Chapter 8
Milk Salts: Technological Significance

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8.1 Introduction

Mammalian milk contains all the essential components to sustain the growth and development of the newborn suckling. Usually, this is taken to mean the protein, fat, and carbohydrate, but it also applies to the mineral components, the milk salts, including the citrate, phosphate, and chloride salts of H^+, K^+, Na^+, Mg^{2+}, and Ca^{2+}, whether as ions in solution or as colloidal species complexed with the caseins. These minerals are essential for bone growth and development, for efficient cellular function, and for maintaining osmolality with increasing carbohydrate (lactose) synthesis. Like the other components, all these mineral species are there for a purpose, and, until weaning, milk may often be the only source of these essential elements.

There have been a number of reviews on the topic of milk salts (Allen 1931; Pyne 1962; Jenness and Patton 1976; Walstra and Jenness 1984; Holt 1985, 1997; Gaucheron 2005; Fox et al. 2015). In this chapter, the term salts will be used to represent substances that are, or can be, present in milk as low molecular weight ions. This group includes both inorganic and organic (e.g., citrate) substances. We can distinguish between the major salt constituents and trace elements and the latter will not be considered in this chapter. The approximate concentration of milk salts is shown in Table 8.1, which approximates to an ionic strength of around 80 mM (Gaucheron 2010). The milk salts have a crucially important impact on many properties of milk, including the formation and stability of the casein micelles, acid–base buffering, and various colligative properties, as well as their key biological role (i.e., providing nutrition for the new-born). In addition, these salts have a powerful

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P. L. H. McSweeney et al. (eds.), *Advanced Dairy Chemistry*,
https://doi.org/10.1007/978-3-030-92585-7_8

Table 8.1 Approximate salt composition in milk (from various sources)

Cationic	Concentration		Anionic	Concentration	
	mg L^{-1}	mmol kg^{-1}		mg L^{-1}	mmol kg^{-1}
Calcium	1040–1280	26–32	Carbonate (including CO_2)	~200	~2
Magnesium	100–150	4–6	Chloride	780–1200	22–34
Potassium	1210–1680	31–43	Citrate	1320–2080	7–11
Sodium	350–600	17–28	Total phosphorus (PO_4) (all forms)	930–1000	30–32
			Inorganic phosphorus (as PO_4)	1800–2180	19–23
			Sulfate	~100	~1

Table 8.2 Approximate distribution of salts between the colloidal and serum phases in milk (from various sources)

	Colloidal (micellar) (%)	Serum (soluble) %
Calcium	69	31
Chloride	≤5	≥95
Citrate	14	86
Inorganic phosphate	53	47
Magnesium	47	53
Potassium	6	94
Sodium	≤5	≥95

influence on protein stability during processing (e.g., rennet coagulation, heat and alcohol stability), the texture of various types of milk protein gels, cheese texture and functionality, and emulsion stability.

Milk is supersaturated with respect to calcium and phosphate ions, and these ions exist in a *dynamic equilibrium* with undissolved or colloidal forms (there is no true equilibrium for the Ca phosphates but some type of *pseudoequilibrium* that is influenced by several factors, including the presence of caseins). It has been recognized since Hammarsten (1879) that this insoluble Ca phosphate fraction is associated with the casein micelles (at that time the micelles were called the Ca caseinogenate). The Ca and phosphate contents vary in proportion to the casein content of milk since much of the Ca and phosphate are associated with the casein micelles. The partition of salts between the colloidal (micellar) and serum (soluble) phases is shown in Table 8.2 (the distribution between these two phases depends on the environmental conditions, including pH, temperature, concentration, etc.). In the serum phase, milk salts may be present as ion pairs (e.g., anions with cations). The Ca and Mg in milk are present at low concentrations as free ions and some as complexes with citrate and phosphate, as well as around 70% associated with casein micelles (Table 8.3). Part of the insoluble calcium is associated with inorganic phosphate to form colloidal calcium phosphate (CCP), which is solubilized at pH values around 5.0. The remaining insoluble calcium (i.e., not in the serum phase) is associated

Table 8.3 Calculated values for the major forms of calcium and magnesium in milk (mainly adapted from Neville 2005)

Binding species	Concentration (mmol L^{-1})
Calcium	
$[Ca^{2+}]$	2.0
$[CaCit^{-}]$	6.9
$[CaPO_4^{-}]$	0.6
Casein	19.4
α-Lactalbumin	0.5
Magnesium	
$[Mg^{2+}]$	0.8
$[MgCit^{-}]$	2.0
$[MgPO_4^{-}]$	0.3
Casein	1.9

directly with caseins; this has sometimes been referred to as caseinate calcium and is only completely released from casein at pH values 3.5–4.0 (Le Great and Brulé 1993). Both Mg and citrate are present in the colloidal phase, which is remarkable since their concentrations (or activities) are not in excess of solubility (Walstra and Jenness 1984). The concentrations of the main salt components in the serum phase have been reported (Jenness and Koops 1962). Theoretical models have been used to calculate the salt equilibria in models of the milk serum phase (e.g., Holt et al. 1981; Mekmene et al. 2009).

This chapter updates and revises the previous version by Lucey and Horne (2009).

8.2 Methods of Analysis

Ashing (e.g., dry heating in a muffle furnace at >500 °C for several hours) of milk is an approximate method of quantifying the inorganic elements (0.7–0.8% in normal milk but values >1.3% can be found in colostrum). However, organic salts are lost during ashing. Some carbonates are lost during ashing (as CO_2) and some types of carbonates are formed from organic compounds. Phosphates from lipids (i.e., phospholipids) also appear in the ash. The sulfur of proteins is oxidized during incineration and appears as sulfate. Oxidation also results in the formation of metal oxides. Wet ashing involves the use of acids like nitric acid. Ashing is routinely used as a pretreatment (by oxidizing organic matter) step for elemental analysis, as the ash can be dissolved with acid and used for quantification of Ca, Fe, etc., by atomic absorption spectroscopy or inductively coupled plasma spectroscopy. The various techniques used for the analysis of milk salts were described by Fox et al. (2015) and Gaucheron (2010). Measurement of the partition of salts between the colloidal and dissolved forms can be achieved by dialysis, ultrafiltration, and the preparation of rennet whey (Davies and White 1960; de la Fuente et al. 1996) although some

adjustments (e.g., to account for excluded volume effects) must be made with these techniques to calculate the serum concentration.

8.3 Secretion of Milk Salts

The biosynthesis of components in milk and milk secretion have been reviewed many times (e.g., Blackwood and Stirling 1932; Petersen 1944; Linzell and Peaker 1971; Larson 1985; McManaman and Neville 2003; Osorio et al. 2016). The cytoplasm of lactating alveolar cells is filled with numerous mitochondria and an extensive rough endoplasmic reticulum network. In addition, there is a well-developed Golgi apparatus, and secretory vesicles containing casein micelles are present in the apical region of the cell. Epithelial cells are connected to each other through an apical junctional complex composed of adherens and tight-junctional elements that function to inhibit direct paracellular exchange of substances between vascular and milk compartments during lactation (McManaman and Neville 2003).

The secretion of milk salts has been reviewed by Holt (1981, 1985), Neville (2005), and Neville et al. (2020). Lactating mammals must supply large amounts of Ca to the mammary gland where it is transported across mammary epithelial cells and into milk. Calcium transfer into milk can be divided into four main steps (Neville et al. 2020): (a) transfer of calcium across the basolateral membrane from the extracellular fluid; (b) intracellular sequestration of calcium in the endoplasmic reticulum to help maintain free cytosolic calcium in the micromolar range; (c) transfer of calcium into the Golgi and secretory compartments where it binds to proteins, phosphate and citrate; and (d) export of calcium into milk across the apical membrane.

Calcium is pumped from the cytoplasm into the Golgi compartment and enters milk via exocytosis of secretory vesicles from the Golgi compartment with a membrane-associated Ca ATPase mediating the transport (Bingham et al. 1993). Circulating Ca concentration must remain relatively constant, i.e., Ca homeostasis; a number of diseases/conditions occur when this is not the case. Such stability relies on cooperation between several organs, principally the parathyroid glands, the kidneys, the skeleton, and the gut. Several important entities are involved in the feedback loop that regulates Ca fluxes to the mammary gland. These control features include an extracellular Ca-sensing receptor (CaR) and parathyroid hormone-related protein (PTHrP) (VanHouten 2005). Very high concentrations of Ca are transferred from the cytoplasm although the cytoplasmic Ca concentration remains relatively constant (in the μM range). This demand for Ca is associated with transient loss of bone mass (in humans), triggered, in part, by the secretion of PTHrP from the mammary gland into the circulation (Ardeshirpour et al. 2006). The CaR is a G-protein-coupled receptor that signals in response to extracellular Ca^{2+} (Ardeshirpour et al. 2006). It is responsible for coordinating Ca homeostasis by regulating both parathyroid hormone secretion and Ca handling in the renal tubules. Calcium activates basolateral CaRs to stimulate its own transport into milk (VanHouten et al. 2004).

The intracellular Na and K concentrations are established by a Na/K-activated ATPase on the basolateral surface of the secretory cell, and there is a dynamic electrochemical equilibrium of these ions across the apical membrane (Holt 1985).

It has been known for a long time that milk is in osmotic equilibrium with blood, i.e., milk is isotonic with blood (van der Laan 1915). Taylor and Husband (1922) were probably the first to suggest that the quantity of lactose produced by the mammary gland controls the daily volume of the milk. Koestler (1920) used the ratio of lactose and chloride as a method to indicate normal and mastitic (abnormal) milk; the Koestler number = (100 × chlorine %)/lactose %. Normal milk has a Koestler number less than 3, while that of mastitic milk is considerable higher (e.g., 15). One of the first studies of the possible mechanisms involved in the secretion of Ca and phosphate in milk was reported by Wright (1928).

The large amounts of phosphate required by the suckling for normal growth and development are also supplied through the milk in at least three chemical forms, namely free inorganic orthophosphate in solution, colloidal (inorganic) phosphate associated with Ca in micellar Ca phosphate, and the ester (organic) phosphate of the caseins. The major pathway for phosphate secretion into milk was believed to be the Golgi vesicle route by a Na^{+}-P_i co-transport mechanism (Shennan and Peaker 2000). However, Holt (1985) described another mechanism by which phosphate is generated in the Golgi lumen by hydrolysis of UDP during lactose synthesis (Kuhn and White 1977). This uridine-nucleotide cycle involves UDP-galactose and glucose. Within the vesicle, these precursors form UDP and lactose. The UDP cannot cross the vesicle membrane unless hydrolyzed to UMP and inorganic phosphate, both of which can re-enter the cytosol, avoiding product inhibition of lactose synthetase. Thus, it should be noted that there is a lot of phosphate released into the Golgi when lactose is synthesized from glucose and UDP-galactose in the Golgi compartment, and this phosphate could be used to phosphorylate casein early in the casein micelle biosynthesis process.

