10 Chemistry and biochemistry of cheese and fermented milks

10.1 Introduction

Cheese is a very varied group of dairy products, produced mainly in Europe, North and South America, Australia and New Zealand and to a lesser extent in North Africa and the Middle East, where it originated during the Agricultural Revolution, 6000–8000 years ago. Cheese production and consumption, which vary widely between countries and regions (Appendices 10A and 10B), is increasing in traditional producing countries (2–4% p.a. for several years) and is spreading to new areas. On a global scale, 30% of all milk is used for cheese; the proportion is about 40% in North America and about 50% in the European Union.

Although traditional cheeses have a rather high fat content, they are rich sources of protein and in most cases of calcium and phosphorus and have anticarigenic properties; some typical compositional data are presented in Table 10.1. Cheese is the classical example of a convenience food: it can be used as the main course in a meal, as a dessert or snack, as a sandwich filler, food ingredient or condiment.

There are at least 1000 named cheese varieties, most of which have very limited production. The principal families are Cheddar, Dutch, Swiss and Pasta filata (e.g. Mozzarella), which together account for about 80% of total cheese production. All varieties can be classified into three superfamilies based on the method used to coagulate the milk, i.e. rennet coagulation (representing about 75% of total production), isoelectric (acid) coagulation and a combination of heat and acid (which represents a very minor group).

Production of cheese curd is essentially a concentration process in which the milkfat and casein are concentrated about tenfold while the whey proteins, lactose and soluble salts are removed in the whey. The acid-coagulated and acid/heat-coagulated cheeses are normally consumed fresh but the vast majority of rennet-coagulated cheeses are ripened (matured) for a period ranging from 3 weeks to more than 2 years, during which numerous microbiological, biochemical, chemical and physical changes occur, resulting in characteristic flavour, aroma and texture. The biochemistry of cheese ripening is very complex and is not yet completely understood.

Table 10.1 Composition of selected cheeses (per 100 g)

Cheese type	Water (g)	Protein (g)	Fat (g)	Cholesterol (mg)	Energy (kJ)
Brie	48.6	19.3	26.9	100	1323
Caerphilly	41.8	23.2	31.3	90	1554
Camembert	50.7	20.9	23.1	75	1232
Cheddar	36.0	25.5	34.4	100	1708
Cheshire	40.6	24.0	31.4	90	1571
Cottage	79.1	13.8	3.9	13	413
Cream cheese	45.5	3.1	47.4	95	1807
Danish blue	45.3	20.1	29.6	75	1437
Edam	43.8	26.0	25.4	80	1382
Emmental	35.7	28.7	29.7	90	1587
Feta	56.5	15.6	20.2	70	1037
Fromage frais	77.9	6.8	7.1	25	469
Gouda	40.1	24.0	31.0	100	1555
Gruyere	35.0	27.2	33.3	100	1695
Mozzarella	49.8	25.1	21.0	65	1204
Parmesan	18.4	39.4	32.7	100	1880
Ricotta	72.1	9.4	11.0	50	599
Roquefort	41.3	19.7	32.9	90	1552
Stilton	38.6	22.7	35.5	105	1701

10.2 Rennet-coagulated cheeses

The production of rennet-coagulated cheeses can, for convenience, be divided into two phases: (1) conversion of milk to curds and (2) ripening of the curds.

10.2.1 Preparation and treatment of cheesemilk

The milk for most cheese varieties is subjected to one or more pre-treatments (Table 10.2). The concentrations of fat and casein and the ratio of these components are two very important parameters affecting cheese quality. While the concentrations of these components in cheese are determined and controlled by the manufacturing protocol, their ratio is regulated by adjusting the composition of the cheesemilk. This is usually done by adjusting the fat content by blending whole and skimmed milk in proportions needed to give the desired fat : casein ratio in the finished cheese, e.g. 1.0 : 0.7 for Cheddar or Gouda. It should be remembered that about 10% of the fat in milk is lost in the whey while only about 5% of the casein is lost (unavoidably, see section 10.2.2).

With the recent commercial availability of ultrafiltration, it has become possible to increase the concentration of casein, thus levelling out seasonal variations in milk composition and consequently in gel characteristics and

Table 10.2 Pre-treatment of cheese milk

Standardization of fat : protein ratio
Addition of skim milk
Removal of some fat
Addition of ultrafiltration retentate
Addition of $CaCl_2$
Adjustment of pH (e.g. by gluconic acid-δ-lactone)
Removal or killing of contaminating bacteria
Thermization (e.g. 65°C × 15 s)
Pasteurization (e.g. 72°C × 15 s)
Bactofugation
Microfiltration

cheese quality. The capacity of a given plant is also increased by pre-concentrating milk by ultrafiltration.

The pH and the concentration of calcium in milk also vary, with consequential effects on the properties of renneted milk gels. The addition of $CaCl_2$ to cheesemilk (0.02%) is widely practised and adjustment and standardization of milk pH by using the acidogen, gluconic acid-δ-lactone (GDL), is recommended and commercially practised on a limited scale.

Although raw milk is still widely used for cheese manufacture, e.g. Parmigiano-Reggiano (Italy), Emmental (Switzerland), Comté and Beaufort (France) and many less well known varieties, both on a factory and farmhouse scale, most Cheddar and Dutch-type cheeses are produced from pasteurized milk (HTST; *c.* 72°C × 15 s). Pasteurization is used primarily to kill pathogenic and spoilage bacteria. However, desirable indigenous bacteria are also killed by pasteurization and it is generally agreed that cheese made from pasteurized milk ripens more slowly and develops a less intense flavour than raw milk cheese, apparently because certain, as yet unidentified, indigenous bacteria are absent. At present, some countries require that all cheese milk should be pasteurized or the cheese aged for at least 60 days (during which time pathogenic bacteria die off). A global requirement for pasteurization of cheesemilk has been recommended but would create restrictions for international trade in cheese, especially for many of those with 'Appellation d'Origine Protégée' status. Research is under way to identify the important indigenous microorganisms in raw milk cheese for use as inoculants for pasteurized milk. While recognizing that pasteurization is very important in ensuring safe cheese, pH (below about 5.2) and water activity (a_w, which is controlled by addition of NaCl) are also critical safety hurdles.

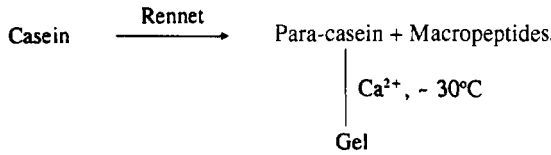
Milk may be thermized (*c.* 65°C × 15 s) on receipt at the factory to reduce bacterial load, especially psychrotrophs, which are heat labile. Since thermization does not kill pathogens, thermized milk is usually fully pasteurized before cheesemaking.

Clostridium tyrobutyricum (an anaerobic spore-former) causes late gas blowing (through the production of H_2 and CO_2) and off-flavours (butanoic acid) in many hard ripened cheeses; Cheddar-type cheeses are major exceptions. Contamination of cheese milk with clostridial spores can be avoided or kept to a very low level by good hygienic practices (soil and silage are the principal sources of clostridia) but they are usually prevented from growing through the use of sodium nitrate ($NaNO_3$) or, less frequently, lysozyme, and/or removed by bactofugation (centrifugation) or microfiltration.

10.2.2 Conversion of milk to cheese curd

Typically, five steps, or groups of steps, are involved in the conversion of milk to cheese curd: coagulation, acidification, syneresis (expulsion of whey), moulding/shaping and salting. These steps, which partly overlap, enable the cheesemaker to control the composition of cheese, which, in turn, has a major influence on cheese ripening and quality.

Enzymatic coagulation of milk. The enzymatic coagulation of milk involves modification of the casein micelles via limited proteolysis by selected proteinases, called rennets, followed by calcium-induced aggregation of the rennet-altered micelles:



If present, the fat globules are occluded in the gel but do not participate in the formation of a gel matrix.

As discussed in Chapter 4, the casein micelles are stabilized by κ-casein, which represents 12–15% of the total casein and is located mainly on the surface of the micelles such that its hydrophobic N-terminal region reacts hydrophobically with the calcium-sensitive α_{s1}-, α_{s2}- and β-caseins while its hydrophilic C-terminal region protrudes into the surrounding aqueous environment, stabilizing the micelles by a negative surface charge and steric stabilization.

Following its isolation in 1956, it was found that κ-casein is the only casein hydrolysed during the rennet coagulation of milk and that it was hydrolysed specifically at the Phe_{105}–Met_{106} bond, producing para-κ-casein (κ-CN f1–105) and macropeptides (f106–169; also called glycomacropeptides since they contain most or all of the sugar groups attached to κ-casein) (Figure 10.1). The hydrophilic macropeptides diffuse into the surrounding medium while the para-κ-casein remains attached to the

Figure 10.1 Amino acid sequence of κ-casein, showing the principal chymosin cleavage site (↓); oligosaccharides are attached at some or all of the threonine residues shown in italics.

micelle core (the macropeptides represent *c.* 30% of κ-casein, i.e. 4–5% of total casein; this unavoidable loss must be considered when calculating the yield of cheese). Removal of the macropeptides from the surface of the casein micelles reduces their zeta potential from about −20 to −10 mV and removes the steric stabilizing layer. The proteolysis of κ-casein is referred to as the **primary (first) phase** of rennet-coagulation.

When about 85% of the total κ-casein in milk has been hydrolysed, the colloidal stability of the micelles is reduced to such an extent that they coagulate at temperatures greater than about 20°C (*c.* 30°C is used in cheesemaking), an event referred to as the **secondary phase** of rennet coagulation. Calcium ions are essential for the coagulation of rennet-altered micelles (although the binding of Ca^{2+} by casein is not affected by renneting).

The Phe_{105}–Met_{106} bond of κ-casein is several orders of magnitude more sensitive to rennets than any other bond in the casein system. The reason(s) for this unique sensitivity has not been fully established but work on synthetic peptides that mimic the sequence of κ-casein around this bond has provided valuable information. The Phe and Met residues themselves are not essential, e.g. both Phe_{105} and Met_{106} can be replaced or modified without drastically changing the sensitivity of the bond – in human, porcine and rodent κ-caseins, Met_{106} is replaced by Ile or Leu, and the proteinase from *Cryphonectria parasitica* (section 10.2.2.2), hydrolyses the bond Ser_{104}–Phe_{105} rather than Phe_{105}–Met_{106}. The smallest κ-casein-like pept-

Table 10.3 Kinetic parameters for hydroloysis of κ-casein peptides by chymosin at pH 4.7 (compiled from Visser *et al.*, 1976; Visser, Slangen and van Rooijen, 1987)

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

[a]pH 6.6.
[b]pH 4.6.

ide hydrolysed by chymosin is Ser.Phe.Met.Ala.Ile (κ-CN f104–108); extending this peptide from its C and/or N terminus increases its susceptibility to chymosin (i.e. increases k_{cat}/K_m); the peptide κ-CN f98–111 is as good a substrate for chymosin as whole κ-casein (Table 10.3). Ser_{104} appears to be essential for cleavage of the Phe_{105}–Met_{106} bond by chymosin, and the hydrophobic residues, Leu_{103}, Ala_{107} and Ile_{108} are also important.

Rennets. The traditional rennets used to coagulate milk for most cheese varieties are prepared from the stomachs of young calves, lambs or kids by extraction with NaCl (*c.* 15%) brines. The principal proteinase in such rennets is chymosin; about 10% of the milk-clotting activity of calf rennet is due to pepsin. As the animal ages, the secretion of chymosin declines while that of pepsin increases; in addition to pepsin, cattle appear to secrete a chymosin-like enzyme throughout life.

Like pepsin, chymosin is an aspartyl (acid) proteinase, i.e. it has two essential aspartyl residues in its active site which is located in a cleft in the globular molecule (molecular mass ~36 kDa) (Figure 10.2). Its pH optimum for general proteolysis is about 4, in comparison with about 2 for pepsins from monogastric animals. Its general proteolytic activity is low relative to its milk-clotting activity and it has moderately high specificity for bulky hydrophobic residues at the P_1 and P'_1 positions of the scissile bond. Its physiological function appears to be to coagulate milk in the stomach of the neonate, thereby increasing the efficiency of digestion, by retarding discharge into the intestine, rather than general proteolysis.

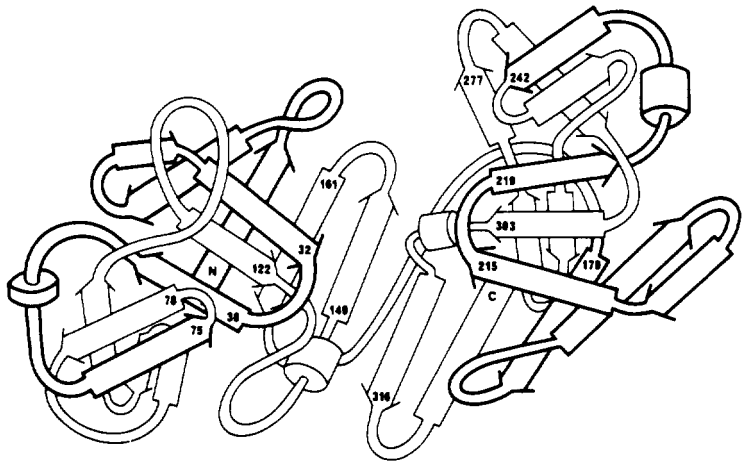


Figure 10.2 Schematic representation of the tertiary structure of an aspartyl proteinase, showing the cleft which contains the active site; arrows indicate β structures and cylinders the α-helices (from Foltmann, 1987).

Due to increasing world production of cheese and the declining supply of young calf stomachs (referred to as vells), the supply of calf rennet has been inadequate for many years. This has led to a search for suitable substitutes. Many proteinases are capable of coagulating milk but most are too proteolytic relative to their milk-clotting activity, leading to a decrease in cheese yield (due to excessive non-specific proteolysis in the cheese vat and loss of peptides in the whey) and defects in the flavour and texture of the ripened cheese, due to excessive or incorrect proteolysis. Only six proteinases are used commercially as rennet substitutes: porcine, bovine and chicken pepsins and the acid proteinases from *Rhizomucor miehei*, *R. pusillus* and *Cryphonectria parasitica*. Chicken pepsin is quite proteolytic and is used widely only in Israel (for religious reasons). Porcine pepsin enjoyed limited success about 30 years ago, usually in admixtures with calf rennet, but it is very sensitive to denaturation at pH values above 6 and may be denatured extensively during cheesemaking, leading to impaired proteolysis during ripening; it is now rarely used as a rennet substitute. Bovine pepsin is quite effective and many commercial calf rennets contain up to 50% bovine pepsin. *Rhizomucor miehei* proteinase, the most widely used microbial rennet, gives generally satisfactory results. *Cryphonectria parasitica* proteinase is, in general, the least suitable of the commercial microbial rennet substitutes and is used only in high-cooked cheeses in which extensive denaturation of the coagulant occurs, e.g. Swiss-type cheeses.

The gene for calf chymosin has been cloned in *Kluyveromyces marxianus* var. *lactis*, *Aspergillus niger* and *E. coli*. Microbial (cloned) chymosins have

given excellent results in cheesemaking trials on various varieties and are now widely used commercially, although they are not permitted in some countries. Significantly, they are accepted for use in vegetarian cheeses. The gene for *R. miehei* proteinase has been cloned in *A. oryzae*; the resultant product, Marzyme GM, is commercially available (Texel, Stockport, UK) and is reported to be a very effective coagulant.

Coagulation of rennet-altered micelles. When *c.* 85% of the total κ-casein has been hydrolysed, the micelles begin to aggregate progressively into a gel network. Gelation is indicated by a rapid increase in viscosity (η) (Figure 10.3). Coagulation commences at a lower degree of hydrolysis of κ-casein if the temperature is increased, the pH reduced or the Ca^{2+} concentration increased.

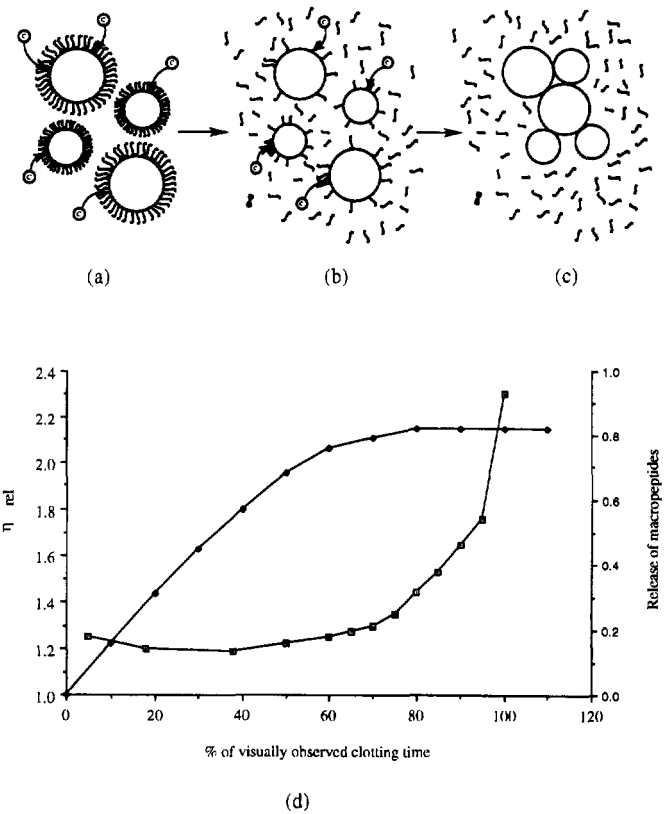


Figure 10.3 Schematic representation of the rennet coagulation of milk. (a) Casein micelles with intact κ-casein layer being attacked by chymosin (C); (b) micelles partially denuded of κ-casein; (c) extensively denuded micelles in the process of aggregation; (d) release of macropeptides (♦) and changes in relative viscosity (⊡) during the course of rennet coagulation.

