constant. Dry-salting combined with stretching in dilute brine has the potential to produce much greater moisture losses than brine-salting, depending on the salting temperature. For example, dry-salting at a curd temperature of 41 ºC may result in losses of 5–6% moisture from the curd before salting to the final cheese. In contrast, losses can be reduced to around 3–4% moisture if the curd is cooled to around 31 ºC before salting, in which case the mixer screw speed may need to be reduced to allow the curd more time to plasticise before kneading.

Mixer screw speed and stretching temperature

Considerable moisture may be squeezed out of LMMC curd during stretching if the shearing forces of the mixer screws are applied before the curd has been fully heated and plasticised. The time–temperature conditions needed to plasticise LMMC curd can vary substantially; therefore, the screw speed and stretching water temperature should be adjusted relative to the curd properties so as deliver enough heat to plasticise the curd before kneading commences in earnest. For example, LMMC curd with very low calcium content (e.g. following a very low draining pH or made by direct acidification [**4**, **149**]) will plasticise at a lower temperature than LMMC containing the same moisture and fat contents but higher calcium content. Thus, the former may be stretched at lower stretching water temperature and/or faster screw speed than the latter. Another example is that of dry salted curd, which, compared with unsalted curd, requires higher temperature to plasticise completely. Therefore, dry salted curd should be stretched at a slower screw speed and/or higher stretching brine temperature in order to minimise moisture losses during stretching. Be careful, however, not to overheat the curd during stretching. Undesirable changes in functional properties, such as increased wetness and aggregation of cheese shreds [**151**], increased expression of watery serum on heating [**148**], and delayed development of a smooth stretchable melted consistency during ripening [**152**] may occur when curd temperatures exceed 60 ºC during stretching.

Changes in moisture content may trigger several important changes in LMMC. In general, higher moisture content results in:

- higher rates of proteolysis by residual rennet (primary proteolysis) and starter bacteria (secondary proteolysis) [**88**];
- increased browning potential during baking due to higher residual lactose and more proteolysis [**163**];
- softer cheese body immediately after manufacture and throughout ageing, which may render the cheese more difficult to shred (reduced machinability) and shreds that are more subject to matting (owing to lower protein density and greater proteolysis);
- increased potential for matting of shreds due to free surface moisture [**151**];
- increased potential for serum separation on melting [**148**];
- increased stretchability and flowability, and decreased chewiness on melting throughout ageing (owing to lower protein density and greater proteolysis);
- overall reduction in the ripening time needed to attain optimum functional properties and a reduction in the window of time during which peak functionality occurs (owing to lower protein density and greater proteolysis).

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151 Shredded cheese tends to mat together into wet aggregates. How may the shreddability of LMMC be improved?

P. S. Kindstedt

The paracasein fibres that form during stretching initially have limited ability to bind water, resulting in the accumulation of loosely entrapped moisture throughout the cheese body [**148**]. This state gives rise to free moisture on newly cut surfaces that causes shreds to clump together. Normally this problem disappears after the first week or so of ageing due to physicochemical changes that cause the water-holding capacity of the cheese to increase [**148**]. However, free surface moisture on shreds may persist longer in cheese with a high moisture-to-protein ratio or cheese that is stretched at high temperature (e.g. 66 ºC), for the same reasons as discussed in the section on serum separation [**148**]. In addition, two other conditions are worth noting.

Incomplete cooling during brining

Brining is used both to salt and to cool low-moisture Mozzarella cheese (LMMC) [**146**]. During brining, salt diffuses into the cheese at the surface and establishes a gradient within the cheese body. Concomitantly, a temperature gradient is established as cooling proceeds from the surface inwards. Incomplete cooling during brining may result in temperature gradients that persist for several days, depending on the size of the block and the extent of the temperature gradient. During ripening, moisture is drawn osmotically from the low salt interior towards the higher salt surface, whereas salt diffuses inwards due to the concentration gradient [**41**, **42**]. This process continues until salt-in-moisture levels equilibrate throughout the cheese. Persistent temperature gradients will also cause water to migrate from the warm cheese centre to the cool surface.

The combined effects of simultaneous gradients of both salt and temperature on the outward migration of moisture can be considerable and result in a very high moisture content at the surface, especially if the average cheese moisture content is at the high end of the normal range (e.g. >50%). Such cheese may become very soft [**160**] and wet at the surface by approximately the end of the first week of ripening, rendering the cheese very difficult to shred. To prevent the problem, the cheese should be adequately pre-cooled before brining, the brining system should have sufficient cooling capacity and circulation to maintain a uniform temperature of around 0–4 ºC, and the cheese should remain in the brine long enough both to salt the cheese adequately and to attain a core temperature of <10 ºC.

High temperature during shredding

The water-holding capacity of LMMC decreases with increasing temperature; therefore, problems with free moisture on newly cut shred surfaces tend to be magnified as the cheese temperature at shredding increases. LMMC is normally

shred at around 0–4 ºC, which maximises water-holding capacity and minimises free moisture. Low cheese temperature also ensures that the fat remains in the solid state, which contributes to a firmer and less sticky body that enhances machinability and resistance of the shreds to matting.

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152 Why does LMMC not develop a smooth stretchable consistency on heating?

P. S. Kindstedt

The thick dense paracasein fibres that form during stretching are initially very hydrophobic, which favours strong protein-to-protein interactions that limit the ability of the cheese to flow (giving the melted cheese a rough appearance) and stretch on heating. During the first week or two of ripening, however, physico-chemical changes cause the paracasein fibres progressively to hydrate [**147**, **148**], which enables the cheese to melt to a smooth and stretchable consistency. Proteolysis also contributes to this transformation [**88**]. Several factors may lengthen the time needed to complete the transformation to a smooth and stretchable melted consistency, including the following:

- High stretching temperature, which causes more soluble calcium to associate with the paracasein fibres and which reduces proteolysis through inactivation of residual coagulant and starter bacteria [**148**]. Low-moisture Mozzarella cheese (LMMC) stretched at high temperature (e.g. 66 ºC) may require 2–4 weeks longer to attain a smooth and stretchable melted consistency than the same cheese stretched at low temperature (e.g. 60 ºC), depending on the moisture and fat content of the cheese.
- High calcium content, which results in more hydrophobic paracasein fibres that require more ripening time to become adequately hydrated. Conditions that favour LMMC with high calcium content include slow acidification/high draining pH [**4**] and heavy fortification of cheesemilk with milk solids.
- High salt content. Although low levels of salt enable the paracasein fibres to hydrate and swell during ripening [**148**], high salt levels compete for water molecules and result in more hydrophobic fibres that require more ripening time to hydrate adequately.
- Stretching at the high end of the pH range, or window, within which the curd plasticises, referred to as stretching the curd when it is 'green'. Higher stretching pH results in higher cheese pH (and also higher calcium content), which favours calcium in the casein-associated state, resulting in more hydrophobic paracasein fibres that take longer to hydrate adequately.
- Low moisture content (more specifically, low moisture-to-protein ratio), which results in a more protein-dense cheese structure that is extensively reinforced by protein-to-protein interactions, and which requires more ripening time to hydrate adequately. Low moisture content also favours lower rates of proteolysis.
- Low fat content, which results in a higher protein density and a less interrupted composite structure [**156**], thus stronger protein-to-protein interactions, and which usually coincides with lower moisture-to-protein ratio and thus reduced proteolysis. Lower fat content also affords less lubrication for contiguous layers of paracasein fibres to slide past one another during melting [**156**, **162**].

- Slow acidification during cheesemaking, such as may be caused by bacteriophage [**17**, **21**], which results in cheese with higher calcium and lower moisture contents.

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153 Why does LMMC become excessively soft and fluid on heating?

P. S. Kindstedt

Flowability is governed primarily by the strength of the protein-to-protein interactions that bind the paracasein fibres together. Very strong interactions restrict flow whereas very weak interactions may cause flow to be excessive and the melted cheese to become too fluid. Protein-to-protein interactions are strong immediately after stretching but weaken during ripening as the paracasein fibres release insoluble calcium and become hydrated [**148**, **149**, **152**], resulting in increased flowability. Proteolysis also weakens protein-to-protein interactions by reducing the length of the casein molecules that constitute the paracasein fibres and increasing the hydration state of the fibres, further enhancing flowability. In general, low-moisture Mozzarella cheese (LMMC) [**146**] will eventually (if aged long enough) become excessively soft and fluid due to the combined effects of proteolysis and increases in paracasein hydration mediated by physicochemical changes. Factors that favour premature development of excessive softness and fluidity include the following:

- High moisture content (more specifically, high moisture-to-protein ratio) which results in a lower protein density and thus weaker protein-to-protein interactions, and which promotes increased proteolysis [**156**].
- High fat content, which results in a lower protein density and a more interrupted composite structure [**156**], thus weaker protein-to-protein interactions, and which usually coincides with higher moisture-to-protein ratio and thus increased proteolysis. Higher fat content also provides greater lubrication for adjacent layers of paracasein fibres to slide past one another during melting [**156**, **162**].
- Low calcium content, such as may be produced by draining the whey at low pH (e.g. 6.0 or lower), which results in more hydrated paracasein fibres with weaker protein-to-protein interactions.
- Low salt content, which results in more hydrated paracasein fibres with weaker protein-to-protein interactions.
- Stretching at the low end of the pH window, referred to as stretching the curd when it is 'ripe'. Lower stretching pH results in lower calcium content and favours calcium in the soluble state, resulting in less hydrophobic paracasein fibres that hydrate more quickly during ageing.
- Elevated proteolysis caused by factors such as the use of a very proteolytic coagulant (e.g. *Cryphonectria parasitica* protease) combined with a low stretching temperature (<60 ºC), or ripening at elevated temperature.

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154 Why does LMMC have poor flowabilty?

P. S. Kindstedt

Conditions that favour poor flowability are the reverse of those that favour excessive softness and fluidity [**153**], namely low moisture and fat contents, high calcium and salt contents, and stretching the curd at the high end of the pH window. Other factors include the following:

- Low cheese pH (<5.0), which causes protein-to-protein interactions to increase steeply as the pH approaches the isoelectric point of casein (i.e. pH 4.6). Stretching at the low end of the pH window (which results in a comparatively low cheese pH immediately after manufacture) combined with high cheese moisture content (which results in higher residual lactose and thus greater production of lactic acid by starter bacteria during ripening) renders low-moisture Mozzarella cheese (LMMC) [**146**] more prone to developing low pH during ripening. Also, LMMC used as an ingredient in refrigerated prepared foods such as refrigerated pizza may decrease in pH owing to the uptake of hydrogen ions from acidic ingredients such as tomato sauce that are in contact with the cheese for extended periods in the unfrozen state.
- Inadequate release of free oil from the cheese on heating. Free oil acts a hydrophobic barrier that impedes dehydration at the cheese surface during baking. Failure to release adequate free oil results in an exposed cheese surface that is subject to rapid dehydration and case-hardening that restricts flow [**162**].
- Poor water holding capacity, characteristic of a young cheese or one stretched at a high temperature [**148**], which results in excessive surface drying and case-hardening during baking.

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155 Why does LMMC brown excessively on cooking?

P. S. Kindstedt

Browning during cooking results from heat-induced Maillard reactions between the carbonyl groups of reducing sugars and the amino groups of peptides and amino acids [**188**]. Reducing sugars in low-moisture Mozzarella cheese (LMMC) consist of unfermented lactose and galactose at concentrations that may vary considerably depending on the sugar-fermenting characteristics of the starter culture used and the extent to which the starter bacteria survive the heat treatment during stretching [**147**]. Short peptides and amino acids in LMMC [**146**] made from high-quality milk occur primarily through secondary proteolysis by the starter bacteria [**23**] and may vary considerably in concentration, depending on the proteolytic characteristics of the starter culture used and the extent of starter inactivation during stretching. Browning reactions occur when adequate concentrations of both reducing sugars and products of proteolysis are present in the cheese during heating. Furthermore, browning reactions are produced more readily in low-moisture environments; therefore, LMMC that dehydrates extensively at the surface during heating will be subject to more intense browning than a comparable cheese that resists dehydration. Strategies for controlling the rate of browning are presented in [**163**]. Excessive browning may result when adequate levels of both reducing sugars and proteolysis products are present in the cheese and when one or more of the following conditions occurs:

- Excessive dehydration at the cheese surface due to inadequate release of free oil [**162**] or to poor water-holding capacity because the cheese is too young or was stretched at a high temperature [**148**].
- Excessive 'blister' formation, which serve as sites for localised dehydration and enhanced browning during pizza baking. Blisters occur when water vapour produced under the layer of melted cheese creates bubbles in the molten cheese. Cheese with high tensile strength will tend to form stable bubbles, the surfaces of which are more exposed to dehydration than the rest of the cheese surface and thus form a skin or blister during baking. Conversely, cheese that produces bubbles that burst shortly after forming is less prone to blister formation. Low moisture, high calcium and high salt contents favour more extensive blistering. Excessive proteolysis also may result in widespread surface dehydration and blistering during baking.
- Excessive secondary proteolysis due to high moisture content or the use of highly proteolytic *Lactobacillus* starter strains.
- Excessive levels of residual sugars in the cheese resulting from the exclusive use of starter strains unable to ferment galactose, high-moisture cheese, or heavy fortification of cheesemilk with lactose-rich milk solids such as non-fat milk powder.

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156 Why and how do the functional properties of LMMC change on heating?

P. S. Kindstedt

The functional properties of low-moisture Mozzarella cheese (LMMC) [**146**] are determined by the underlying cheese structure, which changes dramatically with temperature. At refrigeration temperature (<4 ºC), LMMC possesses a quasi-laminar structure characterised by layers of roughly parallel paracasein fibres interrupted by channels filled with solid fat globules. This composite structure results in rheological properties that are anisotropic in nature, that is, having different properties when evaluated parallel or perpendicular to the fibre direction. The thick dense paracasein fibres impart great tensile strength when LMMC at low temperature is extended in the direction of the fibres. In contrast, when tension is applied perpendicular to the fibre direction, the composite structure disengages and peels along fault lines formed by the channels dispersed throughout the structure. The channels limit the capacity of contiguous planes of paracasein fibres to fuse together tightly because the solid fat globules contained therein interrupt protein-to-protein interactions among fibres. Furthermore, the paracasein gel that forms within the channels as the fibres swell and engulf the fat droplets [**148**] has lower protein density and represents a weak point in the structure. The functional consequence of this quasi-laminar composite structure is especially evident in string cheese, which is notable for its peelability and fibrousness when peeled along the direction of the fibres.