Citrate concentration in milk varies widely throughout lactation and within individual cows (Banks et al. 1984). In general, citrate levels are higher during the grazing season (Holt and Muir 1979) and during early lactation (Braunschweig and Puhan 1999; Garnsworthy et al. 2006). In studies on the goat, Linzell et al. (1976) found that the mammary epithelium is impermeable to citrate in both directions, suggesting that citrate is synthesized within the secretory cells and released into milk after exocytosis of Golgi vesicles. Citrate has an indirect role in fat synthesis by providing reducing equivalents in the form of NADPH, which are required for de novo synthesis of fatty acids (Faulkner and Peaker 1982). Citrate is in equilibrium with iso-citrate, which is converted to α-ketoglutarate in the production of NADPH. Thus, increased de novo synthesis of fatty acids is predicted to lead to a decrease in citrate concentration. Such a correlation was found in the studies of Banks et al. (1984), who used fat supplements to reduce de novo synthesis of fatty acids in the mammary gland and induce increases in milk citrate concentration and was confirmed in the more recent lactational studies of Garnsworthy et al. (2006). The latter authors found a significant correlation between milk citrate and the amounts of acetate required for chain elongation in de novo fatty acid synthesis.

Any change in the citrate concentration of milk would therefore directly influence the Ca^{2+} concentrations (as citrate readily binds Ca^{2+}), which could influence milk behavior, e.g., its rennet coagulation time. Diet-induced changes in the citrate levels in milk would thus alter Ca^{2+} concentrations, and this type of mechanism could account for at least some of the observed diet-related changes in milk functionality. In grass-based milk production systems, there are also seasonal variations (due to stage of lactation as well as feed effects) in the concentrations of minerals, such as calcium and citrate (O'Brien et al. 1999; Dunshea et al. 2019).

It has been proposed that casein-derived phosphopeptides disrupt tight junction integrity, and precipitously cause milk secretion to dry up, i.e., they may help trigger the involution process (Shamay et al. 2002). Serotonin is another likely candidate to trigger this process. It is known that plasmin activity increases near the end of lactation (Politis et al. 1989), and it is possible that some phosphopeptides are produced by this mechanism. Involution involves remodeling of the mammary gland tissue by various proteases (like plasmin) and other mechanisms.

8.4 Factors Influencing the Milk Salts Equilibria

There are numerous dynamic equilibria between the salts in milk, and changes in many environmental conditions influence these equilibria. Some of these changes occur relatively quickly, but those involving CCP can be slow. Mastitic infections of the udder result in a decrease in the concentrations of Ca^{2+} and K^+ in milk but an increase in the concentrations of Na^+ and Cl^- (due to leakage of these ions into milk from blood where their concentrations are much higher than in milk). It should be noted that, during milking and processing, most CO_2 is lost. The impact of various processing techniques on the milk salt equilibria has been regularly reviewed (Holt 1985; de la Fuente 1998; Gaucheron 2005, 2010).

8.4.1 Temperature

Milk as secreted by the cows probably contains about 20 mg of CO_2 per 100 mL (Jenness and Patton 1976). This gas is rapidly lost, and heating and agitation accelerate this loss. The pH of milk decreases as its temperature increases, although few measurements of pH have or can be made at very high temperatures.

The solubility of Ca phosphates decreases at high temperature and during heating heat-induced CCP is formed, which re-solubilizes when milk is subsequently cooled. Jenness and Patton (1976) gave that approximate reaction as:

$$3Ca^{2+} + 2HPO_4^{2-} \rightarrow Ca_3\left(PO_4\right)_2\left(\text{precipitate}\right) + 2H^+$$

The release of H^+ contributes to the decrease in milk pH observed on heating (with extreme heating, there is also the production of organic acids, principally formic from lactose) (Dalgleish 1989). This heat-induced CCP appears to associate with the existing CCP in casein micelles, possibly by increasing the size of the nanoclusters (Holt 1995). The original equilibrium is mostly restored (slowly) after cooling, but there is some hysteresis. Cooling and holding milk at low temperatures result in an increase in the solubility of Ca phosphate and thus a decrease in the concentration of CCP. The Ca^{2+} activity is also mostly restored if sufficient time is allowed for equilibration (Geerts et al. 1983; Augustin and Clarke 1991). At temperatures ≥40 °C, artificial milk serum buffers (or ultrafiltrate) are prone to precipitation. Caseins are effective stabilizers of CCP and usually prevent precipitation of these salts in milk. The absence of casein from these buffers alters the behavior of salts during heating and irreversible precipitation of Ca phosphate occurs (Holt 1995). The deposits found on the surfaces of ultra-high temperature heat exchangers (known as fouling) are rich in Ca phosphate. There are indications that very severe heat treatments (e.g., 120 °C for 15 min) cause a change in the nature of CCP as indicated by an altered acid–base buffering profile (Lucey et al. 1993a), e.g., to form hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$; Visser et al. 1986). There can also be precipitation of Ca phosphate on the heating equipment during severe heating or sterilization, leading to increased fouling, which does not resolubilize upon cooling (Sadeghinezhad et al. 2013). Under these severe heating conditions, caseins are unable to prevent the precipitation of Ca phosphate onto metal surfaces (Holt 1995).

Holding milk at low temperatures causes dissociation of some caseins, especially β-casein (<20% of total β-casein) (Downey and Murphy 1970; Creamer et al. 1977) and some dissolution of CCP (Qvist 1979; Ali et al. 1980). Most of these changes are reversed readily by mild heating, e.g., pasteurization (Qvist 1979).

Freezing of milk is sometimes practiced where milk production is seasonal, e.g., goat and ewe milk for cheesemaking (Wendorff 2001). Freezing and thawing for a sufficient time results in the reversal of most of the changes in the salt equilibrium that may have been caused during freezing. Long-term storage (after several months) of milk at ≤−15 °C can result in protein precipitation (due to the low pH and elevated ionic calcium levels). Ovine and caprine milk stored frozen for a few months had similar levels after thawing of soluble Ca, Mg, and P as in the unfrozen milk (de la Fuente et al. 1997).

8.4.2 pH

Acidification solubilizes CCP, which is an integral part of casein micelles. The extent of solubilization increases markedly below pH 5.6 and is complete at approximately pH 5.0 (Pyne and McGann 1960; Brule et al. 1974; Pierre et al. 1983; van Hooydonk et al. 1986; Dalgleish and Law 1989; Mariette et al. 1993). The pH at which CCP is completely solubilized presumably varies with the conditions (e.g., rate and temperature) of acidification, due to the (slow) kinetics of CCP

solubilization. At pH values <5, milk is unsaturated with respect to most types of calcium phosphates (Lyster 1979). At high pH values (≥6), concentrated milk products (e.g., condensed milk) have an increased likelihood of precipitation of some type of Ca phosphate, especially during heating. Increasing the pH of milk results in the formation of additional CCP. McGann and Pyne (1960) described a method for increasing the CCP content of milk (by up to 200%). Milk pH is increased by the addition of NaOH at about 0 °C followed by exhaustive dialysis against a large excess of the original milk. Ozcan et al. (2011) proposed that, as the pH is increased, the serine phosphates become more negatively charged and less inclined to associate with the CCP. The impact of those changes could be some dissociation of the micelle as well as further precipitation of Ca phosphate causing growth of the nanoclusters, before the dialysis with normal milk restores the original pH value (thereby restoring the calcium-binding activity of the serine phosphate).

Lucey et al. (1996) studied the impact of (cold) acidification and neutralization of milk on the properties of casein micelles. Acidification of milk to pH 5.0 or 4.6, followed by neutralization to pH 6.6, resulted in a reduction in the buffering maximum of milk at pH ~5.1; this buffering peak is caused by the solubilization of CCP. The reduced buffering in reformed (acidified and then neutralized) milk suggests that little reformation of CCP occurs on neutralization; reformed milks also had an elevated Ca^{2+} activity. Acidification of milk to pH >5.5, followed by neutralization to pH 6.6, only slightly reduced buffering (at pH ~5.1), suggesting that either little CCP dissolved on acidification in that pH range or that reformation of CCP occurred on neutralization. Canabady-Rochelle et al. (2007) also reported that milk had a higher soluble Ca level after acidification and neutralization.

Gevaudan et al. (1996) used high-pressure CO_2 to acidify milk reversibly (pH was restored to the original value after depressurization). Acidification to pH ~5 with high-pressure CO_2 resulted in a reduction in the buffering peak at pH ~5.1, but this peak increased during chilled storage of this milk (Raouche et al. 2007).

Heat treatment has little impact on the pH-dependent release of Ca and phosphate from micelles during acidification (Law 1996; Singh et al. 1996).

8.4.3 Concentration of Milk

Concentrating milk by evaporation results in a decrease in milk pH, e.g., a decrease of ~0.3 and 0.5 pH units for 2:1 and 3:1 concentration, respectively (Walstra and Jenness 1984). The $[Ca^{2+}]$ increases with concentration but less than the concentration factor (Walstra and Jenness 1984). Presumably, the slower increase in Ca^{2+} is at least partly due to the formation of additional CCP (even though the pH decreases in evaporated milk). Membrane filtration of milk using either ultrafiltration or microfiltration results in retentates in which CCP is a greater proportion of the total Ca content, as some soluble Ca is lost in the permeate during processing (Lelievre and Lawrence 1988; Srilaorkul et al. 1989; Solanki and Rizvi 2001). In the production of highly concentrated (casein content ≥70%) milk protein powders (e.g., milk

protein concentrates, MPC), extensive diafiltration or washing is required to reduce the lactose content. This extensive washing reduces some of the CCP content and causes some casein dissociation. It is well known that extensive dialysis of casein micelles against water causes dissociation of caseins due to the loss of CCP.

8.4.4 *Effects of Ca Sequestrants (Chelating Agents) and Calcium Addition*

Sequestrants (e.g., citrates and phosphates) combine with polyvalent metal ions (e.g., Ca^{2+} or Mg^{2+}) to form soluble metal complexes. Chelating agents, such as ethylenediaminetetraacetic acid (EDTA), are complexes in which the metal ion is bound to two or more atoms of the chelating agent, usually in the form of a ring-type of structure. The addition of sequestrants or chelating agents to milk disrupts casein micelles by reducing the [Ca^{2+}] and CCP content (Munyua and Larsson-Raznikiewicz 1980; Visser et al. 1986; Udabage et al. 2000), which causes casein micelle dissociation (Morr 1967; Gaucheron 2005). Several studies have reported that some of the CCP crosslinks can be removed from micelles without causing a lot of protein dissociation; higher levels of Ca removal caused micellar disintegration (Lin et al. 1972; Griffin et al. 1988).