The actual reactions leading to coagulation are not known. Ca^{2+} are essential but Ca-binding by caseins does not change on renneting. Colloidal calcium phosphate (CCP) is also essential: reducing the CCP concentration by more than 20% prevents coagulation. Perhaps, hydrophobic interactions, which become dominant when the surface charge and steric stabilization are reduced on hydrolysis of κ-casein, are responsible for coagulation (the coagulum is soluble in urea). The adverse influence of moderately high ionic strength on coagulation suggests that electrostatic interactions are also involved. It is claimed that pH has no effect on the secondary stage of rennet coagulation, which is perhaps surprising since micellar charge is reduced by lowering the pH and should facilitate coagulation. Coagulation is very temperature-sensitive and does not occur below about 18°C, above which the temperature coefficient, Q_{10}, is approximately 16.

Factors that affect rennet coagulation. The effect of various compositional and environmental factors on the primary and secondary phases of rennet coagulation and on the overall coagulation process are summarized in Figure 10.4.

No coagulation occurs below 20°C, due mainly to the very high temperature coefficient of the secondary phase. At higher temperatures (above 55–60°C, depending on pH and enzyme) the rennet is denatured. Rennet coagulation is prolonged or prevented by preheating milk at temperatures above about 70°C (depending on the length of exposure). The effect is due to the interaction of β-lactoglobulin with κ-casein via sulphydryl-disulphide interchange reactions; both the primary and, especially, the secondary phase of coagulation are adversely affected.

Measurement of rennet coagulation time. A number of principles are used to measure the rennet coagulability of milk or the activity of rennets; most measure actual coagulation, i.e. combined first and second stages, but some specifically monitor the hydrolysis of κ-casein. The most commonly used methods are described below.

The simplest method is to measure the time elapsed between the addition of a measured amount of diluted rennet to a sample of milk in a temperature-controlled water-bath at, e.g. 30°C. If the coagulating activity of a rennet preparation is to be determined, a 'reference' milk, e.g. low-heat milk powder reconstituted in 0.01% $CaCl_2$, and perhaps adjusted to a certain pH, e.g. 6.5, should be used. A standard method has been published (IDF, 1992) and a reference milk may be obtained from Institut National de la Recherche Agronomique, Poligny, France. If the coagulability of a particular milk is to be determined, the pH may or may not be adjusted to a standard value. The coagulation point may be determined by placing the milk sample in a bottle or tube which is rotated in a water-bath (Figure 10.5); the fluid milk forms a film on the inside of the rotating bottle/tube but

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

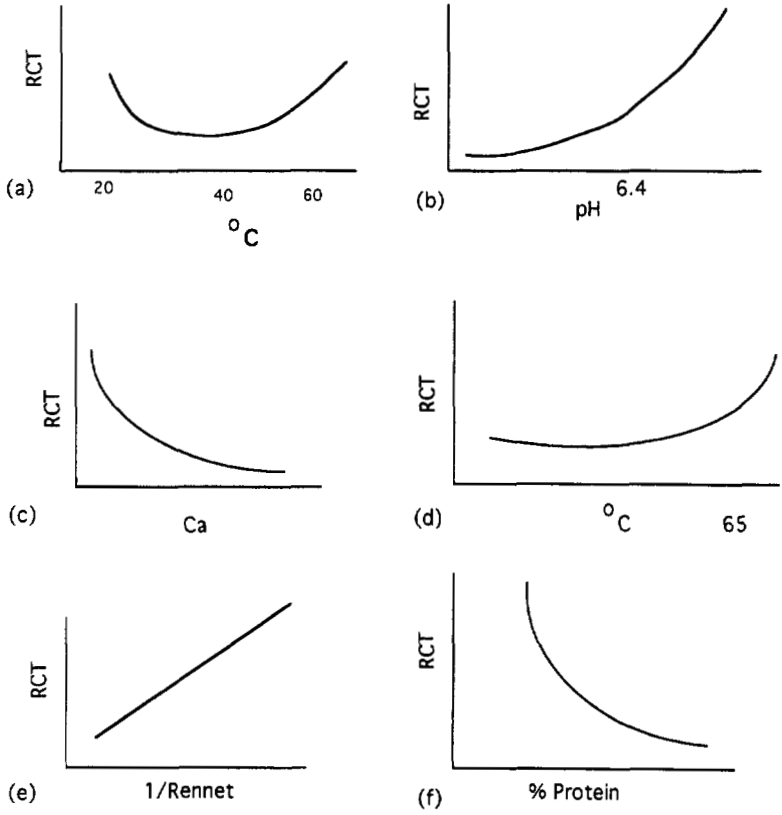


Figure 10.4 Principal factors affecting the rennet coagulation time (RCT) of milk.

flocs of protein form in the film on coagulation. Several types of apparatus using this principle have been described.

As shown in Figure 10.3, the viscosity of milk increases sharply when milk coagulates and may be used to determine the coagulation point. Any type of viscometer may, theoretically, be used but several dedicated pieces

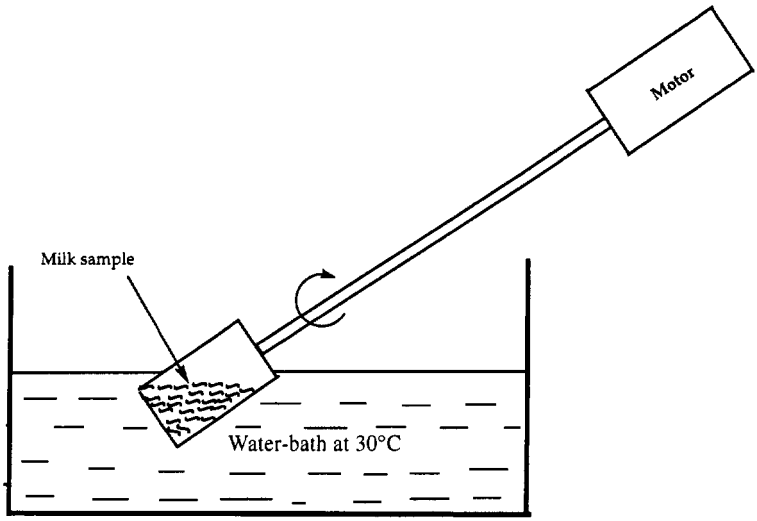


Figure 10.5 Apparatus for visual determination of the rennet coagulation time of milk.

of apparatus have been developed. The most popular of these, although with limited use, is the Formograph (Foss Electric, Denmark), a diagram of which is shown in Figure 10.6a. Samples of milk to be analysed are placed in small beakers which are placed in cavities in an electrically heated metal block. Rennet is added and the loop-shaped pendulum of the instrument placed in the milk. The metal block is moved back and forth, creating a 'drag' on the pendulum in the milk. The arm to which the pendulum is attached contains a mirror from which a flashing light is reflected on to photosensitive paper, creating a mark. While the milk is fluid, the viscosity is low and the drag on the pendulum is slight and it scarcely moves from its normal position; hence a single straight line appears on the paper. As the milk coagulates, the viscosity increases and the pendulum is dragged out of position, resulting in bifurcation of the trace. The rate and extent to which the arms of the trace move apart is an indicator of the strength (firmness) of the gel. A typical trace is shown in Figure 10.6b. A low value of r indicates a short rennet coagulation time while high values of a_{30} and k_{20} indicate a milk with good gel-forming properties.

A recently developed, and apparently industrially useful, apparatus is the hot wire sensor. A diagram of the original assay cell is shown in Figure 10.7a. A sample of milk is placed in a cylindrical vessel containing a wire of uniform dimensions. A current is passed through the wire, generating heat which is dissipated readily while the milk is liquid. As the milk coagulates, generated heat is no longer readily dissipated and the temperature of the

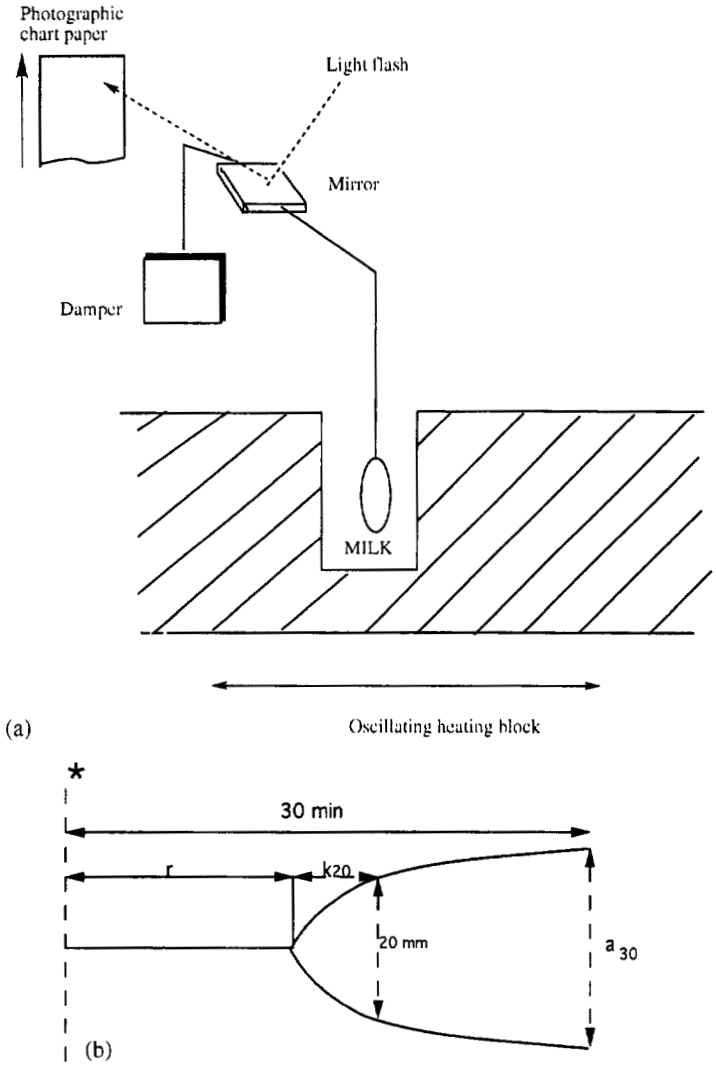


Figure 10.6 (a) Schematic representation of the Formograph apparatus for determining the rennet coagulation of milk. (b) Typical formogram. * Point of rennet addition, r is rennet coagulation time, k_{20} is the time required from coagulation for the arms of the formogram to bifurcate by 20 mm, a_{30} is the extent of bifurcation 30 min after rennet addition (the approximate time at which the coagulum is cut in cheesemaking).

wire increases, causing an increase in its conductivity; a typical trace is shown in Figure 10.7b. The principle has been commercialized by Stoelting Inc. (Kiel, Wisconsin). The wire probe, in a stainless steel shield, is inserted through the wall of the cheese vat. The output from the wire is fed to a computer which can be used to switch on the gel-cutting knife, permitting

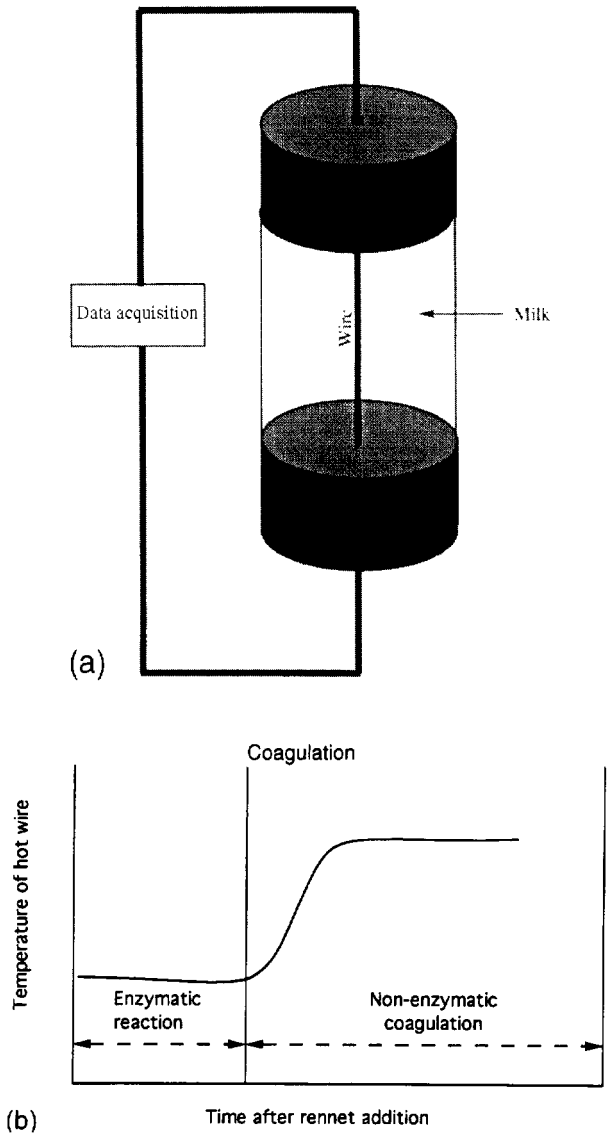


Figure 10.7 (a) Hot wire sensor for objectively measuring the rennet coagulation of milk. (b) Changes in the temperature of the hot wire during the course of the rennet coagulation of milk.

automation and cutting of the gel at a consistent strength, which is important for maximizing cheese yield.

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

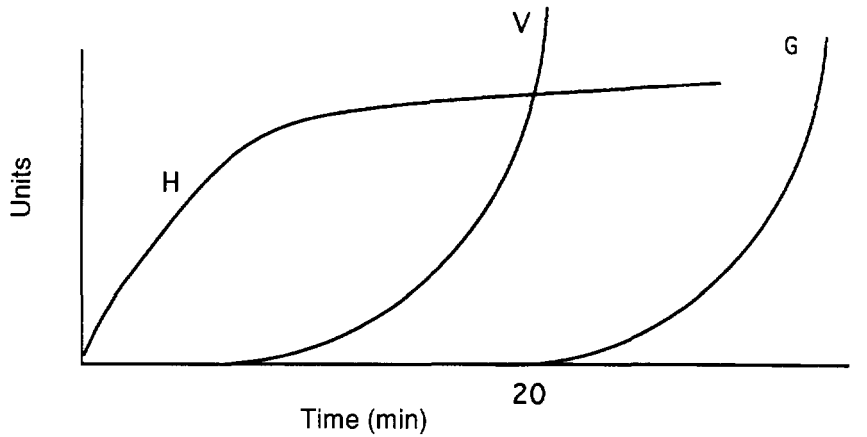


Figure 10.8 Schematic representation of hydrolysis and gel formation in renneted milk; H = hydrolysis of κ-casein; V = changes in the viscosity of renneted milk (second stage of coagulation), G = changes in the viscoelastic modulus (gel formation).

which is slow and cumbersome, or by ion-exchange high performance liquid chromatography (HPLC). The GMP is soluble in TCA (2–12% depending on its carbohydrate content) and can be quantified by the Kjeldahl method or more specifically by determining the concentration of *N*-acetylneuraminic acid or by reversed phase HPLC (RP-HPLC).

The activity of rennets can be easily determined using chromogenic peptide substrates, a number of which are available.

Gel strength (curd tension). The gel network continues to develop for a considerable period after visible coagulation (Figure 10.8). The strength of the gel formed, which is very important from the viewpoints of syneresis (and hence moisture control) and cheese yield, is affected by several factors – the principal ones are summarized in Figure 10.9.

The strength of a renneted milk gel can be measured by several types of viscometers and penetrometers. As discussed on p. 389, the Formograph gives a measure of the gel strength but the data can not be readily converted to rheological terms. Penetrometers give valuable information but are single-point determinations. Dynamic rheometers are particularly useful, allowing the buildup of the gel network to be studied.

Syneresis. Renneted milk gels are quite stable if undisturbed but synerese (contract), following first-order kinetics, when cut or broken. By controlling the extent of syneresis, the cheesemaker can control the moisture content of cheese curd and hence the rate and extent of ripening and the stability of the cheese – the higher the moisture content, the faster the cheese will ripen

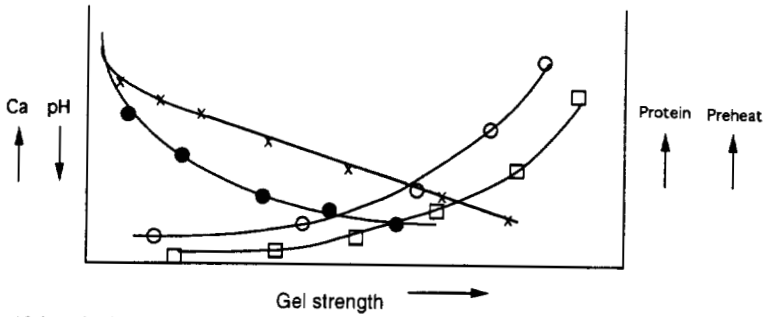


Figure 10.9 Principal factors that affect the strength of renneted milk gels (curd tension); pH (●), calcium concentration (○), protein concentration (□), preheat treatment (×).

