CHAPTER 1

Influence of Processing on Functionality of Milk and Dairy Proteins

Mary Ann Augustin[*,†] and **Punsandani Udabage**[†]

Contents

Abstract

The inherent physical functionality of dairy ingredients makes them useful in a range of food applications. These functionalities include their solubility, water binding, viscosity, gelation, heat stability, renneting, foaming, and emulsifying properties. The suitability of

* School of Chemistry, Monash University, Clayton, VIC 3800, Australia
† Food Science Australia, Werribee, VIC 3030, Australia

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dairy ingredients for an application can be further tailored by altering the structure of the proteins using appropriate processes. The processes discussed include physical modification (heat treatment, acidification, addition of mineral slats, homogenization, and shear), enzymatic modification (renneting, hydrolysis, and transglutamination), and chemical modification (use of chemical agents and the Maillard reaction). Emerging food processes (high pressure and ultrasound) are also discussed. The challenges for using dairy ingredients for the delivery of nutrients and bioactive components, while maintaining physical functionality, are also highlighted. There is a need for continued research into the fundamental aspects of milk proteins and their responses to various stresses for further differentiation of milk products and for the delivery of ingredients with consistent quality for target applications.

I. INTRODUCTION

Milk and dairy ingredients are used in a range of food applications. Their value as food ingredients stems from their ability to impart a range of desirable attributes to food. They contribute to nutritional quality as they are a good source of nutrients. They have roles in influencing the textural and sensory characteristics of the food because of their physical functional properties. These include the ability of milk proteins to hold water and impart viscosity, to form gels, foams, and emulsions and to remain stable during exposure to heating under appropriate conditions. The milk fat component also contributes to the properties of food as it possesses a desirable delicate flavor and can influence the textural properties of food. Although dairy ingredients have traditionally been used for their nutritional and physical functional properties, there is now an increasing interest in the bioactivity of milk components and their potential to have physiological functional roles. This makes them also attractive as ingredients in functional foods that have a role beyond normal nutrition. Although dairy ingredients can potentially provide a range of functionalities, the requirements of dairy ingredients vary with their application. Hence, matching the functionality of the dairy ingredient to their end-use is of paramount importance for their successful incorporation into foods as each application may require one or several functional properties. While dairy ingredients inherently possess several functionalities, their suitability for an application can be further tailored by appropriate processing of milk or ingredients separated from milk and/or modification of the protein (e.g., whey proteins, casein) and nonprotein components (e.g., fat, mineral salt) of a dairy stream (Augustin, 2004; Augustin and Versteeg, 2006).

Both the protein and fat components in milk influence the properties of food, but the ability of the milk to impart desirable properties to food is mostly influenced by the physical functional properties of the milk protein components (Kinsella, 1984; Mulvihill and Fox, 1989). The inherent functionality of milk proteins is related to the structural/conformational properties of protein, which is influenced by both the intrinsic properties of the protein and extrinsic factors. Modification of the protein composition or structure and the organization of the proteins within the dairy ingredient through the application of physical, chemical, or enzymatic processes, alone or in combination, enable the differentiation of the functionality of the ingredient and designing the required functionality for specific applications (Chobert, 2003; Foegeding *et al.*, 2002).

This chapter discusses the influence of processing on the physical functional properties of the milk and milk protein components. The modification of the physical properties of milk, milk powders, and milk protein-based products by the application of various unit processes is the focus of this chapter. Examples are given to demonstrate the effects of various physical, chemical, and enzymatic processes on the structure and functionality of the dairy ingredients. The functionalities that will be covered include solubility, water binding, viscosity, gelation, heat stability, renneting, foaming, and emulsifying. These are the major functionalities that contribute to the physical properties of food (Tables 1 and 2). The potential for the application of emerging food processing