Removal of Ca from milk using an ion-exchange resin resulted in an increase in pH, a reduction in Ca^{2+}, an increase in ethanol stability, and an increase in the rennet coagulation time (Lin et al. 2006).

When comparing the various types of phosphates, the orthophosphates are relatively poor at complexing Ca. Comparing the ability to complex Ca, phosphates and citrates can be ranked in the following order: long-chain phosphates > tripolyphosphate > pyrophosphate > citrate > orthophosphate (Van Wazer and Callis 1958). Figure 8.1 shows a comparison of the [Ca^{2+}] remaining in solution in equilibrium with a 0.01 M solution of a number of sequestering agents (Van Wazer and Callis 1958). This figure demonstrates the relative complexing abilities of the orthophosphates (weak, more free Ca left in solution) with long-chain polyphosphates (strong, little free Ca left in solution).

In well-defined systems, the relative efficiency of sequestrants can be compared by considering the stability constants (formation constant, equilibrium constant) for a given metal (Furia 1972). In general terms, the stability constant of a metal (e.g., Ca^{2+}) complex can be calculated as follows (Furia 1972):

$$K = \frac{[\mathrm{ML}]}{[M][L]}$$

where M = metal ion, L = ligand (sequestrant, chelating agent), ML = metal complex.

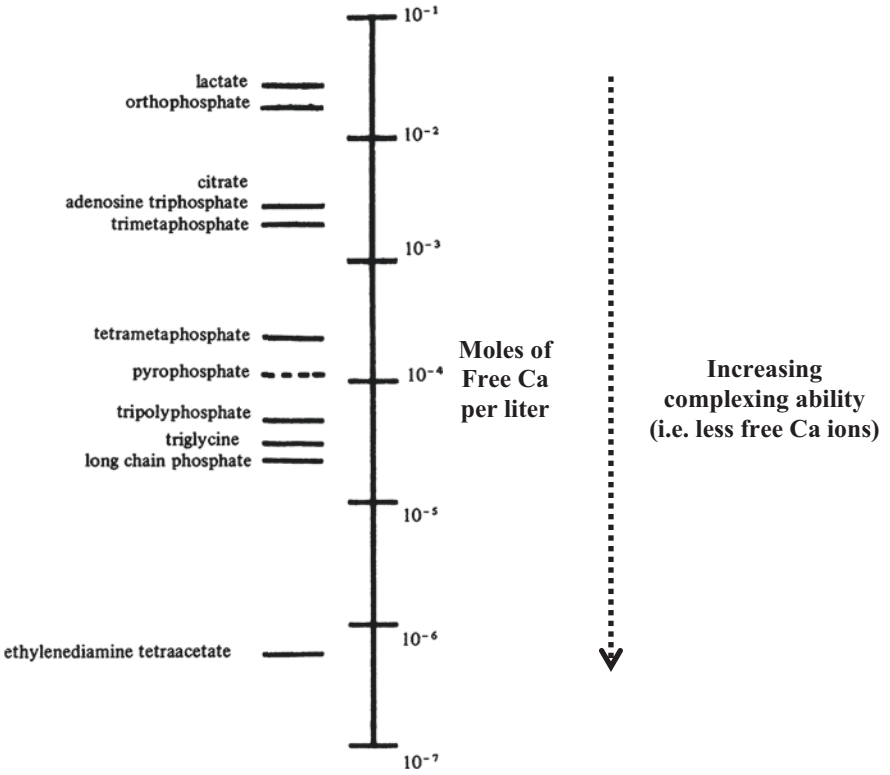

Fig. 8.1 The free calcium concentration (i.e., that not chelated or sequestered) for various types of complexing agents are estimated for the dissociation of a 0.01 M solution of the 1:1 Ca complex. Complexing agents that are lower on the scale (e.g., EDTA) are a stronger chelator for calcium. (Adapted from Van Wazer and Callis 1958)

The (log *K*) stability constants for Ca-chelates with citrate, pyrophosphate, and EDTA are 3.5, 5.0, and 10.7, respectively (Furia 1972). Higher values indicate a stronger tendency to form a complex.

The highly charged anionic nature of polyphosphates causes them to be attracted to, and to orient themselves along, the charged sites of other long-chain polyelectrolytes such as proteins (Van Wazer and Callis 1958). This should increase the charge repulsion between caseins at pH values above their isoelectric point (as in most dairy processing situations). At pH values below the isoelectric point, polyphosphates can induce protein precipitation by cation–anion interactions (Van Wazer and Callis 1958). Casein has been reported to precipitate or aggregate in the presence of phosphates (Fox et al. 1965). Some types of phosphates can crosslink caseins, e.g., pyrophosphates, and these can even induce casein gelation (Mizuno and Lucey 2005, 2007). Phosphate salts have also been used to cause heat-induced aggregation of caseins (Panouillé et al. 2004).

Calcium enrichment of milk is of interest for fortification purposes. Usually this involves adding different types of calcium salts, like those with chloride, lactate, gluconate, or citrate. The impact of calcium addition on milk properties was reviewed by Gaucheron (2010). Excessive levels of calcium addition can cause instability. Insoluble calcium salts like calcium phosphate have the unwanted issue of sedimentation. More common is the use of soluble salts like chloride or lactate. Addition of soluble calcium to milk causes an increase in the insoluble calcium fraction, even though much of the added salt remains in the serum phase; there is also a decrease in milk pH, reduced soluble casein, and an increase in turbidity (Philippe et al. 2003).

8.4.5 High Pressure

High hydrostatic pressure (HP) reduces the light-scattering of milk due to the disruption of casein micelles (Schmidt and Buchheim 1970). HP influences various properties of milk, including a reduction in the size of casein micelles, denaturation of β-lactoglobulin, and a reduction in CCP content (see reviews by Huppertz et al. 2002; López-Fandiño 2006; Munir et al. 2019). HP treatment influences the functional properties of proteins through the disruption of hydrogen bonds and hydrophobic interactions and the separation of ion pairs. The impact on the properties of casein depends not only on the pressure applied but also on factors such as the application time, pH, and temperature. It is well known that HP treatment at ≥300 MPa causes the disintegration of the casein micelles, as observed by a reduction in particle size (Needs et al. 2000; Garcıa-Risco et al. 2003). Micelle size is hardly unaffected, or is slightly increased, by pressures up to 250 MPa (Needs et al. 2000; Huppertz et al. 2004). Concomitant with these size changes, there is dissociation or aggregation (when there is an increase in size) of caseins. Huppertz and de Kruif (2006) proposed that the unfavorable exposure of hydrophobic surfaces at a pressure >200 MPa leads to the formation of larger casein particles from fragments of disrupted casein micelles during prolonged HP treatment. The interactions responsible for this re-association were likely to include van der Waals or hydrophobic interactions.

HP treatment solubilizes some of the CCP in raw (Schrader et al. 1997; López-Fandiño et al. 1998) and heat-treated milk (Gaucheron et al. 1997; Schrader et al. 1997). Some or nearly all of the CCP is restored during subsequent storage of HP-treated milk (Gaucheron et al. 1997: Schrader et al. 1997; Huppertz et al. 2006). Similar trends have been observed for milk of various species, although the magnitude of the changes in the state of the CCP varied (López-Fandiño et al. 1998; Huppertz et al. 2006). Some studies have found hardly any change in the concentration of soluble Ca after HP treatment (Law et al. 1998). It is presumed that, during HP, the solubilization of some of the CCP helps to cause casein micelle disintegration by disrupting one of the key crosslinking agents within micelles. Although pressure release helps to reverse the increase in soluble Ca during pressurization,

the original micelle structure is not reformed (Law et al. 1998). Micellar casein and MPC solutions that were HP-treated exhibited an increase in soluble calcium levels with treatments up to 350 MPa, and thereafter, a slight decrease in soluble calcium was observed (Cadesky et al. 2017).

8.5 Impact of Milk Salts on the Buffering Properties of Milk and Dairy Products

The buffering properties of dairy products have been reviewed by Singh et al. (1997) and Salaün et al. (2005). The affinity of acids and bases for H^+ may be expressed in terms of titration curves and dissociation constants. An acid–base titration curve is a plot of pH versus the amount of acid or base neutralized in the titration. The buffering value (index) at any pH may be determined graphically from the slope of the tangent to the titration curve at that pH. If the added alkali or acid is dB, and the resulting change in pH is dpH, then the average buffering value, i.e., the amount of acid or base required to cause a predetermined change in pH (dpH), is the differential ratio, dB/dpH (Van Slyke 1922), where:

$$\frac{\mathrm{dB}}{dpH} = \frac{\left(\text{ml of acid or base added}\right) \times \left(\text{normality of acid or base}\right)}{\left(\text{average volume of sample}\right) \times \left(pH \text{ change produced}\right)}$$

Apart from casein, the principal buffering components in milk are soluble phosphate, CCP, citrate, and bicarbonate. Srilaorkul et al. (1989) estimated that the contribution of casein, whey proteins and milk salts to the buffering of skim milk was 36.0%, 5.4%, and 58.6%, respectively. Lucey et al. (1993b) reported that, in the pH range 6.7–4.0, soluble salts and whey proteins (i.e., the substances in rennet whey), CCP, and casein contributed approximately 47%, 21%, and 32%, respectively, of the buffering in milk.

