Part I

Dairy product safety and quality

2

The major constituents of milk

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2.1 Introduction

Milk and dairy products are major components of the human diet in Western countries, providing about 30% of dietary proteins and lipids and about 80% of dietary calcium. Current annual production of milk is $\approx 600 \times 10^6$ tonnes, of which ≈85%, 11%, 2% and 2% are bovine, buffalo, caprine and ovine, respectively. Although some raw milk is still consumed, the vast majority of milk is processed to at least some extent. Liquid (beverage) milk is a major food item in all developed dairying countries, representing ≈40% of total milk production. The remainder is processed into one of several thousand products – the dairy industry is probably the most diverse and flexible sector of the food industry. The flexibility of milk as a raw material resides in the chemical and physico-chemical properties of its constituents, many of which are unique. The principal constituents of milk can be modified by enzymatic, chemical and/or physical methods, permitting the production of new products. However, the concentrations and properties of milk constituents are variable and hence the processability of milk and the properties of dairy products are inconsistent, although much of this variability can be eliminated by modern technology, which exploits certain features of milk constituents. Today, most milk is processed in large, highly mechanized and automated factories, where consistency in processing properties is essential. The resulting products are distributed through large wholesale and retail outlets, where consistency is, again, paramount. Consumers expect consistency also. The consistency expected by the processor, distributor and consumer can be achieved only if the properties of milk constituents are understood at the molecular level. This chapter will describe the principal chemical and physico-chemical properties of the major

constituents of milk, i.e., lactose, lipids, proteins and salts, and variations in the concentrations and properties of these constituents.

The natural function of milk is to supply the neonatal mammal, of which there are ≈4500 species, with its complete nutritional and some of its physiological requirements. Because the nutritional requirements are species-specific and change as the neonate matures, the composition of milk shows very large inter-species differences, e.g., the concentrations of fat, protein and lactose range from 1 to 50%, 1 to 20% and 0 to 10%, respectively, and the concentration of each changes during lactation. Inter-species differences in the concentrations of many of the minor constituents are even greater than those of the macro-constituents.

Milk from domesticated animals has been used by humans since at least 8000 BC. Although sheep and goats were the first domesticated dairy animals, because they are more easily managed than cattle, the latter, especially certain breeds of *Bos taurus*, are now the dominant dairy animals. Total recorded world milk production is $\approx 600 \times 10^6$ tonnes per annum, of which ≈85% is bovine, 11% is buffalo and 2% each is from sheep and goats. Small amounts of milk are produced from camels, mares, reindeer and yaks in certain regions with specific cultural and/or climatic conditions. This chapter will concentrate on the constituents and properties of bovine milk. Although the constituents of the milk of the other main dairy species are generally similar to those of bovine milk, they differ in detail and the technological properties of the milk of these species differ significantly.

Milk is a very flexible raw material from which several thousand types of dairy products are produced around the world in a great diversity of flavours and forms, including ≈1000 varieties of cheese. The proportions of total world milk production used for the principal dairy products are: liquid (beverage) milk, ≈39%; cheese, ≈33%; butter, ≈32%; whole milk powder, ≈6%; skimmed milk powder, ≈9%; concentrated milk products, ≈2%; fermented milk products, ≈2%; casein, ≈2%; and infant formulae, ≈0.3%. (The sum value exceeds 100%; this is due to 'double accounting', e.g., butter and skim milk powder, and the standardization of fat content, e.g., for liquid milk, cheese, etc.) This flexibility and diversity are a result of the properties, many of them unique, of the constituents of milk, the principal of which are easily isolated from milk, permitting the production of valuable food ingredients. Milk is free of off-flavours, pigments and toxins, which is a very important feature of milk as a raw material for food ingredients.

The processability and functionality of milk and milk products are determined by the properties and concentrations of its principal constituents: proteins, lipids, lactose and salts. Many of the principal problems encountered during the processing of milk are caused by variability in the concentrations and properties of these constituents arising from several factors, including breed, individuality of the animal, stage of lactation, health of the animal, especially mastitis, and nutritional status. Synchronized calving, as practised in New Zealand, Australia and Ireland to avail of cheap grass, has a very marked effect on the composition and properties of milk (see O'Brien *et al*., 1999a, 1999b,

1999c; Mehra *et al*., 1999). Much of the variability can be offset by standardizing the composition of milk or by modifying the process technology. Genetic polymorphism of milk proteins has a significant effect on the concentration and type of protein in milk. The chemical and physical properties of the principal constituents of milk are well characterized and described, including in the following textbooks: Walstra and Jenness (1984), Wong (1988), Fox (1992, 1995, 1997), Jensen (1995), Fox and McSweeney (1998, 2003) and Walstra *et al*. (1998).

2.2 Lactose

Bovine milk contains about 4.8% lactose. Because lactose is responsible for ~50% of the osmotic pressure of milk, which is equal to that of blood and is nearly constant, the concentration of lactose in milk is independent of breed, individuality and nutritional factors but decreases as lactation advances and especially during mastitic infection, in both cases due to the influx of NaCl from the blood.

Chemical and physico-chemical properties of lactose

Lactose is a reducing disaccharide comprised of glucose and galactose, linked by a β1-4-*O*-glycosidic bond. Among sugars, lactose has a number of distinctive characteristics, some of which cause problems in milk products during processing and storage; however, some of its characteristics are exploited to advantage.

- The aldehyde group on the C-1 of the glucose moiety exists mainly in the hemiacetal form and, consequently, C-1 is a chiral, asymmetric carbon. Therefore, like all reducing sugars, lactose exists as two anomers, α and β, which have markedly different properties. From a functional viewpoint, the most important of these are differences in solubility and crystallization characteristics: α-lactose crystallizes as a monohydrate while crystals of β-lactose are anhydrous.
- The solubility of α- and β-lactose in water at 20ºC is ≈7 g and ≈50 g per 100 ml, respectively. The solubility of α-lactose is much more temperature dependent than that of β-lactose and the solubility curves intersect at ≈93.5ºC.
- At equilibrium in aqueous solution, lactose exists as a mixture of α and β anomers in the approximate ratio 37:63. When an excess of α-lactose is added to water, ≈7 g per 100 ml dissolve immediately, some of which mutarotates to give an α:β ratio of 37:63, leaving the solution unsaturated with respect to both α- and β-lactose. Further α-lactose dissolves, some of which mutarotates to β-lactose. Solubilization and mutarotation continue until two conditions exist, i.e., ≈7 g of dissolved α-lactose per 100 ml and an α:β ratio of 37:63, giving a final solubility of ≈18.2 g per 100 ml.

- When β-lactose is added to water, $\approx$50 g per 100 ml dissolve initially but $\approx$18.5 g of this mutarotate to α-lactose, which exceeds its solubility and therefore some α-lactose crystallizes. This upsets the α:β ratio and more β-lactose mutarotates to α-lactose, which crystallizes. Mutarotation of β-lactose and crystallization of α-lactose continue until $\approx$7 g and $\approx$11.2 g of α- and β-lactose, respectively, are in solution.
- Although lactose has low solubility in comparison with other sugars, once dissolved, it crystallizes with difficulty and forms a supersaturated solution. α-Lactose crystallizes spontaneously from highly supersaturated solutions, but if the solution is only slightly supersaturated, it crysallizes slowly as sharp, tomahawk-shaped crystals. If the dimensions of the crystals exceed $\approx$15 μm, they are detectable on the tongue and palate. Crystals of β-lactose are smaller and monoclinical in shape. In the metastable zone, crystallization of lactose is induced by seeding with finely powdered lactose.
- Since α-lactose is less soluble than the β anomer below 93.5ºC, it is the normal commercial form.
- When concentrated milk is spray-dried, there is not sufficient time for lactose to crystallize and an amorphous glass is formed. If the moisture content of the powder is kept low, the lactose glass is stable, but if the moisture content increases to about 6%, e.g., on exposure of the powder to a high humidity atmosphere, the lactose will crystallize as α-lactose monohydrate. If extensive crystallization occurs, an interlocking mass of crystals is formed, resulting in 'caking', which is a particularly serious problem in whey powders owing to their high content of lactose ($\approx$70%). The problem is avoided by extensive crystallization of lactose before drying, induced by seeding the solution with finely powdered lactose.
- Spray-dried milk powder has poor wettability because the small particles swell on contact with water, blocking the channels between particles. The wettability (often incorrectly referred to as 'solubility') of spray-dried milk powder may be improved by modifying the drying process to produce milk powder with coarser, more easily wetted particles. This is achieved by agglomerating the fine powder particles, in effect by controlling lactose-induced caking; such powders are said to be 'instantized'.
- The crystallization of lactose in frozen milk products results in destabilization of the casein, which aggregates when the product is thawed. In this case, the effect of lactose is indirect. When milk is frozen, pure water freezes and the concentration of solutes in the unfrozen water is increased. Since milk is supersaturated with respect to calcium phosphate ($\approx$66% and $\approx$57% of the Ca and PO_4, respectively, are insoluble and occur in the casein micelles as colloidal calcium phosphate; see Section 2.6), when the amount of water becomes limiting, soluble $Ca(H_2PO_4)_2$ and $CaHPO_4$ crystallize as $(Ca)_3(PO_4)_2$, with the concomitant release of H^+ and a decrease in pH to $\approx$5.8. Unless the temperature is maintained below $-$30ºC, lactose will crystallize as α monohydrate during frozen storage, thus reducing the amount of solvent water and aggravating the problems of calcium phosphate solubility

and pH decline. Thorough crystallization of lactose before freezing alleviates, but does not eliminate, the problem. Pre-heating milk prior to freezing also alleviates the problem, but pre-hydrolysis of lactose to the more soluble glucose and galactose using β-galactosidase appears to be the best solution.

- Although lactose is hygroscopic when it crystallizes, properly crystallized lactose has very low hygroscopicity and, consequently, it is a very useful component of icing sugar.
- Lactose has low sweetness (16% as sweet as sucrose as a 1% solution). This limits its usefulness as a sweetener (the principal function of sugars in foods) but makes it is a very useful diluent, e.g., for food colours, flavours, enzymes, etc., when concomitant sweetness is undesirable.
- Being a reducing sugar, lactose can participitate in the Maillard reaction, with very undesirable consequences in all dairy products, e.g., brown colour, off-flavours, reduced solubility and reduced nutritional value.