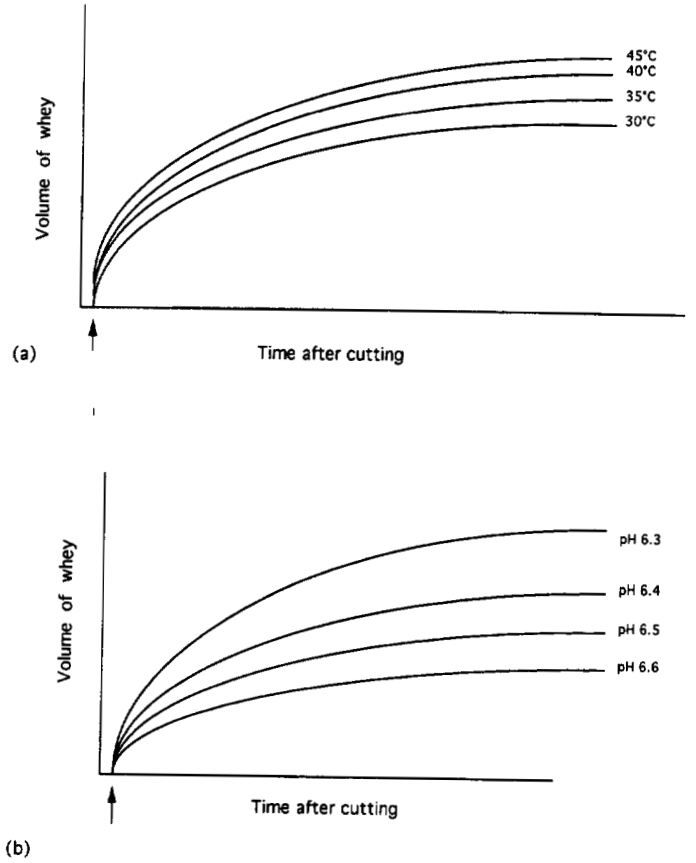


Figure 10.10 Effect of temperature (a) and pH (b) on the rate and extent of syneresis in cut/broken renneted milk gels.

but the lower its stability. Syneresis is promoted by:

- cutting the curd finely, e.g. Emmental (fine cut) versus Camembert (large cut);
- low pH (Figure 10.10b);
- calcium ions;
- increasing the cooking temperature (Camembert, *c.* 30°C; Gouda, *c.* 36°C; Cheddar, *c.* 38°C; Emmental or Parmesan, 52–55°C) (Figure 10.10a);
- stirring the curd during cooking;
- fat retards syneresis, while increasing the protein content (up to a point) improves it; at high protein concentrations, the gel is too firm and does not synerese (e.g. UF retentate).

Gels prepared from heated milk synerese poorly (assuming that the milk does coagulate). Such reduced syneresis properties are desirable for fermented milk products, e.g. yoghurt (milk for which is severly heated, e.g. 90°C × 10 min) but are undesirable for cheese.

Good analytical methods for monitoring syneresis are lacking. Principles that have been exploited include: dilution of an added marker, e.g. a dye, which must not adsorb on to or diffuse into the curd particles, measurement of the electrical conductivity or moisture content of the curd or by measuring the volume of whey released (probably the most commonly used method although only one-point values are obtained).

10.2.3 Acidification

Acid production is a key feature in the manufacture of all cheese varieties – the pH decreases to about 5 (±0.3, depending on variety) within 5–20 h, at a rate depending on the variety (Figure 10.11). Acidification is normally achieved via the bacterial fermentation of lactose to lactic acid, although an acidogen, usually gluconic acid-δ-lactone, alone or in combination with acid, may be used in some cases, e.g. Mozzarella.

Traditionally, cheesemakers relied on the indigenous microflora of milk for lactose fermentation, as is still the case for several minor artisanal varieties. However, since the indigenous microflora varies, so does the rate of acidification and hence the quality of the cheese; the indigenous microflora is largely destroyed by pasteurization. 'Slop-back' or whey cultures (starters; the use of whey from today's cheesemaking as an inoculum for tomorrow's milk) have probably been used for a very long time and are still used commercially, e.g. for such famous cheese as Parmigiano-Reggiano and Comté. However, selected 'pure' cultures have been used for Cheddar and Dutch-type cheeses for at least 80 years and have become progressively more refined over the years. Single-strain cultures were introduced in New Zealand in the 1930s as part of a bacteriophage control programme. Selected phage-unrelated strains are now widely used for Cheddar cheese;

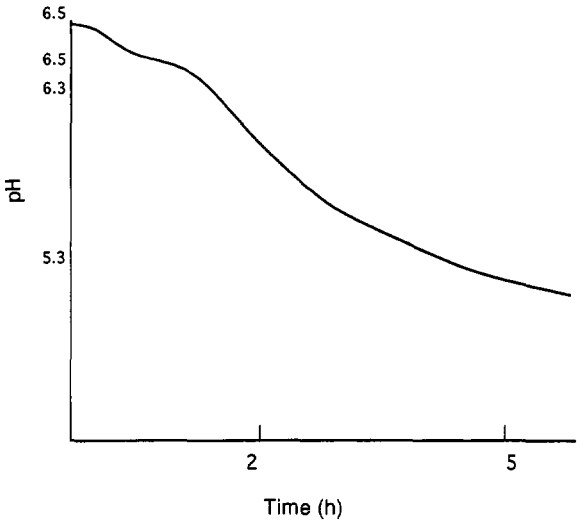


Figure 10.11 pH profile of Cheddar during cheese manufacture.

although selected by a different protocol, highly selected cultures are also used for Dutch and Swiss-type cheeses.

Members of three genera are used as cheese starters. For cheeses that are cooked to a temperature below about 39°C, species of *Lactococcus*, usually *Lc. lactis* ssp. *cremoris*, are used, i.e. for Cheddar, Dutch, Blue, surface mould and surface-smear families. For high-cooked varieties, a thermophilic *Lactobacillus* culture is used, either alone (e.g. Parmesan) or with *Streptococcus salivarius* ssp. *thermophilus* (e.g. most Swiss varieties and Mozzarella). *Leuconostoc* spp. are included in the starter for some cheese varieties, e.g. Dutch types; the function is to produce diacetyl and CO_2 from citrate rather than acid production.

The selection, propagation and use of starters will not be discussed here. The interested reader is referred to Cogan and Hill (1993).

The primary function of cheese starter cultures is to produce lactic acid at a predictable and dependable rate. The metabolism of lactose is summarized in Figure 10.12. Most cheese starters are homofermentative, i.e. produce only lactic acid, usually the L-isomer; *Leuconostoc* species are heterofermentative. The products of lactic acid bacteria are summarized in Table 10.4.

Acid production plays several major roles in cheese manufacture:

- Controls or prevents the growth of spoilage and pathogenic bacteria.
- Affects coagulant activity during coagulation and the retention of active coagulant in the curd.

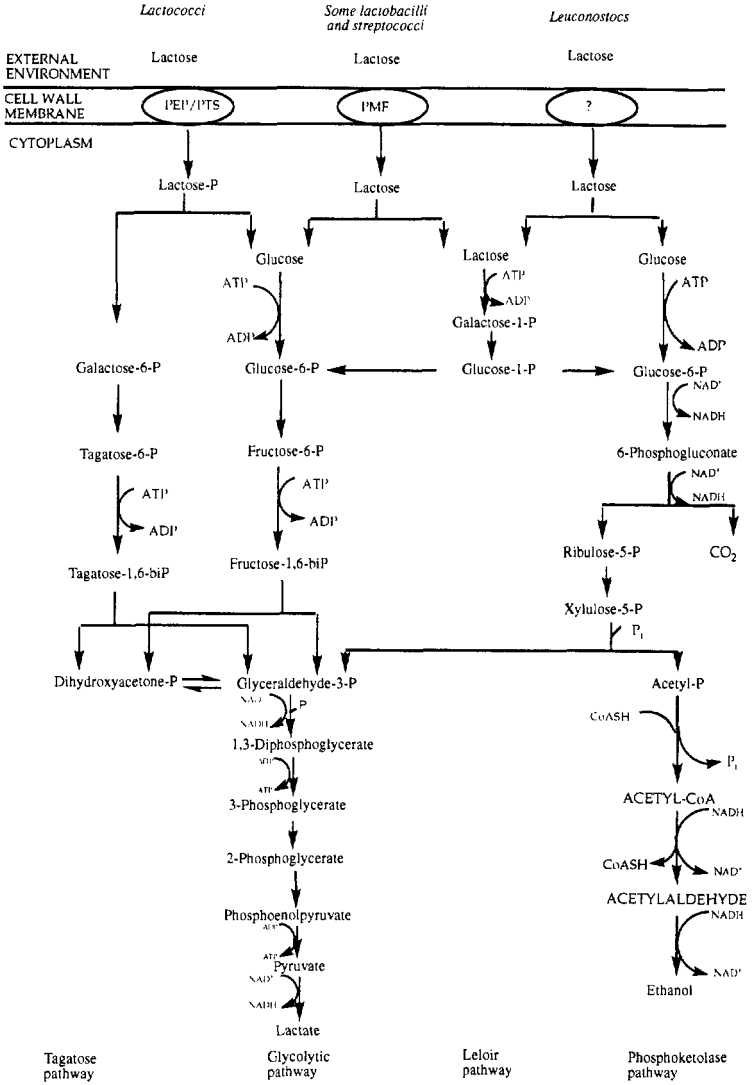


Figure 10.12 Metabolism of lactose by lactic acid bacteria; many *Lactobacillus* species/strains can not metabolize galactose (from Cogan and Hill, 1993).

- Solubilizes of colloidal calcium phosphate and thereby affects cheese texture; rapid acid production leads to a low level of calcium in the cheese and a crumbly texture (e.g. Cheshire) and vice versa (e.g. Emmental).
- Promotes syneresis and hence influences cheese composition.
- Influences the activity of enzymes during ripening, and hence affects cheese quality.

Table 10.4 Salient features of lactose metabolism in starter culture organisms (from Cogan and Hill, 1993)

Organism	Transport[a]	Cleavage[b] enzyme	Pathway[c]	Products (mol mol^{-1} lactose)
Lactococcus spp.	PTS	pβgal	GLY	4 L-Lactate
Leuconostoc spp.	?	βgal	PK	2 D-Lactate + 2 ethanol + 2CO_2
Str. salivarius subsp. *thermophilus*	PMF	βgal	GLY	2 L-Lactate[d]
Lb. delbrueckii subsp. *lactis*	PMF?	βgal	GLY	2 D-Lactate[d]
Lb. delbrueckii subsp. *bulgaricus*	PMF?	βgal	GLY	2 D-Lactate[d]
Lb. helveticus	PMF?	βgal	GLY	4 L- (mainly) + D-lactate

[a]PTS, phosphotransferase system; PMF, proton motive force.
[b]pβgal, phospho-β-galactosidase; βgal, β-galactosidase.
[c]GLY, glycolysis; PK, phosphoketolase.
[d]These species metabolize only the glucose moiety of lactose.

The primary starter performs several functions in addition to acid production, especially reduction of the redox potential (E_h, from about +250 mV in milk to −150 mV in cheese), and, most importantly, plays a major, probably essential, role in the biochemistry of cheese ripening. Many strains produce bacteriocins which control the growth of contaminating micro-organisms.

The ripening of many varieties is characterized by the action, not of the primary starter, but of other micro-organisms, which we will refer to as a secondary culture. Examples are *Propionibacterium* in Swiss-type cheeses, *Penicillium roqueforti* in Blue cheeses, *Penicillium camemberti* in surface mould-ripened cheeses, e.g. Camembert and Brie, *Brevibacterium linens* and yeasts in surface smear-ripened cheese, *Lactococcus lactis* ssp. *lactis* biovar *diacetylactis* and *Leuconostoc* spp. in Dutch-type cheeses. The specific function of these micro-organsims will be discussed in section 10.2.7 on ripening. Traditionally, a secondary culture was not used in Cheddar-type cheeses but there is much current interest in the use of cultures of selected bacteria, usually mesophilic *Lactobacillus* spp. or lactose-negative *Lactococcus* spp., for Cheddar cheese with the objective of intensifying or modifying flavour or accelerating ripening; such cultures are frequently referred to as 'adjunct cultures'.

10.2.4 Moulding and shaping

When the desired pH and moisture content have been achieved, the curds are separated from the whey and placed in moulds of traditional shape and size to drain and form a continuous mass; high-moisture curds form a

Figure 10.13 A selection of cheese varieties, showing the diversity of cheese size, shape and appearance.

continuous mass under their own weight but low-moisture varieties are pressed.

Cheeses are made up in traditional shapes (usually flat cylindrical, but also sausage, pear-shaped or rectangular) and size, ranging from around 250 g (e.g. Camembert) to 60–80 kg (e.g. Emmental; Figure 10.13). The size of cheese is not just a cosmetic feature; Emmental must be large enough to prevent excessive diffusion of CO_2, which is essential for eye development, while Camembert must be quite small so that the surface does not become over-ripe while the centre is still unripe (this cheese softens from the surface to the centre).

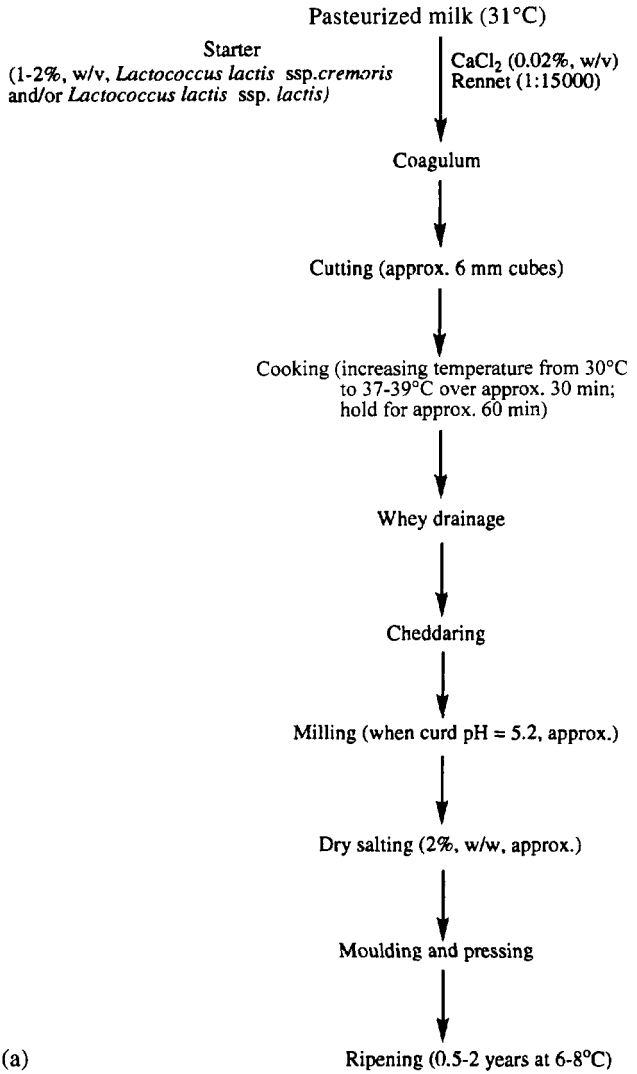
Curds for the *Pasta filata* cheeses, e.g. Mozzarella, Provolone and Halloumi, are heated in hot water (70–75°C), kneaded and stretched when the pH reaches about 5.4; this gives the cheeses a characteristic fibrous structure.

10.2.5 Salting

All cheeses are salted, either by mixing dry salt with the drained curd (confined largely to English varieties), rubbing dry salt on the surface of the pressed cheese (e.g. Romano or Blue cheeses), or by immersion of the pressed cheeses in brine (most varieties). Salt concentration varies from *c.* 0.7% (*c.* 2% salt-in-moisture) in Emmental to 7–8% (*c.* 15% salt-in-moisture) in Domiati.

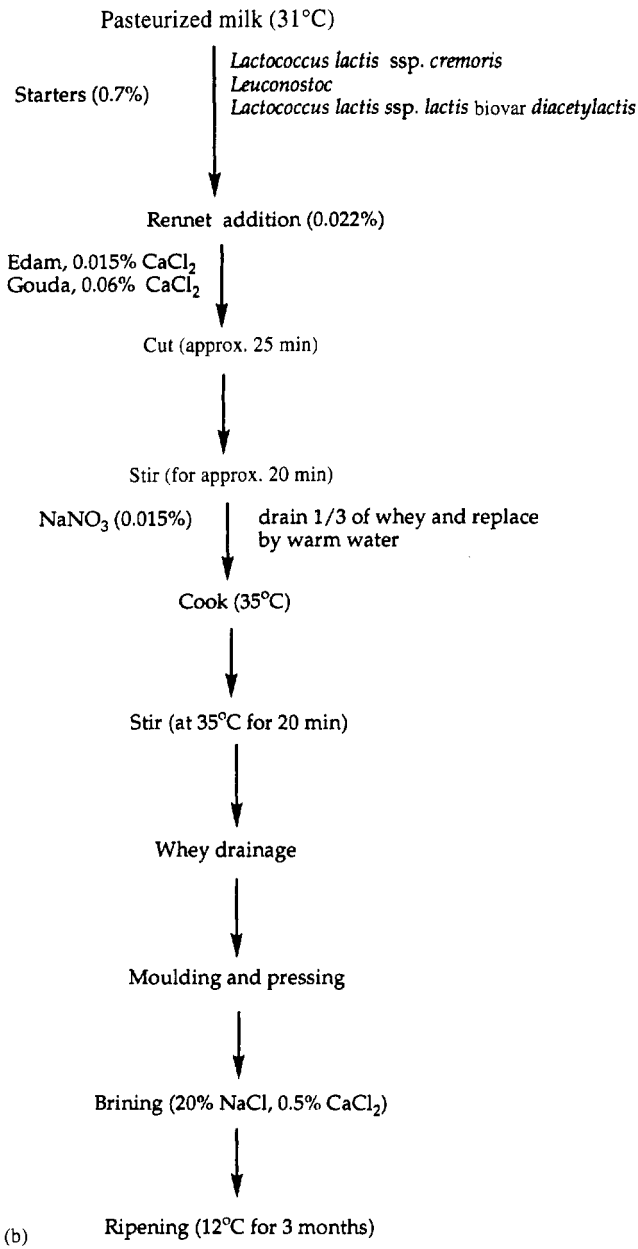
Salt plays a number of important roles in cheese:

- It is the principal factor affecting the water activity of young cheeses and has a major effect on the growth and survival of bacteria and the activity of enzymes in cheese, and hence affects and controls the biochemistry of cheese ripening.