When milk is acidified (Fig. 8.2a), maximum buffering occurs at approximately pH 5.1 but, when acidified milk is back-titrated with base, there is low buffering at pH 5.1 and maximum buffering occurs at pH ~ 6.3. The maximum in the buffering curve at pH ~5.1 is due to the solubilization of CCP, which results in the formation of phosphate ions that combine with H^+ (to form HPO_4^{2-} and $H_2PO_4^-$), resulting in pH buffering (Lucey et al. 1993b). The removal of the CCP from milk, as in CCP-free milk made by the method of Pyne and McGann (1960) (cold acidification of milk to pH ~4.9 and extensive dialysis against normal milk to restore the original pH), results in the absence of the buffering peak at pH 5.1 during acid titration (Fig. 8.2b). When the acidified milk sample is back-titrated with base, buffering is low at pH 5.1, because CCP is already solubilized, but maximum buffering occurs

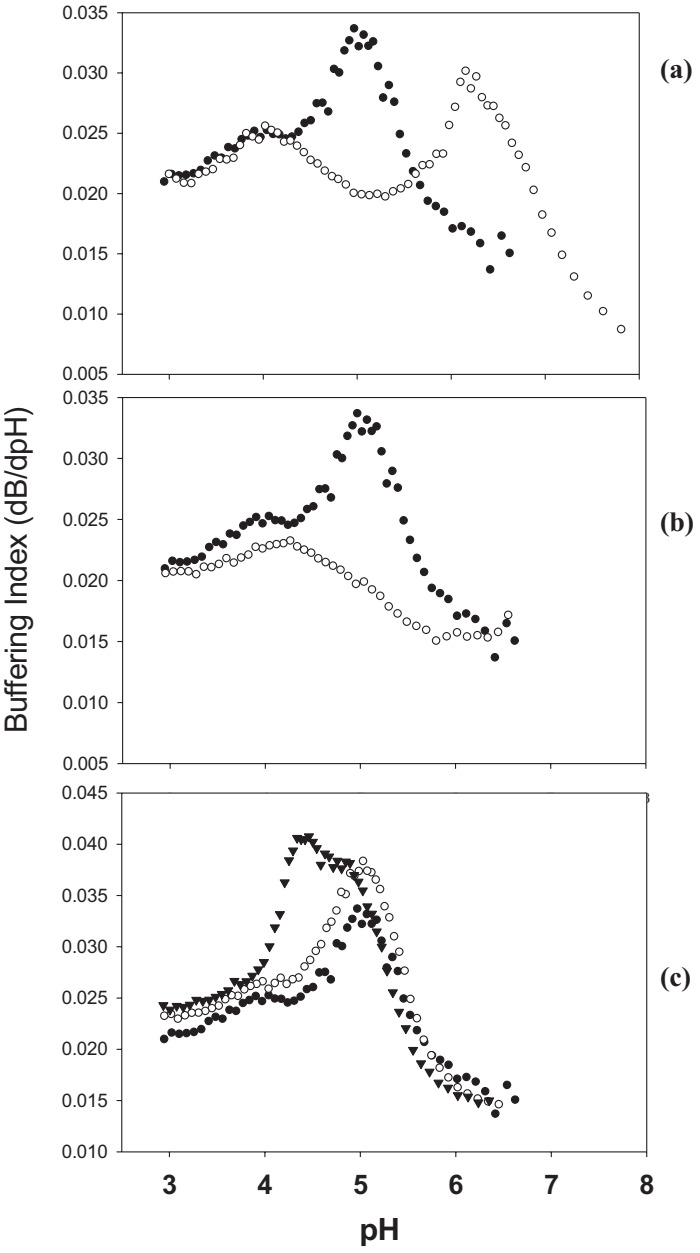

Fig. 8.2 Acid–base buffering curves of (**a**) milk titrated from its initial pH to pH 3.0 with 0.5N HCl (filled circle) and back titrated to pH 8.0 with 0.5N NaOH (open circle); (**b**) milk (filled circle) and colloidal calcium phosphate-free milk (open circle) titrated from the initial pH to pH 3.0 with 0.5N HCl; (**c**) titration of unheated milk (filled circle), milk heated at 100 °C for 10 min (open circle), milk heated at 120 °C for 15 min (filled inverted triangle). Milks were titrated from the initial pH to pH 3.0 with 0.5N HCl. (Adapted from Lucey et al. 1993a, b)

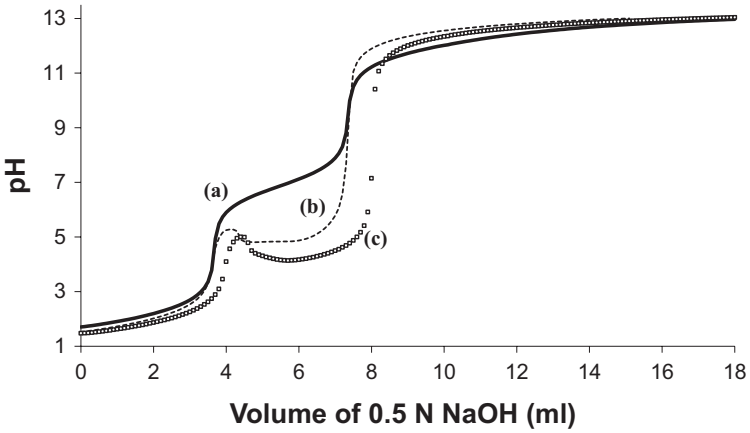

Fig. 8.3 Potentiometric titrations of 400 mg phosphoric acid with 0.5N NaOH in the presence of (a) no calcium, (b) 325 mg $CaCl_2$, (c) 650 mg $CaCl_2$; the method reported by Visser (1962) was used for these titrations (Salim and Lucey, unpublished data)

at pH 6.3, due to the formation of Ca phosphate (precipitation) with the release of H^+ (from HPO_4^{2-} and $H_2PO_4^-$) (Lucey et al. 1993b).

High heat treatments cause an increase in CCP due to the formation of heat-induced CCP (Fig. 8.2c). Some of the heat-induced CCP solubilizes on cooling (depending on the equilibration time allowed), but there is a substantial shift in the type of buffering curve observed during the acidification of very severely heated milk (e.g., 120 °C for 15 min) (Fig. 8.2c).

A strong buffering effect in the pH range 6–7 arises from the formation of Ca phosphate, as can be seen in the titration of phosphoric acid in the presence of Ca (Fig. 8.3). This buffering effect due to precipitation of Ca phosphate has been reported by many investigators, e.g., Visser (1962). Due to the precipitation of Ca phosphate around pH 6, the titration behavior of phosphoric acid in the presence of Ca is completely different compared to when this titration is performed in the absence of Ca (Fig. 8.3). In milk, both Ca and phosphate are present which suggests that this behavior would occur in dairy products. As is shown in Fig. 8.3, the onset of precipitation of Ca phosphate results in the release of H^+. We can speculate that this release of H^+ could also occur during the formation of CCP in the mammary gland and may contribute to the lower pH of milk compared to that of blood.

Acid–base buffering analysis is now widely used to indicate changes in the amount and type of CCP in milk as influenced by various treatments like the addition of chelating salts or heat treatment (e.g., Mizuno and Lucey 2005).

8.6 Interactions Between Milk Salts and Casein

8.6.1 Introduction

Caseins constitute approximately 80% of the protein in bovine milk, with four main types (α_{s1}-, α_{s2}-, β-, and κ-caseins; casein fragments can be produced as a result of proteolysis). Caseins are found in combination with appreciable quantities of micellar or CCP, sometimes called CCP nanoclusters, in the form of aggregates called casein micelles (Holt 1992). Casein plays a critical role in making milk super-saturated with Ca phosphate. As a packaging system, the micelles convert the milk into a free-flowing, low viscosity fluid and provide the means to (safely) transport the high levels of Ca and phosphate at concentrations which would normally precipitate in the mammary gland in the absence of the caseins. The CCP is completely soluble at pH values <5 (Pyne and McGann 1960; Lyster 1979) and the released Ca and phosphate are then available for absorption by the digestive system.

The caseins are a family of phosphoproteins found in the milks of all mammals. They are members of the group of Ca-phosphate-sequestering proteins which include dentine, bone matrix proteins and salivary proteins, among others (Kawasaki and Weiss 2003). Phosphorylation is a posttranslation modification of the caseins, and it occurs at serine residues, or rarely threonine, following a recognized template sequence Ser-X-Y, where X is any amino acid and Y=Glu, SerP or Asp. Due to the placing of the serine residues along the molecular sequences of α_{s1}-, α_{s2}-, and β-caseins, most of the phosphorylated residues are found in clusters. Thus, four of the five phosphorylated serine residues in bovine β-casein are found between positions 15 and 19, with the fifth at position 35. Four of the eight serines in bovine α_{s1}-casein are located between positions 64 and 68, with two more downstream at positions 46 and 48 and one upstream at position 75. Bovine α_{s2}-casein can have a variable level of phosphorylation from 10 to 13 mol P per mole of protein. The most abundant of these, α_{S2}-casein-11P, has three groupings of phosphorylated residues, one cluster of three from residues 8–10, four SerP spread as a group of three from 56 to 58 with the fourth member at 61, with the third cluster of two at positions 129 and 131. The remaining two single Ser-P residues of the total of 11 are located at positions 16 and 135 (Horne 2002). κ-Casein is unique among the caseins due to the absence of phosphoseryl clusters; most molecules of κ-casein contain only one phosphoserine residue, rarely two or three, and all are singlets located in the hydrophilic C-terminal region. The caseins (apart from κ-casein) are therefore sensitive to coagulation or precipitation by Ca. Horne and Dalgleish (1980) demonstrated that the logarithm of this critical coagulation time is a linear function of Q^2, where Q is the net negative charge on the protein, taking into account the binding of Ca to the casein. This linear correlation was also maintained when protein charge was changed following chemical modification of charged residues along the protein chain (Horne 1979, 1983; Horne and Moir 1984).

8.6.2 Casein Micelle Formation

The caseins are sensitive to precipitation due to the presence of approximately 30 mM Ca in milk. However, a key biological purpose of milk is to provide the high concentrations of the essential Ca and phosphate required for the growth of the newborn mammal. So how are these two conflicting factors resolved? The solution involves casein micelle formation, the formation of an insoluble CCP phase within the micelles, and the requirement for one of the caseins (usually κ-casein) to be insensitive to Ca and provide stability against Ca-induced precipitation to the other caseins. We also note that this insoluble CCP phase is never found outside the casein micelle but is an integral part of it. The details of how it forms within on-going micelle synthesis have been the subject of much debate and intensive study. For a discussion of the various casein micelle models, the reader is referred to various reviews (Farrell 1973; Slattery 1976; Rollema 1992; De Kruif and Holt 2003; Farrell et al. 2006; Qi 2007; Fox and Brodkorb 2008). We will focus our explanations on the dual-binding approach for micelle formation as described by Horne (1998, 2002, 2006, 2009), and our perspectives on the nature of casein interactions (Lucey and Horne 2018).