Food applications of lactose

The amount of whey produced annually as a by-product of the manufacture of cheese and casein contains $\approx 8 \times 10^6$ tonnes of lactose. About 400 000 tonnes of lactose are produced per annum. In addition, $\approx$2 000 000 tonnes of whey permeate powder, which serves as a source of lactose for certain applications, e.g., infant formulae, are produced annually.

Owing to many of its properties, especially low sweetness, the market for lactose is limited; it is, therefore, often regarded as a waste product and in the past caused disposal problems. However, some of the properties of lactose make it a valuable ingredient for pharmaceutical and food applications. Lactose is most valuable when used in the pharmaceutical industry where it is widely used as a diluent in pelleting operations.

The principal application of lactose in the food industry is in the humanization of infant formulae – human milk contains $\approx$7% lactose in comparison with $\approx$4.8% in bovine milk. Demineralized whey powder (DWP) is very suitable for this purpose – it is cheaper than lactose and in addition to supplying lactose, DWP supplies whey proteins and adjusts the casein:whey protein ratio to a value closer to that in bovine milk (40:60 compared to 80:20 in bovine milk). It is necessary to demineralize bovine whey since it contains approximately four times as much minerals as human milk.

Lactose is also used as an agglomerating/free-flowing agent in foods, in the confectionery industry to improve the functionality of shortenings, as an anti-caking agent at high relative humidity, in icing mixtures or as a reducing sugar if Maillard browning is required. The low sweetness of lactose is an advantage in many of these applications. Lactose absorbs compounds and may be used as a diluent for food flavours or pigments or to trap food flavours.

Lactose derivatives

A number of more useful and more valuable products may be produced from lactose. The most significant are:

- Lactulose (galactose β1-4 fructose): this sugar, which does not occur in nature, is produced from lactose by heating, especially under slightly alkaline conditions. It is not hydrolysed by intestinal β-galactosidase and enters the large intestine where it promotes the growth of *Bifidobacterium* spp. It is a mild laxative and is used fairly widely for this purpose. More than 20 000 tonnes are produced annually.
- Glucose–galactose syrups, produced by acid or enzymatic (β-galactosidase) hydrolysis: the technology for the production of such hydrolysates has been developed but the product is not cost-competitive with other sugars (sucrose, glucose, glucose–fructose).
- Galactooligosaccharides: β-galactosidase has transferase as well as hydrolytic activity and under certain conditions, the former predominates, leading to the formation of galactooligosaccharides, which have bifidogenic properties and are considered to have promising food applications.
- Ethanol is produced commercially by the fermentation of lactose by *Kluyveromyces lactis*.
- Other derivatives which have limited but potentially important applications include lactitol, lactobionic acid, lactic acid, acetic acid, propionic acid, lactosyl urea and single-cell proteins.

Nutritional aspects of lactose

Lactose is involved in two enzyme-deficiency syndromes: lactose intolerance and galactosemia. The former is due to a deficiency of intestinal β-galactosidase which is rare in infants but common in adults except north-west Europeans and a few African tribes. Since humans are unable to absorb disaccharides from the small intestine, unhydrolysed lactose enters the large intestine where it is fermented by bacteria, leading to flatulence and cramp, and to the absorption of water from the intestinal mucosa, causing diarrhoea. These conditions cause discomfort and may be fatal. Individuals suffering from lactose intolerance avoid the consumption of milk and lactose-containing dairy products. Hydrolysis of lactose by β-galactosidase renders such products suitable for lactose-intolerant individuals. Hydrolysis may be performed at the dairy using soluble or immobilized β-galactosidase or by the consumer at home. Lactose-hydrolysed products enjoy limited commercial success in western countries but have not resulted in a substantial increase in the consumption of dairy products in Asia, which is a very large potential market for dairy products but where lactose intolerance is very widespread.

Galactosemia is caused by the inability to catabolize galactose owing to a deficiency of either of two enzymes, galactokinase or galactose-1P:uridyl transferase. A deficiency of galactokinase leads to the accumulation of galactose which is catabolized via alternative routes, one of which leads to the accumulation of galactitol in various tissues, including the eye, where it causes cataract. A deficiency of galactose-1P:uridyl transferase leads to abnormalities in membranes of the brain and to mental retardation unless galactose is excluded from the diet within a few weeks *post partum*. Both forms of galactosemia occur at a frequency of 1 per $\approx$50 000 births.

Lactose in fermented dairy products

The fermentation of lactose to lactic acid by lactic acid bacteria (LAB) is a critical step in the manufacture of all fermented dairy products. The fermentation pathways are well established (see Cogan and Hill, 1993). Lactose is not a limiting factor in the manufacture of fermented dairy products – only ≈20% of the lactose is fermented in the production of fermented milks. Individuals suffering from lactose intolerance may be able to consume fermented milk products without ill-effects, possibly because LAB produce β-galactosidase and emptying of the stomach is slower than for fresh milk products, thus delaying the release of lactose into the small intestine.

In the manufacture of cheese, most (96–98%) of the lactose is removed in the whey. The concentration of lactose in fresh curd depends on its concentration in the milk and on the moisture content of the curd and varies from ≈1%, w/w, in fresh Cheddar curd to ≈2.5%, w/w, in fresh Camembert. The metabolism of residual lactose in the curd to lactic acid has a major effect on the quality of mature cheese (see Fox *et al*., 1990, 2002). The resultant lactic acid may be catabolized to other compounds, e.g., carbon dioxide and water by the surface mould in Camembert, or to propionic acid, acetic acid and carbon dioxide in Emmental-type cheeses. Excessive lactic acid in cheese curd leads to a low pH, a strong, acidic, harsh taste, and a brittle texture. In Cheddar and related varieties, the L-lactic acid produced by the starter bacteria is racemized to DL-lactic acid; Ca-D-lactate is less soluble than Ca-L-lactate and if its concentration is too high, it will crystallize on the surface of the cheese, giving it an undesirable appearance. Excess residual lactose may also be fermented by heterofermentative lactobacilli, with the production of carbon dioxide, leading to an open texture.

In the manufacture of some cheese varieties, e.g., Dutch cheeses, the curds are washed to reduce their lactose content and thereby regulate the pH of the pressed curd to ≈5.3. In most other varieties, e.g., Cheddar and Emmental, the level of lactose, and hence of lactic acid, in the curd is not controlled by washing. Hence, changes in the concentration of lactose in milk may affect the quality of such cheeses. The concentration of lactose in milk decreases throughout lactation, e.g., from ≈4.8% to <4.0%. When synchronized calving is practised, there is a marked seasonal change in the lactose content of milk and hence of cheese, which may have a significant effect on quality. To overcome seasonal variations in the lactose content of milk, the level of wash water used for Dutch-type cheeses is varied according to the concentrations of lactose and casein in the milk. Ideally, the lactose-to-protein ratio should be standardized, e.g., by washing the curds, to minimize variations in the level of lactic acid, the pH and the quality of cheese.

The curds for acid-curd cheeses are washed free of lactose to improve their keeping quality. Thus, acid-coagulated and mature rennet-coagulated cheeses may be consumed by lactose-intolerant individuals without ill-effects.

2.3 Lipids

Definition and variability

Lipids are defined as those compounds in foods and tissues that are soluble in apolar solvents (ethyl/petroleum ether or chloroform/methanol). The lipid fraction of milk is comprised mainly of triglycerides (98%), with ≈1% phospholipids and small amounts of diglycerides, monoglycerides, cholesterol, cholesteryl esters and traces of fat-soluble vitamins and other lipids. The lipids occur as globules, 0.1–20 μm in diameter, surrounded by the milk fat globule membrane (MFGM), which serves as an emulsifier. The concentration of lipids varies with species, breed, individual animal, stage of lactation, mastitic infection, plane of nutrition, interval between milkings, and point during milking when the sample is taken. Among the principal dairy breeds, Friesian/Holsteins produce milk with the lowest fat content (≈3.5%) and Jersey/Guernsey the highest (≈6%). The fat content varies considerably throughout lactation; when synchronized calving is practised, the fat content of bulk milk varies from ≈3% in early lactation to >4.5% in late lactation. Such large variations in lipid content obviously affect the economics of milk production and the composition of milk products but can be modified readily by natural creaming or centrifugal separation or addition of cream and hence need not affect product quality. Milk lipids exhibit variability in fatty acid composition and in the size and stability of the globules. These variations, especially of the fatty acid profile, are essentially impossible to standardize and hence are responsible for considerable variations in the rheological properties, colour, chemical stability and nutritional properties of fat-containing dairy products.

Fatty acid profile

Ruminant milk fat contains a wider range of fatty acids than any other lipid system – up to 400 fatty acids have been reported in bovine milk fat; the principal fatty acids are the homologous series of saturated fatty acids, $C_{4:0}$–$C_{18:0}$ and $C_{18:1}$ (see Fox, 1995). The outstanding features of the fatty acids in bovine milk fat are a high concentration of short and medium chain acids (ruminant milk fats are the only natural lipids that contain butanoic acid) and a low concentration of polyunsaturated fatty acids (PUFA).

In ruminants, the fatty acids for the synthesis of milk lipids are obtained from triglycerides in chylomicrons in the blood or synthesized *de novo* in the mammary gland from acetate or β-hydroxybutyrate produced by microorganisms in the rumen. The triglycerides in chylomicrons are derived from the animal's feed or synthesized in the liver. Butanoic acid ($C_{4:0}$) is produced by the reduction of β-hydroxybutyrate which is synthesized from dietary roughage by bacteria in the rumen and therefore varies substantially with the animal's diet. All $C_{6:0}$–$C_{14:0}$ and 50% of $C_{16:0}$ are synthesized in the mammary gland via the malonyl-CoA pathway from acetyl-CoA produced from acetate synthesized in the rumen. Essentially 100% of $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ and 50% of $C_{16:0}$ are derived from blood lipids (chylomicrons) and represent ≈50% of total fatty acids in

ruminant milk fat. Unsaturated fatty acids in the animal's diet are hydrogenated by bacteria in the rumen unless they are protected, e.g., by encapsulation.