(a)

Figure 10.14 Protocols for the manufacture of (a) Cheddar, (b) Gouda, (c) Emmental and (d) Parmigiano-Reggiano.



(b)

Figure 10.14 (*Continued*).

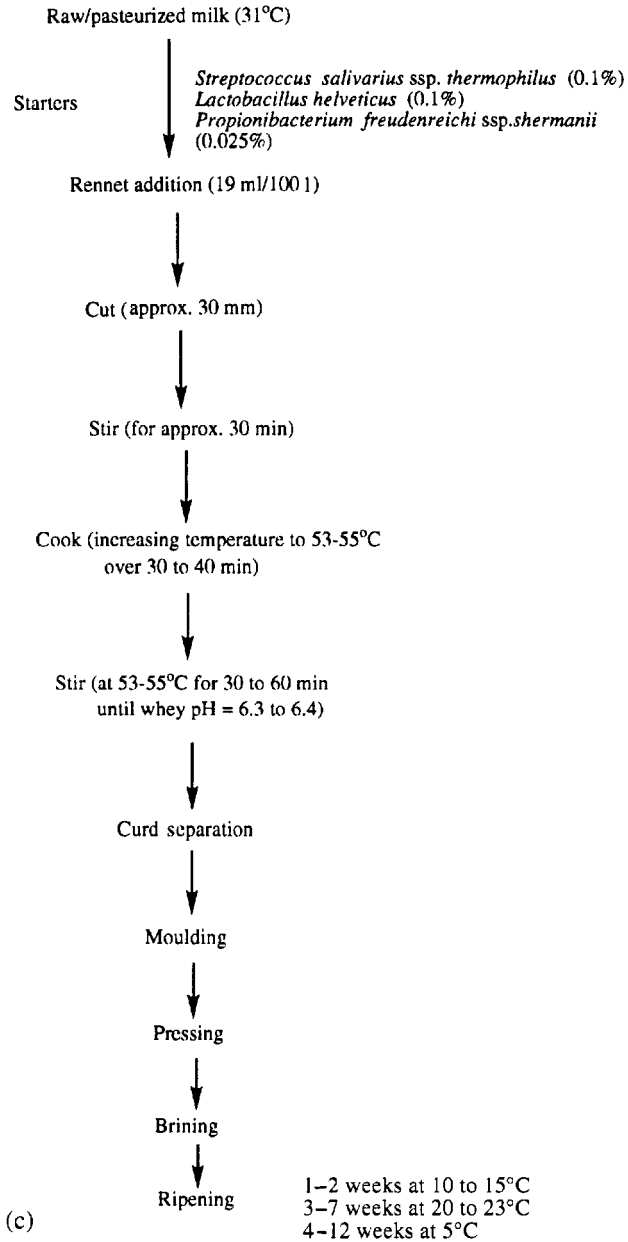


Figure 10.14 (*Continued*).

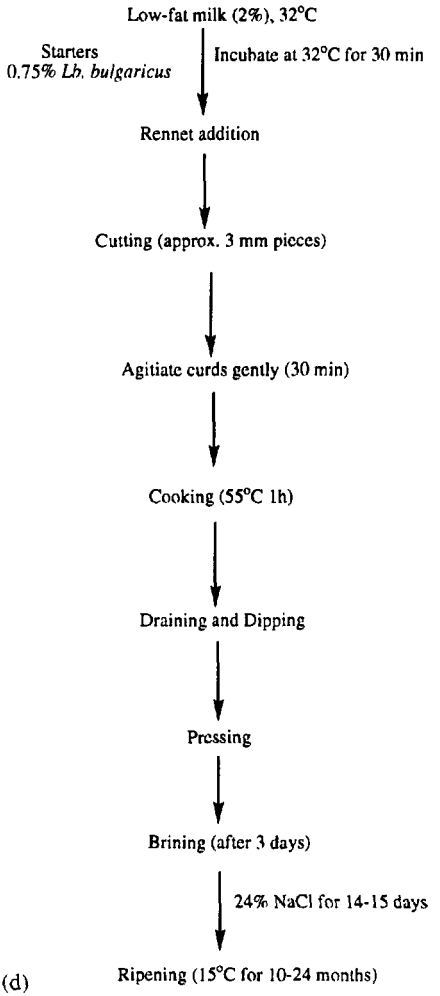


Figure 10.14 (*Continued*).

- Salting promotes syneresis and hence reduces the moisture content of cheese; about 2 kg of water are lost for each kilogram of salt absorbed.
- It has a positive effect on flavour.
- Cheese contributes to dietary sodium, high levels of which have undesirable nutritional consequences, e.g. hypertension and osteoporosis.

10.2.6 Manufacturing protocols for some cheese varieties

The manufacturing protocols for the various cheese varieties differ in detail but many elements are common to many varieties. The protocols for the principal varieties are summarized in Figures 10.14a–d.

10.2.7 Cheese ripening

While rennet-coagulated cheese curd may be consumed immediately after manufacture (and a little is), it is rather flavourless and rubbery. Consequently, rennet-coagulated cheeses are ripened (matured) for a period ranging from about 3 weeks for Mozzarella to more than 2 years for Parmesan and extra-mature Cheddar. During this period, a very complex series of biological, biochemical and chemical reactions occur through which the characteristic flavour compounds are produced and the texture altered.

Four, and in some cheeses five or perhaps six, agents are responsible for these changes:

1. The cheese milk. As discussed in Chapter 8, milk contains about 60 indigenous enzymes, many of which are associated with the fat globules or casein micelles and are therefore incorporated into the cheese curd; the soluble enzymes are largely removed in the whey. Many of the indigenous enzymes are quite heat stable and survive HTST pasteurization; at least three of these (plasmin, acid phosphatase and xanthine oxidase) are active in cheese and contribute to cheese ripening; some indigenous lipase may also survive pasteurization. The contribution of other indigenous enzymes to cheese ripening is not known.
2. Coagulant. Most of the coagulant is lost in the whey but some is retained in the curd. Approximately 6% of added chymosin is normally retained in Cheddar and similar varieties, including Dutch types; the amount of rennet retained increases as the pH at whey drainage is reduced. As much as 20% of added chymosin is retained in high-moisture, low-pH cheese, e.g. Camembert. Only about 3% of microbial rennet substitutes is retained in the curd and the level retained is independent of pH.

   Porcine pepsin is very sensitive to denaturation at pH 6.7 but becomes more stable as the pH is reduced.

   The coagulant is major contributor to proteolysis in most cheese varieties, notable exceptions being high-cooked varieties, e.g. Emmental and Parmesan, in which the coagulant is extensively or totally denatured during curd manufacture.

   A good-quality rennet extract is free of lipolytic activity but a rennet paste is used in the manufacture of some Italian varieties, e.g. Romano and Provolone. Rennet paste contains a lipase, referred to as pre-gastric esterase (PGE), which makes a major contribution to lipolysis in, and to the characteristic flavour of, these cheeses. Rennet paste is considered unhygienic and therefore semi-purified PGE may be added to rennet extract for such cheeses (Chapter 8).
3. Starter bacteria. The starter culture reaches maximum numbers at the end of the manufacturing phase. Their numbers then decline at a rate depending on the strain, typically by 2 log cycles within 1 month. At least some of the non-viable cells lyse at a rate dependent on the strain. As far as is known, the only extracellular enzyme in *Lactococcus*, *Lactobacillus*

and *Streptococcus* is a proteinase which is attached to the cell membrane and protrudes through the cell wall; all peptidases, esterases and phosphatases are intracellular and therefore cell lysis is essential before they can contribute to ripening.

4. Non-starter bacteria. Cheese made from pasteurized, high-quality milk in modern factories using enclosed automated equipment contains very few non-starter bacteria ($<50\,\text{cfu}\,\text{g}^{-1}$) at one day but these multiply to 10^7–$10^8\,\text{cfu}\,\text{g}^{-1}$ within about 2 months (at a rate depending on, especially, temperature). Since the starter population declines during this period, non-starter bacteria dominate the microflora of cheese during the later stages of ripening.

   Properly made cheese is quite a hostile environment for bacteria due to a low pH, moderate-to-high salt in the moisture phase, anaerobic conditions (except at the surface), lack of a fermentable carbohydrate and the production of bacteriocins by the starter. Consequently, cheese is a very selective environment and its internal non-starter microflora is dominated by lactic acid bacteria, especially mesophilic lactobacilli, and perhaps some *Micrococcus* and *Pediococcus*.
5. Secondary and adjunct cultures. As discussed in section 10.2.3, many cheese varieties are characterized by the growth of secondary microorganisms which have strong metabolic activity and dominate the ripening and characteristics of these cheeses.
6. Other exogenous enzymes. An exogenous lipase is added to milk for a few varieties, e.g. pre-gastric lipase (in rennet paste) for Romano or Provolone cheese. In recent years, there has been considerable academic and commercial interest in adding exogenous proteinases (in addition to the coagulant) and/or peptidases to accelerate ripening. The enzymes may be added to the milk or curd in various forms, e.g. free, microencapsulated or in attenuated cells.

The contribution of these agents, individually or in various combinations, has been assessed in model cheese systems from which one or more of the agents was excluded or eliminated, e.g. by using an acidogen rather than starter for acidification or manufacturing cheese in a sterile environment to eliminate non-starter lactic acid bacteria (NSLAB). Such model systems have given very useful information on the biochemistry of ripening.

During ripening, three primary biochemical events occur, glycolysis, lipolysis and proteolysis. The products of these primary reactions undergo numerous modifications and interactions. The primary reactions are fairly well characterized but the secondary changes in most varieties are more or less unknown. An overview of the principal biochemical changes follows.

Glycolysis. Most (about 98%) of the lactose in cheese-milk is removed in the whey as lactose or lactic acid. However, fresh cheese curd contains 1–2%

lactose which is normally metabolized to L-lactic acid by the *Lactococcus* starter within a day for most varieties or a few weeks for Cheddar. In most varieties, the L-lactate is racemized to DL-lactate by NSLAB within about 3 months and a small amount is oxidized to acetic acid at a rate dependent on the oxygen content of the cheese and hence on the permeability of the packaging material.

In cheese varieties made using *Streptococcus salvarius* ssp. *thermophilus* and *Lactobacillus* spp. as starter, e.g. Swiss types and Mozzarella, the metabolism of lactose is more complex than in cheese in which a *Lactococcus* starter is used. In these cheeses, the curd is cooked to 52–55°C, which is above the growth temperature for both components of the starter; as the curd cools, the *Streptococcus*, which is the more heat-tolerant of the two starters, begins to grow, utilizing the glucose moiety of lactose, with the production of L-lactic acid, but not galactose, which accumulates in the curd. When the curd has cooled sufficiently, the *Lactobacillus* spp. grow, and, if a galactose-positive species/strain is used, it metabolizes galactose, producing DL-lactate (Figure 10.15). If a galactose-negative strain of *Lactobacillus* is used, galactose accumulates in the curd and can participate in Maillard browning, especially during heating, which is undesirable, especially in Pizza cheese.

Swiss-type cheeses are ripened at about 22°C for a period to encourage the growth of *Propionibacterium* spp. which use lactic acid as an energy

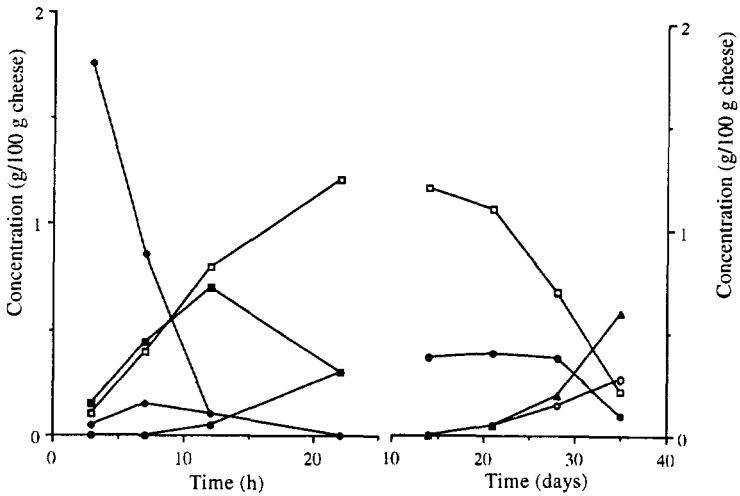


Figure 10.15 Metabolism of lactose, glucose, galactose, D- and L-lactic acid in Emmental cheese. Cheese transferred to hot room (22–24°C) at 14 days. ●, D-lactate; ○, acetate; ■, galactose; □, L-lactate; ♦, glucose; ◇, lactose; ▲, propionate.

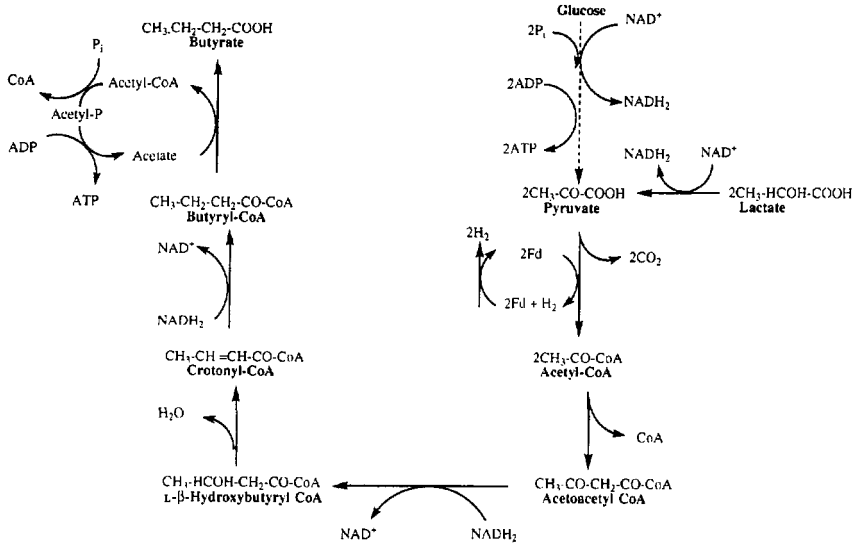


Figure 10.16 Metabolism of glucose or lactic acid by *Clostridium tyrobutyricum* with the production of butyric acid, CO_2 and hydrogen gas.

source, producing propionic acid, acetic acid and CO_2 (Figure 10.15):

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

Lactic acid — Propionic acid — Acetic acid

Propionic and acetic acids probably contribute to the flavour of Swiss-type cheeses, while the CO_2 is responsible for their large characteristic eyes. Lactic acid may be metabolized by *Clostridium tyrobutyricum* to butyric acid, CO_2 and hydrogen (Figure 10.16); butyric acid is responsible for off-flavours and the CO_2 and H_2 for late gas blowing. Clostridia are controlled by good hygienic practices, addition of nitrate or lysozyme, bactofugation or microfiltration. The principal sources of clostridia are soil and silage.

In surface mould-ripened cheeses, e.g. Camembert and Brie, *Penicillium camemberti*, growing on the surface, metabolizes lactic acid as an energy source, causing the pH to increase. Lactic acid diffuses from the centre to the surface, where it is catabolized. Ammonia produced by deamination of amino acids contributes to the increase in pH which reaches about 7.5 at the surface and 6.5 at the centre of the cheese. Ripening of Camembert and Brie is characterized by softening (liquefaction) of the texture from the surface towards the centre. Softening is due to the increase in pH, proteolysis and diffusion of calcium phosphate to the surface, where it precipitates due to the high pH. These events are summarized in Figure 10.17.

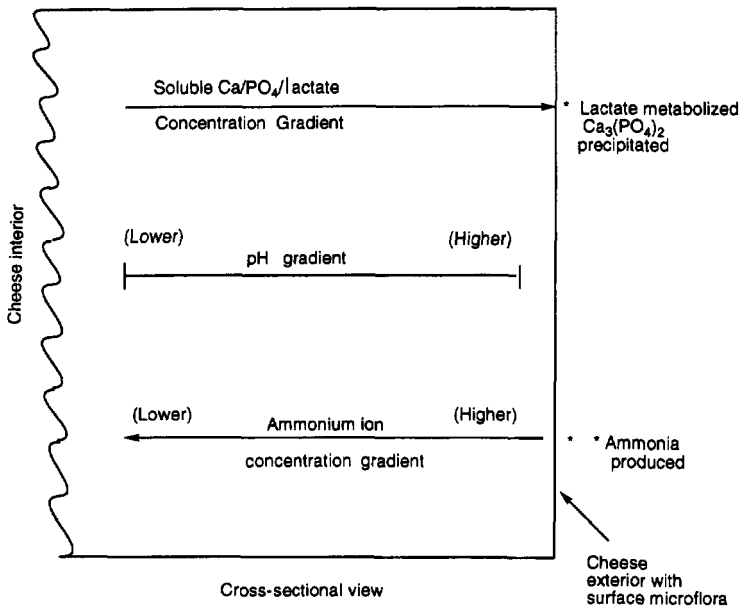


Figure 10.17 Schematic representation of the gradients of calcium, phosphate, lactic acid, pH and ammonia in ripening of Camembert cheese.