In the dual-binding model (Fig. 8.4), micellar assembly and growth take place by a polymerization process involving two distinct forms of bonding/interactions, namely association through clustering of hydrophobic regions/patches of the caseins

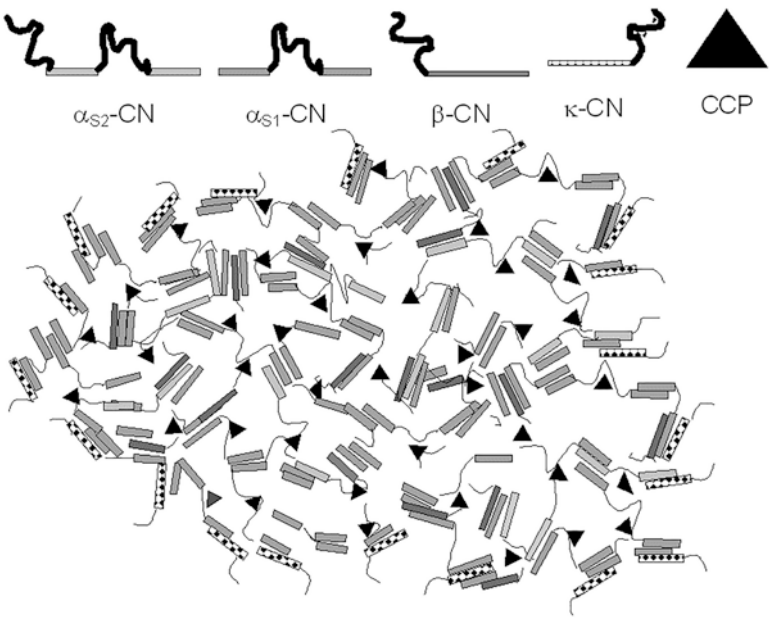

Fig. 8.4 Dual-binding model for the casein micelle. CN is casein and CCP is colloidal calcium phosphate (Horne 1998)

and, secondly, linking of several phosphopeptides into the Ca phosphate nanoclusters (insoluble CCP phase). Central to the model is the concept that bond formation is facilitated and, hence, micellar integrity and stability are maintained, by a local excess of hydrophobic attraction over electrostatic repulsion (otherwise, if the repulsive interactions were too large, little association of casein would occur and micelle formation would not be observed in milk). It should be noted that there are quite different ranges for these interaction components. Compared to hydrophobic interactions, electrostatic repulsion is a long-range force. Clustering of charged groups in specific regions of the protein molecule means that electric dipole moments may be large, so that their effects on interparticle interactions may be rather strong (Piazza 2004).

Each casein molecule effectively functions as a block copolymer, with the hydrophobic region(s) offering the opportunity for a multitude of individual, weak, hydrophobic interactions (driven by the thermodynamically favorable exclusion of water by this type of association). The hydrophilic regions of the casein molecules contain the phosphoserine cluster (or clusters), with the exception of κ-casein which has no such cluster, each offering multiple functionalities for cross-linking. α_{s1}-Casein can polymerize (self-associate) through the hydrophobic blocks, forming a worm-like chain. Further growth is limited by the strong electrostatic repulsion of the hydrophilic regions, but, in the casein micelle, the negative charges of the phosphoserine clusters are neutralized by inserting their phosphate groups into a facet of the Ca phosphate nanocluster.

This has two very important implications for the micelle. Firstly, by removal of a major component of electrostatic repulsion, it increases the propensity for hydrophobic bonding upstream and downstream of the nanocluster link, and thus effectively permits and strengthens those bonds. Secondly, it allows for multiple protein binding to each nanocluster (on different facets), allowing a network to be built up. β-Casein, with only two blocks, a hydrophilic region containing its phosphoserine cluster, and the hydrophobic C-terminal tail, can form polymer links into the network through both, allowing further chain extension through both. α_{s2}-Casein is envisaged in this model as having two of each block, two (possibly three, see below) phosphoserine clusters, and two hydrophobic regions. It is only a small fraction of the total complement of bovine casein but, by being able to sustain growth through all its blocks, it is likely to be bound tightly into the network. α_s-Caseins cannot be essential to micelle formation as human milk contains only trace amounts (~0.06% of total protein) of α_s-caseins (Lönnerdal 2004), and yet micelles are formed. κ-Casein is the most important of the caseins in the dual-binding model of micellar assembly and structure. It can link into the growing chains through its hydrophobic N-terminal block, but its C-terminal block is hydrophilic and cannot sustain growth by linking hydrophobically to another casein molecule. Nor does κ-casein possess a phosphoserine cluster, and therefore, it cannot extend the polymer cluster through a nanocluster link. Thus, chain and network growth are terminated wherever κ-casein joins the chain.

This polymerization process leaves the network with an outer layer dominated by κ-casein although other caseins are also present at, or close to, the surface (Dalgleish 1998). Srinivasan and Lucey (2002) studied the impact of plasmin on the rennet coagulation of skim milk. They found that even partial hydrolysis of β- and α_s-caseins accelerated the rennet coagulation time of milk. Plasmin has very little proteolytic activity against κ-casein. Srinivasan and Lucey (2002) hypothesized that plasmin could have degraded β-casein "hairs" present on the surface of micelles and that this could have reduced the repulsive barrier to aggregation of rennet-altered micelles such that aggregation could occur at a lower degree of κ-casein hydrolysis.

Other evidence that some β-casein may be on, or close to, the micelle surface is that a considerable proportion (up to about 20%) of β-casein can dissociate from the micelle at low temperatures; this also occurs in ovine micelles but to a much lesser extent in porcine milk due to its high CCP content (Umeda 2005; Umeda and Aoki 2005; Umeda et al. 2005). Some CCP also dissolves at low temperatures and that occurrence might also weaken the interactions between β-casein molecules and the rest of the micelle. Low temperature also reduces calcium binding by caseins, thereby enhancing electrostatic repulsion between caseins (Horne and Lucey 2014). How can a considerable proportion of β-casein dissociate at low temperatures while little κ-casein dissociates even though κ-casein is mainly on the surface? It is possible that κ-casein becomes polymerized by S-S bridging between κ-casein molecules (Farrell et al. 1996) after it has terminated the growth of the casein chains. If κ-casein polymers are formed (in vivo), it is likely that these polymers would have greater attachment/linkage to the rest of the micelle structure, making them more difficult to remove. Also, the attractive balance in κ-casein is not very sensitive to changes in phosphoserine involvement in CCP nanoclusters (so the loss of some CCP crosslinks at low temperatures does not have any major impact on its dissociation from the micelle) as they do not interact through CCP crosslinks. In contrast, for β-casein, if some of the CCP crosslinks are dissolved at low temperature, then the exposed negative charge on the phosphoserine residues would make the binding of β-casein to other casein molecules unfavorable. This type of process could allow some of the β-casein to dissociate as temperature is lowered. It is likely that the β-casein that dissociates is closer to the micelle surface, or if not, then the β-casein freed by this process would have some potential chances to re-attach/associate with other caseins as it diffuses through the inner micelle network out to the bulk solution.

κ-Casein-deficient mice, produced by genetic modification, were unable to lactate because of destabilization of the micelles in the lumina of the mammary gland (Shekar et al. 2006). The milk of most species appears to have a κ-casein or Ca-stabilizing casein (i.e., a casein that does not have a phosphate cluster), whereas a few milks contain little or no α_s-caseins (human milk) and various ratios of α_s- to β-caseins.

8.6.3 *Nature of Colloidal Calcium Phosphate and Size of Nanoclusters*

The nature of CCP or micellar Ca phosphate (as it is sometimes called) has been the subject of intense study and debate over the years. There have been several reviews of the nature of CCP (Pyne 1934; McGann and Pyne 1960; Schmidt 1980; van Dijk 1990; Holt 1992, 1995; De Kruif and Holt 2003). Schmidt (1980, 1982) considered CCP to be a ubiquitous coating or "cement" that bonded many casein molecules together. McGann et al. (1983a, b) reported that the CCP depositions in milk systems consist of spherical granules (other later names for this granule include nanoclusters) 2–3 nm in diameter. Such a large entity is incompatible with the small type of CCP structures proposed by van Dijk (1990) or Schmidt (1980) but is smaller than the nanocluster structures of 2.7 nm *radius* recently proposed by Holt et al. (See Bijl et al. 2019, for earlier references and latest developments).

For many years, CCP was believed to a basic Ca phosphate salt (e.g., Pyne and McGann 1960). Pyne and McGann (1960) and McGann et al. (1983a) reported that in CCP, the Ca/P_i ratio is >1.5, which would make it some type of basic salt, like apatite or tricalcium phosphate (e.g., $Ca_3(PO_4)_2$). Citrate and magnesium are also associated with the CCP phase. These studies only consider the inorganic phosphate content of CCP, and, if the ester phosphate content is taken into account, the Ca:P ratio moves closer to the stoichiometry of the mineral brushite (Holt 1997). Evidence that this is maintained across the milks of different species comes from the work of Jenness (1979), who observed linear correlations in plots of total milk calcium and total milk phosphorus versus the casein contents of the milks of 33 species. Assuming a casein monomer in these milks to have a mean molecular weight of 22,500 Da, the slope values of these plots corresponded to 20 Ca^{2+} ions/monomer and 18 mol of micellar phosphate (ester bound + inorganic P)/monomer, that is, close to the 1:1 stoichiometry of dicalcium phosphate. It should be noted that Ca^{2+} ions can also bind to carboxyl groups of the caseins and would then be included as micellar-bound ions in this calculation.

Evidence for the presence of crystalline brushite in the nanoclusters also comes from Holt et al. (1982), who found that the spectrum of milk calcium phosphate obtained by X-ray fine structure absorption was close to that of a brushite sample, but only the earliest peaks in the radial distribution functions could be obtained. Spectra recorded from the lyophilized micelles of pig, goat, rabbit, rat, and human milk samples showed the same short-range environment for Ca and closely resembled that of brushite (Irlam et al. 1985). The lack of longer-range structure in the EXAFS radial distribution functions was taken to imply that the nanoclusters were amorphous, lacking in crystal structure, following the approach of many earlier studies (Pyne and McGann 1960; Knoop et al. 1979; McGann et al. 1983b; Lyster et al. 1984). However, again, these studies only considered basic amorphous calcium phosphates with Ca:P ratios close to 1.5. Lu et al. (2019) carried out a study of the short-range structure of amorphous calcium hydrogen phosphate (ACHP), the acidic salt with Ca:P ratio 1.0. They found the X-ray powder diffraction (XRD)

spectra from these two forms to be typically amorphous and very similar, each with two peaks around the same 2 × theta values (angle between transmitted X-ray beam and the reflected beam), but both quite different from the XRD spectrum of milk CCP published by Wang et al. (2020) which has only one peak and a higher-angle shoulder, both at different 2 × theta values from the Lu et al. (2019) data. Horne et al. (2007) have argued previously that an apparent amorphous XRD spectrum could also be due to the small size of scattering entities, a view upheld by Lenton et al. (2016), who calculated the Ca phosphate XRD spectrum expected as the crystals grew in size, but the spectrum showed an amorphous nature below a 5 nm limit. Most measurements of CCP nanocluster size place the diameter around 2.5 nm (McGann et al. 1983b; McMahon and McManus 1998; Marchin et al. 2007; Kamigaki et al. 2018).