When milk production is seasonal, e.g., in Australia, New Zealand and Ireland, very significant changes occur in the fatty acid profile of milk fat throughout the production season (see Fox, 1995; Fox and McSweeney, 1998). These variations are reflected in the hardness of butter produced from such milk; winter butter is much harder than summer butter. Owing to the lower degree of unsaturation, winter butter should be less susceptible to lipid oxidation than the more unsaturated summer product but the reverse appears to be the case, probably owing to higher levels of pro-oxidants, e.g., Cu and Fe, in winter milk.

Although a ruminant's diet, especially if grass-based, is rich in PUFAs, these are hydrogenated by bacteria in the rumen and, consequently, ruminant milk fat contains very low levels of PUFAs, e.g., bovine milk fat contains ≈2.4% $C_{18:2}$ compared to ≈13% and ≈12% in human and porcine milk fat, respectively. PUFAs are considered to be nutritionally desirable and consequently there has been interest in increasing their concentration in bovine milk fat. This can be done by feeding encapsulated PUFA-rich lipids or crushed PUFA-rich oil seeds to the animal. Increasing the PUFA content also reduces the melting point of the fat and makes butter produced from it more spreadable. However, the lower MP fat may have undesirable effects on the rheological properties of cheese, and PUFA-rich dairy products are very susceptible to lipid oxidation. Although the technical feasibility of increasing the PUFA content of milk fat by feeding protected PUFA-rich lipids to the cow has been demonstrated, it is not economical to do so in most cases. Blending milk fat with PUFA-rich or $C_{18:1}$-rich vegetable oil appears to be much more viable and is now widely practised commercially.

Conjugated linoleic acid

Linoleic acid (*cis*, *cis* Δ 9,12-octadecadienoic acid) is the principal essential fatty acid and has attracted the attention of nutritionists for many years. However, conjugated isomers of linoleic acid (CLA) have attracted very considerable attention recently. CLA is a mixture of eight positional and geometric isomers of linoleic acid which have a number of health-promoting properties, including anticarcinogenic and antiatherogenic activities, reduction of the catabolic effects of immune stimulation and the ability to enhance growth promotion and reduce body fat (see Parodi, 1994, 1997a, 1999; Belury, 1995; Banni and Martin, 1998; Yurawecz *et al*., 1999). Of the eight isomers of CLA, only the *cis* 9, *trans* 11 isomer is biologically active. This compound is effective at very low concentrations, 0.1 g per 100 g diet.

Fat-containing foods of ruminant origin, especially milk and dairy products, are the principal sources of dietary CLA which is produced as an intermediate during the biohydrogenation of linoleic acid by the rumen bacterium, *Butyrivibrio fibrisolvens*. Since CLA is formed from linoleic acid, it is not surprising that the CLA content of milk is affected by diet and season, being highest in summer when cows are on fresh pasture rich in PUFAs (Lock and

Garnsworthy, 2000; Lawless *et al*., 2000) and higher in the fat of milk from cows on mountain than on lowland pasture (Collomb *et al*., 2002). The concentration of CLA in milk fat can be increased 5–7 fold by increasing the level of dietary linoleic acid, e.g., by duodenal infusion (Kraft *et al*., 2000) or by feeding a linoleic acid-rich oil, e.g., sunflower oil (Kelly *et al*., 1998).

A number of other lipids may have anticarcinogenic activity, e.g., sphingomyelin, butanoic acid and ether lipids, but few data are available on these to date (Parodi, 1997a, 1999).

Rheological properties of milk fat

The melting characteristics of ruminant milk fat are such that at low temperatures (e.g., ex-refrigerator) it contains a high proportion of solid fat and has poor spreadability. The rheological properties of milk lipids may be modified by fractional crystallization. Best results are obtained by removing the middle fraction and blending high and low melting point fractions. Fractional crystallization is expensive and is practised to only a limited extent. Securing profitable outlets for the middle melting point fraction is a further problem.

The rheological properties of milk fat may be modified also by increasing the level of PUFAs by feeding PUFA-rich lipids, but this practice is also expensive. The melting characteristics of blends of milk fat and vegetable oils can be varied at will by changing the proportions of the different fats and oils in the blend. This procedure is economical and is widely practised commercially; blending also increases the level of nutritionally desirable PUFAs. The rheological properties of milk fat-based spreads can also be improved by increasing the moisture content of the product; obviously, this is economical and nutritionally desirable in the sense that the caloric value is reduced but the product is less microbiologically stable than butter.

Size and stability of milk fat globules

The average size of the milk fat globules decreases with advancing lactation. Consequently, the separation of fat from milk is less effective in winter than in summer, especially when milk production is seasonal, and this may mean that it is not possible to meet the upper limit for fat content in some products, e.g., casein, during certain periods.

Since lipids are incompatible with aqueous systems, phase separation will occur unless an emulsifier is used to reduce the interfacial tension. In milk, the emulsifier is a membrane, known as the milk fat globule membrane (MFGM). On the inner side of the MFGM is a layer of unstructured lipoproteins acquired within the secretory cells as the triglycerides move from the site of synthesis in the rough endoplasmic reticulum (RER) in the basal region of the cell towards the apical membrane. The fat globules are excreted from the cells by exocytis, i.e., they are pushed through and become surrounded by the apical membrane. Milk proteins and lactose are excreted from the cell by the reverse process: they also are synthesized in the RER and are transferred to the Golgi region, where they are encapsulated in Golgi membrane; the visicles move towards, and fuse

with the apical cell membrane, open and discharge their contents into the alveolar lumen, leaving the visicle (Golgi) membrane as part of the apical membrane, thereby replacing the membrane lost on the excretion of fat globules. Thus, the outer layer of the MFGM is a trilaminar membrane, composed of phospholipids and proteins, with a fluid mosaic structure. The MFGM contains many enzymes which originate mainly from the Golgi apparatus; in fact, most of the indigenous enzymes in milk are concentrated in the MFGM, notable exceptions being plasmin and lipoprotein lipase (LPL), which are associated with the casein micelles. The trilaminar membrane is unstable and is shed during storage into the aqueous phase, where it forms microsomes; it becomes less stable with advancing lactation.

The stability of the MFGM is critical for many aspects of the milk fat system, notably:

- It protects the lipids in the core of the globule against lipolysis by LPL in the skim milk. The MFGM may be damaged by agitation, foaming, freezing and especially by homogenization (during which the natural membrane is replaced by a layer of skim milk proteins, mainly caseins), allowing access for LPL to the core lipids and leading to lipolysis and hydrolytic rancidity which is potentially a major problem in the dairy industry. Milking installations must be properly installed and serviced regularly if damage to the MFGM is to be avoided. Milk should be pasteurized before or immediately after homogenization to inactivate the LPL; in practice, the homogenizer is placed in a loop between the regeneration and final heating sections of an HTST pasteurizer.
- The MFGM is destabilized by freezing, e.g., on bulk tank walls, which may induce lipolysis and related problems.
- The MFGM appears to be less stable in winter/late lactation than in summer/mid lactation; therefore, hydrolytic rancidity is more likely to be a problem in winter than in summer. An aggravating factor is that less milk is usually produced in winter than in summer, especially in seasonal milk production systems, which leads to greater agitation during milking and, consequently, greater damage to the MFGM.
- Damage to the MFGM leads to the formation of non-globular (free) fat, which may be evident as ‘oiling-off’ on tea or coffee and cream plug or age thickening of cream. Problems related to or arising from free fat are more serious in winter than in summer, due to the reduced stability of the MFGM. Homogenization, which replaces the natural MFGM by a layer of skim milk proteins, eliminates problems caused by free fat.

Lipid oxidation

The chemical oxidation of lipids, a major cause of instability in dairy products, is a free radical, autocatalytic process, involving principally the methylene group between a pair of double bonds in PUFAs. The process is initiated and/or catalysed by polyvalent metals, especially copper (Cu) and iron (Fe), UV light,

ionizing radiation or enzymes (in the case of milk by xanthine oxidase, which is a major component of the MFGM). Oxygen is a primary reactant. The principal end-products are unsaturated carbonyls, which cause flavour defects. Polymerization of free radicals and other species leads to the formation of pigmented products and an increase in viscosity, but polymerization is unlikely to be significant in dairy products. Lipid oxidation can be prevented or controlled by:

- Avoiding metal contamination at all stages through the use of stainless steel equipment.
- Avoiding exposure to UV light by using opaque packing (foil or paper).
- Packaging under an inert atmosphere, usually nitrogen (N_2).
- Using scavengers of oxygen (O_2) or free radicals, e.g., glucose oxidase or superoxide dismutase (an indigenous enzyme in milk), respectively.
- Using antioxidants which break the free radical chain reaction; addition of antioxidants to dairy products is not permitted but the level of natural antioxidants, e.g., tocopherols (vitamin E), in milk may be increased by supplementing the animal's feed.

Creaming

Since the specific gravity of lipids and skim milk is 0.9 and 1.036, respectively, the fat globules in milk held under quiescent conditions will rise to the surface under the influence of gravity, a process referred to as creaming. The rate of creaming, V, of fat globules is given by Stokes' equation:

$$V = \frac{2r^2(\rho_1 - \rho_2)g}{9\eta}$$

where r = radius of the globule
ρ_1 = specific gravity of skim milk
ρ_2 = specific gravity of the fat globules
g = acceleration due to gravity
η = viscosity of skim milk.

The values of r, ρ_1, ρ_2 and η suggest that a cream layer would form in milk after $\approx$60 h but milk creams in $\approx$30 min. The rapid rate of creaming is due to the strong tendency of the fat globules to cluster due to the effect of indigenous immunoglobulin M which precipitates onto the fat globules when milk is cooled (and, therefore, is called cryoglobulin). Large globules rise faster than smaller ones, collide with them and form aggregates, an effect promoted by cryoglobulins. The clusters of globules rise rapidly and therefore the creaming process is accelerated as the globules rise and clump. Ovine, caprine or buffalo milk does not contain cryoglobulins and therefore creams much more slowly than bovine milk.

In the past, creaming was a very important physico-chemical property of milk:

- The cream layer served as an index of fat content and hence of the quality of milk to the consumer.

- Creaming was the traditional method for preparing cream from milk for the manufacture of butter. Its significance in this respect declined with the development of the mechanical separator in 1878 but natural creaming is still used to adjust the fat content of milk for some cheese varieties, e.g., Parmigiano-Reggiano. A high proportion of the bacteria in milk becomes occluded in the clusters of fat globules and hence creaming has a sanitizing effect.