The structure and function of LMMC change in two distinct stages on heating. The first stage occurs when the temperature is raised from the initial refrigerated state to about 40–45 ºC. During this period, fat globules are gradually transformed from predominantly solid in nature at <4 ºC to completely liquid at >40 ºC. Solid fat globules at low temperature act as hard spheres that augment the elasticity of cheese structure and contribute to a firm texture. However, fat globules become increasingly deformable as their ratio of solid-to-liquid fat decreases with increasing temperature, which causes the cheese texture to soften. The greater the fat content, the greater the effect of temperature increase on softening. Furthermore, the texture becomes more adhesive or stickier as the proportion of fat in the liquid state increases. Consequently, LMMC becomes more difficult to shred and the shreds are more prone to matting as temperature increases.

The second stage of heat-induced structural and functional changes occurs when the temperature increases to about 45–60 ºC, at which point the cheese undergoes a phase change from solid-like to liquid-like and begins to flow and stretch. During this phase change the paracasein fibres dissociate and begin to collapse and slide past one another, while liquid fat globules coalesce into pools of liquid fat that serve as a lubricant for the displacement of contiguous layers of paracasein fibres. The extent to which fibre layers collapse and slide past one another on melting, and thus the extent of flow and stretch, depends on the strength of the protein-to-protein interactions that reinforce the paracasein

network of fibres against thermal dissociation. Factors that weaken protein-to-protein interactions and promote dissociation result in a smoother, more flowable and stretchable, but less chewy and fibrous, melted consistency [**152**, **153**, **154**].

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157 Why does LMMC become excessively soft and gummy during ripening?

P. S. Kindstedt

Softening during ripening is governed by the same factors that cause flowability to increase [**153**]. High moisture and fat contents, low calcium and salt contents, stretching at the low end of the pH window, and use of very proteolytic coagulant combined with low stretching temperature, or any other cause of elevated proteolysis, favour early onset of excessive softness and gumminess.

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158 What factors affect the functionality of LMMC?

P. S. Kindstedt

With respect to cheese composition, levels of moisture, fat, calcium, salt and pH collectively play a major role in determining the functionality low-moisture Mozzarella cheese [**148**, **150**, **152**, **153**, **155**]. In terms of manufacturing parameters, acidification schedule, temperature conditions during stretching and salting method strongly affect functionality through their effects on composition and physicochemical and proteolytic changes during ageing [**148**, **150**, **152**].

159 LMMC is tough and rubbery; what might be the problem?

P. S. Kindstedt

Conditions that favour tough and rubbery texture in low-moisture Mozzarella cheese [**146**] are primarily the reverse of those that cause softness and gumminess [**157**] and excessive fluidity on melting [**153**], namely, high calcium and salt contents, low fat and moisture contents, high stretching temperature and stretching at the high end of the pH window.

160 What causes the soft rind/soft surface defect in LMMC?

P. S. Kindstedt

Soft rind defect is characterised by a soft, moist, fragile surface layer that is evident in brine-salted low-moisture Mozzarella cheese (LMMC) [**146**] immediately after the cheese is removed from the brine. It is caused by the leaching of calcium from the cheese surface to the brine, which causes the paracasein at the surface to absorb moisture from the brine and swell. The problem is exacerbated when the pH of the brine is higher than that of the cheese. Calcium leaching occurs when the brine is devoid of soluble calcium, such as when new brine is used for the first time. Used brine that is pasteurised to control microbiological contamination can also be problematical because calcium phosphate may precipitate during the heat treatment, leaving the brine depleted of soluble calcium. Soft rind defect can be prevented by adding a food-grade source of soluble calcium, such as calcium chloride, to new brine or newly pasteurised brine to attain a concentration of calcium ions of around 0.05–0.10%. New brine should also be acidified to about pH 5.2 using food-grade acetic or lactic acid.

Soft surface defect is characterised by a soft, pasty, high-moisture surface that develops during ripening. The defect is caused by the cumulative effect of three conditions during brine salting that affect the moisture content at the cheese surface. Firstly, low-temperature brining (0–4 ºC) greatly reduces the loss of moisture from the cheese surface as compared with losses during brining at higher temperatures. Therefore, the surface of LMMC has a comparatively high moisture content immediately after brining. Secondly, the high concentration of salt at the cheese surface creates an osmotic gradient that draws moisture towards the surface during ripening as the salt diffuses inwards. Thirdly, moisture is drawn towards the surface along thermal gradients that may persist within the cheese after brining due to incomplete cooling [**151**]. The defect can be prevented by pre-cooling the cheese before brining, then brining long enough both to salt the cheese adequately and to attain a core temperature of <10 ºC.

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161 What causes soft body defect in LMMC

P. S. Kindstedt

Soft body defect is characterised by excessive softening in the interior of cheese during ripening, which becomes problematical for shredding and probably also affects melting properties. The defect has been associated with high populations of non-starter lactobacilli such as *Lactobacillus casei* subsp. *casei* and *Lb. fermentum* [**56**], which are normally not present in low-moisture Mozzarella cheese (LMMC) [**146**] at high enough levels to cause problems. However, inadequate cooling during brining and biofilm formation in the pasteuriser and on other milk contact surfaces caused by lapses in sanitation may favour high non-starter lactobacilli populations in LMMC.

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162 How may the development of free oil during melting be controlled?

P. S. Kindstedt

During melting, fat globules are gradually transformed from predominantly solid in nature at <4 ºC to completely liquid at >40 ºC. Concurrently, the volume occupied by fat globules increases through thermal expansion, which causes the liquid globules contained within the channels to pack together more closely and coalesce as pools. Pools of liquid fat then flow and merge between collapsing and sliding layers of paracasein fibres as the fibres dissociate at around 45–60 ºC. A portion of the pooled liquid fat finds its way to the cheese surface and is expressed as free oil. Free oil formation, therefore, is governed by two critical factors during melting: (1) the coalescence of fat globules within channels to form pools of liquid fat; (2) the dissociation of paracasein fibres, which causes them to collapse and flow, thereby enabling pools of liquid oil trapped within channels to flow and merge with one another into even larger pools. Factors that can be modulated to control free oil during melting include the following.

Fat-in-dry-matter (FDM)

As the FDM level of low-moisture Mozzarella cheese (LMMC) [**146**] increases, the channels within the cheese structure become larger and more tightly packed with fat globules, which results in greater fat volume expansion, coalescence and pooling of liquid fat during melting. Higher fat content also causes greater interruption of protein-to-protein interactions among fibres, enabling them to collapse and flow more freely on heating, which facilitates the flow and merging of liquid fat pools into even larger pools. Free oil formation in LMMC, therefore, increases with increasing FDM content. It is important to note, however, that free oil increases in an exponential rather than linear manner in relation to FDM (Fig. 1). In general, the rate of production of free oil increases very gradually with increasing FDM between about 10% and 30%, more rapidly between 30% and 40%, and extremely rapidly above 40%. Therefore, LMMC with high FDM is highly prone to the production of excessive free oil on melting. On the other hand, small reductions in FDM in the range of 50–40% can achieve surprisingly large reductions in free oil formation. Alternatively, free oil in LMMC with high FDM can be reduced by using homogenised [**32**] cream to standardise, in part or in total, the fat content of the milk before cheesemaking. Homogenisation of cream produces smaller fat globules that remain more finely dispersed throughout the cheese structure during stretching and are thus less prone to coalesce into pools of liquid fat.

At the low end of the normal FDM range, LMMC generally forms limited free oil on melting because the channels are smaller and less closely packed with fat globules, which limits their coalescence and pooling. The paracasein fibres are also less interrupted and thus more tightly bonded, therefore they collapse and flow less readily. Inadequate release of free oil favours excessive

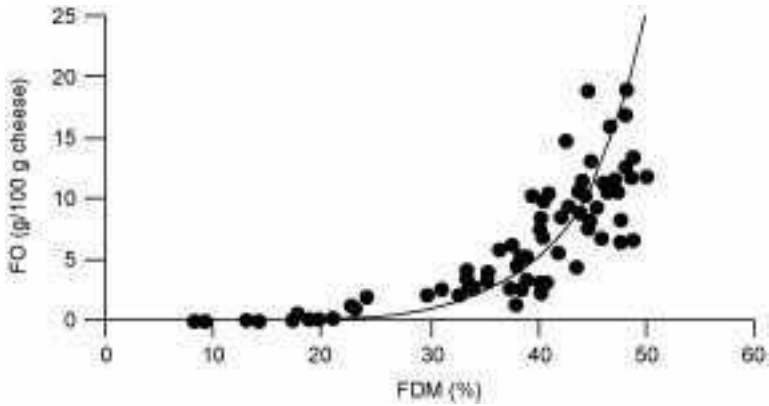

Fig. 1 Relationship between fat-in-dry matter (FDM) content and free oil (FO) formation in Mozzarella cheese. Samples ($n = 144$) varied widely in composition and age and included both commercially and experimentally produced cheeses.

dehydration, case-hardening and excessive browning at the cheese surface during baking [**155**]. Generally, LMMC with >30% FDM produces enough free oil to avoid this problem but excessive dehydration is very common in reduced-fat cheeses with lower FDM. The effects of inadequate free oil formation can be mitigated at the time of baking by spraying a thin layer of edible oil on the cheese surface, which creates a hydrophobic barrier, or by combining a small amount of shredded or comminuted cheese with a high FDM level that oils off readily with the reduced-fat LMMC.

Salt content

Free oil formation decreases in LMMC as the salt content increases. Furthermore, the modulating effect of salt on formation of free oil is magnified as the FDM content increases. LMMC that is both high in FDM and low in salt content, therefore, is exceptionally prone to excessive free oil formation. Thus, it is important to maintain salt levels towards the high end of the normal range when the FDM of LMMC exceeds 40%. The fundamental basis for the modulating effect of salt on free oil is not completely understood but probably relates to increased protein-to-protein interactions at higher salt content which limit the dissociation, collapse and flow of the casein fibres. It has also been hypothesised that higher salt content may give rise to more highly emulsified fat globules that resist coalescence on heating, but this possibility remains uncertain.

Proteolysis

Elevated proteolysis renders LMMC more prone to free oil formation, especially in cheese with FDM content >40%. For example, cheeses made with highly

proteolytic coagulants (e.g. *Cryphonectria parasitica* protease [**29**]) or starter strains combined with low (<60 ºC) stretching temperature, or stored at an elevated temperature, are more prone to elevated proteolysis and excessive free oil formation.

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163 How can the browning rate of LMMC be controlled?

P. S. Kindstedt

Browning rate can be controlled by limiting the concentrations of either residual sugars or peptides and amino acids, or both, in the cheese and by preventing excessive dehydration and blister formation during baking [**155**, **188**]. Specific strategies to reduce browning include:

- using galactose-fermenting starters such as *Lactobacillus helveticus* or adjunct cultures to ferment and limit the accumulation of galactose;
- rinsing the curd with warm water to wash out carbohydrates;
- fortifying the cheesemilk with low-lactose milk solids such as milk protein concentrates (when permitted) in place of lactose-rich milk solids;
- avoiding the use of starter strains that are highly proteolytic;
- producing cheese with adequate water-holding capacity, free oil formation, flow and stretch to limit blister formation [**155**].

Cheeses ripened in brine

164 Introduction

E. Alichanidis

The characteristic of white-brined cheeses is that they are ripened and preserved in brine until consumption. Originally they were produced in countries of the eastern Mediterranean basin, eastern and south-eastern Europe and the Middle East under various names: Feta (Greece), Domiati (Egypt), Telemea or Telemes (Romania, Greece), Beli sir u kriskama (Serbia), Travnik (Bosnia-Herzegovina), Brinza or Brynza (Israel, Russia, Ukraine), Bjalo salamureno sirene or Bjalo sirene (Bulgaria), Beyaz peynir (Turkey), Jerevanskij syr (Armenia), Chanakh (Armenia, Russia), Akawi (Lebanon, Syria), Halloumi (Cyprus), Halloum (Lebanon), Nabulsi (Jordan), Mish (Egypt), Gibna Bayda (Sudan), white cheese, in addition to many other local names.

White-brined cheeses are made from raw, pasteurised [**11**] or thermised [**13**] ewe's, goat's, cow's or buffalo's milk or, often, from mixtures of milks. Nowadays, preconcentration of milk by ultrafiltration [**16**] has gained popularity, especially for the production of cow's milk cheeses. The great majority of these varieties are rennet coagulated and brine-salted cheeses, although for some of them the curd surface is dry-salted (e.g. Feta) or the salt is added directly to the cheesemilk (e.g. Domiati). Various cultures are used as starters: thermophilic (yoghurt) cultures, mesophilic cultures or combinations of mesophilic and thermophilic cultures, or the native microflora of the milk may be used for acidification [**18**].

The colour of the cheeses is, of course, generally white but cow's milk cheeses are off-white to yellowish because of the presence of carotenoids in the fat [**14**]. The cheeses have no rind, and no gas holes or other openings should be

present in the cheese mass except, sometimes, for small mechanical openings [**102**]. The shape varies but usually blocks are rectangular and weigh 250–1000 g or more. The flavour is lightly acid and salty to very salty and, for some varieties, mildly rancid and piquant. Some white-brined cheeses are consumed while fresh (5–10 days old) but most after 2 months or more of ripening in brine.

Cheeses are packed in containers of various sizes and shapes. The most common are tinned or lacquered metal containers or wooden barrels holding 15–16 kg or 40–50 kg of cheese, respectively. The containers are usually filled with brine but, sometimes, cheese whey containing 8–10% or more salt is used. After ripening, cheeses (250–1000 g) may also be packed in plastic bags under vacuum and without brine, for retail marketing.

It is difficult to give an average composition for white-brined cheese owing to the variety of milks and technologies used, but moisture usually lies between 50 and 58%, total nitrogen 2.7–3.1%, fat-in-dry-matter 40–50%, and salt-in-moisture 5.5–9% or above. The pH lies between 4.0 and 5.0 but it is usually 4.2–4.8.

Owing to the low pH, the high salt content and the relatively short ripening period, biochemical changes are not extensive during ripening. Mature cheeses still contain lactose (1%). Usually, the proteolysis index ([water-soluble N/total N] $\times$ 100) lies between 10 and 25% and the total free amino acid content ranges between 1 and 7 g/kg cheese. Levels of free fatty acids range between 2 and 4 g/kg cheese. Acetic acid is the dominant volatile carboxylic acid.

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165 What causes early and late gas blowing in white-brined cheeses?

E. Alichanidis

With a few exceptions (e.g. Emmental, Gruyère, Gouda) for the great majority of cheeses, the formation of holes in cheese mass during curdling, salting or ripening is generally considered a defect [**57**]. The term blowing (or swelling) is used to describe the formation of numerous small (1–2 mm) or larger (3–6 mm) holes in the cheese mass due to the production of unwanted gas (CO_2, H_2 or both). The holes are mostly round and can be formed during the early stages ('early gas blowing') of cheesemaking (curdling, drainage, salting) or after 1–2 months during ripening and storage ('late gas blowing').