TABLE 1 Functional properties of milk proteins

Functionality	Attributes
Water binding	Ability to bind water and swell Dependent on water–protein interactions through peptide bonds or side chains
Solubility	Ability to dissolve Prerequisite for most other desired properties Dependent on pH Proteins are least soluble at their pI
Heat stability	Ability to withstand heat without thickening Essential attribute in many food product applications
Viscosity and gelling	Ability to thicken and form a gel Related to hydration properties and ability to form a network
Emulsifying and foaming	Ability to stabilize interfaces Dependent on the amphiphilic properties of proteins and their ability to unfold at an interface

TABLE 2 Desirable Functional Properties of Dairy Proteins for Food Applications

Application	Major Desirable Functionalities
UHT milk and evaporated milk	Heat stability, emulsifying
Sweetened condensed milk	Viscosity
Cheese	Rennetability
Yoghurt	Water binding, viscosity, gelling
Ice cream	Foaming, emulsifying
Confectionery	Water binding, foaming, emulsifying
Bakery	Water binding, foaming, emulsifying
Manufactured meat and fish products	Water binding, foaming, emulsifying
Chocolate	High ''free-fat''

technologies for modification of dairy ingredient functionality and challenges for using dairy ingredients for the delivery of nutrients and bioactive components are highlighted.

II. PHYSICAL MODIFICATION PROCESSES

Heat treatment of milk has been one of the most common methods used to alter its functionality. Other processing treatments such as the alteration of pH, mineral adjustment, or homogenization or a combination of these can affect the physical functionality of milk. Processes used in the production of dried dairy ingredients also can influence their functional properties, particularly in the manufacture of powders with high protein content.

A. Heat treatment

1. Influence on milk components

The primary purpose of heat treatment is to destroy harmful microorganisms. However, heat treatments induce many other changes in milk, including inactivation of enzymes, denaturation of whey proteins, alteration of the states of association of the casein micelles, chemical modification of amino acid side chains, and changes in the equilibria of the milk salts (Fox, 1989; Holt, 1995). The consequence of heat treatment is the altered functionality of the milk and the dairy ingredient. The extent of the change in functionality depends on the time and temperature of the treatment and the original composition of the dairy stream and the degree of reversibility of the heat-induced changes.

a. Whey protein denaturation Heat treatment of milk above 60 °C causes denaturation of whey proteins. The extent of denaturation depends on the temperature, and pH at the time of heating, with increasing pH above the natural pH of milk increasing the rate of denaturation of β-lactoglobulin and α-lactalbumin (Law and Leaver, 2000). At temperatures up to 90 °C, unfolding of the protein is rate-limiting but further increases in the heating temperature result in only small increases in the rate of denaturation as aggregation of the proteins becomes rate-limiting (Tolkach and Kulozik, 2005).

Increasing pH above the natural pH of milk markedly accelerates the rate of denaturation of β-lactoglobulin. The denatured whey proteins associate with the casein micelles or remain in the serum phase as complexes of denatured whey proteins or denatured whey protein in association with κ-casein.

Generally a decrease in the pH of milk systems prior to heating results in more association of the denatured whey proteins to the casein micelle (Corredig and Dalgleish, 1996; Oldfield *et al.*, 2000; Vasbinder and de Kruif, 2003). Even small changes in pH can shift the distribution of the association of the denatured whey protein with the casein micelle. For example, at a level of 95% whey protein denaturation, there is ∼70% of the denatured whey proteins associated with the casein micelle at pH 6.55 and this is decreased to ∼30% when the pH of milk prior to heating was 6.7. This was reflected in the larger increase in the casein micelle size when milk was heated at the lower pH (Anema and Li, 2003).

Characterization of the aggregates in heated milk revealed that the serum aggregates are mainly disulphide-linked complexes of whey protein and κ-casein (Jean *et al.*, 2006). Increasing the pH of milk from 6.5 to 7.2 produces smaller aggregates with a higher content of κ-casein (Renan *et al.*, 2006). In contrast to the whey proteins, caseins are more stable to heat. However, at high temperatures for long times (120–150 °C up to 60 min), there is aggregation, fragmentation, and dephosphorylation of casein and destruction of some amino acids (Guo *et al.*, 1989).

The heat-induced changes to proteins and their states of association have significant consequences for the functionality of proteins. Heat treatment of milk and dairy streams is often used to manipulate the physical functionality of these ingredients.