Homogenization of milk

Today, creaming is of little general significance. In most cases its effect is negative, and for most dairy products, milk is homogenized, i.e., subjected to a high shearing pressure which reduces the size of the fat globules (average diameter $<1\ \mu$m), increases the fat surface area (4–6 fold), replaces the natural MFGM by a layer of skim milk proteins, denatures cryoglobulins and hence prevents the agglutination and clustering of globules. The valve homogenizer was introduced in France by Gaulin in 1902 and homogenization became widespread after about 1940 (Trout, 1950). Homogenization has several very significant effects on the properties of milk:

- If properly executed, creaming is delayed indefinitely due to the reduced size of the fat globules and the denaturation of cryoglobulins.
- Susceptibility to hydrolytic rancidity is markedly increased because LPL has ready access to the triglycerides; consequently, milk must be heated under conditions sufficiently severe to inactivate LPL before (usually) or immediately after homogenization.
- Susceptibility to oxidative rancidity is reduced because pro-oxidants in the MFGM, e.g., metals and xanthine oxidase, are distributed throughout the milk.
- The whiteness of milk is increased owing to the greater number of light-scattering particles.
- The strength and syneretic properties of renneted-coagulated milk gels for cheese manufacture are reduced; hence, cheese with a higher moisture content is obtained. Milk for cheese manufacture is not normally homogenized; an exception is reduced-fat cheese, in which a higher moisture content improves texture.
- Homogenization reduces the heat stability of whole milk, the effect increasing with fat content and homogenization pressure; homogenization has no effect on the heat stability of skimmed milk.
- The viscosity of whole milk and cream is increased by single-stage homogenization due to the clustering of newly formed fat globules; if desired, the clusters of globules are dispersed by a second homogenization at a lower pressure.

Fat-soluble vitamins

Since the fat-soluble vitamins (A, D, E and K) in milk are derived from the animal's diet, there are large seasonal variations in their concentration in milk;

the breed of cow also has a significant effect: high-fat milk (Jersey and Guernsey) has a higher content of these vitamins than Friesian or Holstein milk (see Fox, 1995). Variations in the concentrations of fat-soluble vitamins in milk have a number of consequences:

- Nutritional: milk contributes a substantial portion of the RDA for these vitamins in Western diets; it is common practice in some countries to fortify milk and butter with vitamins A and D.
- The yellow-orange colour of high-fat dairy products depends on the concentrations of carotenoids and vitamin A, and hence on the animal's diet; fresh, especially clover-rich, pasture is rich in carotenoids.
- Goats, sheep and buffalo do not transfer carotenoids to their milk and products produced therefrom are whiter than corresponding products from bovine milk. The darker colour of the latter may be unattractive to consumers accustomed to caprine or ovine milk products. If necessary, the carotenoids in bovine milk may be bleached (by benzoyl peroxide) or masked (by chlorophyll or TiO_2).
- Vitamin E (tocopherols) is a potent antioxidant and contributes to the oxidative stability of dairy products. The tocopherol content of milk and meat can be increased by supplementing the animal's diet with tocopherols, which is sometimes practised.

2.4 Proteins

Introduction

Technologically, the proteins of milk are its most important constituents (see Fox and McSweeney, 2003, for a comprehensive review of milk proteins). They play important, even essential, roles in all dairy products except butter, ghee and anhydrous milk fat. The roles played by milk proteins include:

- Nutritional: all protein-containing dairy products.
- Physiological: immunoglobulins, lactoferrin, lactoperoxidase, vitamin-binding proteins, protein-derived biologically active peptides.
- Functional:
  - gelation: enzymatically, acid or thermally induced gelation in all cheeses, fermented milks, whey protein concentrates and isolates;
  - heat stability: all thermally processed dairy products;
  - surface activity: caseinates, whey protein concentrates and isolates;
  - rheological: all protein-containing dairy products;
  - water sorption: most dairy products and in food products containing functional milk proteins.

Heterogeneity of milk proteins

It has been known since 1830 that milk contains two types of protein which can be separated by acidification to pH 4.6. The proteins insoluble at pH 4.6 are

called caseins and represent ≈78% of the total nitrogen in bovine milk; the soluble proteins are called whey or serum proteins. It was shown as early as 1885 that the whey proteins were of two types, globulins and albumins, and it was thought that these were transferred directly from the blood (the proteins of blood and whey have generally similar physico-chemical properties and are classified as albumins or globulins), but it was recognized at an early stage that the caseins are distinctly different milk-specific proteins. It is now known that the two principal whey proteins, β-lactoglobulin (β-Lg) and α-lactalbumin (α-La), are also milk-specific.

Evidence began to accumulate in the 1920s that the casein, albumin and globulin fractions are heterogeneous and this was confirmed in the 1930s using analytical ultracentrifugation and free-boundary electrophoresis. Methods for the isolation of the individual proteins were developed and gradually improved so that by about 1970, all the principal milk proteins had been purified to homogeneity.

Bovine milk contains six milk-specific proteins: four caseins, α_{s1}-, α_{s2}-, β- and κ-, representing approximately 38%, 10%, 36% and 15%, respectively, of whole casein, and β-Lg and α-La, which represent approximately 40% and 20%, respectively, of total whey proteins. It also contains several minor whey proteins, including bovine serum albumin (BSA) and immunoglobulins (Ig), which are transferred from the blood; each represents about 10% of the whey proteins in mature bovine milk. The remaining 10% is mainly non-protein nitrogen and trace amounts of several proteins, including approximately 60 indigenous enzymes.

The application of electrophoresis in starch or polyacrylamide gels, which were introduced about 1960, showed that the milk protein system is very heterogeneous due to:

- Genetic polymorphism, usually involving substitution of one or two amino acids
- Variations in the degree of phosphorylation of the caseins
- Variations in the degree of glycosylation of κ-casein
- Intermolecular disulphide bond formation in α_{s1}- and κ-caseins
- Limited proteolysis by plasmin, especially of β- and α_{s1}-caseins; the resulting peptides include the γ- and λ-caseins and proteose peptones.

The concentration of total protein in milk is affected by most of the same factors that affect the concentration of fat, i.e., breed, individuality, nutritional status, health and stage of lactation, but with the exception of the last, the magnitude of the effect is less than for milk fat. The concentration of protein in milk decreases very markedly during the first few days *post partum*, mainly due to the decrease in Ig from ≈10% in the first colostrum to 0.1% within about one week. The concentration of total protein continues to decline more slowly thereafter to a minimum after about four weeks and then increases until the end of lactation. Data on variations in the groups of proteins throughout lactation have been published (see Mehra *et al*., 1999) but there are few data on variations in the concentrations of the principal proteins individually.

Table 2.1 Characteristics of the principal proteins in cow's milk protein

	Molecular mass	AA residues			PO_4	Concentration (g/L)	
		Total	Pro	Cys			
α_{s1}-Casein	23 164	199	17	0	8	10.0	A,B,C,D,E,F,G,H
α_{s2}-Casein	25 388	207	10	2	10–13	2.6	A,B,C,D
β-Casein	23 983	209	35	0	5	9.3	A^1,A^2,A^3,B,C,D,E,F,G
κ-Casein[a]	19 038	169	20	2	1	3.3	A,B,C,E,F^s,F^I,G^S,G^S,H,I,J
β-Lactoglobulin	18 277	162	8	5	0	3.2	A,B,C,D,E,F,H,I,J
α-Lactalbumin	14 175	123	2	8	0	1.2	A,B,C

[a] Glycosylated to variable extent.

Molecular properties of milk proteins

The principal lactoproteins are very well characterized at the molecular level; the principal properties are summarized in Table 2.1. The most notable features of the principal milk-specific proteins are:

- All milk proteins are quite small molecules, ≈15–25 kDa.
- All the caseins are phosphorylated but to different and variable degrees; the phosphate groups are esterified as monoesters of serine residues.
- β-Casein is the only one of the principal milk proteins that is glycosylated. The sugar moieties are galactose, galactosamine and *N*-acetylneuraminic acid (sialic acid), which occur as tri- or tetra-saccharides. Zero to four oligosaccharides are attached to the polypeptide via serine residues in the C-terminal region of the molecule.
- The primary structures of the principal milk proteins and of many of their variants are known. The caseins have a rather uneven distribution of polar and apolar residues along their sequences, creating hydrophobic and hydrophilic patches; this structural feature bestows the caseins with very good surface activity, giving them very good emulsifying and foaming properties.
- The two principal caseins, α_{s1}- and β-, are devoid of cysteine or cystine residues; the two minor caseins, α_{s2}- and κ-caseins, contain two intermolecular disulphides. β-Lg contains two intramolecular disulphides and one sulphydryl group which is buried and unreactive in the native protein but becomes exposed and reactive when the molecule is denatured; it reacts via sulphydryl–disulphide interactions with other proteins, especially κ-casein, with major consequences on many important properties of the milk protein system, especially heat stability and cheesemaking properties. α-La has four intramolecular disulphides.
- All the caseins, especially β-casein, contain a high level of proline, which disrupts α- and β-structures; consequently, the caseins are rather unstructured molecules and are readily susceptible to proteolysis. However, theoretical calculations suggest that the caseins may have a considerable level of secondary and tertiary structures; to explain the differences between the experimental and theoretical indices of higher structures, it has been suggested that the caseins have very mobile, flexible structures and are referred to as rheomorphic.
- In contrast, the whey proteins are highly structured and compact, with high levels of α-helices, β-sheets and β-turns. In β-Lg, the β-sheets are in an antiparallel arrangement and form a β-barrel calyx. This is a member of the lipocalin family of proteins to which a Special Issue of the journal *Biochimica et Biophysica Acta* has been devoted (Akerstrom *et al.*, 2000).
- The caseins are often regarded as rather hydrophobic proteins but they are not particularly so; however, they do have a high surface hydrophobicity owing to their open structure; in globular proteins, the hydrophobic residues are buried within the molecule but they are exposed in the caseins.