In surface smear-ripened cheeses, e.g. Munster, Limburger, Tilsit, Trapist, the surface of the cheese is colonized first by yeasts which catabolize lactic acid, causing the pH to increase, and then by *Brevibacterium linens*, the characteristic micro-organism of the surface smear but which does not grow below pH 5.8, and various other micro-organisms, including *Micrococcus*, *Arthrobacter* and coryneform bacteria.

Lipolysis. Some lipolysis occurs in all cheeses; the resulting fatty acids contribute to cheese flavour. In most varieties, lipolysis is rather limited (Table 10.5) and is caused mainly by the limited lipolytic activity of the starter and non-starter lactic acid bacteria, perhaps with a contribution from indigenous milk lipase, especially in cheese made from raw milk.

Extensive lipolysis occurs in two families of cheese in which fatty acids and/or their degradation products are major contributors to flavour, i.e. certain Italian varieties (e.g. Romano and Provolone) and the Blue cheeses. Rennet paste, which contains pre-gastric esterase (PGE) rather than rennet extract, is used in the manufacture of these Italian cheeses. PGE is highly specific for the fatty acids on the *sn*-3 position of glycerol, which, in the case of milk lipids, are predominantly highly flavoured short-chain fatty acids (butanoic to decanoic). These acids are principally responsible for the characteristic piquant flavour of these Italian cheeses.

Table 10.5 Free fatty acids in a selection of cheese varieties (Woo and Lindsay, 1984; Woo, Kollodge and Lindsay, 1984)

Variety	FFA (mg kg^{-1})	Variety	FFA (mg kg^{-1})
Sapsago	211	Gjetost	1658
Edam	356	Provolone	2118
Mozzarella	363	Brick	2150
Colby	550	Limburger	4187
Camembert	681	Goats' milk	4558
Port Salut	700	Parmesan	4993
Moneterey Jack	736	Romano	6743
Cheddar	1028	Roquefort	32453
Gruyere	1481	Blue (US)	32230

Blue cheeses undergo very extensive lipolysis during ripening; up to 25% of all fatty acids may be released. The principal lipase in Blue cheese is that produced by *Penicillium roqueforti*, with minor contributions from indigenous milk lipase and the lipases of starter and non-starter lactic acid bacteria. The free fatty acids contribute directly to the flavour of Blue cheeses but, more importantly, they undergo partial β-oxidation to alkan-2-ones (methyl ketones; ($R{-}\overset{\overset{O}{\|}}{C}{-}CH_3$) through the catabolic activity of the mould (Figure 10.18). A homologous series of alkan-2-ones from C_3 to C_{17} is formed (corresponding to the fatty acids from C_4 to C_{18}), but heptanone and nonanone predominate; typical concentrations are shown in Table 10.6. The characteristic peppery flavour of Blue cheeses is due to alkan-2-ones. Under anaerobic conditions, some of the alkan-2-ones may be reduced to the corresponding alkan-2-ols (secondary alcohols), which cause off-flavours.

Proteolysis. Proteolysis is the most complex, and perhaps the most important, of the three primary biochemical events in the ripening of most cheese varieties. In internal, bacterially ripened cheeses, e.g. Cheddar, Dutch and Swiss varieties, it is mainly responsible for the textural changes that occur during ripening, i.e. conversion of the tough rubbery texture of fresh curd to the smooth, pliable body of mature cheese. Small peptides and free amino acids contribute directly to cheese flavour and amino acids serve as substrates in several flavour-generating reactions, e.g. decarboxylation, deamination and desulphuration. Amino acids may also react chemically with carbonyls via the Maillard reaction and Strecker degradation, with the production of a great diversity of sapid compounds (Chapter 2). Excessive amounts of hydrophobic peptides may be produced under certain circumstances and may lead to bitterness which some consumers find very objectional; however, at an appropriate concentration, and when properly balanced by other compounds, bitter peptides probably contribute positively to cheese flavour.

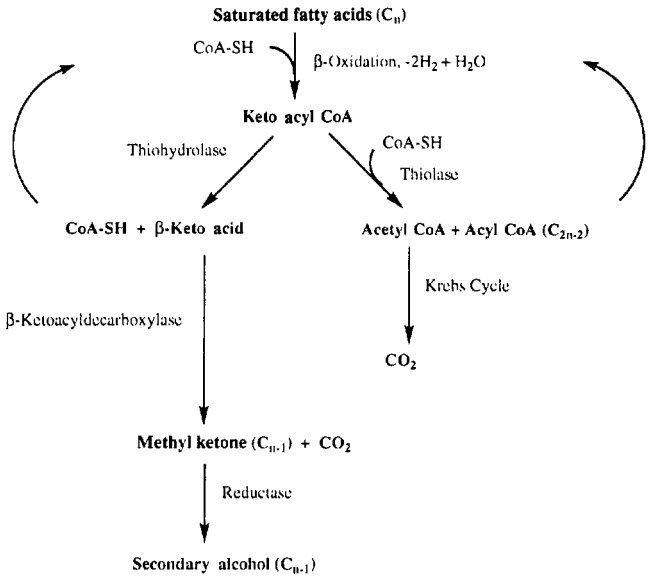


Figure 10.18 β-Oxidation of fatty acids to methyl ketones by *Penicillium roqueforti* and subsequent reduction to secondary alcohols.

Table 10.6 Typical concentrations of alkan-2-ones in Blue cheese (from Kinsella and Hwang, 1976)

	μg per 10 g dry Blue cheese							
2-Alkanone	A[a]	B[a]	C[a]	D[b]	E[b]	F[b]	G[c]	H[c]
2-Propanone	65	54	75	210	–	0	60	T[d]
2-Pentanone	360	140	410	1022	367	51	372	285
2-Heptanone	800	380	380	1827	755	243	3845	3354
2-Nonanone	560	440	1760	1816	600	176	3737	3505
2-Undecanone	128	120	590	136	135	56	1304	1383
2-Tridecanone	–	–	–	100	120	77	309	945
Total	1940	1146	4296	5111	1978	603	9627	9372

[a]Commercial samples of ripe Blue cheese.
[b]Samples D, E and F of Blue cheese ripened for 2, 3 and 4 months, respectively.
[c]Samples G and H of very small batches of experimental Blue cheese ripened for 2 and 3 months, respectively.
[d]Trace.

The level of proteolysis in cheese varies from limited (e.g. Mozzarella) through moderate (e.g. Cheddar and Gouda) to very extensive (e.g. Blue cheeses). The products of proteolysis range from very large polypeptides, only a little smaller than the parent caseins, to amino acids which may, in turn, be catabolized to a very diverse range of sapid compounds, including amines, acids and sulphur compounds.

Depending on the depth of information required, proteolysis in cheese is assessed by a wide range of techniques. Electrophoresis, usually urea–PAGE, is particularly appropriate for monitoring primary proteolysis, i.e. proteolysis of the caseins and the resulting large polypeptides. Quantifying the formation of peptides and amino acids soluble in water, at pH 4.6, in TCA, ethanol or phosphotungstic acid, or the measurement of free amino groups by reaction with ninhydrin, *o*-phthaldialdehyde, trinitrobenzene or fluorescamine, is suitable for monitoring secondary proteolysis. Reversed phase HPLC is especially useful for fingerprinting the small peptide profile in cheese and is now widely used. High-performance ion-exchange or size exclusion chromatography are also effective but are less widely used.

Proteolysis has not yet been fully characterized in any cheese variety but considerable progress has been made for Cheddar and, as far as is known, generally similar results apply to other low-cook, internal bacterially ripened cheeses (e.g. Dutch types). Proteolysis in Cheddar will be summarized as an example of these types of cheese.

Urea–PAGE shows that α_{s1}-casein is completely hydrolysed in Cheddar within 3–4 months (Figure 10.19). It is hydrolysed by chymosin, initially at Phe_{23}–Phe_{24} and later at Leu_{101}–Lys_{102}, and to a lesser extent at Phe_{32}–Gly_{33}, Leu_{98}–Lys_{99} and Leu_{109}–Glu_{110}. Although β-casein in solution is readily hydrolysed by chymosin, in cheese β-casein is very resistant to chymosin but is hydrolysed slowly (*c.* 50% at 6 months) by plasmin at Lys_{28}–Lys_{29}, Lys_{105}–His/Gln_{106} and Lys_{107}–Glu_{108}, producing γ^1, γ^2- and γ^3-caseins, respectively, and the corresponding proteose–peptones (PP5, PP8 slow and PP8 fast; Chapter 4). Chymosin and, to lesser extent, plasmin

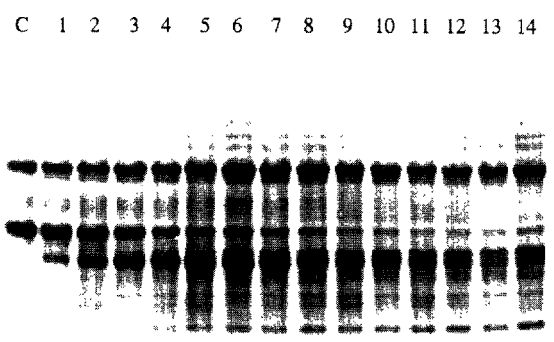


Figure 10.19 Urea-polyacrylamide gel electrophoretograms of Cheddar cheese after ripening for 0, 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18 or 20 weeks (lanes 1–14); C, sodium caseinate. (Supplied by S. Mooney.)

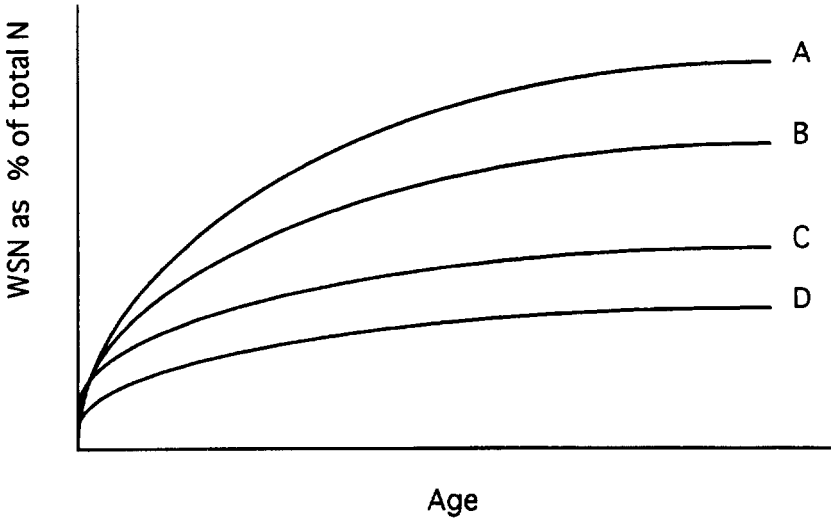


Figure 10.20 Formation of water-soluble nitrogen (WSN) in: (A) Cheddar cheese with a controlled microflora (free of non-starter bacteria); (B) controlled microflora chemically-acidified (starter-free) cheese; (C) controlled microflora, rennet-free cheese; (D) controlled microflora, rennet-free, starter-free cheese.

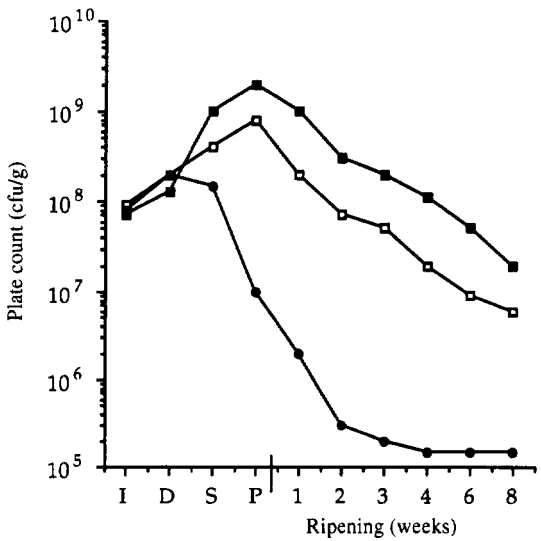


Figure 10.21 Changes in the population of starter cells in cheese made using different single strain starters. I, Inoculation; D, whey drainage; S, salting; P, after pressing.

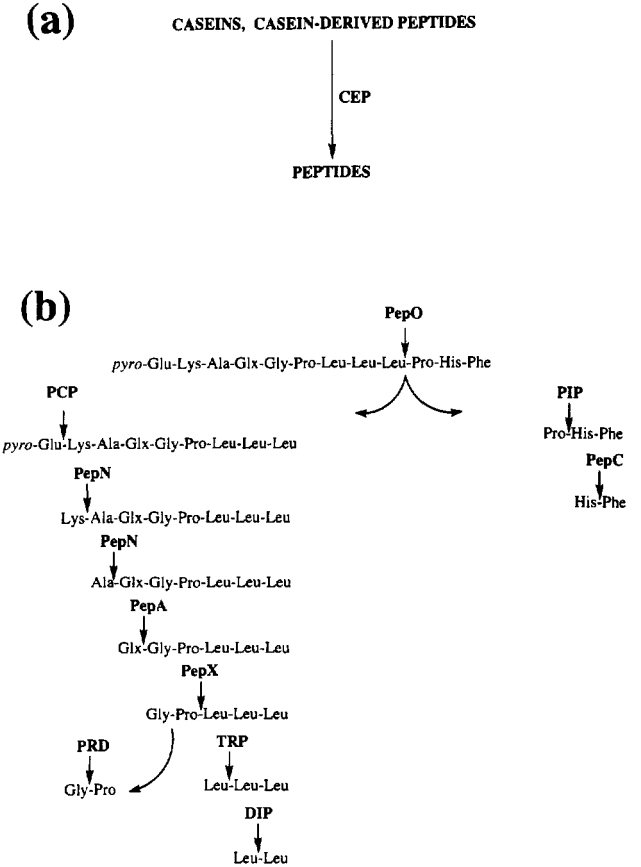


Figure 10.22 Schematic representation of the hydrolysis of casein (a) by lactococcal cell envelope proteinase (CEP), and (b) degradation of an hypothetical dodecapeptide by the combined action of lactococcal peptidases: oligopeptidase (PepO), various aminopeptidases (PCP, PepN, PepA, PepX), tripeptidase (TRP), prolidase (PRD) and dipeptidase (DIP).

are mainly responsible for primary proteolysis, i.e. the formation of water (or pH 4.6)-soluble N, as summarized in Figure 10.20.

Although *in vitro*, the cell wall-associated proteinase of the *Lactococcus* starters is quite active on β-casein (and that from some strains on α_{s1}-casein also), in cheese, they appear to act mainly on casein-derived peptides, produced by chymosin from α_{s1}-casein or by plasmin from β-casein.

The starter cells begin to die off at the end of curd manufacture (Figure 10.21); the dead cells may lyse and release their intracellular endopeptidases (Pep O, Pep F), aminopeptidases (including Pep N, Pep A, Pep C, Pep X), tripeptidases and dipeptidases (including proline-specific peptidases) which produce a range of free amino acids (Figure 10.22). About 150 peptides have

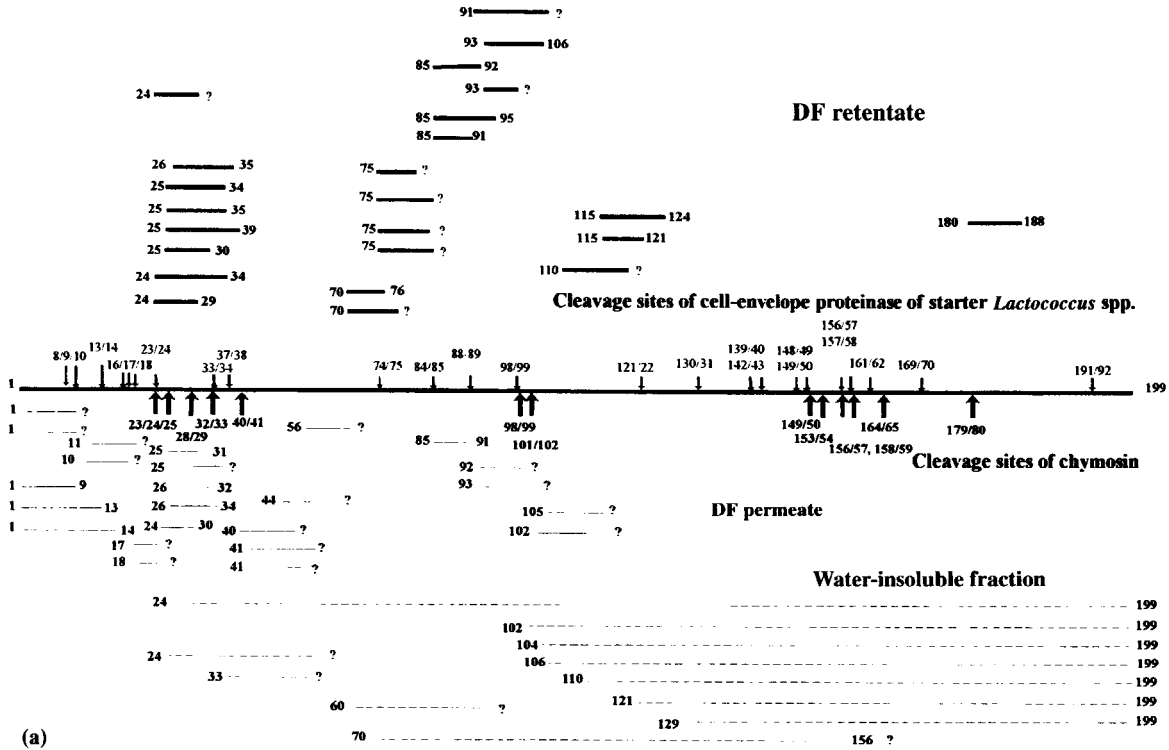


Figure 10.23 Water-insoluble and water-soluble peptides derived from α_{s1}-casein (A), α_{s2}-casein (B) or β-casein (C) isolated from Cheddar cheese; DF = diafiltration. The principal chymosin, plasmin and lactococcal cell-envelope proteinase cleavage sites are indicated by arrows (data from T.K. Singh and S. Mooney, unpublished).