Early gas blowing

Early gas blowing is, by far, the most common defect for white-brined cheeses. It occurs very rapidly, usually 22–48 h after curdling and the visible outcome is the presence of numerous, generally small, holes within the curd mass/cheese mass. In most cases, the problem is not apparent until the cheese blocks are cut. However, in some cases, intense gas formation can cause a deformation of the cheese block and significant swelling, resulting in a sponge-like cheese. This defect is caused by the multiplication of milk microorganisms during cheesemaking. It is much more frequent in cheeses made from raw milk of poor bacteriological quality and it is amplified when the temperature of the environment is high, and cooling facilities do not exist to lower the temperature of draining and salting rooms.

Coliform bacteria are generally responsible for early gas blowing, particularly *Enterobacter aerogenes*, *Escherichia coli* and *Klebsiella aerogenes*, which ferment lactose and, besides acid, produce CO_2 and H_2. H_2 is very weakly soluble in the water phase and favours blowing. The characteristic of coliform-related blowing is the appearance of very numerous and very small holes (1–2 mm) within the cheese mass (Fig. 1b).

When small holes are accompanied by larger ones (3–6 cm), yeasts are usually also involved (Fig. 1c,d). Yeasts alone do not frequently cause early gas blowing and, in cases where they do, the defect is not severe, since only a few species can ferment lactose and produce CO_2. Yeasts that may be involved in early gas blowing include *Kluyveromyces lactis, Dekkera anomala* and *Torulospora delbrueckii*, depending on the local factory; microflora and species vary from country to country.

The presence of coliforms and yeasts in cheesemilk does not necessarily lead to this defect in cheese. Early gas blowing appears and is serious when two factors coexist: (1) the initial number of microorganisms associated with this defect is relatively large ($>10^5$–10^6/ml milk) and (2) the rate of curd acidification by the starter culture is very slow or insufficient to suppress their growth.

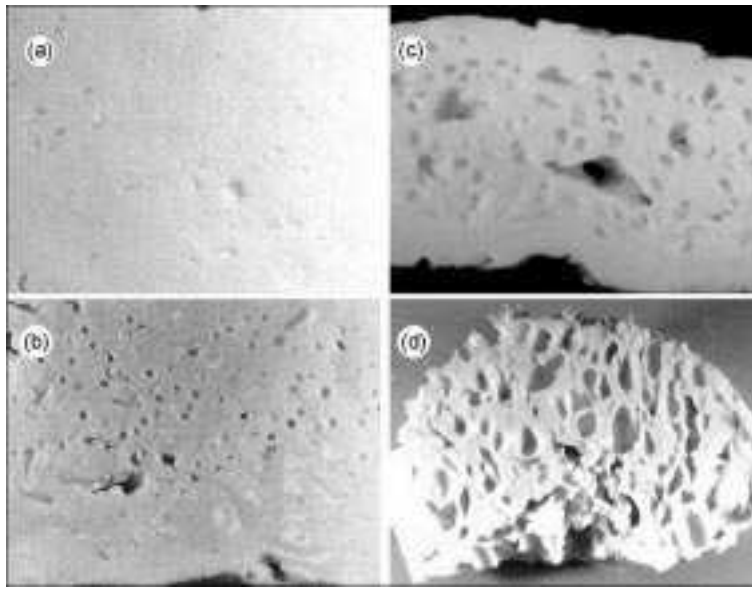

Fig. 1 Early gas blowing of white-brined cheese: (a), normal cheese; (b), cheese infected by coliforms; (c) and (d), cheese infected by coliforms and yeasts.

Early gas blowing can be prevented through the following measures:

- Microorganisms responsible for the defect can be eliminated by pasteurisation of milk [**11**]. Recontamination is avoided by sterilisation of all equipment used for cheesemaking and good manufacturing practice.
- The key to avoid early blowing is acid production at the appropriate rate and time [**17**], in order to suppress the growth of defect-causing microorganisms. So, starter cultures should be active and free from bacteriophage [**21**] and unwanted microorganisms and cheesemilk should be free from antibiotics [**19**]. The cheesemaker should be careful because although lactic acid production during curdling and draining is of vital importance, an excessive acidification leads to excessive whey drainage [**36**]. Yield decreases and the cheese obtained is dry, hard and grainy and without cohesion, especially when goat's and cow's milk are used.
- The use of milk free from antibiotics is essential for several reasons. Cheesemilk should be checked before cheesemaking using a commercially available test kit. The cost of the above test may be high for small enterprises. An alternative practical test is the 'yoghurt test', which is very simple and cheap, although not as sensitive and reliable: the milk is heated at ~85 ºC for 5 min, cooled to 42–43 ºC and inoculated with a freshly prepared yoghurt culture (3%). After fermentation for 2.5–3 h, the yoghurt gel is inspected. Milk containing no antibiotics should give a firm gel with a good strength; otherwise, the gel is very weak or gelation may not occur at all.

Measures to cure early blowing once it has occurred are very limited and not very effective. At first notice of the defect during draining, the temperature of the room should be lowered immediately. Salting should be done earlier and at lower temperature than usual and the brine used for salting should be more concentrated (>18–20%, w/w). Care should be taken for salt to penetrate quickly and be distributed evenly within the whole cheese mass.

Cheeses need special attention while in brine. Because of gas formation, cheese blocks may float in brine-baths and their upper surface must be salted with coarse dry salt. During salting, the brine solution becomes weaker, especially at junctions between cheese blocks, because of excreted whey. A way to stir up the brine is by pushing from time to time the cheese blocks down into the brine. Also, after some time, salt should be added to the brine solution to maintain its concentration.

Late gas blowing

The term late gas blowing ('late gas swelling', 'butyric acid swelling') is used to describe the formation of unwanted gas holes in the cheese mass after 1–2 months from cheesemaking (during ripening and storage). Late gas blowing is a most unusual defect for white-brined cheeses being, actually, a defect of semi-hard and hard cheeses, caused mainly by the outgrowth of *Clostridia* (principally, spores of *Clostridium tyrobutyricum* originating mostly from silage). The spores fully survive the high-temperature short-time (HTST) treatment normally applied to pasteurise the cheesemilk. They germinate in cheese, where they can grow at >7 ºC under anaerobic conditions, producing principally butyric acid, CO_2 and H_2 from the breakdown of lactic acid. The optimal pH for their growth is 5.8 but they can grow in a pH range of 4.5–7.5. At optimal pH they tolerate as much as 5.5–6% salt.

The environment of the white-brined cheeses is unfavourable for the growth of *Clostridia* to numbers enabling them to cause a defect, since the pH of the cheese falls quickly (in 24–48 h) below 5.0 and approaches 4.6 after 2–3 weeks. Furthermore, the salt-in-moisture content of the white-brined cheese is usually 5.5–10%. During salting, the salt penetrates quickly through the whole cheese mass as these cheeses have no rind, the cheese blocks are small, weighing 250–1000 g, and they are stored in brine containing more than 8% salt. Only rarely, when the cheese pH is well above 5.0 and, at the same time, the salt-in-moisture is <5%, cheeses may show this defect after 1 or 2 months' storage.

Although this is a very rare defect for white-brined cheese, some general rules may apply to eliminate clostridial spores contaminating milk. The most radical measure is to limit the initial milk contamination at the stage of production by using good quality silage and, to avoid contamination during milking (teat cleaning, discarding the first milk, etc.). A great part of clostridial spores may be removed by bactofugation or microfiltration. Alternatively, their growth may be prevented by using KNO_3 or $NaNO_3$ (10–20 g/100 l milk) or lysozyme (where allowed).

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166 What causes blowing of the white-brined cheese containers?

E. Alichanidis

A type of gas blowing, which is sometimes confused with late blowing [**91**], is the swelling of the white-brined cheese containers, but not of the cheese, which is of good quality and no offensive odour or taste are detected.

The problem is related to post-pasteurisation contamination by several groups of microorganisms producing gases, which cause the inflation of tins or the ballooning of the plastic bags used for cheese packaging. Usually, the defect produced by heterofermentative lactic acid bacteria [**56**] is not severe, although some of these organisms, besides lactose, also ferment citrate to produce CO_2. Sometimes *Bacillus* spp. also cause this defect. The defect is also caused by some yeasts originating mainly from the brine used for salting the cheese. The defect in this case is more serious as the yeasts can withstand well the harsh environment of the brine. When contamination is not severe, and the containers are kept under constant low temperatures (<4 ºC), this defect may remain unnoticed. Usually, it shows up when the containers are exposed to ambient temperature during transportation and marketing of the cheese. This defect can also appear when the containers are sealed before the intense cheese fermentation is completed; in that case, blowing is more pronounced and appears earlier during ripening.

To prevent this defect, post-pasteurisation contamination should be avoided by improving plant sanitation. Walls, floors and ceilings should be cleaned and the equipment sterilised. If contamination with yeasts is suspected, a good practice is to pasteurise the salting-brine from time to time. If the cheese pieces are intended to be individually packed in plastic bags, packaging should be applied after the cheese is fully ripe and as much air as possible should be removed during vacuum sealing.

If the tin is completely sealed, a small hole should be made in the lid and, simultaneously, the sides of the container should be knocked by hand to allow gasses to escape. Then, the container should be resealed. In the case of film-packed cheeses, returned back from the retailer, the cheese should be re-packed in a new plastic pouch after having been washed with brine. However, this is not a good approach; it is much better that the blowing be prevented as described above.

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167 How may mouldiness in white-brined cheese be avoided?

E. Alichanidis

Although certain moulds are used as secondary cultures in the manufacture of mould-ripened cheeses [**18**, **128**, **137**], their growth on the surface of the white-brined cheeses is considered a defect. Mould growth on theses cheeses may result in several problems:

- surface discoloration (yellowish, blueish, greenish);
- off-flavours including ester-like odours, 'plastic' or 'kerosene' odours. If sorbates are used as preservatives (when allowed), sorbate-resistant moulds are able to metabolise them and form plastic-like or 'kerosene' odours;
- rotting; and, most importantly,
- formation of mycotoxins (e.g. aflatoxins).

The moulds found most frequently belong to the genera *Penicillium*, *Aspergillus*, *Mucor*, *Cladosporium*, *Alternaria* and *Geotrichum*.

Mouldiness in white-brined cheese is very rare and will never occur if the cheeses are continuously and completely submerged in the brine of the package. Moulds contaminate the surface of the cheese mainly during the period after the curd is formed into blocks (i.e. after draining and salting) and before packing. So, care should be taken to ensure that atmospheric contamination is minimal by using (a) antifungal paints for ceiling and walls (if not covered by tiles), (b) UV lamps and (c) sterilisation of the equipment. When the cheese is stored in wooden barrels, the barrels should be thoroughly washed and steam-sterilised (especially the upper lid) and left upside-down to drain the condensed steam before filling with cheese pieces. If the cheese pieces are intended to be individually packed in plastic bags, they should be packaged after the cheese is fully ripe and as much air as possible should be removed during vacuum sealing.

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168 What causes softening of the cheese body in white-brined cheeses?

E. Alichanidis

Cheeses become soft by taking up water from the brine in the package and the volume of the cheese blocks expands. The cheese blocks may stick together, making it sometimes impossible to take them out of the container one at a time without damaging them. In extreme cases and if the cheeses are left unattended, the defect proceeds further and the cheese body becomes very soft like a thick mud and the cheeses start to rot. The colour of their surface changes from white to yellowish and progressively to pale brown or even to brown due to the growth of yeasts and moulds. The smell of the cheeses is very bad, like a rotten egg, and of course they are unsuitable for consumption.

Softening of cheese with normal pH and moisture

Softening of white-brined cheeses with normal pH (~4.6) and moisture (~56%) is very rare. It occurs only when the salt concentration in the brine in the package is lower than the salt-in-moisture content of the cheese. To prevent the appearance of this defect, the salt concentration in the brine added to fill the final package should be at least 2% higher than the salt-in-moisture of the cheese.

When the defect is not severe, it can be cured by adding salt to the brine in the package. A better, and recommended, alternative is to replace the brine in the package by a solution with a higher salt concentration, containing ~0.1 or 0.2% $CaCl_2$ and pH adjusted to ~4.6 with acetic or citric acid.

Softening of cheese with insufficient acidity and high moisture content

Softening becomes serious when cheeses with insufficient development of acidity and with high moisture content are prematurely transferred from the warm room (16–18 ºC, used for intense ripening) to the cold room (4–5 ºC) for slow ripening and storage. At the lower temperature, a further development of acidity is very slow. Cheeses cannot expel whey [**34**, **36**] and the pH and moisture remain high. In such conditions (high pH, high moisture) proteolysis proceeds faster and, besides coagulant, starter and secondary flora enzymes, plasmin is also expected to take part and the cheese mass absorbs water from the brine. The situation becomes worse if fresh brine is added to the final package (i.e. no pH adjustment, containing low levels of soluble calcium) and its salt concentration is low.

The final result is that the cheeses become softer, the blocks expand and may stick together, which makes it sometimes impossible to remove them from the package without damage. When the package is opened, an odour like boiled cabbage (due to sulphur compounds) is often noticed.

Up to this stage, the defect may be curable (at least partly). But if the cheeses

are left unattended, their pH increases further, reaching values over 5.5 or even higher and the cheeses start to rot. Cheeses absorb ('drink') part or all the brine, their body softens greatly and becomes like mud, and their surface colour, especially that of the blocks of the upper layer, becomes brownish owing to the growth of yeasts and moulds, and the smell becomes horrible, like a rotten egg. In that case, the defect is not curable and the cheeses should be carefully removed from the factory and discarded.

To prevent softening of white-brined cheese (except for Domiati and Halloumi-type cheeses), it is essential that about 24 h after coagulation, the pH is lower than 5.0, the moisture ~58% or lower and the salt-in-moisture content is ~2.5%. Also, the cheeses should remain in the warm room (16–18 ºC) for long enough (usually 2–3 weeks) to complete their intense fermentation stage and attain a pH value ~4.6 or lower, a moisture level of ~55% and a salt-in-moisture content 5.5–6.0% before being transferred to the cold room (4–5 ºC) for further ripening and storage. A sufficient quantity of brine should be added to the containers to ensure that all cheese blocks are submerged. The salt content of the brine should be at least 2% higher than the salt-in-moisture content of the cheese.

It is a good practice, while the cheeses are in the cold room, to open a few containers from each batch periodically to check the level of the brine and the pH of the cheese. If the level of the brine is lower than desired and the pH of the cheese is higher than that when cheeses were transferred to the cold room (>5.0), this is a sign that the cheeses are prone to spoilage and it would be good to take action immediately.