- Also due to their open structure, the caseins are quite susceptible to proteolysis, which accords with their putative function as a source of amino acids for the neonate. However, their hydrophobic patches give them a high propensity to yield bitter hydrolysates, even in cheese which undergoes relatively little proteolysis. In contrast, the highly structured whey proteins are very resistant to proteolysis in the native state and may transverse the intestinal tract of the neonate intact.
- Probably because of their rather open structures, the caseins are extremely heat stable, e.g., sodium caseinate can be heated at 140ºC for 1 h without obvious physical effects. The more highly structured whey proteins are comparatively heat labile, although in comparison with many other globular proteins, they are quite heat stable; they are completely denatured on heating at 90ºC for 10 min.
- Under the ionic conditions in milk, α-La exists as monomers of MW $\approx$14.7 kDa. β-Lg exists as dimers (MW $\sim$36 kDa) in the pH range 5.5–7.5; at pH values <3.5 or >7.5 it exists as monomers, while at pH 3.5–5.5 it exists as octamers. The caseins exist as very complex structures, known as casein micelles, which are described below.
- The function of the caseins appears to be to supply amino acids to the neonate. They have no biological function *sensu stricto* but their Ca-binding properties enable a high concentration of calcium phosphate to be carried in milk in a 'soluble' form; without the 'solubilizing' influence of casein, $Ca_3(PO_4)_2$ would precipitate in the ducts of the mammary gland and cause atopic milk stones (see Holt, 1994).
- β-Lg binds several hydrophobic molecules; it binds and protects retinol *in vitro* and perhaps functions as a retinol carrier *in vivo*. In the intestine, it may exchange retinol with a retinol-binding protein. It also binds fatty acids and thereby stimulates lipase – perhaps this is its principal biological function. All members of the lipocalin family have some form of binding function (see Akerstrom *et al*., 2000).
- α-La is a metalloprotein – it binds one calcium atom per molecule in a peptide loop containing four Asp residues. The apoprotein is quite heat labile but the metalloprotein is rather heat stable; the difference in heat stability between the halo- and apoprotein is exploited in the isolation of α-La on a potentially industrial scale.
- α-La is a specifier protein in lactose synthesis; it makes UDP-galactose transferase highly specific for glucose as an acceptor of galactose, resulting in the synthesis of lactose.

Casein micelles

α_{s1}-, α_{s2}- and β-caseins, which together represent approximately 85% of total casein, are precipitated by calcium at concentrations above 6 mM at temperatures above 20ºC. Since milk contains $\approx$30 mmol/L Ca, it would be expected that most of the caseins would precipitate in milk. However, κ-casein is soluble in

Table 2.2 Average characteristics of casein micelles

Characteristic	Value
Diameter	120 nm (range: 50–600 nm)
Surface area	8×10^{-10} cm^2
Volume	2.1×10^{-15} cm^3
Density (hydrated)	1.0632 g/cm^{-3}
Mass	2.2×10^{-15} g
Water content	63%
Hydration	3.7 g H_2O per g protein
Voluminosity	44 cm^3/g
Molecular weight (hydrated)	1.3×10^9 Da
Molecular weight (dehydrated)	5×10^8 Da
Number of peptide chains	5×10^3
Number of particles per ml milk	10^{14}–10^{16}
Surface of micelles per ml milk	5×10^4 cm^2
Mean free distance between micelles	240 nm

high concentrations of calcium and it reacts with and stabilizes the Ca-sensitive caseins through the formation of casein micelles.

The micelles are spherical colloidal particles, with a mean diameter of ≈120 nm (range 50–600 nm). They have a mean particle mass of $\approx 10^8$ Da, i.e., there are about 5000 casein molecules (20–25 kDa) in an average micelle. On a dry weight basis, the micelles contain ≈94% protein and ≈6% non-protein species, mainly calcium and phosphate, with smaller amounts of magnesium (Mg) and citrate and traces of other metals; these are collectively called colloidal calcium phosphate (CCP). Under the conditions that exist in milk, the micelles are hydrated to the extent of ≈2 g water per g protein. There are $\approx 10^{15}$ micelles per ml milk, with a surface area of $\approx 5 \times 10^4$ cm^2; the micelles are about 240 nm apart (see Table 2.2). Owing to their very large surface area, the surface properties of the micelles are of major significance, and because they are quite closely packed, even in unconcentrated milk, they collide frequently due to Brownian, thermal convection and mechanical motion.

The micro-structure of the casein micelle has been the subject of considerable research, especially during the past 50 years, i.e., since the discovery and isolation of the micelle-stabilizing protein, κ-casein; however, there is still a lack of general consensus. Numerous models have been proposed, the most widely supported being the sub-micelle model first proposed by Morr in 1966 and refined several times since. Essentially, this model proposes that the micelle is built up from sub-micelles (MW $\approx 5 \times 10^6$ Da) held together by CCP and surrounded and stabilized by a surface layer rich in κ-casein but with some of the other caseins exposed also (Fig. 2.1). It is proposed that the hydrophilic C-terminal region of κ-casein protrudes from the surface, creating a hairy layer around the micelle and stabilizing it through a zeta potential of about −20 mV and steric stabilization. The principal direct experimental support for this model is provided by electron microscopy, which indicates a

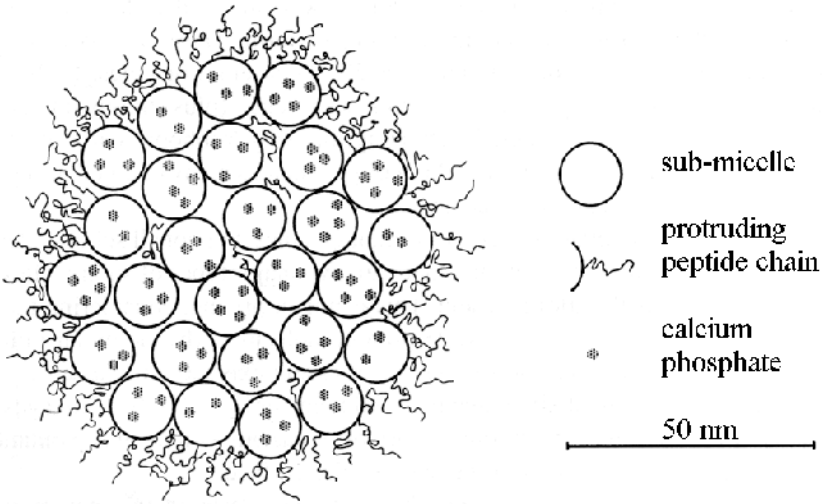

Fig. 2.1 Schematic model of a cross-section through a casein micelle (from Walstra, 1999).

non-uniform electron density which has been interpreted as indicating sub-micelles.

However, several authors have expressed reservations about the sub-unit model and several alternatives have been proposed. In one of these (Holt, 1992), it is proposed that the Ca-sensitive caseins are linked by micro-crystals of CCP and surrounded by a layer of κ-casein with its C-terminal region protruding from the surface (Fig. 2.2). In the dual-binding model of Horne (2003), it is proposed that individual casein molecules interact via hydrophobic regions in their primary structures, leaving the hydrophilic regions free and with the hydrophilic C-terminal region of κ-casein protruding into the aqueous phase (Fig. 2.3). Thus, the key structural features of the sub-micelle model are retained in both alternatives, i.e., the integrating role of CCP and a surface layer consisting predominantly of κ-casein.

The micelles disintegrate when the CCP is solubilized, e.g., by acidification, citrate or oxalate, followed by dialysis; about 60% of the CCP can be removed without disintegration of the micelles. The micelles can also be dispersed by raising the pH to ≈9.0, which does not solubilize the CCP and presumably causes disintegration by increasing the net negative charge. Urea at >5M or sodium dodecyl sulphate (SDS) also dissociates the micelles, suggesting that hydrogen and/or hydrophobic bonds are important for micelle integrity. At 20ºC, the micelles are precipitated by ethanol or other low MW alcohols at approximately 35% or over, but if the temperature is increased above about 70ºC, surprisingly, the precipitated casein dissolves and the solution becomes quite clear, indicating dissociation of the micelles (O'Connell *et al.*, 2001a, 2001b). Micelle-like particles reform on cooling and these form a gel at about 4ºC. It is not known whether the sub-particles

Fig. 2.2 Schematic diagram of a casein micelle showing a generally uniform protein matrix and calcium phosphate nanoclusters (from Fox and McSweeney, 1998).

formed on treating milk with acid, urea, SDS or ethanol correspond to casein sub-micelles.

There have been few studies on variations in micelle size throughout lactation and these have failed to show consistent trends. No studies on variability in the microstructure of the casein micelle have been published. There are no reported studies on the effects of the nutritional and health status of the animal on the structure of the casein micelles, although their stability and behaviour are

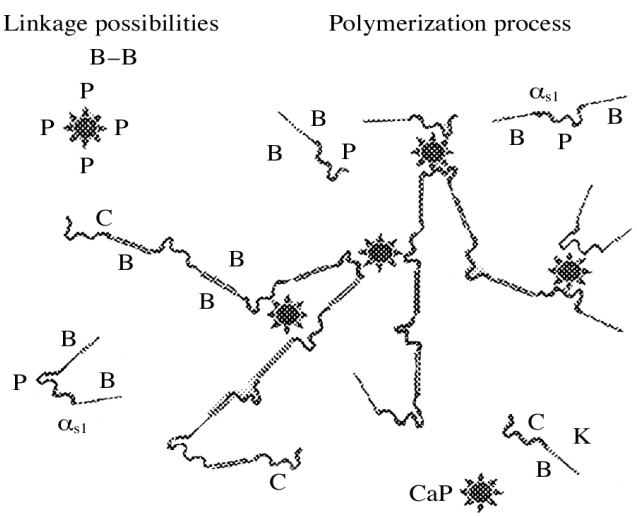

Fig. 2.3 Dual binding model of a section of the casein micelle showing interactions between α_{s1}-, β- and κ-caseins (from Horne, 2003).

strongly dependent on pH, milk salts and whey proteins, which are affected by such factors.

2.5 Minor proteins

In addition to the caseins and the two principal whey proteins, milk contains several proteins at low or trace levels. The significance of most of these proteins has been largely overlooked by dairy technologists but many of them are biologically active (see Schrezenmeir *et al*., 2000); some are now regarded as highly significant and have attracted considerable attention as neutraceuticals. When ways of increasing the value of milk proteins are discussed, the focus is usually on these minor proteins but they are, in fact, of little economic value to the overall dairy industry. They are found mainly in the whey but some are also located in the fat globule membrane. The principal minor proteins are listed in Table 2.3 and will be described briefly; reviews of the minor proteins include Fox and Flynn (1992) and Haggarty (2003).