All of these observations are accommodated in the model for nanocluster structure and formation developed by Horne et al. (2007) and refined in Horne (2009, 2014, 2020) and Lucey and Horne (2018). In this model, the ester phosphates of the caseins are viewed as integrated into a brushite crystalline lattice structure. In this, they must have a surface location. We see the formation of nanoclusters as forming via a biomineralization mechanism, with the phosphate centers of the caseins as a facet of the nanocluster first initiating the reaction through the binding of calcium to serine (organic) phosphate. This is stronger than the calcium bond with an isolated (inorganic) phosphate anion and is therefore favored (Mekmene and Gaucheron 2011; Bijl et al. 2019). This structure then accumulates further phosphate and calcium ions in a brushite lattice framework, which is closed off to further growth and completed by other nearby phosphoserine clusters. These clusters can come from the same casein molecule or from other casein molecules in the vicinity. These, in turn, may be part of other phosphate nanoclusters in an extended interlinked network. A minimum of four phosphate centers or facets is envisaged for each nanocluster, giving a tetrahedral structure, though six such facets in a bi-pyramid is another possibility. Such structures preserve the stoichiometry between ester and inorganic phosphate required to allow the Ca:P_i ratio to be 1.5 and Ca:(P_i + P_{Ser}) to be 1.0. Their small size falls within the limits observed in electron microscopy studies and their molecular weights are in the range estimated for excised CCP nanoclusters by Choi et al. (2011).

Holt et al. (see Lenton et al. 2020 for references) provided an alternative mechanism for the creation of calcium phosphate nanoclusters, suggesting that the phosphate centers of the caseins inhibit the growth of a core of nucleating mineral calcium phosphate. The latest update of this concept has calcium ions binding to the phosphoserines before sequestering the mineral nuclei (Bijl et al. 2019) While this recognizes that the calcium binding to the serine phosphate is stronger than the bond to inorganic phosphate (Bijl et al. 2019; Mekmene and Gaucheron 2011), it raises the question of where the system finds the calcium ions necessary to grow the mineral nucleating calcium phosphate core. This is the question persistently raised by Horne (Horne 2006, 2009, 2014, 2020; Horne et al. 2007; Lucey and Horne 2018), all arising from the observation that in mixtures of α_{S1}-casein, phosphate, and calcium precipitate at far higher rates than would be predicted in the absence of protein

(Horne 1982). That the phosphate centers should simply coat a mineral core to form the nanoclusters is akin to suggesting that Shakespeare have the eponymous hero of his tragedy, Hamlet, appear for the first and only time in the last scene of the final act to close the curtains. This argues instead for the active role we have suggested above; growth of the nanoclusters is initiated, controlled and finally terminated by the phosphoserines.

The pK_a values for phosphate reported in chemistry textbooks are 2.1, 6.9, and 12.0, and Walstra and Jenness (1984) suggested that, on comparing the pH of milk (~6.7) with the pK_{a2} of phosphoric acid, one would expect $CaHPO_4$ to be the form of CCP. However, upon addition of NaOH, in the presence of Ca, all phosphates (pK_{a2} and pK_{a3}) are titrated around pH 6–7 due to the precipitation of Ca phosphate (Fig. 8.3). That observation, and the strong buffering at pH ~5.1 during acid titration of milk (Fig. 8.2a) caused by the protonation of the released phosphate ions (from CCP), suggests that the form of CCP in milk is less likely to be an acidic form (like $CaHPO \cdot 2H_2O$) and may be a more basic form (e.g., tricalcium phosphate). Other titration studies, including those with oxalate (Pyne and Ryan 1950; Jenness 1973), have indicated that most P_i in CCP is in the form of PO_4^{3-} (i.e., tricalcium phosphate). Holt (1985) suggested that there may be a difficulty in titration studies if it is assumed that the exposed phosphoserine groups, after the dissolution of CCP, do not contribute to some part of the titration. This suggestion by Holt (1985) does not explain the acid–base buffering behavior shown in Fig. 8.2a, as back-titration with base indicated that CCP does indeed contribute to the buffering at pH ~5. Removal of CCP resulted in the elimination of the buffering peak at pH ~5 (Fig. 8.2b). Any calculation of milk salt equilibria needs to take into account the unexpected pK_{a2} and pK_{a3} values of phosphate in milk/serum, although this does not appear to have always been the case.

8.7 Functional Properties of Milk Products

Milk salts greatly influence the functional properties of milk and various dairy products primarily by influencing the structural integrity of micelles or the sensitivity to aggregation of caseins. There have been a number of reviews on the effects of salts on the functionality of milk products (e.g., Augustin 2000).

8.7.1 Rennet-Induced Gels

Generally, it is thought that Ca does not directly affect the enzymatic phase of rennet gelation of milk, although addition of $CaCl_2$ does reduce milk pH, which accelerates the hydrolysis reaction of rennet on κ-casein (Lucey and Fox 1993). Rennet-altered micelles will aggregate only in the presence of free Ca^{2+}, and gelation occurs only if there is sufficient CCP present (i.e., it needs some type of casein

micellar structure, as sodium caseinate does not form a rennet-induced gel, even though there is release of the macropeptide). Addition of (<50 mM) Ca reduces the rennet gelation time, even at a constant milk pH, and flocculation occurs at a lower degree of κ-casein hydrolysis. Addition of Ca increases the rate of firming of renneted milk gels, mainly by neutralization of the negatively charged groups on the micelle surface and possibly by the formation of Ca bridges. Addition of high concentrations of Ca (e.g., >0.1 M) reduces the rate of gel firming, probably by increasing the effective (positive) surface charge on the micelles. Other alkali earth metals (like Sr and Mg) have similar impacts on rennet gelation to Ca addition (Cooke and McSweeney 2014).

Addition of up to 10 mM Ca increases the strength of rennet-induced gels (Lucey and Fox 1993). Low levels (≤0.02% $CaCl_2$) are often added by cheesemakers to help standardize the gelation process (e.g., cutting time); increased firmness and lower meltability are two other impacts likely in the cheese. Milk with high pH values (e.g., late-lactation, mastitic or non-coagulating individual milks) has poor renneting behavior that can be improved by addition of calcium chloride. Combinations of pH, NaCl, and $CaCl_2$ have been used to cause specific modification in the casein micelles in order to optimize rennet gelation properties (Lazzaro et al. 2020).

Reduction of the CCP content of casein micelles by ~30% prevents gelation unless [Ca^{2+}] is increased (Shalabi and Fox 1982). Udabage et al. (2001) investigated the effects of mineral salts and Ca sequestrants or chelating agents on the gelation of renneted skim milk. They found that, depending on the level of chelating agent, addition of citrate or ethylenediaminetetraacetic acid (EDTA) reduced the storage modulus (G') of rennet-induced gels, and, above a certain concentration, (rennet gelation was completely inhibited 10 mmol kg^{-1} milk).

Choi et al. (2007) demonstrated that the concentration of insoluble Ca phosphate (CCP) associated with the casein micelles had an important influence on the properties of rennet-induced gels. Removal of some CCP from milk prior to gelation using a Ca-chelator lowered the storage modulus of rennet-induced gels due to the reduction in the amount of CCP crosslinking in casein micelles. A reduction in the CCP content prior to rennet-induced gelation resulted in gels with higher loss tangent (LT) values, indicating greater bond mobility.

The swelling, hydration, and solubility of casein micelles in renneted milk are greatly increased in the presence of NaCl but markedly reduced if the brine solution contains Ca (Lucey and Fox 1993). The addition of high concentrations of NaCl causes a reduction in rennet coagulation time. In some cheese varieties, salt is added to the cheesemilk (e.g., Domiati), resulting in a slower set and weaker curd (Fahmi and Shahara 1950). These changes are largely reversible on removal of the excess NaCl by exhaustive dialysis against bulk milk (Huppertz 2007).

Casein concentrates (both liquid and dried forms) made using extensive diafiltration often require calcium addition to have adequate rennet coagulation properties. The diafiltration process removes soluble components, including Ca^{2+}, that are needed for renneting.

8.7.2 Acid-Induced Milk Gels

Acid-induced casein gels can be made from sodium caseinate, indicating that the presence of CCP is not a requirement for the formation of acid casein gels (Lucey et al. 1997). Since CCP is completely soluble at pH ≤5, CCP crosslinks do not contribute to the final (or at least at pH values <5) stiffness of acid milk gels. The rate and extent of CCP solubilization during the gelation process is an important variable influencing acid milk gel properties. In particular, the solubilization of CCP after gelation in acid gels made from heated milk results in an increase in the loss tangent (LT) value (which is the ratio of viscous to elastic moduli). Acid gels made from unheated milk do not exhibit this maximum in the LT behavior, due to their lower pH of gelation, because CCP had mostly dissolved prior to gelation.

The addition of Ca-chelating agents to milk has been reported to increase firmness of acid milk gels made with glucono-δ-lactone (GDL) (Johnston and Murphy 1992). Addition of EDTA also caused an increase in the LT value in acid-heat-induced skim milk gels (Goddard and Augustin 1995). Ozcan-Yilsay et al. (2007) studied the effect of trisodium citrate (TSC) on the rheological, physical properties, and microstructure of set yogurt. The storage modulus of gels increased significantly on addition of low levels of TSC, and highest values were observed in samples with 10–20 mM TSC; higher (>20 mM) TSC concentrations resulted in a large decrease in stiffness and longer gelation times. No maximum in LT was observed in yogurts made with ≥25 mM of TSC as CCP was dissolved completely prior to gelation. Partial removal of CCP resulted in an increase in the LT at pH 5.1. Ozcan-Yilsay et al. (2007) suggested that, at low TSC levels, the removal of CCP crosslinks may have facilitated greater rearrangement and molecular mobility of the micelle structure, which may have helped to increase the storage modulus and LT of gels by increasing the formation of crosslinks between strands. Ozcan-Yilsay et al. (2007) also concluded that the LT maximum observed in yogurts made from heated milk was due to the presence of CCP, as the modification of the CCP content altered this peak and removal of CCP eliminated this feature in the LT profiles. Ramasubramanian et al. (2008) reported that neither calcium addition nor chelation by citrate significantly altered the viscosity of stirred yogurt, although Kamal et al. (2017) indicated that addition of $CaCl_2$ did increase the firmness of acid set gels (made with GDL).

Ozcan et al. (2011) studied the effect of increasing the CCP content of heated milk on yogurt gelation properties; there was no major change in the storage modulus values when the CCP was increased to 116%, but the gelation pH did increase.