Usually at this stage of the defect the cheese body has started to soften and expand but cheese blocks are not stacked together and their flavour is still acceptable. To prevent the defect becoming worse, transfer containers to 8–10 ºC and, if necessary, add new brine to cover the cheese blocks. The added brine should have a pH value of ~4.6, a salt content 3–4% higher than salt-in-moisture of the cheese and should contain 0.1% $CaCl_2$. After adding the brine, invert the containers several times to mix the old and the new brines and leave the containers at 8–10ºC for about a week. Then move the containers to the warm room (16–18 ºC) and leave them until the cheeses develop sufficient acidity. If the starter culture is still active, the pH of the cheese will drop to the proper value in a week or two, cheeses will expel water and their body will shrink, and the level of brine will rise. Then, the cheeses should be transferred to the cold room but it is advisable to market the cheeses as soon as possible. If the cheeses are not able to develop acidity by themselves, they should be treated as described below.

The defect proceeds further than described above

When the defect proceeds further all phenomena described above are enhanced. Thus, the pH of the cheese rises further reaching values well over 5.0, cheese blocks stick together, most of the brine is absorbed and, in many instances, it is insufficient to cover the cheese. A light smell of boiled cabbage is noticed when

the containers are opened. This is the last chance for immediate action in order to save the cheese, at least partly.

To solve the defect at this stage (if permitted), remove cheese blocks from the containers (inevitably, some cheese will be lost), wash them with brine using a soft brush and expose them to the air for 3–6 h to allow volatile sulphur compounds to be lost. Prepare new brine containing 10–12% salt, 0.1–0.2% $CaCl_2$ and adjust the pH to about 4.5 with acetic or citric acid. Repack the cheese, placing a parchment paper between cheese block layers and fill the container with enough brine to cover completely the cheese. This treatment will stop the defect proceeding further very soon, but cheeses should be marketed immediately.

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169 Why is the brine surrounding my white-brined cheese ropy?

E. Alichanidis

Ropiness is a defect of the brine surrounding the cheese in the package and is not usually associated with undesirable organoleptic properties, although it affects the appearance of the cheese, making it unappealing to the consumer. The characteristic of this defect is that, when cheese blocks are removed from the package, the surrounding brine forms strands and does not run freely away from the cheese surface (Fig. 1). The increase in the brine viscosity is due to exopolysaccharides (EPS), compounds produced by some strains of mesophilic or thermophilic lactic acid bacteria, which contaminate cheesemilk, cheese surface or brine. Sometimes, however, the starter culture used for cheesemaking contains strains capable of producing EPS.

Organisms responsible for this defect, under some circumstances, include some strains of *Lactobacillus plantarum* or *Lb. pseudoplantarum*, *Alcaligenes* spp. and also strains of yoghurt cultures (*Lb. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*). All measures should be taken to avoid the occurrence of this defect, since only few remedies can be done afterwards. To prevent ropiness:

- Carefully select starter and adjunct cultures [**18**], especially yoghurt cultures to avoid strains that produce EPS (such strains are often used to improve body, mouthfeel and prevent wheying-off in fermented milks and stirred yoghurts).
- Take any measure to avoid contamination of the cheesemilk after pasteurisation [**11**], the cheese itself, and the brine by cleaning and disinfecting (e.g. by steam) the facilities of the cheese plant. Also, good quality drinking water should be used to prepare the brine of the package.
- Brine for salting the cheese [**41**] may be a source of contaminating bacteria (e.g. *Lb. plantarum*). These organisms do not produce this defect, even in old brines, because they cannot tolerate the high salt content (16–18%). Although they cannot grow in such an environment, they can contaminate the cheese blocks and be transferred to the brine of the final package, which usually contains 8–9% salt. Some strains can grow in this new environment and may

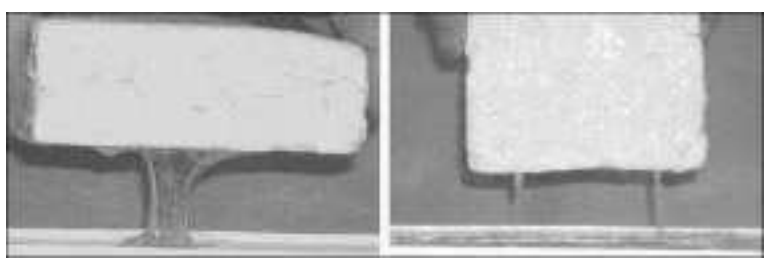

Fig. 1 Ropiness development in brine used for storage of white-brined cheese.

produce the defect, especially if the pH of the brine of the package was not adjusted and the pH of the cheese is high (>4.8). For cheese with a low pH (close to 4.0), adjustment of the pH of brine and salt content of >10% may substantially retard or even eliminate the defect but, in such cases, the cheese will be very acid and salty. So, if the brine is suspected as the source of contamination, periodic change (which is expensive) or good pasteurisation of the brine will be beneficial.

To cure the appearance of ropiness, if permitted, remove the cheeses from the package, wash them with brine using a soft brush, leave the blocks for 1–2 h on a bench covered with cheese-cloth (and repeat washing). Clean the brush frequently and thoroughly with hot water. Repack cheeses in clean new containers; the old containers cannot be cleaned and steam-sterilised effectively, especially the inner part of the upper lid, joints and corners. Prepare a fresh brine containing 10–12% salt and adjust its pH to 4.5 or lower using acetic acid. Fill the containers with sufficient brine, taking care to cover the cheese blocks completely. This treatment will retard early reappearance of the defect, but it is advisable to market the cheeses as soon as possible.

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Acid and acid/heat-coagulated cheeses

170 Introduction

N. Y. Farkye

Acid and acid-heat coagulated cheeses [**81**, **83**] are typically fresh (unripened) soft cheese varieties produced by the coagulation of milk, cream or whey or blends thereof via direct chemical acidification, culture acidification or a combination of chemical acidification and high heat treatment.

Because of their physical and rheological consistencies, many acid- and acid/heat-coagulated cheeses are classified as soft cheeses [**83**] – which are defined as containing minimum of 50% milk fat in the cheese solids (fat-free substance or fat-in-dry-matter, FDM) and unspecified moisture content, according to the US Code of Federal Regulations. The FAO/WHO specify >67% water in the fat-free cheese matter (Wff) Teubner (1998) and Robinson and Wilbey (1998) define soft cheeses as containing >61% Wff and 10–50% FDM. Data on the composition of various soft cheeses in the literature (Kosikowski and Mistry, 1997) reveal moisture contents in the range of 50–80%.

Acid- and acid/high heat-coagulated cheeses may be manufactured from whole milk, skim milk, cream, whey or combinations thereof. Because most acid- and acid/heat-coagulated cheeses are consumed fresh or shortly after manufacture, and also to eliminate the risk of food poisoning [**58**], it is important that the milk or other dairy ingredients used for soft cheese manufacture be adequately pasteurised (72 °C × 15 s) [**11**]. In several countries, the use of raw milk for cheesemaking is still prevalent. In the US, cheese manufactured from raw milk must be stored at a minimum of 1.7 °C for at least 60 days before consumption. This regulation limits the manufacture and sale of unripened soft cheeses from raw milk.

Table 1 Examples of varieties of acid and acid/heat-coagulated cheeses

Cheese category	Example	Manufactured from	Moisture content	Starter type/method of acidification	Secondary flora
Unripened soft cheese (acid coagulated)	Cottage Quarg Bakers	Skim milk	≤80%	*L. lactis* subsp. *cremoris*; *L. lactis* subsp. *lactis* Phosphoric acid and gluconic acid δ-lactone (GDL) for direct acid Cottage cheese	*Lc. mesenteroides* subsp. *cremoris* Citrate-positive strains of *L. lactis*
	Cream Neufchatel	Cream	55% (max.) 76% (max.)	*L. lactis* subsp. *cremoris* *L. lactis* subsp. *lactis*	Citrate-positive strains of *L. lactis* *Lc. mesenteroides* subsp. *cremoris*
	Yoghurt cheese	Milk		*S. thermophilus* + *Lb. delbrueckii* subsp. *bulgaricus*	
Unripened soft cheese (high heat + acid coagulated)	Ricotta Mascarpone Queso Blanco Paneer	Whey Cream Whole milk	<55–80	No starter. Acidification by food-grade organic acid (e.g. citric, acetic)	

Types of acid-coagulated and acid/heat-coagulated cheeses

Table 1 provides a list of acid-coagulated cheeses (e.g. Cottage, Cream, Quarg, Fromage Blanc) and those coagulated by the action of acid and heat (e.g. Mascarpone, Ricotta, Queso Blanco and Paneer). These cheeses may be manufactured from skim milk, whole milk, cream or whey.

Most of the acid-coagulated cheeses are manufactured by culture acidification using mesophilic starter [**18**] comprising *Lactococcus lactis* subsp. *lactis* or *Lactococcus lactis* subsp. *cremoris* along with flavour and aroma-producing cultures consisting of citrate-positive *Lactococcus lactis* subsp. *lactis* (formerly *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*) or *Leuconostoc mesenteroides* subsp. *cremoris*. Cottage cheese may also be manufactured by direct acidification principles using a combination of food-grade acids, often phosphoric acid and gluconic acid-δ-lactone (GDL).

The acid/heat-coagulated-type cheeses are manufactured by first heating the raw ingredient, usually whole milk, cream, whey or milk/whey blends to high temperatures (85 °C $\times$ 30 min) or equivalent to denature the whey proteins [**2**, **11**] causing them to coagulate with the caseins on acidification with a food-grade organic acid (e.g. citric, acetic, lactic). Unlike the acid coagulated cheeses, coagulation in the acid/heat-coagulated cheeses occurs at higher pH (e.g. >5.3) compared to pH 4.6 for the former cheeses. While most acid curd cheeses require small quantities of rennet to aid coagulation and curd firmness, rennet is not used in cheeses made by acid and acid/heat coagulation

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171 How may wheying-off (spontaneous syneresis) in Quarg be avoided?

N. Y. Farkye

The process of wheying-off or syneresis is the expulsion of whey or serum phase from milk gel or curd [**34**]. Syneresis is part of the process of transforming milk into Quarg (Quark) and occurs as a result of shrinkage of casein network due to pH reduction and increased temperature during manufacture.

Wheying-off of Quarg [**35**, **36**] may be due to:

- pH reduction during acidification and storage – rapid acidification leads to delayed casein network rearrangement during curd formation;
- temperature abuse during gel formation and storage of cheese – high incubation temperatures promote rapid acidification and delayed casein network rearrangement;
- proteolysis of caseins by bacteria and proteolytic enzymes in the cheese;
- disturbance of package during transportation and storage.

Quarg is made from pasteurised (or highly heated, e.g. 85 ºC × 10–40 min) skim milk, cooled to ~20–23 ºC and inoculated with 1–2% type O starter (typically comprising *Lactococcus lactis* subsp. *lactis* or *L. lactis* subsp. *cremoris*) and a small quantity of rennet (~0.5–1.0 ml/100 l) added after the cultured milk reaches pH 6.1–6.3. The cultured milk is held for 14–18 h until pH 4.4–4.5 when the acid coagulum is broken by stirring. When rennet is used, a firmer coagulum occurs at a higher pH. Addition of rennet helps to facilitate whey drainage. If rennet is not used, desired firmness occurs at a lower pH leading to over-acidification and, therefore, to wheying-off. The use of too much rennet may result in premature syneresis before the desired pH for breaking the curd is reached. Increasing incubation temperature during acidification can result in higher pH values, which lead to coarse gels and spontaneous wheying-off.

Wheying-off after packaging also occurs when the storage temperatures are high owing to contraction of the casein matrix. Also, proteolysis caused by microorganisms in cheese during storage and disturbance of product caused by movement during transportation can result in syneresis.

Some factors known to prevent syneresis of acid gels include: increasing the level of total solids in the milk (fortification of milk with milk powder), homogenisation, lowering curd-forming temperature, and rapid cooling of finished cheese to prevent further pH decrease during storage and addition of hydrocolloids.

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172 Why is Quarg dry and grainy?

N. Y. Farkye

Dryness in Quarg occurs when the moisture content is too low; typical moisture content of Quarg is ~82%. When Quarg is made with low moisture content, it tends to be dry. When the Quarg curd is broken at pH 4.6, it is gently and slowly stirred into a smooth mass before curd separation. Insufficient acid development and breaking the coagulum at high pH can lead to a grainy texture. When the coagulum is rapidly stirred without adequate cooling before curd separation, the resultant curd will be grainy. Use of excess rennet and insufficient acidity at the time of draining the curd cause this defect. Protein denaturation due to air drying of curd can also lead to a grainy texture.

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173 How may over-acid and bitter flavor defects in Quarg be avoided?

N. Y. Farkye

High acid (low pH) in Quarg is due to the use of high starter inoculation, high incubation temperature (hence rapid acidification) [**17**], and inadequate cooling of the curd during storage – leading to increased microbial activity. Careful selection of starters is important to prevent bitterness [**89**]. Some strains of lactic acid bacteria used as starters in cheesemaking are known to produce bitter peptides. Psychrotrophic bacteria (e.g. *Pseudomonas* spp.) can grow in cheese [**7**], causing proteolysis and bitterness. Also, it has been suggested that increasing the calcium content of Quarg may result in bitterness.

Bitterness in Quarg is due to excessive residual rennet (chymosin) activity in the curd during storage [**28**]. A small amount of chymosin (0.5–1.0 ml single strength per 100 l) is used for manufacture of Quarg. High residual rennet activity at low pH results in excessive proteolysis, sometimes leading to the formation of bitter peptides. Therefore, excessive rennet use must be avoided and good whey drainage helps to reduce residual rennet levels in cheese.

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174 How may the viscosity of Cream cheese be controlled?

N. Y. Farkye

Cream cheese is a soft, unripened cheese made from cream or mixtures of cream, milk or skim milk standardised to ~11% fat and 8% non-fat milk solids. Cream cheese made from mix with less than 11% fat tends to be dry, crumbly, mealy and grainy. Increasing moisture content improves the dry crumbly conditions, but too much moisture leads to wheying off. Efficient single stage homogenisation (~140 MPa) of the mix helps improve the smoothness of Cream cheese and drainage of whey. Single stage homogenisation is preferable because of cluster formation of fat globules, which increases viscosity. pH at breaking the curd also affects viscosity and texture properties of the cheese. Cream cheese is crumbly, grainy, mealy and dry when the coagulum is broken (stirred) at pH > 4.9. Best results are obtained when the coagulum is broken at pH 4.6–4.7. Low pH leads to curd that is smooth but soft, sticky and undesirable. The temperature of heating curd is 46–54 ºC. High cooking temperature gives the same effects as high pH at breaking. Similarly, a decrease in cooking temperature gives similar characteristics as breaking coagulum at low pH.