2.5.1 Immunoglobulins

It has long been recognized that colostrum contains a very high (≈10%) concentration of immunoglobulins (Ig) and that this level declines rapidly after parturition to ≈0.1%. IgG1 is the principal Ig in bovine milk, with lesser amounts of IgG2, IgA and IgM. IgA is the principal Ig in human milk. The cow,

Table 2.3 Some properties of minor proteins in bovine milks

Protein	Molecular mass (daltons)	Concentration (mg/L)	Source
Immunoglobulins	150 000–1 000 000	200	Blood, mammary
Blood serum albumin	66 433	100–400	Blood
β_2-Microglobulin	11 636	9.5	Monocytes
Osteopontin	60 000	3–10	Mammary
Proteose peptone 3	28 000	300	Mammary
Folate-binding protein	30 000	6–10	—
Vitamin D-binding protein	52 000	16	Blood
Vitamin B_{12}-binding protein	43 000	0.1–0.2	—
Angiogenin-1	14 577	4–8	Mammary
Angiogenin-2	14 522	—	—
Kininogen	68 000/17 000	—	Blood
Lactoferrin	82 000	20–350	Mammary
Transferrin	77 000	—	Blood
Ceruloplasmin	132 000	—	Mammary
α_1-Acid glycoprotein	40 000	<20	Blood
Prosaposin	66 000	6.0	Mammary
Enzymes (~60)	Various	Trace	Blood, mammary

sheep, goat and some other species do not transfer Ig to the foetus *in utero*; the neonate is born devoid of serum Ig and, consequently, it is very susceptible to bacterial and viral infection, with a very high risk of mortality. The young of these species can absorb Ig from the intestine for several days after birth and thereby acquire passive immunity until they synthesize their own Igs within a few weeks. Some species, including the human, transfer Ig *in utero* and the offspring are born with a broad spectrum of antibodies. Although the young of these species cannot absorb Ig from the intestine, the ingestion of colostrum is still very important because the Igs it contains prevent intestinal infection. Some species, e.g., the horse, transfer Ig both *in utero* and via colostrum.

The modern dairy cow produces colostrum far in excess of the requirements, even the consumption capacity, of its calf. Therefore, colostrum is available surplus to the requirements of the calf and there is commercial interest in the recovery of Ig and other nutriceuticals therefrom (Pakkanen and Aalto, 1997). There is also considerable interest in hyperimmunizing cows against certain human pathogens, e.g., rota virus, for the production of antibody-rich milk for human consumption, especially by infants; the Ig could be isolated from the milk and presented as a 'pharmeutical' or consumed directly in the milk.

2.5.2 Bovine serum albumin (BSA)

About 1–2% of the protein in bovine milk is BSA which enters by leakage through intercellular junctions. BSA represents ≈50% of the protein in bovine blood in which it performs several functions. As befits its physiological importance, BSA is very well characterized (see Carter and Ho, 1994). It is a single polypeptide of 582 amino acid residues with a calculated MW of 66 433 Da. Its primary structure has 17 intramolecular disulphide bridges that hold the molecule in nine loops, which form three equally sized globular domains; it has one sulphydryl group. BSA has no known biological function in milk, and considering its very low concentration, it probably has no technological significance in milk.

2.5.3 Metal-binding proteins

Milk contains several metal-binding proteins, of which the caseins are quantitatively the most important. The significance of calcium in the structure of α-La has been discussed. Several enzymes are metallo-proteins, e.g., xanthine oxidase (Fe, Mo), alkaline phosphatase (Zn, Mg), lactoperoxidase (Fe), catalase (Fe) and glutathione peroxidase (Se).

The most significant metallo-protein is lactoferrin (Lf), a non-haem iron-binding glycoprotein (see Lonnerdal, 2003). It is a member of a family of iron-binding proteins, which includes transferrin and ovotransferrin (conalbumin). In spite of its name, it is not milk-specific, being present in several body fluids, including saliva, tears, sweat and semen. Bovine Lf consists of 689 amino acid residues, has a MW of 77 kDa (a glycoprotein) and three disulphide bonds; it

binds two atoms of Fe per molecule. Lf has several potential biological functions, of which improving the bioavailability of Fe and a bacteriostatic effect (by sequestering Fe and making it unavailable to intestinal bacteria) are the best established, at least *in vitro*. Other possible functions include antioxidant, antibacterial, antiviral, anti-inflammatory, immunomodulatory and anticarcinogenic activity. Human milk contains a much higher level of Lf (≈20% of total N) than bovine milk and this has stimulated interest in fortifying bovine milk-based infant formulae with Lf. The pI of Lf is ≈9.0, i.e., it is cationic at the pH of milk whereas most milk proteins are anionic. This difference in pI is the principle of an industrial-scale method for the isolation of Lf. Hydrolysis of Lf by pepsin yields peptides called lactoferricins, which are more bacteriostatic than Lf; their activity is independent of iron status.

Bovine milk also contains transferrin, which is identical to serum transferrin. It is a single polypeptide chain with a calculated MW of 75 830 Da; it has one N-linked glycan and can bind two moles of iron per mole.

A copper-binding glycoprotein, ceruloplasmin, also known as ferroxidase (EC 1.16.3.1), has been identified in the milk of several species, including cattle (see Wooten *et al*., 1996). Serum ceruloplasmin is a single chain, copper-binding α_2-globulin with a MW of ~126 000 Da. It can bind six atoms of copper per molecule and may play a role in delivering essential copper to the neonate.

2.5.4 β_2-Microglobulin

β_2-Microglobulin occurs free in body fluids and on the surface of all nucleated cells; it is a component of the immune system (see Groves and Greenberg, 1982). β_2-Microglobulin, initially called lactollin, was first isolated from bovine acid-precipitated casein by Groves *et al*. (1963). Lactollin was reported to have a MW of 43 000 Da but was subsequently found to be a tetramer of β_2-microglobulin, which consists of 98 amino acids, with a calculated MW of 11 636 Da. Apparently, β_2-microglobulin is produced from the cellular fraction in milk, probably monocytes, by proteolysis mainly within the mammary gland. No significance has been attached to β_2-microglobulin in milk.

2.5.5 Osteopontin

Osteopontin (OPN) is a highly phosphorylated acidic glycoprotein consisting of 261 amino acid residues with a calculated MW of 29 283 (total MW of the glycoprotein, ≈60 000 Da). It contains 27 phosphoserine and one phosphothreonine residue and has three O-glycosylated threonines. OPN has 50 potential calcium-binding sites, about half of which are saturated under normal physiological concentrations of calcium and magnesium.

OPN occurs in bone (it is one of the major non-collagenous proteins in bone), in many other normal and malignant tissues and in milk and urine, and can bind to many cell types. It is believed to have a diverse range of functions (Denhardt and Guo, 1993; Bayless *et al*., 1997), including:

- An adhesive and/or signalling role in injury-related events
- Mineralization and the resorption of bone matrix
- Calcium-dependent or calcium-mediated processes
- Inhibition of the growth of calcium oxalate crystals.

The role of OPN in milk is not clear. It may be important in calcium binding but, considering its low concentration in milk, its total Ca-binding capacity is small in comparison with casein. It is not known whether OPN, or peptides derived from it, are absorbed from the gastrointestinal tract and, if so, whether they retain their biological function and are transported to possible sites of activity within the body.

2.5.6 Proteose peptone 3

Bovine proteose peptone 3 (PP3) is a heat-stable phosphoglycoprotein that was first identified in the proteose peptone fraction of milk. Unlike the other peptides in this fraction, which are proteolytic products of the caseins, PP3 is an indigenous milk protein, synthesized in the mammary gland. Bovine PP3 is a single polypeptide chain of 135 amino acids with five phosphorylation and three glycosylation sites. The constituent sugars are fucose, mannose, galactose, *N*-acetylglucosamine, *N*-acetylgalactosamine and sialic acid. The calculated MW of the apoprotein is 15 304 Da. When isolated from milk, the PP3 fraction contains at least three components of MW $\approx$28, 18 and 11 kDa, the largest of which is PP3 while the smaller components are fragments thereof generated by plasmin (see Girardet and Linden, 1996). Initially, PP3 was considered to be an exclusively whey protein, but subsequent immunochemical studies showed that it is present in the MFGM also. Clearly, the term proteose peptone is a misnomer and it has been proposed to change the name to *lactophorin* or *lactoglycophorin* (Girardet and Linden, 1996).

PP3 cDNA from bovine mammary gland has 56% homology with mouse and rat glycosylation-dependent cell-adhesion molecule 1, GlyCAM-1, which is involved in the adhesion of lymphocytes to endothelial cells. However, the glycan moieties of PP3 and GlyCAM-1 differ and PP3 is unable to bind to L-selectin, which is essential for the function of murine GlyCAM-1.

PP3 has excellent foaming and emulsifying properties. Owing to its strong surfactant properties (Campagna *et al*., 1998), it can prevent contact between milk lipase and its substrates, thus preventing spontaneous lipolysis. Although its amino acid composition suggests that PP3 is not a hydrophobic protein, it behaves hydrophobically, possibly owing to the formation of an amphiphilic α-helix, one side of which contains hydrophilic residues while the other side is hydrophobic. PP3 has been referred to as the *hydrophobic fraction of proteose peptone*.

The biological role of PP3 is unknown; proposed functions include stimulation of the growth of bifidobacteria and calcium ion binding via the phosphorylated *N*-terminus of the molecule.

2.5.7 Vitamin-binding proteins

Milk contains binding proteins for at least the following vitamins: retinol (vitamin A), vitamin D, folic acid, riboflavin and cobalamin (vitamin B_{12}). The precise role of these proteins is not clear but they may improve the absorption of vitamins from the intestine or act as antibacterial agents by rendering vitamins unavailable to bacteria. The concentration of these proteins varies during lactation but the influence of factors such as individuality, breed and nutritional status is not known. The activity of these proteins is reduced or destroyed on heating at temperatures slightly higher than HTST pasteurization. As discussed earlier, β-Lg binds and perhaps acts as a carrier for retinol, although this may not be its function *in vivo*.