Roefs and van Vliet (1990) reported that increasing the concentration of NaCl added to cold-acidified skim milk samples resulted in a decrease in the dynamic moduli of the gels formed when these samples were warmed. This indicated that electrostatic interactions are important for particle interactions. At high ionic strengths, charged groups on casein particles would be screened, thereby weakening interactions between particles, which would result in a slower rate of increase of the storage moduli. In preparing Na caseinate gels by cold acidification, the addition of at least 0.1 M NaCl (to the acidified sample) was necessary to prevent precipitation

during the warming up procedure (Roefs and van Vliet 1990). Possibly, the primary effect of NaCl was to reduce rearrangement during the aggregation stage of gel formation. The addition of a high concentration of NaCl (>0.24 mol L^{-1}) to cold acidified milk prevented gel formation when it was subsequently heated to a higher temperature for gelation (Roefs and van Vliet 1990). Lucey et al. (1997) studied the impact of NaCl on the properties of acid casein gels. They found that the pH at gelation was lower, ≤5.0, in gels made with added NaCl than in gels made without added NaCl (pH ~5.1).

Low-methoxyl pectin is often used as a stabilizer in acid milk gel systems. Harte et al. (2007) proposed that, during the acidification of milk, the release of Ca^{2+} arising from the solubilization of CCP induces the formation of pectin-pectin complexes and, at lower pH values, these complexes interact with the casein particles. For acid casein gels made in the absence of Ca ions, a substantial reduction in the storage modulus was detected at pectin concentrations as low as 0.01–0.02% (w/v), and there was a significant increase in gelation time at pectin concentrations ≥0.05% (w/v) (Matia-Merino et al. 2004). Complete inhibition of acid-induced gelation of casein was noted at ≥0.8% (w/v) pectin. Addition of Ca at low pectin contents (<0.2%) reduced the modulus of acid milk gels but there was a large increase in the storage modulus at higher levels of pectin (≥0.2%, w/v).

8.7.3 Heat-Induced Whey Protein Gels

Salts have a major effect on the type, as well as the mechanical/sensory properties, of whey protein gels formed as a result of heat treatment. It is generally recognized that the addition of $CaCl_2$ to dialyzed samples of whey protein concentrate (WPC) or whey protein isolate (WPI) results in an increase in gel strength. Above a level of 10–20 mM $CaCl_2$, gel firmness starts to decrease (Schmidt et al. 1979; Kuhn and Foegeding 1991). It has been speculated that excessive Ca causes rapid protein aggregation (due to decreased protein stability), which limits protein unfolding and network formation (Mangino 1992). Caussin et al. (2003) reported that the addition of Ca to whey proteins resulted in the formation of very large protein aggregates during heating. Most commercially available WPC products probably have a Ca content that is higher than that required for optimal gel strength (Mangino 1992). There is considerable variability in the thermal aggregation behavior of commercial whey products and some of these differences could be removed by dialysis of these samples to a common ionic strength (McPhail and Holt 1999). The concentrations of divalent cations are higher in WPC made from cheese whey than in WPC made from acid whey, and these cations are not easily removed by dialysis, suggesting some binding by the whey proteins (Havea et al. 2001). Membrane filtration of acid whey WPC at low pH values resulted in a greater extent of demineralization. WPC made from acid whey is a superior heat-gelling product compared with cheese whey WPC (Veith and Reynolds 2004). These differences could be due to the absence of GMP and the low Ca concentration in acid whey WPC. Adjusting cheese whey to

low pH values prior to filtration/diafiltration can also cause flocculation of residual lipoprotein complexes, which has been used as a method to remove residual lipids from whey (Breslau et al. 1975).

8.7.4 Cold-Set Whey Protein Gels

Whey protein gels can also be produced using a two-step process that involves heat treatment at low ionic strength and/or far from the isoelectric point, followed by an increase in ionic strength and/or adjustment of pH (Barbut and Foegeding 1993; Britten and Giroux 2001). These gels are called cold-set gels, as the initial heat treatment produces a polymerized solution and gelation can occur at low temperatures (≤ ambient) if the repulsive forces are screened by the addition of mono- or poly-valent cations (e.g., Ca^{2+}) or a decrease in pH (e.g., through the addition of GDL or bacterial fermentation). To obtain gels via the cold-set gelation method, it is necessary to first prepare a solution of heat-denatured proteins, with a protein concentration below the critical gelation concentration. Heating (e.g., 80 °C for 30 min) results in the formation of soluble, denatured whey protein aggregates. Whey protein fibril-type gels are formed at very low pH values (e.g., 2) and cold-set fibril gels can also be made by the addition of Ca^{2+} (Bolder et al. 2006).

8.7.5 Emulsions

Caseins, especially caseinates, are widely used as emulsifiers (Dickinson 1997). The aggregation state of casein greatly influences surface activity, with sodium caseinate (non-micellar) having greater surface activity than micellar or Ca caseinate (Mulvihill and Murphy 1991). Dalgleish (1987) reported that emulsions prepared with α_s- or β-casein were sensitive to precipitation by Ca but that emulsions prepared with κ-casein did not aggregate on Ca addition. The phosphoserine residues in β-casein helped that molecules maintain a thick steric stabilizing monolayer on emulsion interfaces (Dickinson 1997). Increasing ionic strength by the addition of electrolytes screens out the double-layer repulsion and therefore reduces the electrostatic stabilization of proteins. Therefore, emulsions prepared with commercial milk protein ingredients of high salt content may be more susceptible to flocculation than model systems prepared with pure proteins dissolved in low ionic strength buffer solutions (Dickinson 1997). Calcium ions influence the stability of sodium caseinate-stabilized emulsions (Ye and Singh 2001). Addition of $CaCl_2$ before or after homogenization caused a decrease in the creaming stability of emulsions made with 0.5% sodium caseinate. In contrast, addition of $CaCl_2$ up to ~10 mM increased the creaming stability of emulsions made with 3% sodium caseinate, although the stability decreased again >20 mM $CaCl_2$. There was an increase in the surface

protein concentration with an increase in the level of $CaCl_2$, which was due to enhanced adsorption of the α_s-caseins (Ye and Singh 2001).

8.7.6 Foaming and Rehydration Properties After Spray Drying

Milk exhibits improved foam expansion when treated with EDTA (Ward et al. 1997), probably due to disruption of the micellar structure following the chelation of calcium from CCP. The Ca concentration influences the interactions of β-casein at the air–water interface; in the absence of Ca, a weak interfacial gel forms, whereas, with Ca addition, a strong interfacial gel forms quickly (Vessely et al. 2005). Added calcium chloride increases, and calcium-chelating agents decreases, the foam stability of skim milk (Kamath et al. 2011), presumably by altering casein–calcium interactions that contribute to the stabilization of the foam structure. The foamability of reconstituted skim milk powder increased as the NaCl concentration was increased from 0 to 0.8 M due to the gradually increasing dissociation of casein micelles (Zhang et al. 2004). The foamability of whey protein isolate increased when NaCl concentration was increased from 0 to 0.1 M, but decreased at higher NaCl concentrations (Zhang et al. 2004).

The addition of citrate or phosphate solutions to micellar casein suspensions before drying considerably increased rehydration rates, and this was related to the destruction of the micelle structure (Schuck et al. 2002). Water uptake in casein suspensions was improved by adding NaCl during rehydration. The addition of $CaCl_2$ considerably affected micelle organization and led to the formation of insoluble structures during spray drying. Partial demineralization, NaCl addition, or calcium chelating agents have been used to improve the solubility of high protein MPC.

8.7.7 Stability of Caseins

8.7.7.1 Ethanol

The stability of milk to various concentrations of added ethanol has been used as a milk quality index and is important in the production of drinks, such as cream liqueurs. Figure 8.5 is an attempt to illustrate the impact of pH on the ethanol and heat stability of caseins. Low pH values reduce stability, and stability increases sigmoidally with pH. The inflection point (pK) depends on the properties of individual milks. For a more complete description of this profile/behavior, see Horne (2003). Horne and Parker (1981) found that the addition of Ca or Mg to milk samples caused a shift of the ethanol stability (ES)/pH profile to more alkaline pH values. The addition of phosphate or citrate had little or no effect on the ES/pH profile, although addition of EDTA, a stronger sequestrant, caused a shift in the profile to more acidic pH.

The studies of Horne and Parker on ethanol stability, reviewed by Horne (2003), emphasized the role of the inorganic components of the milk serum, reinforcing the conclusions of Sommer and Binney (1923) that salt balance, the excess of Ca and Mg over citrate and phosphate in milk serum, was critical in alcohol-induced coagulation. Decreasing the salt balance ratio thus caused a shift in the ES/pH profile to acidic pH, whereas increasing the salt balance ratio shifted the profile to more alkaline values. The mechanism, proposed by Horne (1987) to explain these observations, suggests that ethanol has two competing effects on the micellar system, destabilization through loss of the hairy layer, and shifts in the Ca phosphate equilibria, first noted by Pierre (1985). If the ethanol promotes the precipitation of Ca phosphate external to the micelle, it would first reduce the concentration of free Ca, reduce the level of caseinate-bound Ca, and disrupt the binding through Ca phosphate nanoclusters. Moderate losses would increase the negative charge on the caseins and increase the thickness of the steric stabilizing layer. The higher the alcohol concentration, the faster and more extensive would be the precipitation of Ca phosphate. The ensuing adjustment in protein charge and conformation, although relatively rapid, still requires a finite response time. Countering these changes are the effects of ethanol as a non-solvent for the proteins, promoting cross-linking and collapse of the hairy layer. When the coagulation reaction occurs faster than the adjustment of charge and conformation resulting from shifts in Ca phosphate equilibria, or the extent of the latter is limited by insufficient ethanol, the aggregation reaction dominates and precipitation of micelles follows.

The origin of the sigmoidal ethanol stability/pH profile (Fig. 8.5) can also be explained through the effect of pH on Ca phosphate precipitation. Increasing pH brings about increased Ca phosphate precipitation, possibly further enhanced by the ethanol, which means that more ethanol is required to precipitate the protein, i.e., to overcome the increased energy barrier being erected following the transfer of Ca phosphate from the nanocluster state. Conversely, decreasing pH acts to diminish the influence of ethanol-induced precipitation of Ca phosphate by titrating away negative charge and reducing electrostatic repulsion between protein species. Other

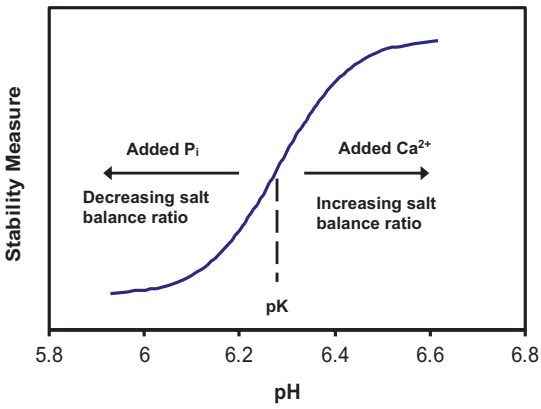

Fig. 8.5 Stability phase diagram of milk as a function of pH or other treatments; schematic, meant to indicate trends for both ethanol and heat stability behavior of milk. (Adapted from Horne 2003)

effects of milk serum composition, of forewarming the milk, and of modifying milk concentration and ionic strength can all be explained in similar fashion (Horne 2003).