The protein content of the mix contributes to the viscosity of Cream cheese. High protein content increases viscosity of Cream cheese. High shearing rate and time during processing tends to reduce viscosity. In hot pack Cream cheese, locust bean gum is used at the rate of 0.35% to prevent syneresis.

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175 Free oil forms in Cream cheese at the outlet of the heat exchanger. How can this problem be resolved?

N. Y. Farkye

During Cream cheese manufacture, the Cream cheese mix is pasteurised (63–85 ºC × 30 min or equivalent) and homogenised (~14.0 MPa, single stage) before fermentation and curd formation. Homogenisation destroys the original milk fat globule membrane [**32**], freeing milk fat which is coated by newly formed membranes comprising casein and whey proteins. Consequently, homogenisation of the mix minimises fat losses when whey is separated from the curd. When the mix is not properly homogenised and the fat is not properly emulsified, the mix is destabilised during pumping and stirring, leading to fat separation, churning and free oil formation.

To prevent free oil formation, care must be taken to avoid over pumping, over mixing and churning of the milk fat. Ensure that the mix is adequately homogenised.

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176 Why is the coagulum of Cottage cheese weak with poor syneresis?

N. Y. Farkye

Syneresis is the physical expulsion of whey or serum phase from milk gel after the coagulum (gel) is cut [**34**]. Syneresis also occurs during stirring and cooking of the curd [**36**]. Both occurrences are normal parts of the cheesemaking process. Syneresis can also occur in finished Cottage cheese after packaging. This form of syneresis, also called wheying-off, is undesirable.

When skim milk for Cottage cheese manufacture is high-heat treated instead being subjected to minimal pasteurisation, whey proteins (mostly β-lactoglobulin) denature and form a complex with κ-casein [**11**]. The associated whey proteins coagulate with the caseins on acidification; coagulation occurs at a higher pH. The coagulum is weak and retains moisture, resulting in a soft curd and poor syneresis during cooking. A weak coagulum also occurs when the total solids level is low (<9% dry matter content), and calcium chloride and rennet are not used to aid coagulation. During Cottage cheesemaking, ~0.02% $CaCl_2$ and very low levels of rennet (<0.5 ml double strength chymosin/454 kg milk) are often added. The coagulum firmness is also influenced by the setting temperature. The setting temperature for short-set Cottage cheese (curd ready to cut in 4–6 h) is 30–32 ºC, while that for long-set Cottage cheese (coagulum ready to cut in 12–16 h) is 22–25 ºC. Setting at temperatures lower than recommended may result in delayed curd formation and a weak curd that shatters readily.

After cutting the curd, the rate of cooking (increasing temperature during stirring) affects the rate of whey expulsion [**36**]. Suggested rate of cooking is 0.11 ºC each 5 min for the first 30 min and about 0.21 ºC each 5 min until a cook temperature of 52–54 ºC is reached about 2 h after the start of cooking, with continuous and steady stirring to prevent matting of the curd. When the rate of heating is too rapid, the outer surface protein layer of the curd cooks fast and forms a semipermeable shell around the curd particles [**38**]. This phenomenon causes whey to be trapped inside the curd – leaving the interior of the curd particles soft, hence, poor syneresis. Also, curd particles allowed to matt during cooking tend to hold water resulting in poor syneresis.

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177 What is agglutination of starter bacteria and how do I avoid sludge formation?

N. Y. Farkye

Agglutination is the term used to describe clumping of bacterial cells due to the presence of antibodies or agglutinins that are specific for those strains. The sticking action of agglutinins causes the bacteria to flocculate and sediment to the bottom of the vat where they continue to produce acid, causing over-acidification and precipitation of the caseins and leading to sludge formation [**20**]. Agglutinins are natural inhibitors in milk and are primarily immuno-globulins that act as antibodies against specific antigens – often bacteria.

Agglutination may be prevented by renneting milk immediately after adding starter, causing the starter cells to become enclosed in the paracaseinate network. Homogenisation of starter bacteria or addition of lecithin causes dispersion of starter chains and prevents them settling on the bottom of the vat, thereby preventing agglutination.

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178 How do I solve the 'floating curd' defect in Cottage cheese?

N. Y. Farkye

Floating curd is caused by entrapped gas (especially CO_2) in the curd. Starter bacteria such as citrate-positive *Lactococcus lactis* metabolise citrate to produce CO_2 [**18**]. At low cooking temperatures, the gas is still formed but is in solution. Curd containing too much gas not only floats but also tends to mat together during early stages of cooking. The gas bubbles cause the curd particles to rupture, resulting in shattering and yield losses. Interestingly, starters that metabolise citrate to produce diacetyl, which gives Cottage cheese most of its buttery flavour and aroma, produce CO_2. Therefore, to prevent curd floating, careful selection of starters that produce little or no CO_2 is necessary. Floating is more frequently encountered when non-fat dry milk is used to fortify skim milk or used as a sole source of milk for Cottage cheese manufacture as some starters produce more gas in high-solids than in low-solids milk.

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179 Why are the curd particles for Cottage cheese slick and slimy?

N. Y. Farkye

Slick and slimy curd particles are due to microbial spoilage and when the pH of the cheese is high. The main causative agents are psychrotrophic bacteria [**7**], e.g. *Pseudomonas* spp., *Alcaligenes* spp. and *Flavobacterium* spp. that grow rapidly at refrigeration temperatures. These organisms are proteolytic – hydrolysing caseins to cause liquefaction of the curd. *Alcaligenes* spp. grow to produce slime. Also, the use of alkaline wash water can result in slick curd owing to the formation of sodium caseinate on the surface of the curd particles.

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180 Why is there whey separation from my Cottage cheese after packaging?

N. Y. Farkye

Separation of whey or 'free whey' in Cottage cheese occurs when the cream dressing curdles as a result of acid produced by starter bacteria [**17**]. When Cottage cheese curd is undercooked, it retains moisture and residual lactose which promote the growth of starter bacteria when the storage temperature is high (>21 ºC). Starter activity causes pH reduction, which leads to clotting of the dressing and wheying off. High storage temperatures also promote the growth and proteolytic activity of spoilage microorganisms, which hydrolyse caseins, leading to wheying off. Also, over-stabilisation of dressing can result in wheying off in Cottage cheese.

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181 What strategies should be adopted to improve the yield of Cottage cheese?

N. Y. Farkye

The typical yield of Cottage cheese is 15–17 kg cheese at 80% moisture per 100 kg skim milk. All methods that increase protein content of the skim milk will increase yields and, conversely, yield losses occur due to excessive proteolysis of caseins by starter bacteria and rennet. Several methods listed below have been reported to increase the yield of Cottage cheese [**48**, **49**, **50**, **51**, **52**]. However, the commercial success of each individual method is unknown.

Methods for increasing yield of Cottage cheese include the following:

- Heating skim milk to temperatures higher than minimum high-temperature short-time (HTST) heat treatment of 72 °C × 15 s. This causes denaturation of whey proteins that form a complex with caseins and cause them to be trapped in the curd [**11**, **12**]. The drawback for this approach is that the coagulum is soft at cutting and the curd may be too soft (and mushy) due to moisture entrapment.
- Increasing total solids in cheese milk by fortification with skim-milk powder or using skim milk concentrated by membrane filtration technologies (e.g. ultrafiltered (UF) [**16**] or microfiltered (MF) skim-milk retentate). This approach increases the protein (casein and whey protein) content of cheese-milk and also traps more whey proteins in the curd.
- Addition of sodium hexametaphosphate or carrageenan plus sodium hexametaphosphate to milk.

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182 What are the likely causes of surface discoloration, off-flavors and bitterness in Cottage cheese?

N. Y. Farkye

Bitterness is caused by psychrotrophic bacteria [**7**], e.g. *Pseudomonas putrefaciens*. These bacteria have active proteolytic enzymes that hydrolyse caseins to pitter peptides. *P. putrefaciens* is also known to cause surface taint and off-odours owing to the liberation of some organic acids, especially isovaleric acid. Others, such as *P. fluorescens*, cause surface discoloration owing to the production of water-soluble fluorescent pigments (which glow under UV light). Also, curds that are cooked too fast during the early stages tend to trap moisture [**38**], resulting in over-acidification and increased rennet activity. Poor quality skim milk and the use of bitter starters can lead to the development of off-flavours and bitterness [**89**]. The use of wash water containing high organic matter content and high chlorine (>5 ppm) may result in unclean medicinal off-flavours in Cottage cheese.

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183 How may the shelf-life of Cottage cheese be extended?

N. Y. Farkye

Cottage cheese has a shelf-life of 2–3 weeks under refrigerated storage. Good quality skim milk and ingredients as well as establishing critical control points and strict adherence to good manufacturing practices and sanitation standards operating procedures to ensure that cheesemaking equipment, utensils and environment (e.g. air in processing and packaging areas) are clean will improve product quality and shelf-life.

After cooking and whey removal, Cottage cheese curd is washed successively in water to firm up and cool the curd, and remove residual lactose. Hence, controlling quality and pH of wash water are important in enhancing shelf-life. Wash water must be potable and preferably at slightly acidic or neutral pH. Hard water, alkalinated water and poor quality well water are unacceptable. Wash water may be chlorinated to give 5 ppm available chlorine prior to use. Hard water and water at alkaline pH may be acidified to pH $\leq$ 6.5 with food-grade acids (e.g. phosphoric or citric acid). Acidic conditions enhance the effectiveness of chlorine. To increase shelf-life of dry cottage cheese curd, it can be stored in cold brine until it is time for cream addition and packaging.

The shelf-life of creamed Cottage cheese can be increased by adding preservatives such as propionates and sorbates (~0.075%, w/w) when permitted by law. These products are usually added to the cream dressing. Commercially available natural bacteriocin-like compounds that are effective in inhibiting Gram-negative psychrotrophs, yeasts and moulds may be added to cream dressing to extend shelf-life. Also, lactic acid bacteria that produce bacteriocins may be used as starters for cheesemaking. In addition, technologies to inject CO_2 directly into the dressing or flushing the headspace of packaged Cottage cheese with pure CO_2 can control the growth of psychrotrophic spoilage bacteria and extend shelf-life.

Packaging and lids should be stored in clean dry areas. Also, it is important to prevent temperature abuse during manufacture, distribution and storage (both in warehouse and in refrigerated cases in supermarkets) to help increase shelf-life. It is important to keep wash water temperature less than 4 ºC, and the curd should be creamed and packaged immediately after washing. Temperatures less than 4 ºC must be maintained during transportation and storage. Temperatures close to 0 ºC will extend shelf-life.

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184 What are harsh and green flavour defects in Cottage cheese?

N. Y. Farkye

Cottage cheese has a mild acid taste due to the presence of lactic acid and, to a lesser extent, formic and acetic acids. The buttery aroma in Cottage cheese is due mostly, to diacetyl which is produced by citrate metabolism by citrate-positive strains of *Lactococcus lactis*. An acceptable level of diacetyl in Cottage cheese is about 2 ppm. However, the ratio of diacetyl to acetaldehyde determines whether the flavour is 'harsh' or 'green'. Good Cottage cheese flavour occurs when the ratio of diacetyl to acetaldehyde is 3–5. When the ratio of diacetyl to acetaldehyde is greater 5, Cottage cheese flavour is described as 'harsh'; and when the ratio is less than 3, the flavour is described as 'green'.

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185 How may the mouthfeel of Queso Blanco be improved?

N. Y. Farkye

Queso Blanco is a soft cheese manufactured by direct acidification of highly heated (85–90 ºC) milk using food-grade organic acids, e.g. acetic, citric, lactic acids. Fruit juices such as lemon juice have also been used. The strength of acid used is typically 1–3%. After whey drainage, the curd is salted, hooped and packaged. The texture and mouthfeel of Queso Blanco depend on the curd characteristics, which are governed by the severity of heat treatment given to milk, acid type used for manufacture and mode of clotting. When the acid used is too strong and the finished pH of the cheese is too low, the cheese has a grainy texture.

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186 What approaches may be used to control the texture of Queso Blanco?

N. Y. Farkye

The texture of Queso Blanco depends on the heat treatment given to milk, coagulation conditions (i.e. temperature, acid type, strength and rate of addition) and cooling conditions. When the milk is coagulated at a higher temperature, the resultant cheese has a firmer texture due to reduced moisture content. The type of acid used for manufacture also affects moisture levels and, hence, texture. Queso Blanco made using acetic acid has a firmer texture than that made with citric or lactic acid. Also, the strength of acid and the mode of delivery are important. The use of higher acid concentrations not only imparts an acid flavour but also gives a softer cheese. All the acid needed for coagulation must be added in a short time with minimal stirring to allow the curd to sink to the bottom of the vat. Too much stirring causes occlusion of air and results in floating and non-cohesive curd.

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Cheese as a food ingredient

187 Introduction

P. L. H. McSweeney

Cheese may be produced in a great variety of flavours and forms and has been enjoyed for centuries. While much cheese is consumed on its own, or with bread or crackers, a large and increasing proportion of cheese produced is used as an ingredient in other food products. The use of cheese as an ingredient in cooking dates back to Roman times and culinary applications of cheese have resulted in a wide range of dishes, including omelettes, quiches, sauces, chicken cordon bleu and various pasta dishes.

Industrial applications of cheese include the production of shredded cheese, cheese blends, combination products and various cheese-based ingredients including processed cheese, cheese powders and enzyme-modified cheeses [**83**, **189**, **197**]. Certain varieties have traditionally been used mainly as ingredients (e.g. low moisture part-skim Mozzarella [**146**] as a topping on pizzas or grated Parmesan used as a condiment on pasta [**96**]) but increasingly other varieties are being tailor-made for ingredient applications. When manufacturing cheese for an ingredient application, one must consider a range of properties, including the ability to crumble/slice/shred, flowability of the shredded product, ability to 'cream' when sheared, nutritional value and flavour. In addition, if the cheese is to be heated, a range of parameters associated with the melted cheese must be considered including meltability, flow resistance, stretchability, chewiness when baked/grilled, degree of free oil formation, viscosity and extent of Maillard browning [**188**]. By varying the manufacturing protocols, cheesemakers can control many of these properties and thus produce natural cheese with the desired properties for ingredient applications.

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188 How may browning of heated cheese be controlled?

P. L. H. McSweeney

Much cheese manufactured now is used for ingredient applications [**187**] and hence cheese may be exposed to heat during processing or cooking. Browning of heated cheese is due to the Maillard pathway, which is a very complex series of reactions involving amino groups of free amino acids, peptides and proteins and a reducing sugar, and which is favoured by intermediate water activities and high temperatures. A certain degree of Maillard browning may be desirable in certain applications of cheese as an ingredient (e.g. slight browning of low-moisture part-skim Mozzarella used as a pizza topping) or it may be undesirable.