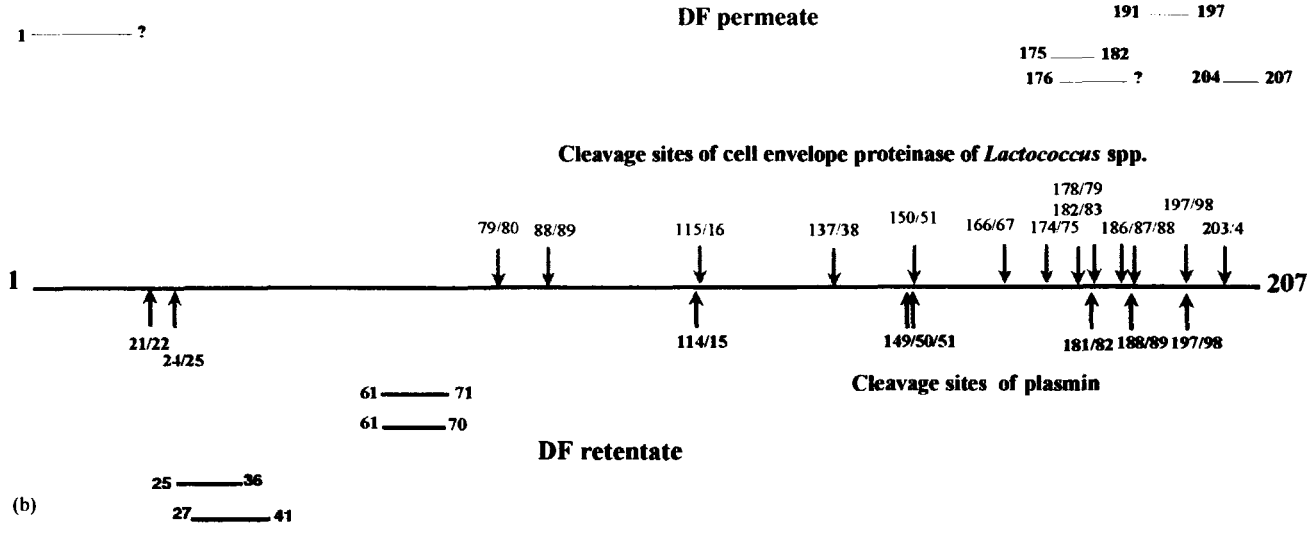


Figure 10.23 (*Continued*).

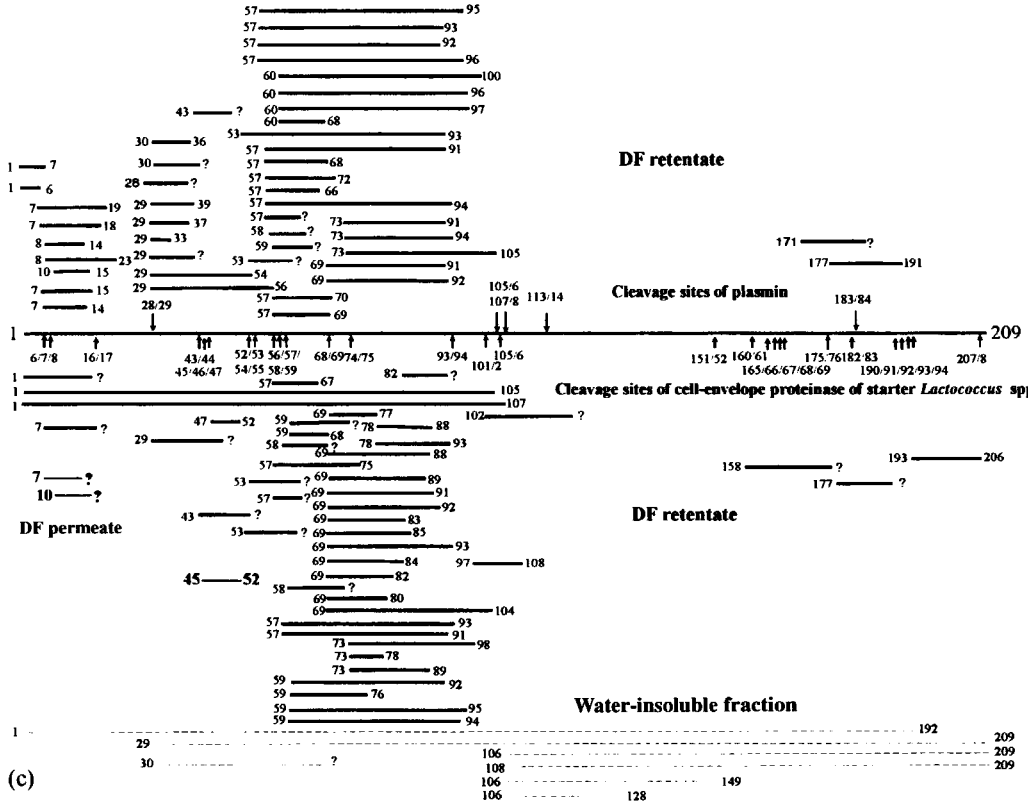


Figure 10.23 (*Continued*).

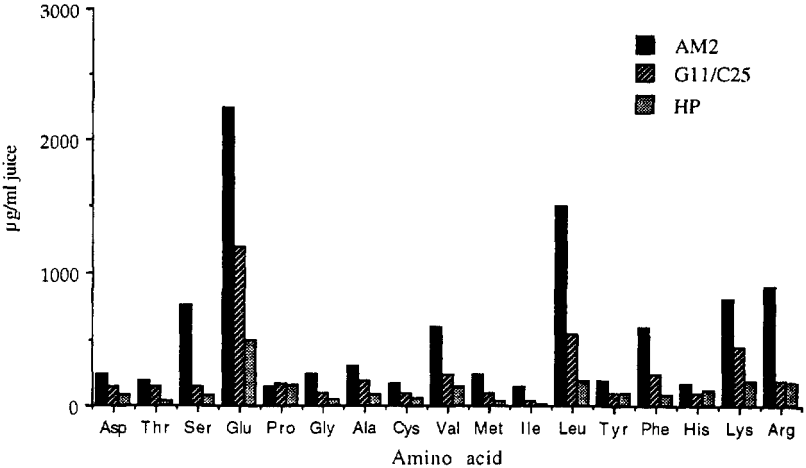


Figure 10.24 Concentration of individual amino acids in 60-day-old Cheddar cheese, made with a single-strain starter *Lactococcus lactis* ssp. *cremoris* AM_2, G11/C25 or HP (from Wilkinson, 1992).

been isolated from the water-soluble fraction of Cheddar, and characterized (Figure 10.23). These show that both lactococcal proteinase and exopeptidase contribute to proteolysis in cheese. The proteinases and peptidases of the NSLAB (mainly mesophilic lactobacilli) appear to contribute little to proteolysis in Cheddar, except in the production of amino acids.

The principal amino acids in Cheddar are shown in Figure 10.24.

10.2.8 Cheese flavour

Although interest in cheese flavour dates from the beginning of this century, very little progress was made until the development of gas liquid chromatography (GC) in the late 1950s, and especially the coupling of GC and mass spectrometry (MS). More than 200 volatile compounds have been identified in cheese by GC–MS (principal compounds are listed in Table 10.7). The volatile fraction of cheese may be obtained by taking a sample of headspace but the concentration of many compounds is too low, even for modern GC–MS techniques. The volatiles may be concentrated by solvent extraction or distillation. In the former, a large solvent peak may mask important constituents while the latter may generate artefacts, even at moderately low temperatures. Trapping of volatiles, e.g. on adsorbants or in cold traps, is probably the most satisfactory method for concentration.

The taste of cheese is concentrated in the water-soluble fraction (peptides, amino acids, organic acids, amines, NaCl) while the aroma is mainly in the volatile fraction. Initially, it was believed that cheese flavour was due to one

Table 10.7 Volatile compounds which have been identified in Cheedar cheese (modified from Urbach, 1993)

Acetaldehyde	Dimethyl disulfide	2-Methylbutanol
Acetoin	Dimethyl trisulfide	3-Methylbutanol
Acetone	δ-Dodecalactone	3-Methyl-2-butanone
Acetophenone	Ethanol	3-Methylbutyric acid
1,2-Butanediol	Ethyl butanol	2-Nonanone
n-Butanol	2-Ethyl butanol	δ-Octalactone
2-Butanol	Ethyl butyrate	*n*-Octanoic acid
Butanone	Ethyl hexanoate	2-Octanol
n-Butyl acetate	2-Heptanone	2,4-Pentanediol
2-Butyl acetate	*n*-Hexanal	*n*-Pentanoic acid
n-Butyl butyrate	*n*-Hexanoic acid	2-Pentanol
n-Butyric acid	*n*-Hexanol	Pentan-2-one
Carbon dioxide	2-Hexanone	*n*-Propanol
p-Cresol	Hexanethiol	Propanal
γ-Decalactone	2-Hexenal	Propenal
δ-Decalactone	Isobutanol	*n*-Propyl butyrate
n-Decanoic acid	Isohexanal	Tetrahydrofuran
Diacetyl	Methanethiol	Thiophen-2-aldehyde
Diethyl ether	Methional	2-Tridecanone
Dimethyl sulfide	Methyl acetate	2-Undecanone

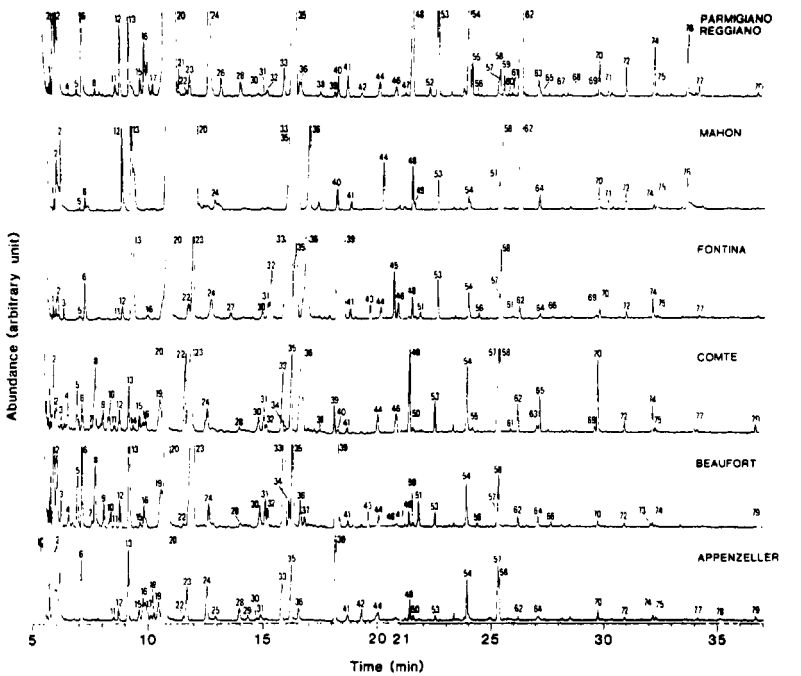


Figure 10.25 GC-MS chromatograms of the headspace volatiles of six cheese varieties (from Bosset and Gauch, 1993).

or a small number of compounds, but it was soon realized that all cheeses contained essentially the same sapid compounds. Recognition of this led to the component balance theory, i.e. cheese flavour is due to the concentration and balance of a range of compounds. Although considerable information on the flavour compounds in several cheese varieties has been accumulated, it is not possible to fully describe the flavour of any variety, with the possible exception of Blue cheeses, the flavour of which is dominated by alkan-2-ones.

Many cheeses contain the same or similar compounds but at different concentrations and proportions; chromatograms of some cheese varieties are shown in Figure 10.25. The principal classes of components present are aldehydes, ketones, acids, amines, lactones, esters, hydrocarbons and sulphur compounds; the latter, e.g. H_2S, methanethiol (CH_3SH), dimethyl sulphide ($H_3C\text{-}S\text{-}CH_3$) and dimethyl disulphide ($H_3C\text{-}S\text{-}S\text{-}CH_3$), are considered to be particularly important in Cheddar cheese. The biogenesis of flavour compounds has been reviewed by Fox *et al.* (1993, 1996a) and Fox, Singh and McSweeney (1995).

10.2.9 Accelerated ripening of cheese

Since the ripening of cheese, especially low moisture varieties, is a slow process, it is expensive in terms of controlled atmosphere storage and stocks. Ripening is also unpredictable. Hence, there are economic and technological incentives to accelerate ripening, while retaining or improving characteristic flavour and texture.

The principal approaches used to accelerate cheese ripening are:

1. Elevated ripening temperatures, especially for Cheddar which is now usually ripened at 6–8°C; most other varieties are ripened at a higher temperature, e.g. around 14°C for Dutch types or 20–22°C for Swiss types and Parmesan, and hence there is little or no scope for increasing the ripening temperature.
2. Exogenous enzymes, usually proteinases and/or peptidases. For several reasons, this approach has had limited success, except for enzyme-modified cheeses (EMC). These are usually high-moisture products which are used as ingredients for processed cheese, cheese spreads, cheese dips or cheese flavourings.
3. Attenuated lactic acid bacteria, e.g. freeze-shocked, heat-shocked or lactose-negative mutants.
4. Adjunct starters, especially mesophilic lactobacilli.
5. Use of fast-lysing starters which die and release their intracellular enzymes rapidly.
6. Genetically modified starters which super-produce certain enzymes; unfortunately, the key enzymes are not yet known.

The lack of definitive information on the key flavour-generating reactions in cheese is hampering efforts to accelerate ripening, which are, at present, empirical. Considerable in-depth information on the biochemistry of cheese ripening is now becoming available which will facilitate the genetic engineering of starter cultures with improved cheesemaking properties. Acceleration of cheese ripening has been reviewed by Fox *et al.* (1996b).

10.3 Acid-coagulated cheeses

On acidification to pH 4.6, the caseins coagulate, which is the principle used to manufacture of a family of cheeses which represent about 25% of total cheese consumption and are the principal cheeses in some countries (Appendix 10B). Acidification is traditionally and usually achieved by *in situ* fermentation of lactose by a *Lactococcus* starter but direct acidification by acid or acidogen (gluconic acid-δ-lactone) is also practised. The principal

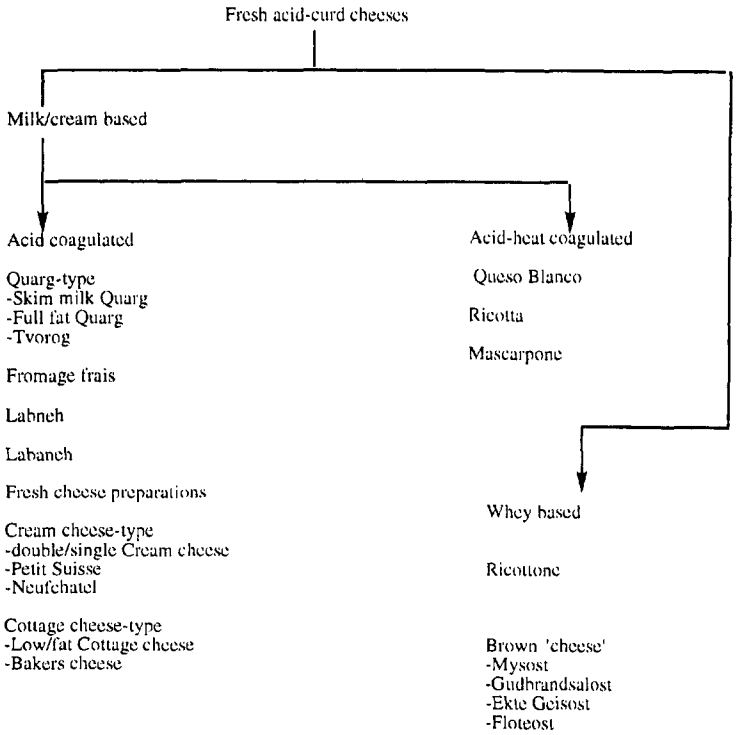


Figure 10.26 Examples of acid-coagulated or heat-acid coagulated or whey-based cheese varieties (from Fox *et al.*, 1996a).