2. Effects on heat stability

Milk and dairy streams need to be stable during heat processing as it is an integral step in the manufacture of dairy products. The heat stability of milk, which is the ability of milk to withstand heat treatment without excessive thickening or coagulation, has therefore been a subject that has attracted a lot of interest over many years.

a. Milk and concentrated milks Single strength milk is heat stable at its natural pH (6.7). Concentration of milk decreases heat stability and shifts the pH of maximum heat stability to lower pH. For example, skim milk (20% solids) has significantly lower heat stability than normal strength milk even at its pH of maximum heat stability at pH 6.4–6.6 (Singh, 2004).

Most milk has maximum heat stability at pH 6.7 and minimum heat stability at pH 6.9. When the pH is increased above 6.9, there is an increase in heat stability. The maintenance of micellar integrity is important for heat stability. The decrease in heat stability of single strength milk at pH 6.9 is often linked to the dissociation of κ-casein from the micelle (Singh and Fox, 1985). This is because the κ-casein-depleted micelles are more susceptible to calcium-induced precipitation. The stabilizing effect of β-lactoglobulin on the heat stability of milk at the pH of minimum heat stability has been related to its ability to reduce the dissociation of κ-casein (Singh and Fox, 1987). O'Connell and Fox (2001) suggested that the role β-lactoglobulin at the pH of minimum heat stability may be linked to its effect on sensitizing casein micelles to heat-induced precipitation of calcium phosphate by increasing the hydrophobicity of the micelle. In addition, they found that the heat-induced precipitation of calcium phosphate as a function of pH appears to be inversely related to the heat stability of milk.

The change in heat stability of milk as a function of pH and concentration has been related to the states of the association of the milk proteins and the equilibria between the milk proteins. Hence, it is not surprising that the common treatments used for improving heat stability of concentrated milks have involved giving milk a preheat treatment prior to concentration. Newstead and Baucke (1983) investigated the effect of different preheat treatments (10–240 s at 90–140 °C and from 10–2700 s at 90 °C) of raw skim milk on heat stability of concentrated milks. The treatment at 110–120 °C for 120–240 s was found to be most effective for improving heat stability. Another lever that has been used to improve the heat stability of concentrated milk is the pH adjustment of the skim milk prior to preheating. The heat stability of recombined concentrated milk was generally improved by lowering the pH of the skim milk by 0.05–0.10 units prior to processing (Newstead and Conaghan, 1978). An alternative strategy for improving heat stability of concentrated milk is to alter the mineral balance of the system. This is discussed in Section II.C.2.

b. Modified milks Modification of the ratio of casein to whey protein or protein standardization of milk with other milk fractions expands the range of dairy products. The addition of whey protein concentrate or individual whey proteins (α-lactalbumin or β-lactoglobulin) to milk generally causes a reduction in heat stability (Rattray and Jelen, 1997). Standardization of the protein content of skim milk with ultrafiltered permeates from

various dairy sources (skim milk, sweet whey, and acid whey) produced standardized milk with different heat stability. This depended on the source used for preparation of the permeate. Increased heat stability was obtained with addition of permeate from skim milk or sweet whey, whereas heat stability decreased when permeate from acid whey was used. The detrimental effects on heat stability were related to the higher level of soluble calcium in permeate from acid whey (Rattray and Jelen, 1996).

c. Whey Whey proteins are inherently unstable to heating. The stability of whey to heat-induced precipitation can be reduced by the addition of phosphates or citrates. These complexing agents mask the effect of calcium on precipitation of proteins (de Rham and Chanton, 1984).