Control of Maillard browning in heated cheese involves removing one of the necessary reactants and/or providing conditions unsuitable for browning to occur. Browning is favoured at high temperatures, pH above ~6 and at intermediate a_w. However, it is often impractical to vary these conditions to avoid browning and hence control of Maillard browning in cheese usually involves eliminating one of the reactants. Although extensive proteolysis may promote Maillard browning, since a high level of protein is always present in cheese, the rate-determining reactant for Maillard browning is the reducing sugar. In the context of cheese, the sugar involved in Maillard browning is usually lactose or galactose. Most of the lactose remaining in the curd after pressing is rapidly metabolised by the starter [**18**] but lactose may persist in cheese if the salt-in-moisture (S/M) content inhibits starter activity [**55**]. Hence, careful control of S/M may help to reduce levels of residual lactose and hence browning. Lactose that persists after S/M has increased to an inhibitory level is usually metabolised during ripening by non-starter lactic acid bacteria (NSLAB) [**56**] and changes to the NSLAB may cause lactose to persist. Another approach to reducing lactose levels is to wash the curd in water, as is practised during the manufacture of Cottage cheese. When using thermophilic starters, it is important that the *Lactobacillus* strain used is capable of metabolising the galactose moiety of lactose since the other component of the starter, *Streptococcus thermophilus*, is unable to metabolise galactose (Gal^-). The absence of lactobacilli or the use of Gal^- strains will lead to the accumulation of galactose in the cheese and hence to the risk of Maillard browning when the cheese is heated.

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Processed cheese

189 Introduction: what is processed cheese?

T. P. Guinee

Pasteurised process cheese products (PCPs) are cheese-based foods produced by comminuting, blending and melting one or more natural cheeses and optional ingredients into a smooth homogeneous blend with the aid of heat, mechanical shear and (usually) emulsifying salts (ES). Optional ingredients, which are determined by the product type, include water, dairy ingredients, emulsifying salts, flavours, colours, preservatives and condiments. The development of PCPs in the period from 1910 to the 1920s was inspired by the possibility of increased cheese trade through the manufacture of products that were physicochemically and microbiologically more stable and transportable than the natural cheeses from which they were made.

There are various types of PCPs, the type or category of which depend on the particular region where they are produced. In the USA, the code of Federal Regulations defines four types based on permitted ingredients and composition: pasteurised process cheese, pasteurised process cheese food, pasteurised process cheese spread and pasteurised blended cheese. The percentage of natural cheese in PCPs ranges from a minimum of 51% in processed cheese spreads and foods to ~95% in processed cheese.

The manufacture of PCPs essentially involves the following major steps:

- cleaning and shredding of the natural cheese;
- formulation;
- blending of cheese with emulsifying salts, water and optional ingredients;

- processing of the blend;
- hot packing and cooling.

Formulation involves selection of the correct type and quantity of natural cheeses, ES, water and optional ingredients to give a PCP with the desired composition, textural and functional properties [**192**, **193**, **194**]. Processing refers to the heat treatment of the blend, by direct or indirect steam, with constant agitation until it is molten and uniform in consistency. In batch processing, the temperature–time combination varies (70–95 ºC for 4–15 min) depending on the formulation, extent of agitation, desired product texture and shelf-life characteristics. In continuous cooking, the blend is heated to 130–145 ºC for a few seconds, flash cooled to 90 ºC and held for a further 4–15 min. Processing has two main functions:

1. It kills any potential pathogenic and spoilage microorganisms in the blend, and thereby extends the shelf-life of the product.
2. It facilitates the interaction of the different blend ingredients and the physicochemical and microstructural changes necessary to transform the blend into a physicochemically stable end product.

The processed blend is conveyed from the cooker to the filling machine where it is packed hot prior to cooling and storage at 4 ºC. Numerous packaging formats are possible through the use of specialised filling/moulding machines including individually wrapped portions (e.g. foil-wrapped triangles), blocks, sausage-shapes, cans, tubes and slices.

Added ES plays a crucial role in the formation of PCPs. The ES usually contain a monovalent cation and a polyvalent anion, with the sodium salts of citric acid and/or phosphoric acid being the most common types. In their absence, processing would generally lead to the formation of a heterogeneous, gummy, pudding-like mass that undergoes extensive oiling-off and moisture exudation during manufacture and on cooling. These defects are associated with heat- and shear-induced physical damage of the fat globule membranes in the natural cheese, liquefaction and coalescence of non-globular fat, and aggregation of the protein (paracasein) phase of the natural cheese in the blend. The addition of ES at levels of 1 to 3% (w/w) prevents such defects. While they are not emulsifying agents per se, the ES convert the insoluble cheese protein (paracasein) to sodium paracaseinate, which binds water and emulsifies the dispersed free oil droplets during processing. This conversion is mediated by two important functions of the ES:

1. Upward adjustment and stabilisation (buffering) of the blend pH (from ~5.0–5.3 to 5.8–6.1).
2. Sequestration of calcium (Ca^{2+}) from the cheese protein by ion-exchange with the Na^{+} ion of the ES (Fig. 1).

The combined effect of ES, heating and shearing then leads to structural transformation from a ‘loose’ oil-in-water (O/W) emulsion physically encased

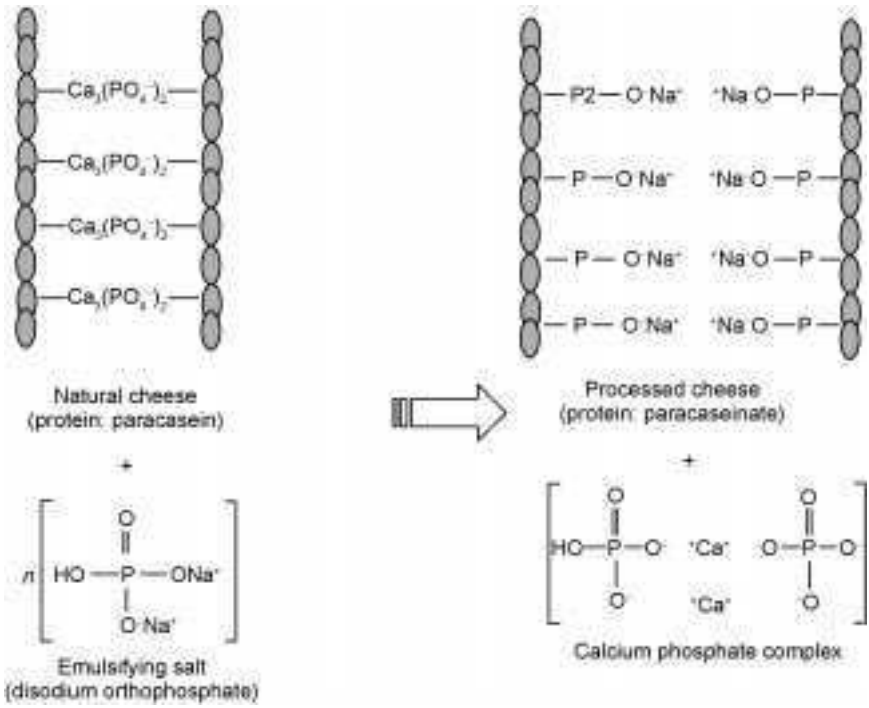

Fig. 1 Schematic showing the ion exchange function of added emulsifying salt. The emulsifying salt exchanges sodium for calcium on the insoluble cheese protein (paracasein) and thereby results in the destruction of calcium phosphate crosslinks between the strands of the casein matrix in the cheese.

within a particulate cheese paracasein matrix in the natural cheese to a 'finer' oil-in-water emulsion in a concentrated paracasein(ate) dispersion in PCPs.

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190 Why does processed cheese sometimes have a gummy pudding-like texture and oil-off?

T. P. Guinee

A gummy pudding-like texture with oiling-off in processed cheese [**189**] is a defect linked to partial phase separation of the fat as a result of coalescence or aggregation of oil droplets. Coalescence may be due to:

- incomplete emulsification of the free fat droplets created during heating and shearing of the blend of natural cheeses and other materials; or
- de-emulsification of fat coinciding with 'over emulsification' and protein-dehydration.

Factors that cause incomplete emulsification or de-emulsification of fat, and hence the defect, are summarised in Table 1.

However, a broader understanding of the problem and its causes necessitates an examination of what happens to natural cheese when it is subjected to heating and shearing, as applied during processed cheese manufacture. The application of heat (70–90 ºC) and shear to the natural cheese results in the formation of a heterogeneous, gummy, pudding-like mass which undergoes extensive oiling-off and moisture exudation during manufacture and subsequent cooling. These defects are due to (i) de-emulsification of the naturally emulsified milk fat globules in the natural cheese and subsequent coalescence of the liquefied oil droplets as a result of shearing of native fat globule membranes, and (ii) partial dehydration/aggregation and shrinkage of the paracasein matrix due to hydrophobic and other interactions. Here, the free fat formed is essentially non-emulsified and the protein portion of the product has a dull, heavy, porous, coarse appearance.

When emulsifying salts (ES) are added to the natural cheese prior to processing, the above problems do not normally occur provided that the formulation (blend materials, composition) and processing conditions (temperature, shear, time) are suitable. The main effect of the ES is to facilitate the hydration and solubility of the cheese protein (paracasein) (Fig. 1) and thereby enable it to behave as a water binding and emulsifying agent (paracaseinate). They accomplish this effect by their ability to sequester calcium from the protein and to increase the negative charge on the protein via buffering at a high pH. In the presence of ES, heat and shear, the fat released on processing the natural cheese is dispersed by shear into fine droplets (5–20 μm) that are coated by the hydrated paracasein and thereby emulsified. Consequently, the addition of the ES results in the formation of a smooth, homogeneous, stable processed cheese product. Microstructurally, the product is a concentrated emulsion of discrete, rounded fat droplets of varying size (typically ~1–10 μm) in a hydrated protein matrix.

Prolonged holding of the molten processed cheese product at 70–90 ºC can result in the development of a short, stiff, heavy, pudding-like consistency and dull appearance in high-moisture processed cheese spreads. In processed cheese slices and blocks, it creates the appearance of an 'orange-peel'-like surface and

Table 1 Factors causing gumminess and oiling-off in processed cheese*

Factors associated with incomplete fat emulsification	Factors associated with de-emulsification of fat
Insufficient fat dispersion during processing • Inadequate size reduction of natural cheese particle size • Inadequate shear mixing of cheese, ES and water **Incomplete emulsification of free fat by the cheese protein (paracasein) (undercreaming)** • Inadequate degree of paracasein hydration o Low level of ES o Use of emulsifying salts with low calcium chelating capacity and protein hydration characteristics (e.g. exclusive use of trisodium citrate) o pH buffering range too low, e.g. pH < 5.6 (as for example with sodium dihydrogen orthophosphate, or with mono- and disodium citrates) o Processing temperature or time inadequate to give desired degree of chemical interaction between emulsifying salts and cheese • Excessive proteolysis of casein in cheese o Quantity of aged cheese in blend too high o Addition of exogenous proetinases, with high proteolytic activity, to cheese • Protein-to-fat ratio of formulation too low o Use of cheese with high fat and low protein o Addition of excessive fat/oil to the blend, e.g. butteroil	**Protein dehydration and simultaneous aggregation/coalescence of emulsified particles** (overcreaming) and increase in density of para-network in finished processed cheese • Prolonged holding of the hot processed cheese in the cooker at high temperature (> 70–90 ºC) **Increasing the degree of fat emulsification beyond the critical emulsification point** (where all the 'available' protein in the system is not sufficient to cover the available fat surface) • Excessive shear o High shear rate mixing o Long processing time • High level of intact casein in blend o Use of high proportion of very young cheese in blend o Use of rennet casein • Use of emulsifying salts with strong calcium chelating and casein-hydration tendencies, e.g. pyro- and sodium tripolyphosphates • Low protein-to-fat ratio of formulation, especially if degree of emulsification is high

* Usually incomplete emulsification (sometimes denoted *undercreaming*) and de-emulsification (sometimes denoted *overcreaming*) are due to the interactive effects of several factors.

the development of an over-firm and heavy pudding-like (coarse) structure that exudes beads of free oil and, in extreme cases, leaks moisture especially on cooling. Simultaneously, the resultant PCP is firmer and on heating exhibits a higher viscosity and lower degrees of fluidity and flow/spread [**192**]. The defect, known as *overcreaming*, is accompanied by a decrease in nitrogen solubility

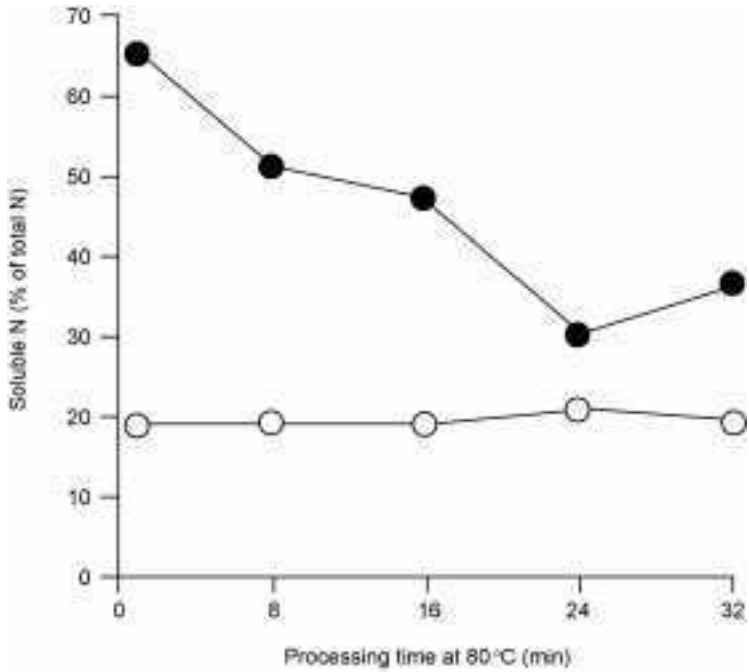

Fig. 1 Changes in water soluble N in processed Cheddar cheese (●) as a function of processing time at 80 ºC. Identical processed cheese blends (cheese, water and emulsifying salt) were formulated, processed to 80 ºC over 4 min, and then held for different times at 80 ºC while continuously heating and shearing; samples were removed after these times, packed, stored for 36–48 h at 4 ºC and analysed. The water-soluble N content of the cheese in each blend prior to processing (◯) was also analysed.