Most of the folate and its derivatives in raw bovine milk are bound to a folate-binding protein (FBP) which is present at a level of $\approx$10 mg/L. Bovine protein milk FBP is a single chain of 222 amino acid residues with a calculated MW of 25 825 Da. It contains eight disulphide bridges and two *N*-linked carbohydrate moieties (Asp 49 and 141) which represent $\approx$10% of the mass of the molecule. The principal sugars are *N*-acetylglucosamine, *N*-acetylgalactosamine, fucose, mannose, galactose and sialic acid; the glycoprotein has a MW of 30 000 Da. FBP tightly binds one mole of folate per mole in the pH range 5.5 to 8.0. The folate is released at acidic pH values but is rebound on neutralization. FBP increases the retention and bioavailability of folate. It may also protect folate from folate-requiring intestinal bacteria, and sequester folate synthesized by other microorganisms in the large intestine. Thus, milk FBP may contribute in several ways to the maintenance of the folate economy of the neonate (see Parodi, 1997b).

A vitamin D-binding protein (DBP) is present in the plasma of most vertebrates. It is a glycoprotein with a MW of 52 000 Da. It has high structural homology with BSA and, like BSA, can bind long-chain fatty acids. DBP has been detected in the milk of several species at ~2% of the level in blood serum. The concentration of DBP is higher in bovine colostrum and early milk than in mature milk; the protein is probably derived from serum as no synthesis of DBP has been observed in the mammary gland.

Three proteins are required for the uptake of vitamin B_{12} (cobalamin) in mammals. Gastric intrinsic factor (GIF) binds the free vitamin released from foods on digestion. The GIF–cobalamin complex enters the ileal mucosal cells by a receptor-mediated mechanism by which the vitamin is transferred to another protein, transcobalamin (TC). The TC–cobalamin complex and unsaturated TC are released into the portal plasma along with the third cobalamin-binding protein, haptocorrin (HC), the function of which is not clear but which may bind and remove from circulation cobalamin analogues that could interfere with the function of cobalamin. HC has been identified in the milk of several species (human, rat, pig and rabbit) where it exists mainly in an unsaturated form. Its function appears to be to sequester cobalamin released from food, to facilitate its absorption and prevent it being taken up by vitamin B_{12}-requiring intestinal microorganisms. It may also protect the

neonate against pathogenic bacteria that require cobalamin. However, the vitamin B_{12}-binding protein found in bovine milk is TC (Fedosov *et al*., 1996), which has been isolated in two molecular forms: a low MW protein (43 000 Da) and its aggregated form (280 000 Da) which dissociates on treatment with urea.

A riboflavin-binding protein (RfBP), with a MW of ≈38 000 Da, has been partially purified from raw bovine milk. The RfBP–riboflavin complex has good antioxidant properties, similar to those of the protein isolated from avian eggs where it has an important nutritional role. The RfBP in milk may be derived from serum; its physiological function has not been established.

2.5.8 Angiogenins

Angiogenins induce the growth of new blood vessels, i.e., angiogenesis. They have high sequence homology with members of the RNaseA superfamily of proteins and have ribonucleolytic activity. Angiogenesis is a complex biological process of which the ribonucleolytic activity of angiogenins is only one of a number of essential biochemical steps that lead to the formation of new blood vessels (Strydom, 1998).

Two angiogenins (angiogenin-1 and angiogenin-2) have been identified in bovine milk and blood serum. Both strongly promote the growth of new blood vessels in a chicken membrane assay. Bovine angiogenin-1 (ANG-1) is a highly basic non-glycosylated, single-polypeptide protein containing 125 amino acids and three disulphide bonds. Its calculated MW is 14 577 Da. ANG-1 has 64% sequence identity with human angiogenin and 34% identity with bovine RNase A.

ANG-2 is a single polypeptide chain of 123 amino acids, with three disulphide bonds and a calculated MW of 14 522 Da. Unlike ANG-1, ANG-2 has one *N*-linked glycosylation site (Asp 33). Glycosylation may have a major effect on the function of ANG-2. The amino acid sequence of bovine ANG-2 has 57% identity with that of bovine ANG-1 and it has lower RNase activity than ANG-1.

The function(s) of the angiogenins in milk is unknown. They may be part of a repair system to protect either the mammary gland or the intestine of the neonate and/or part of the host-defence system, due to their high transfer RNase activity.

2.5.9 Kininogen

Two forms of kininogen have been identified in bovine milk, a high MW form (>68 000 Da) and a low MW form (16 000–17 000 Da) (Wilson *et al*., 1989). Bradykinin, a biologically active peptide containing nine amino acids released from the high MW kininogen by the action of the enzyme kallikrein, has been detected in the mammary gland, and is secreted into milk, from which has been isolated. The forms of kininogen in milk are apparently different from those in bovine plasma.

Plasma kininogen is an inhibitor of thiol proteases and has an important role in blood coagulation. Bradykinin has several functions: it affects smooth muscle contraction, induces hypertension and is involved in natriuresis and diuresis. The biological significance of bradykinin and kininogen in milk is unknown, although a fragment of bovine milk kininogen promotes the proliferation of osteoblastic MC3T3-E1 cells and may play a role in bone formation.

2.5.10 Glycoproteins

Many of the minor proteins discussed above are glycoproteins; in addition, several other minor glycoproteins have been found in milk and colostrum but their identity and function have not been elucidated fully. Some of these glycoproteins, which have been isolated, belong to a family of closely related, highly acidic glycoproteins called M-1 glycoproteins. Their average MW is 10 000 Da and they contain galactose, glucosamine, galactosamine, sialic acid and other sugars. Some glycoproteins stimulate the growth of bifidobacteria, presumably via their aminosugars.

One of the M-1 glycoproteins in colostrum, but which has not been detected in milk, is orosomucoid (α_1-acid glycoprotein), a member of the lipocalin family. It has a MW of $\approx$40 000 Da and contains five *N*-linked glycan groups. α_1-Acid glycoprotein is thought to modulate the immune system; its concentration in blood increases during inflammatory diseases, malignancy and pregnancy.

One of the high molecular weight glycoproteins in bovine milk is prosaposin, a neurotrophic factor which plays an important role in the development, repair and maintenance of the nervous system (Patton *et al*., 1997). It is a precursor of saposins A, B, C and D, which are sphingolipid activator proteins, but saposins are not detected in milk. Prosaposin is present in whey as a monomer of about 66 000 Da. The amino acid sequence, determined from cDNA, consists of 525 amino acids with a calculated MW of 58 051 Da. The physiological role of milk prosaposin in the neonate or the mammary gland is not known, although the potent biological activity of saposin C, released by digestion, could be important for the growth and development of the young.

2.5.11 Growth factors

A great diversity of protein growth factors (hormones), including epidermal growth factor, insulin, insulin-like growth factors 1 and 2, three human milk growth factors (α1, α2 and β), two mammary-derived growth factors (I and II), colony stimulating factor, nerve growth factor, platelet-derived growth factor and bombasin, are present in milk (see Fox and Flynn, 1992). It is not clear whether these factors play a role in the development of the neonate or in the development and functioning of the mammary gland or both. There is little information on the variability of these hormones or the effect of animal or husbandry factors. Studies on such variability appear warranted.

2.5.12 Indigenous milk enzymes

Milk contains about 60 indigenous enzymes, which represent a minor but very important part of the milk protein system. The principal indigenous enzymes in milk have been isolated and well characterized; the extensive literature has been reviewed (see Fox *et al*., 2003). The enzymes originate from the secretory cells or the blood and enter milk as a result of the mechanisms by which the constituents of milk, especially the fat globules, are exported from the mammocytes. With the possible exception of bile salts-activated lipase in human milk and that of a few other species, the indigenous enzymes probably have no direct function in milk. Many of the indigenous enzymes are concentrated in the MFGM and originate in the Golgi membranes of the cell or the cell cytoplasm, some of which may becomes entrapped as crescents inside the encircling membrane during exocytosis. A few enzymes, notably plasmin and lipoprotein lipase, are associated with the casein micelles and several are present in the milk serum; many of the latter are derived from the MFGM which is shed as the milk ages. The indigenous enzymes are significant for the following reasons.

Technological

- Plasmin causes proteolysis in milk and some dairy products; it may be responsible for age gelation in UHT milk and contributes to proteolysis in cheese during ripening, especially in varieties that are cooked at a high temperature and in which the coagulant is extensively or completely denatured, e.g., Emmental, Parmesan and Mozzarella.
- Lipoprotein lipase may cause hydrolytic rancidity in milk and butter but contributes positively to the ripening of raw milk cheese.
- Acid phosphatase can dephosphorylate casein and modify its functional properties; it may contribute to cheese ripening.
- Xanthine oxidase is a very potent prooxidant and may cause oxidative rancidity in milk; it reduces nitrate to nitrite, which prevents the growth of clostridia in cheese.
- Lactoperoxidase is a very effective bacteriocidal agent in the presence of a low level of H_2O_2 and SCN^- and is exploited for the cold-sterilization of milk.

Indices of milk quality and history

The standard assay for the adequacy of HTST pasteurization of milk is the inactivation of alkaline phosphatase. Proposed assays for super-pasteurization of milk are based on the inactivation of γ-glutamyltranspeptidase or lactoperoxidase.

The concentration/activity of several enzymes in milk increases during, and may be used as an index of, mastitic infection, e.g., catalase, acid phosphatase and especially *N*-acetylglucosaminidase. These increases reflect the breakdown of mammary tissue during infection and the increase in the number of leucocytes (somatic cells) in milk.

Antibacterial
Milk contains several bactericidal agents, two of which are the enzymes, lysozyme and lactoperoxidase.

Considering the routes through which enzymes enter milk, it is not surprising that there is considerable variation in the activity of all enzymes in milk for which data are available. However, the data are limited to the most significant enzymes and even for these, only a few causes of variation have been investigated. For those enzymes for which data are available, e.g., plasmin, lipoprotein lipase, xanthine oxidase and superoxide dismutase, there is considerable inter-breed and inter-cow variability, although the consistency of this variability is not certain; usually, the data are from one-off analysis. The activity of most enzymes increases markedly during a mastitic infection and with advancing lactation. Physiological and nutritional stress cause an increase in LPL activity in milk and probably of other enzymes also.