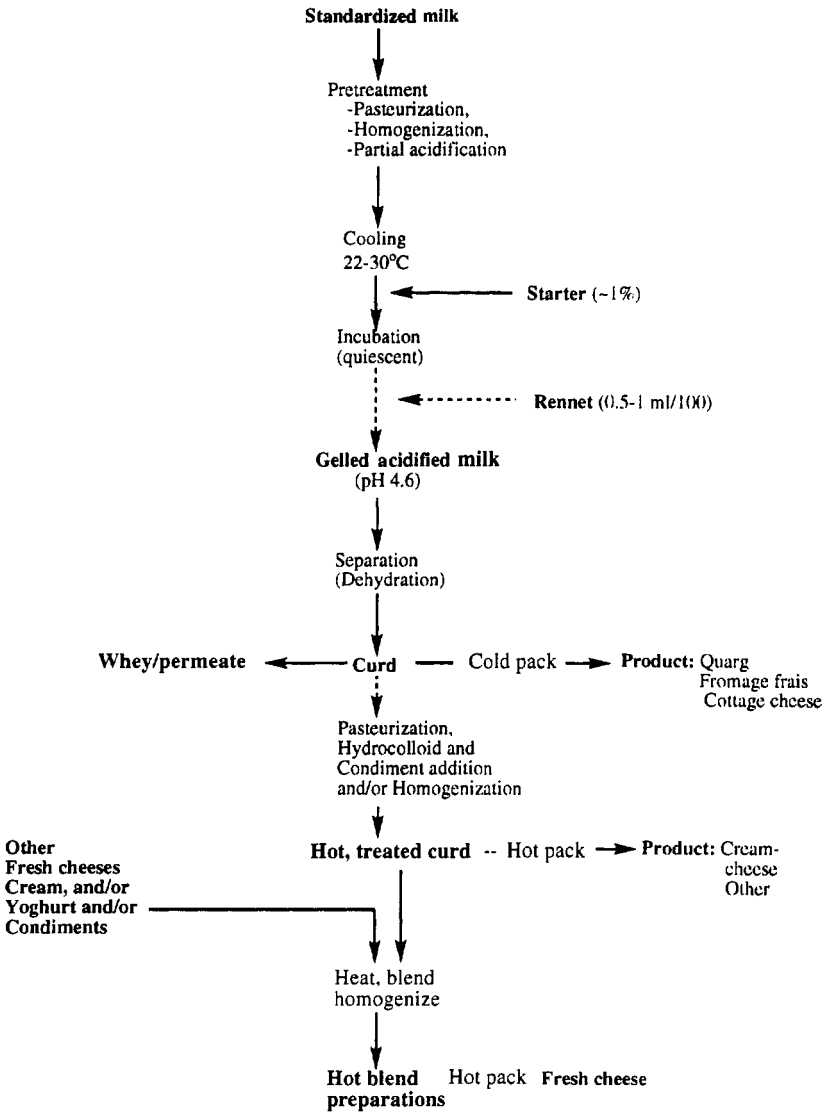


Figure 10.27 Protocol for the manufacture of fresh acid-coagulated cheese (from Fox *et al.*, 1996a).

families of acid-coagulated cheeses are illustrated in Figure 10.26 and a typical manufacturing protocol is shown in Figure 10.27.

Acid-coagulated cheeses are usually produced from skim milk and are consumed fresh. Major varieties include quarg, (American) cottage cheese, cream cheese and petit suisse. These cheeses may be consumed in salads, as

food ingredients and serve as the base for a rapidly expanding group of dairy products, i.e. fromage frais-type products.

The casein may also be coagulated at a pH above 4.6, e.g. about 5.2, by using a higher temperature, e.g. 80–90°C. This principle is used to manufacture another family of cheeses, which include Ricotta (and variants thereof), Anari, and some types of Queso Blanco. These cheeses may be made exclusively from whey but usually from a blend of milk and whey and are usually used as a food ingredient, e.g. in lasagne or ravioli.

10.4 Processed cheese products

Processed cheese is produced by blending shredded natural cheese of the same or different varieties and at different degrees of maturity with emulsifying agents and heating the blend under vacuum with constant agitation until a homogeneous mass is obtained. Other dairy and non-dairy ingredients may be included in the blend. The possibility of producing processed cheese was first assessed in 1895; emulsifying salts were not used and the product was not successful. The first sucessful product, in which emulsifying salts were used, was introduced in Europe in 1912 and in the USA in 1917 by Kraft. Since then, the market for processed cheese has increased and the range of products expanded.

Although established consumers may regard processed cheeses as inferior products compared to natural cheeses, they have numerous advantages compared to the latter:

1. A certain amount of cheese which would otherwise be difficult or impossible to commercialize may be used, e.g. cheese with deformations, cheese trimmings or cheese after removal of localized mould.
2. A blend of cheese varieties and non-cheese components may be used, making it possible to produce processed cheeses differing in consistency, flavour, shape and size.
3. They have good storage stability at moderate temperatures, thus reducing the cost of storage and transport.
4. They are more stable than natural cheeses during storage, which results in less wastage, a feature that may be especially important in remote areas and in households with a low level of cheese consumption.
5. They are amenable to imaginative packing in various conveniently sized units.
6. They are suitable for sandwiches and fast food outlets.
7. They are attractive to children who generally do not like or appreciate the stronger flavour of natural cheeses.

Today, a wide range of processed cheese products is available, varying in composition and flavour (Table 10.8).

Table 10.8 Compositional specifications and permitted ingredients in pasteurized processed cheese products[a] (modified from Fox *et al.*, 1996a)

Product	Moisture (%, w/w)	Fat (%, w/w)	Fat in dry matter (%, w/w)	Ingredients
Pasteurized blended cheese	≤43	–	≥47	Cheese; cream, anhydrous milk fat, dehydrated cream (in quantities such that the fat derived from them is less than 5% (w/w) in finished product); water; salt; food-grade colours, spices and flavours; mould inhibitors (sorbic acid, potassium/sodium sorbate, and/or sodium/calcium propionates), at levels ≤0.2% (w/w) finished product
Pasteurized processed cheese	≤43	–	≥47	As for pasteurized blended cheese, but with the following extra optional ingredients: emulsifying salts (sodium phosphates, sodium citrates; 3% (w/w) of finished product), food-grade organic acids (e.g. lactic, acetic or citric) at levels such that pH of finished product is ≥5.3
Pasteurized processed cheese foods	≤44	≥23	–	As for pasteurized blended cheese, but with the following extra optional ingredients (milk, skim milk, buttermilk, cheese whey, whey proteins – in wet or dehydrated forms)
Pasteurized processed cheese spreads	40–60	≥20	–	As for pasteurized blended cheese, but with the following extra optional ingredients: food-grade hydrocolloids (e.g. carob bean gum, guar gum, xanthan gums, gelatin, carboxymethylcellulose, and/or carageenan) at levels <0.8% (w/w) of finished products; food-grade sweetening agents (e.g. sugar, dextrose, corn syrup, glucose syrup, hydrolysed lactose)

[a]Minimum temperatures and times specified for processing are 65.5°C for 30 s.

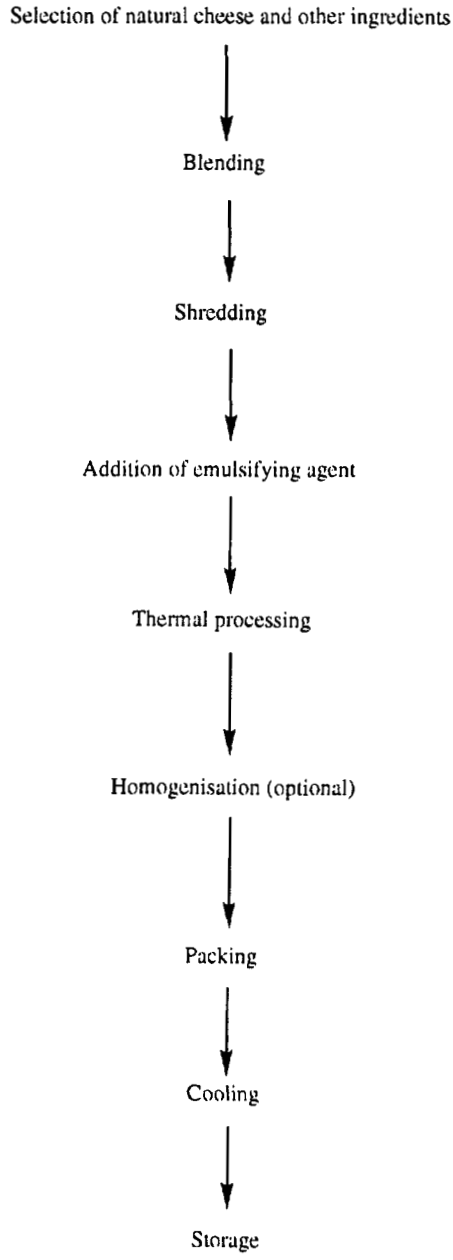


Figure 10.28 Protocol for the manufacture of processed cheese.

10.4.1 Processing protocol

The typical protocol for the manufacture of processed cheese is outlined in Figure 10.28.

The important criteria for selecting cheese are type, flavour, maturity, consistency, texture and pH. The selection is determined by the type of processed cheese to be produced and by cost factors.

A great diversity of non-cheese ingredients may be used in the manufacture of processed cheese (Figure 10.29).

Emulsifying salts are critical in the manufacture of processed cheese with desirable properties. The most commonly used salts are orthophosphates, polyphosphates and citrates but several other agents are used (Tables 10.9 and 10.10). Emulsifying salts are not emulsifiers in the strict sense, since they are not surface active. Their essential role in processed cheese is to supplement the emulsifying properties of cheese proteins. This is accomplished by sequestering calcium, solubilizing, dispersing, hydrating and swelling the proteins and adjusting and stabilizing the pH.

The actual blend of ingredients used and the processing parameters depend on the type of processed cheese to be produced; typical parameters are summarized in Table 10.11.

One of the major advantages of processed cheese is the flexibility of the finished form, which facilitates usage. The texture may vary from firm and sliceable to soft and spreadable. These cheeses may be presented as large blocks (5–10 kg), suitable for industrial catering, smaller blocks, e.g. 0.5 kg,

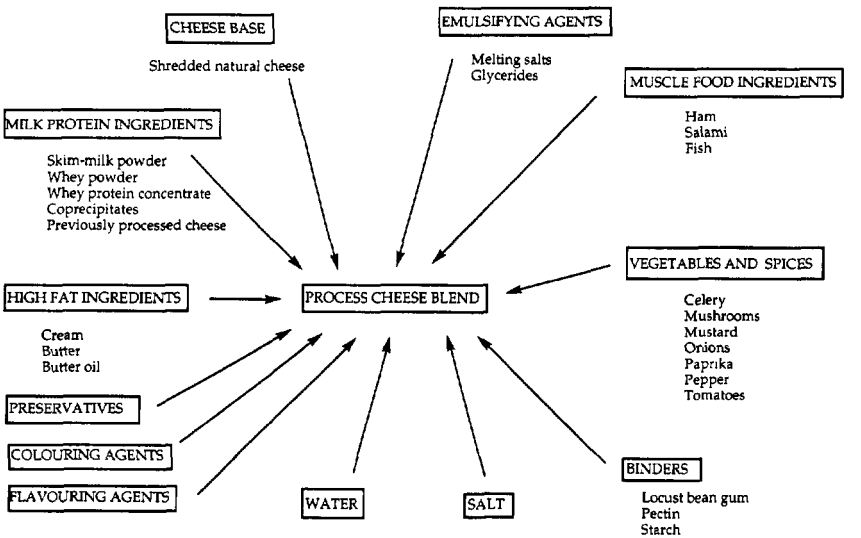


Figure 10.29 Examples of non-cheese ingredients used in processed cheese (from Caric and Kalab, 1987).

Table 10.9 Properties of emulsifying salts for processed cheese products (from Caric and Kalab, 1987)

Group	Emulsifying salt	Formula	Solubility at 20°C (%)	pH value (1% solution)
Citrates	Trisodium citrate	$2Na_3C_6H_5O_7.1H_2O$	High	6.23–6.26
Orthophosphates	Monosodium phosphate	$NaH_2PO_4.2H_2O$	40	4.0–4.2
	Disodium phosphate	$Na_2HPO_4.12H_2O$	18	8.9–9.1
Pyrophosphates	Disodium pyrophosphate	$Na_2H_2P_2O_7$	10.7	4.0–4.5
	Trisodium pyrophosphate	$Na_3HP_2O_7.9H_2O$	32.0	6.7–7.5
	Tetrasodium pyrophosphate	$Na_4P_2O_7.10H_2O$	10–12	10.2–10.4
Polyphosphates	Pentasodium tripolyphosphate	$Na_5P_3O_{10}$	14–15	9.3–9.5
	Sodium tetrapolyphosphate	$Na_6P_4O_{13}$	14–15	9.0–9.5
	Sodium hexametaphosphate (Graham's salt)	$Na_{n+2}P_nO_{3n+1}$ ($n = 10$–25)	Very high	6.0–7.5
Aluminium phosphates	Sodium aluminium phosphate	$NaH_{14}Al_3(PO_4)_8.4H_2O$	–	8.0

Table 10.10 General properties of emulsifying salts in relation to cheese processing (from Fox *et al.*, 1996a,b)

Property	Citrates	Orthophosphates	Pyrophosphates	Polyphosphates	Aluminium
Ion exchange (calcium sequesterization)	Low	Low	Moderate	High–very high	Low
Buffering action in the pH range 5.3–6.0	High	High	Moderate	Low–very low	–
para-Caseinate dispersion	Low	Low	High	Very high	–
Emulsification	Low	Low	Very high	Very high ($n = 3$–10) –low	Very low
Bacteriostatic	Nil	Low	High	High–very high	–

Table 10.11 Chemical, mechanical and thermal parameters as regulating factors in the cheese processing procedures (from Caric and Kalab, 1993)

Process conditions	Processed cheese block	Processed cheese slice	Processed cheese spread
Raw material			
a. Average of cheese	Young to medium ripe, predominantly young	Predominantly young	Combination of young, medium ripe, overipe
b. Water-insoluble N as a % of total N	75–90%	80–90%	60–75%
c. Structure	Predominantly long	Long	Short to long
Emulsifying salt	Structure-building, not creaming, e.g. high molecular weight polyphosphate, citrate	Structure-building, not creaming, e.g. phosphate/citrate mixtures	Creaming, e.g. low and medium molecular weight polyphosphate
Water addition	10–25% (all at once)	5–15% (all at once)	20–45% (in portions)
Temperature	80–85°C	78–85°C	85–98°C (150°C)
Duration of processing (min)	4–8	4–6	8–15
pH	5.4–5.7	5.6–5.9	5.6–6.0
Agitation	Slow	Slow	Rapid
Reworked cheese	0–0.2%	0	5–20%
Milk powder or whey powder 5–12%		0	0
Homogenization	None	None	Advantageous
Filling (min)	5–15	As fast as possible	10–30
Cooling	Slowly (10–12 h) at room temperature	Very rapid	Rapidly (15–30 min) in cool air

for household use, small unit packs, e.g. 25–50 g, or slices which are particularly suited for industrial catering and fast food outlets.

10.5 Cheese analogues

Cheese analogues represent a new range of cheese-like products which probably contain no cheese. The most important of these are Mozzarella (Pizza) cheese analogues which are produced from rennet casein, fat or oil (usually vegetable) and emulsifying salts. The function of emulsifying salts is essentially similar to those in processed cheese, i.e. to solubilize the proteins. The manufacturing protocol is usually similar to that used for processed cheese, bearing in mind that the protein is dried rennet casein rather than a blend of cheeses (Figure 10.30).

The main attributes required of cheese analogues used in pizzas are meltability and stretchability; flavour is provided by other ingredients of the

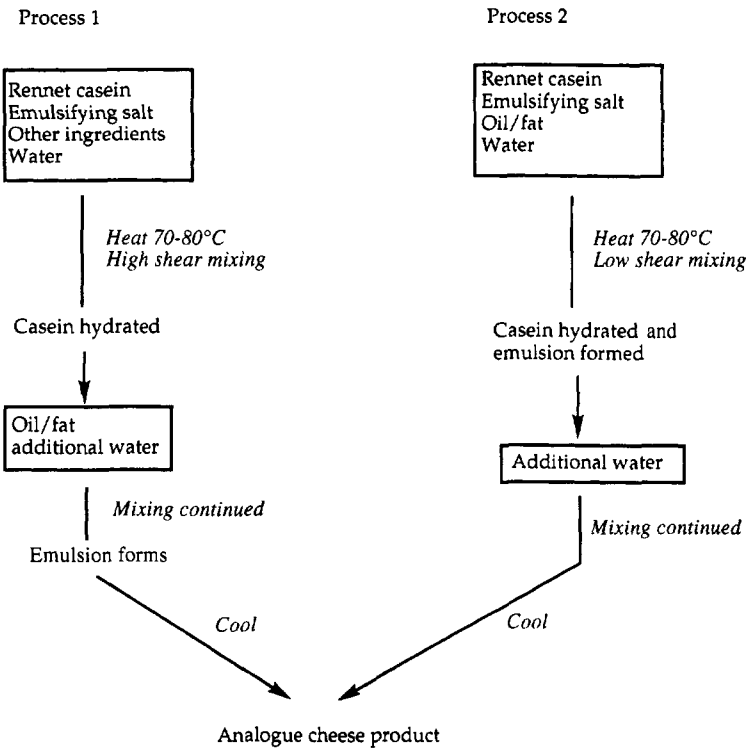


Figure 10.30 Typical protocols for the manufacture of cheese analogue from rennet casein.

pizza, e.g. tomato paste, sausage, peppers, spices, anchovies, etc. It may be possible to produce analogues of other cheeses by adding biochemically or chemically generated cheese flavours. Apart from the use of some casein (rennet or acid) in processed cheese blends, cheese analogues, other than Mozzarella, are not widely used at present. As discussed in section 10.2.8, the flavour and texture of natural cheeses are very complex and cannot be simulated readily. The usual approach is to accelerate the ripening of natural cheese (section 10.2.9), although this approach has enjoyed limited success to date.