(Fig. 1), suggesting that it may be associated with hydrophobic-induced aggregation of paracasein on prolonged holding at high temperature. The consequent loss in paracasein hydration would diminish its ability to stabilise the emulsified fat globules, which tend to coalesce. Moreover, an increase in degree of aggregation of the protein would coincide with an increase in the rigidity of the protein matrix of the product, an occurrence conducive to the short, heavy, pudding-like structure.

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191 Why does processed cheese sometimes have a soapy flavour?

T. P. Guinee

Soapiness is a flavour defect that occurs infrequently in processed cheese products [**189**]. The presence of this defect is probably due to the formation of soaps such as sodium palmitate or potassium oleate as a result of the interaction of the cations of emulsifying salts (e.g. Na^+, K^+) and medium to long chain fatty acids. The formation of these compounds is favoured by the high pH and high temperature during cheese processing. Their perception will depend on the pH of the processed cheese, the concentration present and the ability of other flavour/odour compounds present (e.g. natural cheese flavours, sodium chloride) to mask or accentuate them.

Soapiness in processed cheese is most frequently associated with the use of sodium or potassium phosphates, especially orthophosphates, as emulsifying salts. The soapy flavour associated with orthophosphates is probably due in part to their strong buffering capacity and ability to increase the pH of the processed cheese blend to relatively high values (≥ 6) compared with salts with lower buffering capacity (e.g. sodium salts of polyphosphoric acids or citric acid). The higher pH with sodium orthophosphates favours the formation of soap compounds. The dissociation constants, pK_a, for phosphoric acid (H_3PO_4) are 2.14, 6.86 and 12.4 at 25 ºC. From the Henderson–Hasselbalch equation:

$$\text{pH} = pK_a + \log \frac{[\text{salt (e.g. } Na_2HPO_4)]}{[\text{acid (e.g. } NaH_2PO_4)]}$$

the pK_a values correspond to the pH values at which the concentrations of the acid and salt forms of the compound are present at equal concentrations. The acid–salt (sodium) forms of H_3PO_4 are H_3PO_4 and NaH_2PO_4 (pK_a 2.14), NaH_2PO_4 and Na_2HPO_4 (pK_a 6.86), and Na_2HPO_4 and Na_3PO_4 (pK_a 12.4). The corresponding pK_a values for citric acid are 3.0, 4.5 and 4.9, respectively. Owing to its ability to buffer the processed cheese blend to high pH values, the use of a high level of trisodium orthophosphate (Na_3PO_4) as an emulsifying salt increases the risk of soapy flavour, especially if high levels of free fatty acids are present in the natural cheese being processed [**90**]. In contrast to phosphates, citrates impart a clean flavour to processed cheese products. This may be expected because of their inability to increase the pH to values as high as that obtained with orthophosphates; the pK_a values for citric acid are much lower than those of phosphoric acid. Soapiness in processed cheese may be also due the carry-over of soapy flavours from the natural cheese.

Soapy flavour has been reported in several cheese types including Blue-type [**137**], Camembert [**128**] and Cheddar [**100**]. Its incidence is associated with high levels of free fatty acids, especially capric ($C_{10:0}$) and lauric ($C_{12:0}$) acids. The prevalence of soapy flavours in cheese is increased by the addition of lipolytic agents such as moulds (e.g. *Aspergillus* species) and/or lipolytic enzymes to the cheese milk and/or by homogenisation of cheesemilk or cream [**31**, **32**].

Homogenisation increases the susceptibility of milk fat to break down into free fatty acids by the lipolytic agents in the milk or cream.

Soapiness in processed cheeses may be reduced by:

- avoiding the use of natural cheese with soapy off-flavour;
- avoiding the use of cheese or other materials (e.g. hydrolysed butter oil, cheese flavours) with high levels of free fatty acids;
- reducing the pH of the processed cheese;
- reducing the level of orthophosphate emulsifying salts, especially trisodium orthophosphate (ideally use a blend of sodium phosphates and trisodium citrate);
- reducing the processing temperature;
- avoiding the use of 're-worked' processed cheese with soapy off-flavour; and/or
- reducing the levels of fat and/or moisture of the product

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192 How is the firmness and spreadability of processed cheese controlled?

T. P. Guinee

The firmness of processed cheese [**189**] may be defined as the force or stress (force per unit area) required to deform the cheese by a given amount (e.g. when compressing between the molars during ingestion) while spreadability refers to the displacement from original dimensions on the application of a shear stress (e.g. when spreading a piece of cheese on a cracker). The firmness and spreadability of processed cheese products are influenced by many factors, which are summarised in Table 1. Most of these factors exert their effects through their influence on casein hydration (CH), level of intact casein (IC), degree of fat emulsification (DEF) and/or extent of 'creaming', which refers to the thickening of the processed blend during manufacture. These effects are more easily understood by considering the interactive relationships of IC, CH and DEF on processed cheese, as discussed below.

During processing of cheese, the combined effects of added emulsifying salts (ES), heat and shear increase the hydration of the insoluble cheese protein (paracasein) [**190**], which is thereby partly transformed to an effective surface active agent (paracaseinate) that emulsifies the dispersed droplets of free fat. The paracaseinate membrane of the emulsified fat droplets enables them to interact with, and become an integral part of, the new structural protein matrix of the processed cheeses. The contribution of fat to structure building therefore increases with DEF, as the size of the emulsified fat globules becomes smaller and specific surface area increases. The interaction of the ES with the cheese protein and the concomitant increases in CH and viscosity of aqueous phase during processing favour an increase in DEF. Consequently, the processed cheese blend (cheese, ES, water and optional ingredients) becomes progressively thicker and 'creamier' with processing time in the cooker; in practice, this thickening is referred to as 'creaming'. Simultaneously, the final product becomes firmer, more rigid, shorter, less adhesive and less spreadable.

'Creaming' is necessary, especially in high-moisture processed cheese spreads where it transforms a 'liquidy', 'runny' product to one with a thick, creamy, viscous consistency. However, prolonged processing of the processed cheese blend in the cooker at high temperatures is undesirable as it leads to too much 'stand-up' of product during filling, loss of spreadability, increased firmness and ultimately a heavy pudding-like textured mass which exudes free oil/water [**190**]. This defect, known as 'overcreaming', may be due to the reduction in protein hydration (as reflected by a decrease in the level of water-soluble N) [**190**], an occurrence expected to be paralleled by an increase in heat-induced protein aggregation and some associated de-emulsification and coalescence of fat. However, 'overcreaming' is not normally an issue except where processing times or holding time of manufactured product in pre-filling line buffer tanks are prolonged, e.g. in the event of a plant breakdown.

Table 1 General effect of different parameters on the firmness and spreadability of processed cheese products (PCP)

Formulation	Firmness*	Spreadability*	Effect
Increasing emulsifying salt level	↑	↓	Higher casein hydration (CH) and degree of emulsification of fat (DEF)
Emulsifying salt type			
Replacing sodium orthophosphates with sodium polyphosphates	↑	↓	Higher DEF
Replacing trisodium citrate with sodium orthophosphates or polyphosphates	↑	↓	Higher DEF
Cheese			
Increasing protein-to-fat ratio	↑	↓	Higher intact casein (IC) for fat emulsification and structure building
Increasing proportion of young cheese in the blend	↑	↓	Less proteolysis and more IC
Substitution of rennet-curd cheese by:			
Reworked processed cheese[†]	↑	↓	Higher 'creaming effect' and higher DEF
Cheese base[‡]	↑	↓	Higher IC and DEF
Acid-heat coagulated cheeses	↑	↓	Higher IC and DEF
Dairy ingredients			
Calcium co-precipitate	↑	↓	Higher IC
Skim milk powder	↓	↑	High lactose level and protein solubility
Rennet casein	↑	↓	Higher IC and DEF
Composition of PCP			
Increasing moisture content	↓	↑	Lower IC, higher moisture-lubrication
Increasing levels of lactose or fat in dry matter	↓	↑	Increased protein solubility and greater system fluidity
Increasing protein in dry matter	↑	↓	Higher IC and DEF
Increasing pH	↓	↑	?
Processing conditions			
Increasing processing temperature (70–90 °C)	↑	↓	Increased hydrophobic-induced interaction between proteins
Holding time at maximum temperature	↑	↓	

* Arrows: ↑ and ↓ indicate increases and decreases, respectively.
[†] Rework is processed cheese that is the 'left-overs' in cookers and filling machines, damaged packs, and batches that have 'overcreamed' (thickened excessively) and are too viscous to pump/fill.
[‡] Cheese base is milk ultrafiltrate which is diafiltered, inoculated with starter culture (and/or rennet) until the pH reaches ~5.2–5.8, pasteurised and concentrated to a dry matter content of ~60% (w/w).

'Overcreaming' may also occur within normal processing schedules if the emulsifying capacity of the cheese and/or other ingredients in the blend is excessive. For example, young cheese, rennet casein or sodium caseinate, all of which have a high IC level (i.e. a low level of protein hydrolysis) can predispose the blend to overcreaming. Moreover, in the case of young cheese, the high IC content leaves sufficient unhydrated casein to contribute to structure and rigidity of the processed cheese product. As protein is hydrolysed in natural cheese during ripening, it has less propensity to confer a firm, elastic texture on the finished processed cheese. Consequently, processed cheeses prepared from aged natural cheeses are generally softer and more spreadable than those made using young cheese, with the level of the effect depending on processing conditions and type/level of emulsifying salts.

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193 Why does processed cheese have a dry, short, crumbly texture?

T. P. Guinee

A dry, short crumbly texture could be indicative of a number of basic problems with the processed cheese [**189**]: an over-acid product; very low levels of moisture or fat; insufficient hydration of cheese protein (paracasein), excessive creaming with a high degree of fat emulsification with little, or no, free oil; and/or heterogeneous 'unknitted' structure. The factors that contribute to these problems are summarised in Table 1.

A homogeneous plastic consistency in processed cheese products (PCPs) requires sufficient degrees of protein hydration and fat emulsification. The flexibility of casein strands, as influenced by the increase in the hydration of paracasein during processing, enables the strands to assemble in a new uniform structural continuum. Simultaneously the hydrated paracaseinate emulsifies the dispersed free fat droplets formed during the heating and shearing of the natural cheese. The ability of emulsified fat particles (coated with paracaseinate) in the hot molten processed cheese blend to flow and deform facilitates the formation of the processed cheese structure, which may be envisaged as a network of paracasein and paracaseinate strands incorporating the emulsified fat globules. The pliability of the liquid fat globules, which may be considered as connecting loci/junctions, assists the 'knitting' of isolated, dangling pieces of paracasein/caseinate network into a structural continuum during the setting of the processed cheese after packing.

Probably the main cause of a dry, short, crumbly cheese is the use of an emulsifying salt (ES) blend which gives too low a pH in the final product. This can occur when using ES such as mono- and disodium citrates and monosodium dihydrogen orthophosphates on their own or in blends at high levels. However, a low pH could also occur as a result of using a high proportion of low pH ingredients such as acid-curd cheeses [**170**] (e.g. Quarg, low-fat fresh cheeses) and acid casein in the formulation. The effect of pH probably stems from its influences on protein hydration, protein aggregation and structural topography of the protein matrix, which in turn affects the sensory perceptions received on contact between the product (processed cheese) and the consumer. Other factors contributing to low protein hydration and hence shortness in PCPs include too low a concentration of ES, lack of adequate mixing and heating, and lack of interaction of ES with cheese. Conversely, factors contributing to a high degree of fat emulsification (e.g. increases in shear, processing time and intact casein content, ES with strong calcium binding and buffering characteristics) may also promote overcreaming of the blend and shortness in the final product. A high degree of emulsification leads to smaller paracasein-covered fat globules in the molten processed cheeses, a situation conducive to a lower fat globule pliability and a higher degree of interaction between the paracasein covered fat globule and the paracasein network. Moreover, overcreaming coincides with a reduction in casein hydration [**190**], a factor that also favours a short, dry texture.

Table 1 Factors that cause a dry, short, crumbly texture in processed cheese

Root causes	Contributory factors	Remedy
Over-acid/low pH (< 5.6)	High proportions of, or exclusive use of, emulsifying salt (ES) that: • buffer at low pH such as mono- or disodium citrates, monosodium dihydrogen orthophosphate • have low buffering capacity such as polyphosphates	Reformulate the ES blend to include sufficient levels of salts that buffer and stabilise the pH of the product to 5.9 to 6.1, such as trisodium citrate, di- and trisodium orthophosphates
	Use of high proportion of low-pH cheeses such as acid curd cheeses (e.g. Quarg), Feta, Cheshire	Include more higher pH cheeses (e.g. Gouda, Cheddar, Swiss). Increase the levels of ES and/or reformulate ES blend to get desired pH of 5.9 to 6.1
Moisture level too low	Insufficient water addition High proportion of low-moisture cheeses in formulation, especially if the cheeses are dry, short and hard (e.g. Parmesan and Romano-types)	Increase moisture level of formulation by inclusion of higher-moisture cheeses or addition of water
Low level of free fat	A strong *creaming* effect as promoted by: • high level of young cheese	Increase proportion of mature cheese in formulation
	• high proportion of ES that give high calcium sequestration, water binding and fat emulsification such as pyrophosphates, tripoly-phosphates, and other short-chain polyphosphates	Increase level of ES Use a high proportion of ES that give lower degree of fat emulsification such as trisodium citrate, di- and trisodium orthophosphates.
	• excessive pre-blending, shear/ processing time/holding excessively long prior to filling/slow cooling	Optimise processing conditions
	A low level of fat in the natural cheese or a low protein-to-fat ratio of the formulation	Increase fat content of formulation by inclusion of higher-fat cheeses and/or addition of butter/butter oil
Heterogeneous product structure	Inadequate dissolution, mixing and/or interaction of materials due to: insufficient shear, heat, processing time	Optimise processing conditions

Dryness in processed cheese may also be associated with low levels of moisture and fat, both of which act as lubricants, and thereby facilitate relative movement of adjoining layers of the matrix during mastication and shearing. Hence, a minimum level of free fat, or fat that easily becomes free during consumption and/or shearing of the product, is also necessary to reduce the risk of a dry, crumbly texture.

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194 Why does processed cheese have a soft, inelastic, adhesive and spreadable texture?