The presence of caseins in combination with whey proteins at the time of heating protects the whey protein against precipitation. Dickinson and Parkinson (2004) found that substitution of 10% of the β-lactoglobulin with caseinate improved the heat stability of emulsions (10 vol % oil, 2 wt % protein). O'Kennedy and Mounsey (2006) showed that α_{s1}/β-casein improved the stability of whey protein isolate suspensions (0.5 wt %, pH 6.0) heated at 85 °C for 10 min. The ratio of whey protein isolate: α_{s1}/β-casein required for complete suppression of heat-induced aggregation was 1:0.1 (w/w). The presence of the caseins did not alter the extent of denaturation but inhibited denatured whey protein aggregation. Micellar casein could also be used to stabilize whey proteins against heat-induced aggregation. Although whey protein denaturation was promoted in the presence of micellar casein, aggregation of the denatured whey proteins was controlled down to pH 5.4. Thus, although both α_{s1}/β-casein mixtures and micellar casein can stabilize whey proteins, the mechanisms of their actions are different.

3. Effects on gelling properties at neutral pH

Heat treatment of whey protein dispersions results in the formation of precipitates, gels, or soluble polymer dispersions depending on pH, ionic strength, protein concentration, and the presence of salts (Donovan and Mulvihill, 1987; Foegeding *et al.*, 2002). By controlling the conditions of the heat treatment, whey-based gels of varying textures may be obtained. Most research on gels of dairy proteins at approximately neutral pH has been done on whey proteins. The literature on the effects of gelling of whey proteins suggests that the states of protein created by the heat treatment, which can be manipulated by a variety of factors including pH, time and temperature of heating, presence of salts, concentration of protein at time of heating, and order of processing, dictate the properties of heat-induced whey protein gels.

As whey proteins are heated, they denature. A gel is formed when there is a sufficient interaction between the denatured protein molecules. A precipitate, gel, or soluble whey aggregates is obtained depending on

protein concentration, temperature and time of heating, pH, and ionic strength. The gels obtained can be particulate, fine-stranded, or have a mixed network (Bottcher and Foegeding, 1994; Mangino, 1992; Mulvihill and Donovan, 1987). Where there is a strong electrostatic repulsion between the protein particles, fine-stranded gels are formed (i.e., when the protein is at a pH much above the isoelectric point and at low ionic strength). Particulate gels are formed when the pH is nearer the isoelectric point and in high ionic strength environments where the electrostatic charge is screened. Particulate gels possess a loose network of large protein particles and these gels have low water-binding capacity and are usually opaque. Fine-stranded gels have a network caused by the association of strands and these have good water-binding activity and are usually translucent (Langton and Hermansson, 1992).

The texture, opacity, and water-holding properties of the whey gels can be further modulated by salts as they influence protein–protein interactions. When heated, whey protein isolate dispersions (10% w/w; pH 6.9; 80 °C/15 min) were made with different concentrations of NaCl, the heated dispersions did not form a gel and remained transparent at low NaCl concentration (<60 mM NaCl). However, they formed gels with increasing elastic modulus and high water-binding capacity with increasing NaCl concentration (60–150 mM NaCl). At higher salt concentrations (150–200 mM), gels had decreased elastic modulus and lower water-binding capacity (Chantrapornchai and McClements, 2002).

An alternative approach to the formation of whey gels is to predenature whey proteins under carefully controlled conditions to form aggregates, followed by cold gelation in the presence of salts (Bryant and McClements, 2000). The formation of these cold-set gels involves a heat treatment of native whey dispersions at about neutral or alkaline pH under conditions of low ionic strength and at low enough protein concentration to avoid gelation during heating. This causes unfolding of the native whey proteins and the formation of disulphide cross-linked aggregates. The conditions of heating (e.g., pH and whey protein concentration) as well as the rate of cold gelation can be manipulated to give cold-set gels with different properties (Alting *et al.*, 2003; Bryant and McClements, 2000).