10.6 Cultured milks

Acidified (cultured) milk products may very well be the oldest dairy products. If removed aseptically from a healthy udder, milk is essentially sterile but, in practice, milk becomes contaminated by various bacteria, including lactic acid bacteria (LAB) during milking. During storage, these contaminants grow at rates dependent on the temperature. LAB probably dominate the microflora of uncooled milk expressed by hand. Since LAB are well suited for growth in milk, they grow rapidly at ambient temperature, metabolizing lactose to lactic acid and reducing the pH of the milk to the isoelectric point of caseins (about pH 4.6), at which they form a gel under quiescent conditions, thus producing cultured milks. Such products have existed since the domestication of dairy animals and some form of cultured milk is produced throughout the world; the principal products are

Table 10.12 Some typical examples of starter cultures employed in the manufacture of fermented milks (from Robinson and Tamime, 1993)

Type of culture	Product	Micro-organisms involved
Mesophilic	Taetmojolk	*Lactococcus lactis* subsp. *lactis*
	Folkjolk	*Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* *Leuconostoc mesenteroides* subsp. *cremoris*
	Ymer	*Lc. lactis* subsp. *cremoris* *Lc. lactis* subsp. *lactis* biovar. *diacetylactis*
	Kefir	Kefir grains – thermophilic lactobacilli and *Kluyveromyces marxianus*
Typical fermentation temperature 20–22°C		
Thermophilic	Yoghurt	*Streptococcus salvarius* subsp. *thermophilus* *Lactobacillus delbrueckii* subsp. *bulgaricus*
	Yakult	*Lactobacillus casei* subsp. *casei*
	Acidophilus milk	*Lactobacillus acidophilus*
	A/B milk	*Lb. acidophilus* *Bifidobacterium bifidum*
	A/B yoghurt	As above plus yoghurt culture
Typical fermentation temperatures 37–42°C		

listed in Table 10.12 (Tamime and Robinson, 1985); yoghurt in its various forms, is probably the most important type but consumption varies widely (Table 1.6).

The production of fermented milks no longer depends on acid production by the indigenous microflora. Instead, the milk is inoculated with a carefully selected culture of LAB and for some products with LAB plus lactose-fermenting yeasts (Table 10.12). The principal function of LAB is to produce acid at an appropriate rate via the pathways summarized in Figure 10.12. The yoghurt fermentation is essentially homofermentative but the characteristic flavour of cultured buttermilk is due mainly to diacetyl which is produced from citrate by *Lactococccus lactis* ssp. *lactis* biovar *diacetylactis*, which is included in the culture for this product (Figure 10.31).

Kefir and Koumiss contain about 1 and 6% ethanol, respectively, which is produced by lactose-fermenting yeasts, usually *Kluyveromyces marxianus*. The ethanol modifies the flavour of the products and the CO_2 produced in the fermentation affects both their flavour and texture. Koumiss, which is produced traditionally from mares' milk, mainly in Russia and surrounding areas of Asia, is not in fact coagulated.

The technology of fermented milks will not be discussed in detail and the interested reader is referred to Tamime and Robinson (1985), Tamime and Marshall (1997) and Marshall and Tamime (1997). A flow diagram of the manufacturing protocol of yoghurt is presented in Figure 10.32. Depending on the product, the milk used may be full-fat, partially skimmed or fully skimmed. If it contains fat, the milk is homogenized at 10–20 MPa to prevent creaming during fermentation. For yoghurt, the milk is usually supplemented with skim-milk powder to improve gel characteristics. Acid milk gels are quite stable if left undisturbed but if stirred or shaken, they synerese, expressing whey, which is undesirable. The tendency to synerese is reduced by heating the milk at, for example, 90°C × 10 min or 120°C × 2 min. Heating causes denaturation of whey proteins, especially β-lactoglobulin, and their interaction with the casein micelles via κ-casein. The whey protein-coated micelles form a finer (smaller whey pockets) gel than that formed from unheated or HTST pasteurized milk, with less tendency to synerese.

In some countries, it is common practice to add sucrose to the milk for yoghurt, to reduce the acid taste. It is also very common practice to add fruit pulp, fruit essence or other flavouring, e.g. chocolate, to yoghurt, either to the milk (set yoghurt) or to the yoghurt after fermentation (stirred yoghurt).

In the manufacture of Labneh and other Middle Eastern fermented milks, the fermented product is concentrated by removing part of the serum (whey). This was done traditionally by stirring the yoghurt and transferring it to muslin bags to partially drain. Concentration can now be achieved by ultrafiltration, before, but preferably after, fermentation.

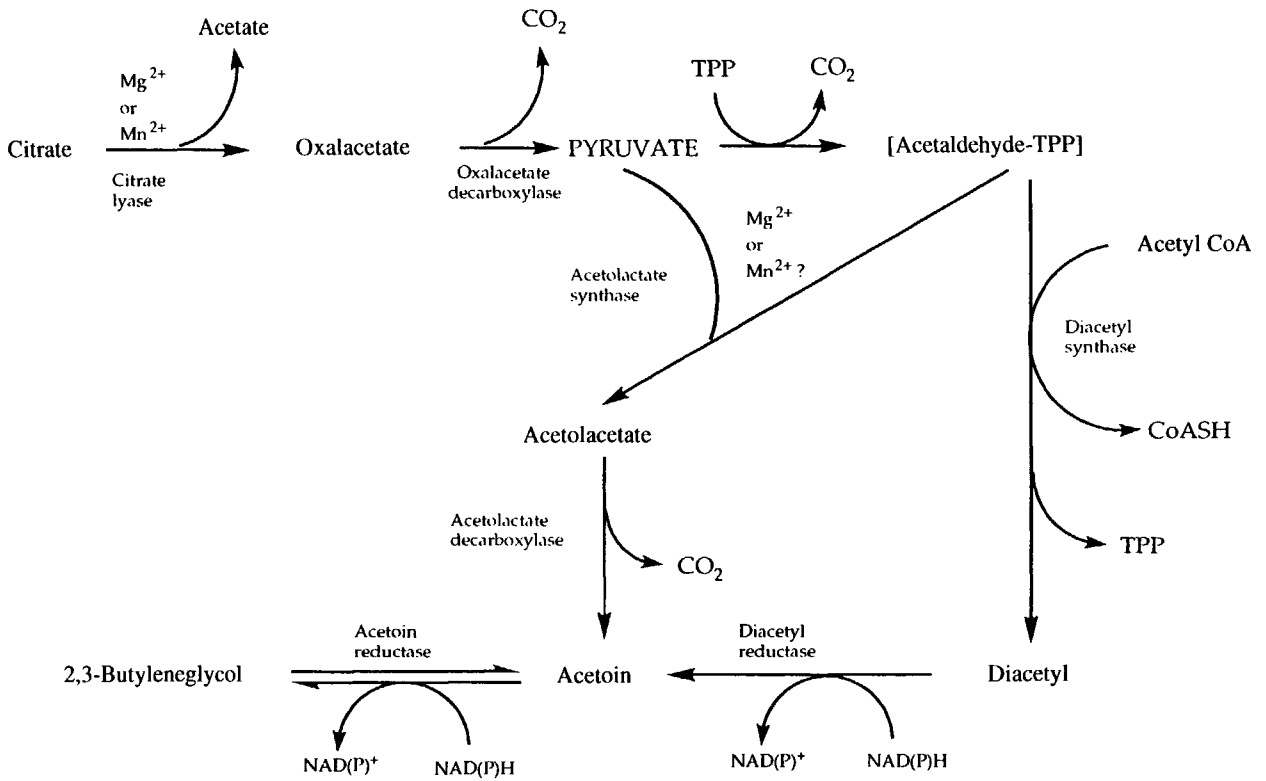


Figure 10.31 Citrate metabolism by *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* or *Leuconostoc* spp. (from Cogan and Hill, 1993).

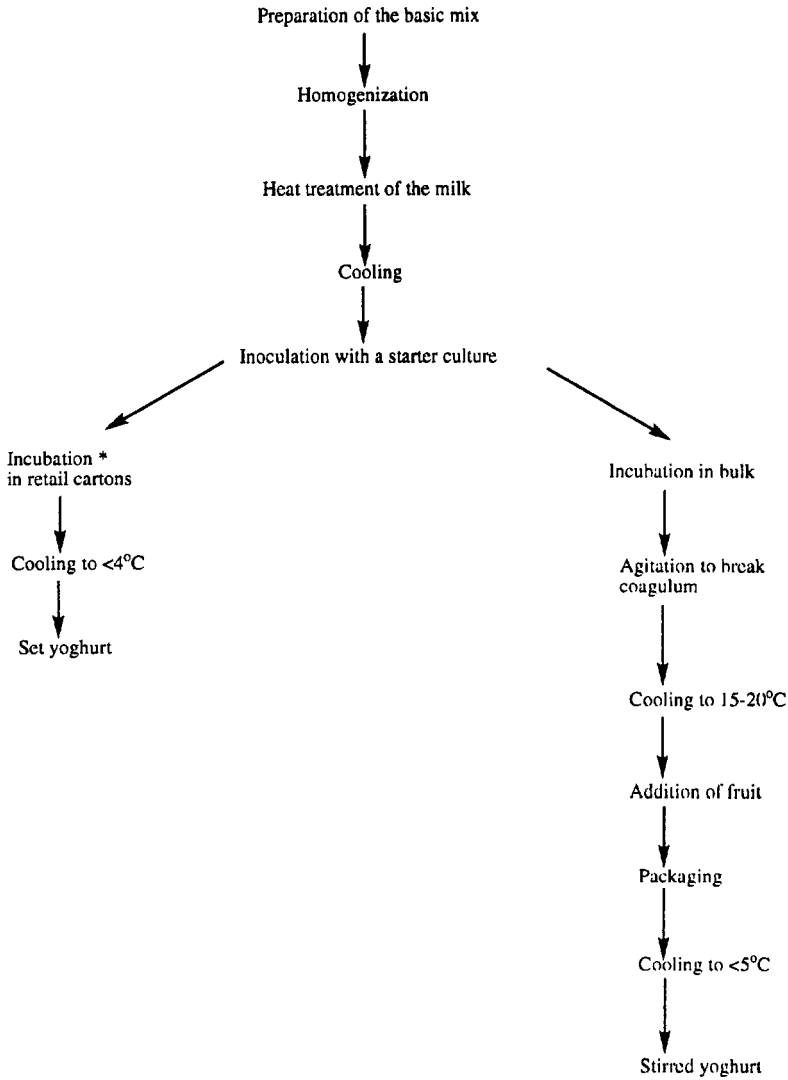


Figure 10.32 Protocol for the manufacture of yoghurt. *, Sucrose and/or fruit (fruit flavours) may be added at this point. (From Robinson and Tamime, 1993.)

Fermented milk products exhibit thixotropic rheological properties, i.e. the viscosity (resistance to flow) decreases as the rate of shear increases; a typical relationship is shown in Figure 10.33. The rheological properties are major parameters of quality and are controlled by varying the total solids content of the milk, the heat treatment and homogenization of the milk and the use of hydrocolloids, e.g. gelatin or carageenan.

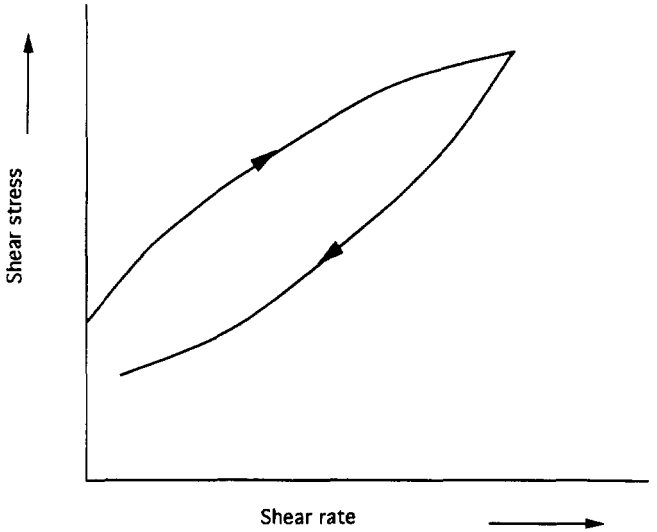


Figure 10.33 Representation of shear stress as a function of shear rate for yoghurt displaying rheological hysteresis.

Fermented milk products developed by chance but the increased storage stability and desirable organoleptic properties of such products were soon appreciated. Special therapeutic properties of yoghurt were claimed by Metchnikoff in 1910 and have been a controversial subject since. It is now generally accepted that fermented milk products have nutritional benefits above those of their gross chemical constituents. It has been documented that some *Lactobacillus* spp., and in particular *Bifidobacterium* spp., contained in yoghurt can colonize the large intestine, reduce its pH and control the growth of undesirable micro-organisms. Some of these bacteria also produce probiotics. Yoghurts containing such cultures, often referred to as bioyoghurt, are enjoying considerable commercial success. Legislation in many countries specifies a minimum number of viable micro-organisms in yoghurt.

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Appendices

Appendix 10A World cheese production, 1994 (FAO, 1994)

(See facing page)

Country	Cheese production (tonnes)	Country	Cheese production (tonnes)
World	**14 880 089**	Jordan	4612
Africa	**495 298**	Japan	98 000
Algeria	1045	Kazakhstan	93 000
Angola	1007	Kyrgyzstan	25 000
Botswana	1498	Lebanon	14 744
Egypt	333 950	Mongolia	1764
Eritrea	216	Myanmar	27 622
Ethiopia	4600	Oman	411
Kenya	210	Syria	78 638
Mauritania	1664	Tajikistan	16 000
Morocco	6947	Turkey	139 177
Namibia	70	Turkmenistan	7000
Niger	12 064	Uzbekistan	46 000
Nigeria	7022	Yemen	9155
South Africa	38 000	**Europe**	**7 075 705**
Sudan	72 479	Albania	15 400
Tanzania	1200	Austria	109 600
Tunisia	7060	Belarus	109 000
Zambia	1069	Belgium–Luxembourg	68 000
Zimbabwe	5197	Bosnia–Hercegovina	13 500
North and Central		Bulgaria	66 000
America	**3 861 927**	Croatia	16 701
Canada	305 100	Czech Republic	117 449
Costa Rica	5960	Denmark	288 100
Cuba	14 600	Estonia	23 000
Dominican Republic	2500	Finland	92 193
El Salvador	2580	France	1 562 496
Guatemala	11 700	Germany	1 371 174
Honduras	8310	Greece	210 300
Mexico	116 360	Hungary	77 496
Nicaragua	5318	Iceland	2050
Panama	4500	Ireland	91 250
USA	3 385 000	Italy	919 373
South America	**613 158**	Latvia	18 000
Argentina	330 000	Lithuania	4000
Bolivia	6738	Macedonia, FYR of	8100
Brasil	60 150	Malta	83
Chile	44 599	Moldova Republic	30 500
Colombia	51 000	Netherlands	647 640
Ecuador	6288	Norway	80 300
Peru	19 983	Poland	296 200
Uruguay	20 400	Portugal	64 400
Venezuela	74 000	Romania	51 204
Asia	**873 757**	Russian Federation	708 000
Afghanistan	15 600	Slovakia	42 202
Armenia	14 750	Slovenia	10 000
Azerbaijan	43 000	Spain	159 000
Bangladesh	1000	Sweden	138 854
Bhutan	2021	Switzerland	134 640
China	164 646	United Kingdom	362 000
Cyprus	5600	Ukraine	308 770
Georgia	54 600	Yugoslavia, FR	60 000
Iran	200 089	**Oceania**	**423 625**
Iraq	24 733	Australia	233 635
Israel	85 944	New Zealand	190 000

The following countries are included in FAO (1994) but no data for cheese production are available: Burkina Faso, Burundi, Chad, Madagascar, Guinea, Rwanda, Senegal, Somalia, Swaziland, Jamaica, Trinidad and Tobago, Suriname, India, Indonesia, Republic of Korea, Malaysia, Nepal, Pakistan, Philippines, Saudi Arabia, Sri Lanka, Thailand, United Arab Emirates and Fiji.

Appendix 10B Consumption of cheese (kg per caput, 1993) (IDF, 1995)

Country	Ripened cheese[a]	Fresh and cottage cheese	Total
France	15.5	7.3	22.8
Italy	13.4	6.7	20.1
Belgium	15.1	4.7	19.8
Germany	10.5	8.0	18.5
Iceland	11.9	5.2	17.1
Switzerland	13.6	2.8	16.4
Sweden	15.5	0.9	16.4
Netherlands	14.1	1.7	15.8
Denmark	14.5	0.9	15.4
Finland	12.0	2.3	14.3
Norway	14.0	0.2	14.2
Canada	12.4	0.9	13.3
USA	11.9	1.3	13.2
Austria	7.5	3.9	11.4
Estonia	4.4	5.6	10.0
Australia	8.7	0.8	9.5
United Kingdom	—— 8.3 ——		8.3
Spain	—— 8.1 ——		8.1
New Zealand	—— 8.1 ——		8.1
Hungary	4.6	3.3	7.9
Ireland[b]	—— 5.6 ——		5.6
South Africa	1.5	0.1	1.6
Japan	—— 1.4 ——		1.4

[a]Including processed cheese.
[b]Data for Ireland 1991.