The indigenous enzymes in milk have the potential to cause considerable changes in milk lipids and proteins and hence in the processability of milk and in the quality of dairy products. Some of the enzymes are inactivated by HTST pasteurization but most are quite stable; although most are present at quite low levels, they can cause considerable change during a prolonged storage period. Plasmin activity in milk is actually increased by HTST pasteurization, probably due to inactivation of indigenous inhibitors.

The activity of some indigenous enzymes is strongly suppressed in milk but full activity may be realized under certain circumstances. For example, the triglycerides in milk are protected from LPL, which is associated mainly with the casein micelles, by the MFGM, and very little lipolysis normally occurs. However, if the MFGM is damaged by agitation, foaming, etc., lipolysis will occur rapidly. Some plasmin activity occurs in all milk but it is relatively low because most (80%) of the potential activity occurs as plasminogen, the activation of which by indigenous activators is inhibited by indigenous inhibitors of the activators; inhibitors of plasmin are also present in milk. Both sets of inhibitors are in the serum phase whereas plasmin, plasminogen and plasminogen activators are associated with the casein micelles. If the micelles are separated from the serum and redispersed in buffer, very rapid and extensive proteolysis occurs.

2.5.13 Biologically active peptides

One of the most exciting recent developments in milk proteins is the discovery that all milk proteins contain sequences that possess biological/physiological activities following specific proteolysis (see Gobbetti *et al*., 2002, Pihlento-Leppala, 2003; FitzGerald and Meisel, 2003). The best studied and perhaps the most important of these peptides are:

- Phosphopeptides
- Angiotensin-converting enzyme inhibitory peptides

- Platelet-modifying peptides
- Opiate peptides
- Immunomodulating peptides.

The rennet coagulation of milk involves the specific hydrolysis of the Phe_{105}–Met_{106} bond of the micelle-stabilizing protein, κ-casein. The C-terminal part of κ-CN (residues 106–169), known as the caseinomacropeptide (CMP) or glycomacropeptide (GMP) or caseinoglycomacropeptide, diffuses into the whey while the *N*-terminal part (*para*-κ-CN; κ-CNf1-105) remains in the cheese curd. The CMPs represent ≈30% of κ-CN (≈4% of total casein) and 15–20% of the total N in cheese whey. Thus, large quantities of CMP are readily available from whey from which it can be isolated fairly readily (see Brody, 2000).

The CMP has a number of interesting biological properties (see Brody, 2000):

- It contains no aromatic amino acids and therefore is suitable for the nutrition of individuals suffering from phenylketonuria; unfortunately, it lacks other essential amino acids, i.e., Cys, His, Trp and Tyr as well as Phe.
- It inhibits the binding of cholera toxin and *E. coli* enterotoxins.
- It inhibits bacterial and viral adhesion.
- It suppresses gastric secretions.
- It promotes the growth of bifidobacteria.
- It modulates immune system responses.

Smaller peptides derived from the CMP by proteolysis also have interesting properties, e.g.:

- Antithrombotic (κ-CN f106–116 and especially 113–116) (Maubois *et al*., 1991)
- Growth-promoting activity for *Lc. lactis* ssp. *lactis* (Bouhallab *et al*., 1993).

2.5.14 Protein-related aspects of quality

With the exception of butter, ghee and anhydrous milk fat, the properties and functionality of the proteins have major effects on the quality, even the existence, of dairy products. The most significant protein-rich dairy products are liquid (beverage) milk (HTST pasteurized and UHT sterilized), cheese (rennet- or acid-coagulated), milk powders, concentrated-sterilized milk, functional milk protein products (caseins, caseinates, whey protein concentrates, whey protein isolates), fermented milk products, ice cream and infant foods. Many of these products will be considered in some detail in the following chapters. The importance of protein functionality depends on the product, e.g.:

- Rennet coagulability
- Acid coagulability
- Heat stability

- Thermal gelation
- Surface activity
- Water binding.

For comprehensive discussions of these subjects, the reader is referred to Fox and McSweeney (2003). The significance of milk proteins as food ingredients was discussed by Fox (2001). The functionality of the milk proteins, especially the caseins, is strongly affected by the ions in milk, especially H^+ and Ca^{2+}. The salts in milk are discussed below.

2.6 Salts

When milk is heated in a muffle furnace at 500ºC for ≈5 h, a residue, ash, derived mainly from the inorganic salts of milk and representing ≈0.7%, w/w, of the milk, remains. However, the elements are changed from their original forms to oxides or carbonates and the ash contains P and S derived from caseins, lipids, sugar phosphates or high-energy phosphates. The organic salts, the most important of which is citrate, are oxidized and lost during ashing; some volatile metals, e.g., sodium, are also partially lost. Thus, ash does not accurately represent the salts of milk. However, the principal inorganic and organic ions in milk can be determined directly by potentiometric, spectrophotometric or other methods. The typical concentrations of the principal elements, often referred to as macro-elements, are shown in Table 2.4. There is considerable variability in reported values, due, in part, to poor analytical methods and/or to samples from cows in very early or late lactation or suffering from mastitis. Milk also contains 20–25 elements at very low or trace levels (Table 2.5). These micro-elements are very important from a nutritional viewpoint and some, e.g., Fe and Cu, are very potent lipid prooxidants (see Fox and McSweeney, 1998).

Some of the salts in milk are fully soluble but others, especially calcium phosphate, exceed their solubility under the conditions in milk and occur partly in the colloidal state, associated with the casein micelles; these salts are referred to as colloidal calcium phosphate (CCP), although some magnesium, citrate and traces of other elements are present also. The typical distribution of the principal organic and inorganic ions between the soluble and colloidal phases is summarized in Table 2.4. The actual form of the principal species can be either determined or calculated, after making certain assumptions; typical values are shown in Table 2.4.

The solubility and ionization status of many of the principal ionic species are interrelated, especially H^+, Ca^{2+}, PO_4^{3-} and citrate^{3-}. These relationships have major effects on the stability of the caseinate system and consequently on the processing properties of milk. The status of various species in milk can be modified by adding certain salts to milk, e.g., [Ca^{2+}] by PO_4^{3-} or citrate^{3-}; addition of $CaCl_2$ to milk affects the distribution and ionization status of calcium, phosphate and the pH of milk.

Table 2.4 Concentration and distribution of the principal ions in bovine milk (modified from Fox and McSweeney, 1998)

Species	Concentration (mg/L)	Soluble		Colloidal
		%	Form	%
Sodium	500	92	Ionized	8
Potassium	1450	92	Ionized	8
Chloride	1200	100	Ionized	—
Sulphate	100	100	Ionized	—
Phosphate	750	43	10% bound to Ca and Mg 51% H_2PO^- 39% HPO_4^{2-}	57
Citrate	1750	94	85% bound to Ca and Mg 14% $Citr^{3-}$ 1% $HCitr^{2-}$	
Calcium	1200	34	35% Ca^{2+} 55% bound to citrate 10% bound to phosphate	66
Magnesium	130	67	Probably similar to calcium	33

The precise nature and structure of CCP are uncertain. It is associated with the caseins, probably via the organic phosphate residues; it probably exists as micro-crystals which include PO_4 residues of casein. The simplest stoichiometry is $Ca_3(PO_4)_2$ but spectroscopic data suggest that $CaHPO_4$ is the most likely form.

The distribution of species between the soluble and colloidal phases is strongly affected by pH and temperature. As the pH is reduced, CCP dissolves and is completely soluble below approximately pH 4.9; the reverse occurs when the pH is increased. These pH-dependent shifts mean that some acid-precipitated products, e.g., acid casein and acid-coagulated cheeses, have a very low concentration of Ca, which has nutritional significance.

The solubility of calcium phosphate decreases as the temperature is increased. Consequently, soluble $CaPO_4$ is transferred to the colloidal phase, with the release of H^+ and a decrease in pH:

$$CaHPO_4/Ca(H_2PO_4)_2 \leftrightarrow Ca_3(PO_4)_2 + 3H^+$$

These changes are quite substantial but are at least partially reversible on cooling. Since milk is supersaturated with calcium phosphate, concentration of milk by evaporation of water increases the degree of supersaturation and the transfer of soluble calcium phosphate to the colloidal state, with the concomitant release of H^+. Dilution has the opposite effect.

Milk salts equilibria are also shifted on freezing. Since pure water freezes, the concentrations of solutes in the unfrozen liquid are increased. Soluble calcium phosphate precipitates as $Ca_3(PO_4)_2$, releasing H^+ (the pH of the liquid phase may decrease to 5.8). The crystallization of lactose as a monohydrate aggravates the situation by reducing the amount of solvent water.

Table 2.5 Mineral composition (mg/L or μg/L) in mature human or bovine milk (modified from Flynn and Power, 1985)

	Mature human milk		Bovine milk	
Constituent	Mean	Range	Mean	Range
Sodium (mg)	150	110–200	500	350–900
Potassium (mg)	600	570–620	1500	1100–1700
Chloride (mg)	430	350–550	950	900–1100
Calcium (mg)	350	320–360	1200	1100–1300
Magnesium (mg)	28	26–30	120	90–140
Phosphorus (mg)	145	140–150	950	900–1000
Iron (μg)	760	620–930	500	300–600
Zinc (μg)	2950	2600–3300	3500	2000–6000
Copper (μg)	390	370–430	200	100–600
Manganese (μg)	12	7–15	30	20–50
Iodine (μg)	70	20–120	260	—
Fluoride (μg)	77	21–155		30–220
Selenium (μg)	14	8–19	—	5–67
Cobalt (μg)	12	1–27	1	0.5–1.3
Chromium (μg)	40	6–100	10	8–13
Molybdenum (μg)	8	4–16	73	18–120
Nickel (μg)	25	8–85	25	0–50
Silicon (μg)	700	150–1200	2600	750–7000

The concentration of macro-elements, their distribution between the soluble and colloidal phases and their ionization status have been investigated extensively, e.g., White and Davies (1958), Keogh *et al*. (1982) and O'Brien *et al*. (1999c). The changes are the consequence of stage of lactation, nutrition, genetic effects and perhaps other factors. The distribution and ionization status are strongly affected by pH, which increases during lactation. Changes in salts equilibria have major effects on the stability of milk proteins, and to a lesser extent of milk lipids, and consequently on the processability of milk. The changes are particularly significant for the production, yield and quality of cheese, of heat-sterilized products (UHT and in-container sterilized) and of milk powders.

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