Mleko and Foegeding (2000) prepared whey protein polymers by heating whey protein isolate dispersions (4% w/v; pH 8) at 80 °C and adjusting pH (6.0–8.0). Weak gels were formed at pH 6.0 and 6.5, while highly viscous solutions were obtained at pH 7.0–7.5. Yet another approach has been the development of cold gelling-derivatized whey protein isolate powders. The preparation of these powders involved heating whey proteins to form gels and subsequent drying of the preparations. Reconstitution of the powders in water at ambient or refrigeration temperatures results in viscous solutions or weak gels (Firebaugh and Daubert, 2005; Hudson *et al.*, 2000; Resch and Daubert, 2002). Other approaches that have been examined to manipulate gelling properties

include a two-stage heat-induced polymerization and aggregation process. Whey protein isolate dispersions subjected to a double heating step (30 min at 80 °C at pH 8 followed by 30 min at 80 °C at pH 7.0) were compared to those subjected to a single heating step (30 min at 80 °C and pH 7.0). Significant increases in the elastic modulus of gels could be obtained by using a double heating process compared to a single heating step (Glibowski *et al.*, 2006).

There are novel uses for gelling dairy ingredients as demonstrated by the use of cold-set β-lactoglobulin gels for delivery of iron. This has been achieved by the addition of Fe^{2+} to preheated β-lactoglobulin dispersions to form gels with entrapped iron with different microstructures and iron: protein ratios (Remondetto *et al.*, 2002).

4. Effects on surface properties

The effects of heat treatment on functional properties other than heat stability or gelling have received less attention. Heat treatment and pH at the time of heat treatment have an impact on the surface properties of proteins.

The amount of denaturation and aggregation induced by various heat treatments has to be controlled to optimize the surface properties of proteins. An increase in surface hydrophobicity of whey protein concentrates with heat treatments has been correlated with an improvement in emulsifying and foaming capacity (Moro *et al.*, 2001). A heat treatment is required to expose buried hydrophobic groups of proteins. This is required for improving surface properties. However, if the heat treatment is too harsh, aggregation can occur and this decreases surface hydrophobicity leading to a decrease in surface properties. Zhu and Damodaran (1994) suggested that the ratio of monomeric to polymeric protein in whey protein isolates exposed to heat treatments (70 or 90 °C) influenced their foaming properties.

When heat treatment of whey protein concentrates was carried out at 84 °C for 30 s (pH 6.0, 6.5, and 7.0), the improvement in emulsion stability was greatest when pH was 6.0 (Moon and Mangino, 2004). However, when the heat treatment was applied to the whey prior to ultrafiltration for preparation of whey protein concentrates, the emulsifying properties of the resultant whey protein concentrate were improved by heat treatment (70 °C for 2 min) when pH was increased from pH 6 to 7 but a higher heat treatment (80 °C for 2 min) decreased emulsifying properties at pH 7 (Fachin and Viotto, 2005). The different effects of heating, depending on the dairy stream and heating conditions, highlight the need to control the protein species in each of the heated streams.

B. Acidification

Acidification results in an alteration of the protein and mineral equilibria with consequent effects on the physical, chemical, and functional properties of milk and ingredients.

1. Influence on milk components

Acidification of milk results in the formation of a gel. Gelation of milk is primarily due to the charge neutralization of the protein particles in milk as the isoelectric point of the milk protein particles is approached. The micelles maintain their original size until the first signs of gelation.

The nature of the casein particles in milk is changed as a consequence of lowering pH because of the complex equilibrium that dictates the distribution of minerals and caseins between the serum and colloidal phases of milk. Acidification causes the solubilization of colloidal calcium phosphate, resulting in an increase in Ca^{2+} and phosphate activity (Dalgleish and Law, 1989; Van Hooydonk *et al.*, 1986a). The solubilization of colloidal calcium phosphate is accompanied by a release of caseins from the micelles. Approximately about 14% of the calcium is still present in the micellar phase even after virtually all the colloidal inorganic phosphate is solubilized at pH 5.3 and 30 °C (Van Hooydonk *et al.*, 1986a). The calcium that remains is regarded as the calcium directly bound to casein through phosphoseryl and carboxylate residues. The amount of caseins released at a given pH decreases with increasing temperature. The percentage of (β + γ)-casein released at a given pH is greater than that of other caseins at a fixed temperature (Dalgleish and Law, 1988). The pH at which the maximum dissociation of casein occurs also depends on the temperature, with the pH of the maximum decreasing with decreasing temperature. This occurs at pH 5.1 at 4 °C, 5.4 at 20 °C and 5.5–5.6 at 30 °C (Dalgleish and Law, 1988; Van Hooydonk *et al.*, 1986a).