Tsioulpas et al. (2007) reported that there is an inverse nonlinear relationship between free Ca ion concentration and ethanol stability ($r = 0.84$), confirming the earlier observations of Davies and White (1958). Citrate found naturally in milk acts as a stabilizing factor, as it slightly improved milk stability (Tsioulpas et al. 2007). Ethanol stability values for milks during lactation were reported to have a mean value of 83.2 ± 12.6% (range 62–100%) (Tsioulpas et al. 2007). Chavez et al. (2004) found that ethanol stability was positively correlated with the concentrations of chloride, potassium and ionic Ca in milk. Horne and Parker (1983) reported that the addition of NaCl reduced the ethanol stability of unconcentrated milk, primarily at pH > 6.5, which they suggested is a result of increased ionic strength. O'Kennedy et al. (2001) demonstrated that α_{s1}- and β-caseins were only minor components of the ethanol-induced precipitate, whereas α_{s2}- and κ-casein were the main proteins susceptible to aggregation.

8.7.7.2 Heat

The heat stability of milk has been regularly and extensively reviewed (Fox and Morrissey 1977; Singh and Creamer 1992; O'Connell and Fox 2003; Singh 2004; Dumpler et al. 2020). Older literature was reviewed by Pyne (1962). The effects of raising temperature on the status of the various Ca phosphate species have already been discussed above (Sect. 8.4.1). Heat stability is the ability of milk or concentrates to resist severe heat treatments without thickening, gelation, or coagulation (Augustin 2000).

Discussions on heat stability are complicated by the knowledge that heat-induced coagulation as a function of pH can follow two distinct profiles. In a Type A heat coagulation time (HCT) vs. pH profile, the time to induce coagulation at a fixed temperature first increases with pH, then enters a minimum before stability increases again at more alkaline pH values. In the Type B profile, heat coagulation time increases progressively with pH. Individual milks which follow type A behavior predominate in most countries, while all bulk milks show type A behavior (O'Connell and Fox 2003). When milk is heated, several competitive and often interdependent reactions occur, not all of them directly involving the milk salts. Fox (1981) listed a selection of these, but it is now generally agreed that the presence of the minimum in a type A profile is associated with the heat-induced formation of a complex between β-lactoglobulin and κ-casein. Such chemical reactions are outside the scope of this chapter and are covered in the reviews of Fox and Morrissey (1977), Singh and Creamer (1992), O'Connell and Fox (2003), and Singh (2004). However, milks showing type A characteristics can be converted into type B profiles and vice versa. For a list of methods and a discussion of these observations, see Horne and Muir (1990).

Interestingly, several of these methods involve manipulating the levels of milk salts, particularly Ca and phosphate. For many years, it was considered that

differences in the heat stability of milk were due to variations in the composition of milk salts, and this led Sommer and Hart (1919) to propose the salt balance theory referred to above in our discussion of ethanol stability. O'Connell and Fox (2003) have suggested that subsequent attempts to correlate heat stability with natural variations in the composition of milk salts are due to the original studies being based on deliberate additions of salts to milk at levels outside natural variability. This overlooks the fact that the experiments of Sommer and co-workers employed a different protocol for the heat stability assay, namely a measurement of the heat coagulation temperature, the temperature at which milk instantaneously coagulates (i.e., effectively coagulates within a short time, <2 min). Because this is a measure of instantaneous coagulation, it is unaffected by changes that occur on prolonged heating. Instead, the response to changing pH, as observed by Miller and Sommer (1940), is remarkably similar to the sigmoidal ethanol stability/pH profile. Moreover, the addition of Ca shifts this profile to more alkaline values while the addition of phosphate has the opposite effect of producing an acidic shift, just like the response of ethanol stability profiles. Horne and Muir (1990) suggested that such behavior indicated that heat-induced coagulation as measured by this assay might follow a similar, if not identical, pathway to alcohol-induced coagulation as described above, involving the precipitation of Ca phosphate and a decrease in Ca activity with increasing pH. Such a scenario also ties in with the observation that the amount of free Ca^{2+} has been associated by various authors with the heat stability of milk, powdered milk, and recombined milk (Augustin and Clarke 1990; Singh and Creamer 1992; Williams et al. 2005). Addition of Ca to milk results in a decrease in heat stability due to the increase in free [Ca^{2+}] (Philippe et al. 2004). Seasonal changes in milk salts (soluble Ca) have been correlated with changes in the heat stability of milk (Kelly et al. 1982). Salts, such as orthophosphates, are often added to milk concentrates (or ultra-high temperature sterilized milks) during processing to improve heat stability. Orthophosphates reduce the Ca^{2+} activity, which is mainly responsible for the improved heat stability (Augustin and Clarke 1990). O'Connell and Fox (2001) suggested that heat-induced precipitation of CCP is involved in the thermal coagulation of milk and that the specific effect of β-lactoglobulin at the pH of maximum stability may be related to its ability to chelate Ca.

Crowley et al. (2014) reported that Ca-ion activity of MPC suspensions increased with increasing protein content of MPC powers. During the manufacture of high-protein MPC powders, the extensive diafiltration involved removed many soluble salts including citrates. When the MPC is reconstituted into water, some CCP dissolves into the serum phase, but a higher proportion of serum Ca is in the form of ionic calcium due to the reduced concentration of anions available to form soluble complexes. Crowley et al. (2014) also reported that, at pH < 6.8, the heat stability of MPC suspensions decreased with increasing protein content of the MPC powders, due to the high Ca-ion activity (which could also help explain the protein aggregation and increasing insolubility observed during powder storage).

8.7.8 Cheese Texture and Functionality

The importance of calcium and phosphate interactions for cheese manufacturing properties, as well as textural properties, has been reviewed (Lucey and Fox 1993; McMahon and Oberg 1998; Lucey et al. 2003; Johnson and Lucey 2006). Processed cheese manufacture is based on the use of citrate or phosphate salts to sequester some of the Ca from the residual CCP, which solubilizes caseins that can then emulsify the released fat. The acidity of whey at drainage and rate of acid development are recognized as important parameters that determine the mineral content, acidity, and quality of cheese. Schulz (1952) developed a classification of cheese varieties based on their Ca contents. Monib (1962) was one of the first investigators to study the Ca phosphate–casein complex in cheese and concluded that very dilute cheese extracts did not represent cheese-like conditions and that their use would lead to incorrect conclusions about serum Ca concentrations (i.e., excessive dilution resulted in the dissolution of more insoluble Ca).

By the 1980s, it was recognized that acid development during manufacture determines the overall loss of Ca, which determines the basic structure of cheese (e.g., Lawrence et al. 1983). In the early 1990s, it became accepted that much of the residual Ca in cheese is associated with casein and that much of the CCP was not dissolved during cheesemaking (Lucey and Fox 1993). Previously, it was thought that almost all the CCP in cheese had dissolved, at least in most cheeses due to their low pH values (<5.3) since by this pH most of the CCP in milk is dissolved. It was also recognized that the insoluble Ca component is an important structural unit influencing cheese texture (Lucey and Fox 1993). Many subsequent studies have demonstrated the importance of pH and Ca content on the functional properties of cheese (e.g., Yun et al. 1993; Guinee et al. 2002; Joshi et al. 2002). It is now accepted that, during ripening, there are important changes in the amount of insoluble Ca (e.g., Guo and Kindstedt 1995; Hassan et al. 2004) and that these shifts in the Ca equilibrium contribute to textural changes during ripening (Lucey et al. 2005; O'Mahony et al. 2005). These initial (first few weeks) textural changes include increased fusion of milled curd, reduced rigidity ("curdiness"), and increased meltability. The proportion of insoluble Ca in cheese has been estimated by the expression of some of the aqueous phase ("juice") under high hydraulic pressure (Morris et al. 1988; Lucey and Fox 1993), centrifugation to extract some expressible serum in young high-moisture cheeses (Guo and Kindstedt 1995), measurement of acid–base buffering (Lucey and Fox 1993; Hassan et al. 2004) and water extraction methods (Metzger et al. 2001).

8.8 Other Uses/Applications of Milk Salts

Milk minerals (typical composition: <5% protein, <9% lactose, >70% ash, 25% Ca, 14% phosphorus) are produced by concentrating, alkalization to precipitate calcium and phosphates, and drying of de-proteinized whey. Acid whey contains higher

levels of milk salts like calcium and phosphate, making it an attractive starting material for the manufacture of milk minerals. Liquid–solid hydrocylones have been recently explored to remove the larger, more abrasive calcium phosphate precipitates often formed during the alkalization step (Crowley et al. 2019). This ingredient is often used for mineral fortification purposes in a range of food products. Milk minerals (or permeate powders) have a salty taste and have been used as a replacement for NaCl in foods.

A number of biologically active peptides are released during digestive breakdown of caseins, and they play a physiological role in newborn mammals (Kitts 2006). Casein phosphopeptides (CPPs) are resistant to further hydrolysis by mammalian digestive enzymes and accumulate in the small intestine. CPPs render Ca^{2+} in a relatively soluble form for potentially enhanced bioavailability by paracellular (passive) mechanisms. CPPs are produced commercially by a number of dairy companies and used as nutritional ingredients to enhance mineral absorption as well as provide anticariogenic benefits (Reynolds 1999; Tsuchita et al. 2001).

8.9 Concluding Remarks

Milk salts play a critical role in the formation and stability of casein micelles. They influence many of the important functional properties of dairy products, including gelation, protein stability, emulsification, foaming, and cheese texture. The concentration of milk salts can be varied by processing conditions including acidification or the addition of metal chelators/sequestrants. The nature and structure of CCP are still being investigated, but the main features are known. The manipulation of the amount of insoluble Ca in cheese is the major focus of ongoing studies related to controlling cheese performance. There is growing awareness of the nutritional benefits of Ca and P, which has resulted in the fortification of dairy products like dairy beverages, and cheese with Ca.

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