T. P. Guinee

Soft, inelastic, adhesive and spreadable texture is desirable in processed cheese spreads [**189**]. Formulation factors contributing to these characteristics in processed cheese spreads include a high moisture content in the final product (e.g. 50–60%, w/w), the use of a medium-to-high proportion of well-matured cheese with a high level of proteolysis (and low level of intact casein) in the formulation, a relatively high pH (6.1–6.2), and the use of emulsifying salts promoting a good creaming effect such as sodium tripolyphosphates or sodium pyrophosphates. Additionally, processing conditions favouring the formation of high-moisture processed cheese spreads with this texture include a high shear rate (agitation speed) and a long processing time. These conditions promote a desired 'creaming' reaction, which is paralleled by sufficiently high degrees of protein hydration and water binding and a desired thick creamy texture and spreadability. In the absence of a strong creaming reaction, high-moisture processed cheese formulations have an undesirable 'runny' and liquid consistency. On completion of cooking and filling, the well-creamed processed cheese mass is then rapidly cooled to minimise hydrophobically induced interactions between the proteins. Such interactions are favoured by slow cooling and are undesirable in spreadable processed cheeses as they are conducive to 'overcreaming' of the product and a loss of spreadability and creaminess.

In contrast to processed cheese spreads, a soft, spreadable, adhesive texture is undesirable in processed cheese slices and blocks where it may be considered as a defect. The main causes of this texture defect in the latter products are probably excessive moisture in the formulation, the use of a high proportion of natural cheese that is over ripe and has a very low content of intact casein (a high level of proteolysis). Other contributory factors are:

- high pH (e.g. >6.1, as affected by the use of an emulsifying salts with unsuitable buffering capacity and/or the use of high pH cheese in the blend);
- a high level of substitution of young cheese or rennet casein (insoluble calcium paracasein) with a more hydrated casein (such as sodium caseinate, skim milk powder, ultrafiltered milk retentate); and
- rapid cooling.

The latter factors promote an excessively high degree of casein hydration and, consequently, a lower degree of casein aggregation, which in turn favours a less elastic, more spreadable product.

This defect in block and sliced processed cheese products may be remedied by reducing the water content, increasing the proportion of young cheese in the blend, substituting mature cheese with rennet casein where young cheese is not available, reducing the pH of the product to < 6.0 by use of an appropriate emulsifying salt blend, increasing processing shear and time, and/or slow cooling of the product. While increases in processing shear and time generally

coincide with increases in the elasticity and firmness of block products, a lower degree of shear and shorter processing times may sometimes be more effective in minimising this defect in block processed cheeses that are poorly formulated with a high proportion of over-ripe cheese. This is because lower shear and shorter processing times favour a lower degree of interaction of emulsifying salt with the cheese protein and hence lower degrees of calcium sequestration and protein hydration.

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195 What causes crystals in processed cheese and how can this problem be minimised?

T. P. Guinee

Crystals in processed cheese products (PCPs) [**189**] can form from various species, usually salts, present at concentrations that exceed their solubility product. Several crystal types have been identified using techniques such as electron microscopy, X-ray diffraction analysis and infrared spectroscopy. These include calcium pyrophosphate dihydrate, disodium phosphate dodecahydrate, unreacted emulsifying salts (ES), tyrosine, calcium citrates, calcium lactate, lactose and complexes of various materials such as calcium, fatty acids, free amino acids, protein and lactose. The crystals can vary in shape and dimensions, e.g. 30 μm for calcium phosphate aggregates, and up to 80 μm in diameter for calcium lactate crystals. Crystals are undesirable as they may cause a gritty/sand texture, white spots throughout the mass of processed cheese and/or a whitish mould-like surface appearance on the surfaces of slices or blocks.

The main causes of crystallisation are discussed below.

The carry-over of crystals from natural cheeses, such as insoluble tyrosine in Swiss cheese or calcium lactate from Cheddar cheese [107]

When such cheeses are processed, crystals will generally be carried over into the processed cheese, as less water is available for their solubility than in the natural cheese. However, if only small quantities of the natural cheese with crystals are used in the formulation, then their level in the processed cheese may be too low to be detected.

The formation of insoluble calcium phosphate crystals as a result of the interaction between the anion of the ES and the Ca of paracasein

This is most likely when hard/semi-hard cheeses with high calcium levels such as Gruyere [**117**] (~0.80%), Hergardsost (~0.84%), half-fat Cheddar (~0.95%), Parmesan [**97**] (~0.98%) and Emmental (1.02%) are used in the processed cheese blend. The inclusion of other high calcium ingredients such as rennet casein (~2.6% Ca) and calcium caseinate (2% Ca) in the formulation predisposes the product to this defect also, especially if used at relatively high levels. Substituting rennet casein or high-calcium hard cheeses with cheeses with a lower calcium level, such as Gouda [**108**] or full-fat Cheddar [**100**] (~ 0.75%, w/w), reduces the formation of these crystals.

The incomplete dissolution of ES, especially when added to the processed cheese blend at high levels (e.g. up to 3%, w/w)

Unlike natural cheese, much of the water (up to 70%) in PCPs is not free and, presumably, is not available for solution of ES or other solutes such as lactose

and amino acids. Hence, the actual concentration of ES in the moisture phase of the processed cheese may be much higher than the apparent concentration. Reducing the level of added ES lowers the incidence of insoluble ES in the product.

High pH of processed cheese product

The pH has a major influence on the susceptibility to crystal formation, especially where sodium orthophosphates are used as the ES. This is because the pH determines the level of dissociation of the ES and hence the ratio of salt-to-acid forms of the ES, according to the Henderson–Hasselbalch equation:

$$\mathrm{pH} = \mathrm{p}K_\mathrm{a} + \log \frac{[\text{salt (e.g. } Na_2HPO_4)]}{[\text{acid (e.g. } NaH_2PO_4)]}$$

where pK_a is the dissociation constant.

The salt and acid forms differ markedly in their solubility in aqueous solution and hence their ratio determines the likelihood of crystallisation at a given concentration of ES. At the pH of PCPs (5.5–6.0), NaH_2PO_4 and Na_2HPO_4 are the major forms present, irrespective of the type of orthophosphate added, since the pK_a values for H_3PO_4, are 2.14, 6.86 and 12.4 at 25 ºC. The ratio of Na_2HPO_4 to NaH_2PO_4 varies depending on the pH. In the finished processed cheese, Na_2HPO_4 occurs mainly as the dodecahydrate disodium salt ($Na_2HPO_4{\cdot}12H_2O$) and it solubility (~1.5–2.5% w/v) is much lower than that of the monosodium salt (>45%, w/v). Hence, in processed cheeses made using sodium phosphates, $Na_2HPO_4{\cdot}12H_2O$ is the predominant crystalline species. Its tendency to crystallise is markedly reduced by small reductions in the pH in range 5.5–6.5. However, there is an inverse relationship between the pH required in the processed cheese to prevent the formation of crystals and the phosphate content of the PCP; the higher the phosphate content, the lower the pH required in order to prevent crystallisation.

The use of excess lactose (e.g. by adding skim milk or whey powders) in the processed cheese formulation

At high levels of addition (e.g. ≥3%, w/w), the lactose concentration in the free water may be saturated (>15%, w/v at 21 ºC), leading to the formation of lactose crystals which may then act as nuclei for the crystallisation of various mineral species.

Other treatments

Other treatments that lead to dehydration of the cheese, such as smoking, are also conducive to crystal growth on the surface of the processed cheese.

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Cheese-like products

196 Introduction: what are analogue cheeses?

T. P. Guinee

Analogue cheeses (ACs) are substitutes or imitations of natural cheeses [**83**] or processed cheeses [**189**] that are manufactured by blending various edible oils/fats, proteins, other ingredients and water into a smooth homogeneous blend with the aid of heat, mechanical shear and emulsifying salts. ACs were developed in the USA in the early 1970s, the main impetus being the desire to generate cheap cheese substitutes for use in the industrial and catering cheese sectors. ACs may be classified as imitation or substitute cheeses depending on the composition and nutritional status. FDA regulations in the USA specify that a cheese analogue is an imitation if it substitutes and resembles another cheese but is nutritionally inferior (i.e. reduction in content of essential nutrients), and a substitute if it is not nutritionally inferior.

The most common types of ACs are substitutes of low-moisture Mozzarella [**146**], Cheddar [**100**], Monterey Jack or pasteurised processed Cheddar and are made from vegetable fat, dairy proteins (principally rennet casein), flavours and other ingredients (Table 1). They are primarily used as cheese toppings on frozen pizza, but also as slices in cheeseburgers, components of grated cheese blends, and ingredients in formulated /assembled foods such as processed meat products, cheese sauces, cheese dips, and ready-prepared meals. Some advantages of ACs compared to natural cheeses are the lower cost; the ease of forming products with customised textural, cooking, and/or with nutritional (low salt, fat and cholestrol; mineral enrichment) attributes, as affected by altering formulations and processing conditions; and the relatively high stability of their textural and cooking properties during storage at refrigerated temperature.

Table 1 Ingredients used in formulation of analogue cheeses[a]

Ingredient type	Example/effect	Typical addition level (%, w/w)
Fat	Butter, anhydrous milk fat, native or partially hydrogenated soya bean oil, corn oil, palm kernel oil (gives desired composition, flavour and texture)	22–28
Milk proteins	Casein, caseinates (contributes to formation of physicochemical stable product, desired texture/cooking properties)	18–24
Starches	Native and modified forms of maize, rice, potato starches (partial substitution for casein/cost reduction)	0.0–3.0
Stabilisers		
Emulsifying salts	Sodium phosphates and sodium citrates (assist in the formation of physicochemically stable product; affect textural and functional properties)	0.5–2.0
Hydrocolloids	Hydrocolloids: guar gum, xanthan gum, carageenans (enhance product stability affect texture and functional properties)	0.0–0.3
Acidifying agents	Food-grade organic acids, e.g. lactic, acetic, citric or phosphoric acids (assist control of pH of final product)	0.2
Flavours and flavour enhancers	NaCl, enzyme-modified cheese, starter distillate, smoke extracts yeast extracts (flavour source/accentuation)	0.5–3.0
Colours	Annato, paprika, artificial colours (impart desired colour)	0.04
Preservatives	Nisin, potassium sorbate, calcium/sodium propionate (retard mould growth; prolongs shelf-life)	0.1
Minerals and vitamin preparations	Magnesium oxide, zinc oxide, iron, vitamin A palmitate, riboflavin, thiamine, folic acid (improve nutritive value)	0.0–0.5

[a]The ingredients permitted are subject to the regulations prevailing in the region of manufacture.

The manufacturing technology for ACs is very similar to that for pasteurised processed cheese products. It involves:

- formulation, deciding on the different types and levels of ingredients to be included;
- blending of ingredients;
- processing (heating and shearing) of the blend in a cooker (kettle);

- addition of food-grade acids to adjust pH downwards from ~9 to ~6.0;
- hot packing, prior to cooling and storage.

The order of adding ingredients varies with plant practices, the hydration properties of the casein, type and level of starch in the formulation, cooker type, overall plant design, duration of cooking, and the end-product characteristics. The addition of the acid at the end of manufacture rather than at the beginning ensures a high pH (~8–9) in the blend during processing. This in turn favours efficient sequestration of calcium from the rennet casein by the sodium phosphate emulsifying salts and confers a high negative charge on the casein; both these factors mediate the conversion of the rennet casein (paracasein) to paracaseinate which binds water and emulsifies the vegetable oil. The addition of flavours toward the end of processing minimises the loss of flavour volatiles.

The principles of manufacture of ACs are also similar to those for pasteurised processed cheese products involving:

- upward pH adjustment of the blend by the added ES;
- the sequestration of Ca from the rennet casein by added ES at the high temperatures (typically ~80–84 ºC);
- conversion of the insoluble rennet casein into a hydrated paracaseinate, an effective water-binding and emulsifying agent;
- dispersion of added fat by the shear and its emulsification by the hydrated paracaseinate;
- setting and structure formation during cooling.

The degrees of casein hydration and fat emulsification are major determinants of texture and cooking properties of the finished product.

Further reading

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197 What is enzyme-modified cheese?

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Enzyme-modified cheese (EMC) is cheese curd that has been treated with enzymes to produce a concentrated cheese flavour ingredient. Manufacturing protocols for EMC (Fig. 1) are very variable and are usually proprietary to individual companies but the first step generally involves the formation of a paste by blending freshly made cheese curd, and perhaps other ingredients such as other sources of fat and protein, with water and emulsifying salts. The paste is then pasteurised (e.g. 72–80 ºC for 10–20 min) to inactivate microorganisms and enzymes and may be homogenised to increase the surface area of the fat available for lipolysis. A blend of enzymes (e.g. proteinases, peptidases and lipases) is then added, sometimes together with starter organisms, and the paste is incubated for ~1–4 days at pH 5–7 and 25–45 ºC. After the desired flavour has developed, the paste is heat-treated to inactivate the added enzymes and to stabilise the product. The paste may then be homogenised to minimise phase separation before being formulated and packaged for sale (EMC paste) or being dried to give an EMC powder. EMCs may be made by a one-step process (in which the substrate is acted upon simultaneously by proteinases and lipases) or a two-step process in which the substrate is hydrolysed initially by proteinases, heated and then incubated separately with lipases.

EMCs are used to provide a cheese flavour note to a wide range of products including processed and analogue cheese, cheese powders, soups, sauces, dips, crackers, salad dressings and in coatings for snack foods. The flavour profile of EMCs may be quite different from that of cheese (indeed many are quite bitter) but when added in low levels to a relatively bland base, they provide the desired cheesy note to the finished product. EMCs have approximately 15–30 times the

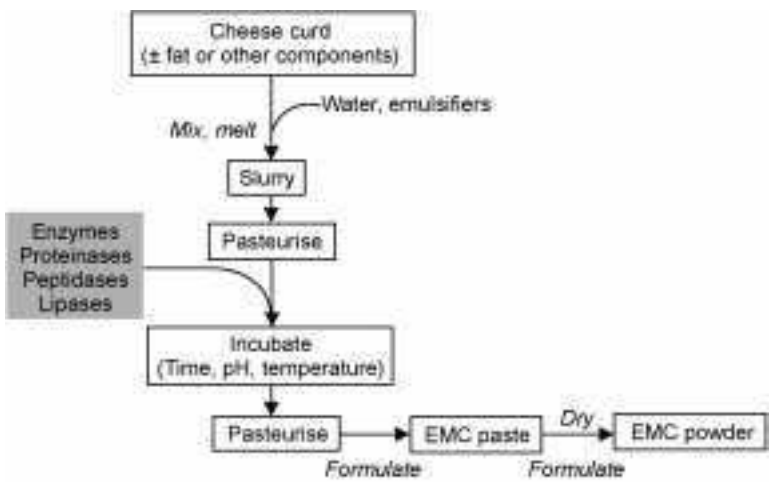

Fig. 1 Outline of the manufacturing process for enzyme-modified cheese.

flavour intensity of natural cheese and they can be made to mimic the flavours of a range of varieties (e.g. Cheddar, Blue, Romano, Parmesan, Camembert, Gouda and Gruyère).

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