The gelation pH increases with increasing temperature, occurring at pH 5.0 at 15 °C and pH 5.1 at 20 °C. Heat treatment of milk also increases gelation pH (Banon and Hardy, 1991). For example, milk heated at 80–90 °C has a gelation pH of ∼5.4. The gelation pH has been related to the percentage of total β-lactoglobulin in the heated milk (Vasbinder *et al.*, 2003).

2. Acidified milk gels and yoghurts

Heating of milk prior to addition of cultures is known to increase the firmness of yoghurt and reduce syneresis (Augustin *et al.*, 1999; Dannenberg and Kessler, 1988a,b). Heat treatment of milk prior to acidification with glucono-δ-lactone has been shown to increase the firmness of the acid gels, to increase the pH at which gelation occurs and to reduce syneresis (Lucey *et al.*, 1997, 1998). The higher pH at gelation of heated milks was considered to be due to the alteration of the casein micelle surface because of the attachment of denatured whey protein to the casein micelle. However, by using confocal scanning electron microscopy with separate staining of the proteins in milk, it has been shown that the denatured whey proteins that remain in the serum phase also gel at the

same time as the whey-coated casein micelles (Vasbinder *et al*., 2004). Guyomarc'h *et al*. (2003) suggested that the soluble aggregates containing denatured whey proteins in heated milk have a greater effect on increasing gelation pH than whey proteins bound to casein micelles.

a. Effects of milk solids concentration The properties of acid and yoghurt gels may be altered by changing the concentration and distribution of proteins in the milk. However, the effects obtained depend on the milk composition and conditions used during the heat treatment. This is because both these factors affect the association of the milk proteins. Yoghurts made with higher milk solids generally have improved properties, but these effects are dependent on the source of milk solids used. Yoghurts made from milks fortified with ultrafiltered milk solids were firmer that those fortified with skim milk powder (Becker and Puhan, 1989).

b. Effects of alteration of the casein:whey ratio Changing the ratio of casein:whey protein in yoghurt milk results in yoghurts with different textures. Yoghurts had increased gel strength and reduced syneresis when the protein content of yoghurt milk was kept constant and the ratio of whey protein to casein was increased. This was attributed to differences in microstructure of the yoghurts where increasing whey protein led to a finer structure and a denser network of protein aggregates (Puvanenthiran *et al*., 2002). Augustin *et al.* (2003) showed that increasing the protein content of yoghurt milk by partial substitution (20%) of skim milk solids with whey protein concentrates increased the firmness of yoghurts. However, the increase was obtained only in cases where the yoghurt milk was stable to the heat treatment applied prior to the addition of cultures. When there was excessive thickening during the heat treatment of yoghurt milk, the properties of the yoghurt were compromised, suggesting that the state of aggregation of the proteins in the heated milk affected yoghurt properties.

c. Effect of heating conditions Altering the pH of the heat treatment prior to acidification, which alters the states of association of whey proteins with the casein micelles, may be used to manipulate the properties of milk gels. The change in gelation properties of milks heated at different pH prior to acidification has been related to the differences in proportions of denatured whey proteins associated with the casein micelle and soluble complexes in the serum.

Adjustment of milk pH (6.9–6.35) prior to heat treatment and readjustment to pH 6.7 followed by acidification markedly change the gelation properties of milk. The differences in gelation properties are related to the

pH dependence of casein–whey protein interactions, which results in different protein structures being formed at the time of heating. At pH > 6.6, there is partial coverage of casein micelles and separate whey protein aggregates, whereas at pH < 6.6, the whey proteins are attached to the micelles. The different protein structures formed during heating at various pH influence their gelation properties on acidification (Vasbinder and de Kruif, 2003).

Anema *et al*. (2004) found that increasing the pH at heating from 6.5 to 7.1, which increases the amount of aggregates in the serum, followed by readjustment to pH 6.7 prior to addition of glucono-δ-lactone, increased the pH of gelation, decreased gelation time, and increased the firmness (elastic modulus) of the gels. Large differences in gel properties were obtained, even though there were small changes in the extent of denaturation of whey proteins. Their results suggest that the decreased association of the denatured whey proteins with casein micelles and increased levels of soluble whey protein complexes obtained with increase in pH at heating improved gelation properties. The importance of the soluble complexes in heated milk on the structure of acid gels has been confirmed by others who showed that increasing the amounts of soluble complexes in milk by increasing the pH of heat treatment of milk from 6.5 to 7.1 gave rise to stronger acid gels. However, heat treatment of milk at higher pH (7.2), which further increases the amount of soluble material, weakens the acid gels formed from these milks (Rodriguez del Angel and Dalgleish, 2006).

Schorsch *et al.* (2001) examined the effects of denaturation of whey proteins in the presence and absence of casein micelles on gel properties. Heat treatment sequence was found to influence the acid gelation properties of casein–whey mixtures. Denaturation of whey proteins in the absence of casein micelles induced more rapid gelation on addition of acid. Gels made from these milks had a more particulate gel structure than gels made from casein–whey mixtures which were heated without prior denaturation of the whey proteins.

3. Acidified whey gels

These types of gels are prepared by heating whey proteins and acidifying the heated solutions. The presence of calcium ions in solutions at the time of heating affects the acid gels subsequently formed. Britten and Giroux (2001) made gels by preheating whey at pH 6.5–8.5 in the absence or presence (up to 4 mM) of calcium at 90 °C for 15 min prior to acidification. Opaque particulate gels were formed and their gel strength was dependent on the type of whey polymers formed. Where polymers with high intrinsic viscosity were produced on heating, these generally resulted in strong gels. The ability to manipulate acid gelation properties of whey

polymers enables their incorporation into yoghurt formulations (Britten and Giroux, 2001).

C. Addition of mineral salts

The addition of mineral salts to alter protein and mineral equilibria in milk is a strategy that has been used to manipulate milk functionality, either alone or in combination with other processing treatments, such as alteration of pH, ultrafiltration, diafiltration, heating and cooling, or static high-pressure treatment.

1. Influence on milk components

In milk, the caseins and minerals are in dynamic equilibrium between the micellar (colloidal) and the serum phase. When the native environment of caseins and minerals is altered by the addition of mineral salts, the partitioning of minerals and caseins between the serum and colloidal phases is altered.

Altering the composition of milk with the addition of mineral salts at constant pH induces shifts in the mineral and casein partition, causing the establishment of new positions of equilibria. In general, addition of calcium or inorganic phosphate causes transfer of serum calcium or inorganic phosphate into the colloidal phase. The Ca^{2+} activity is also affected, increasing on the addition of calcium and decreasing on the addition of inorganic phosphate (Rose, 1968; Tessier and Rose, 1958; Udabage *et al.*, 2000; Van Hooydonk *et al.*, 1986c).

The content of colloidal calcium phosphate is also changed on addition of salts. Disintegration of casein micelles is observed with addition of calcium chelating agents (EDTA or citrate) as a result of the solubilization of the colloidal calcium phosphate and the micellar casein (Griffin *et al.*, 1988; Holt, 1982; Lin *et al.*, 1972; Rollema and Brinkhuis, 1989). Solubilization of colloidal calcium phosphate beyond a critical level causes the disintegration of casein micelles and a loss of micellar integrity (Udabage *et al.*, 2000).

The alteration of mineral and casein equilibria is reflected in changes to the physical properties of milk. The addition of citrate and different types of phosphates (ortho-, pyro-, or hexameta) to milk protein concentrate solutions, which alters the distribution of calcium and inorganic phosphate between the colloidal and serum phases of milk, affects its turbidity and buffering capacity (Mizuno and Lucey, 2005). The turbidity is affected because dissolution of colloidal calcium phosphate is accompanied by release of caseins into the serum.

The changes in the equilibria affect many of the functional properties including heat and ethanol stability, renneting, solubility, foaming, and

emulsifying. The effects of altering mineral equilibria on functionality of milk have been previously reviewed (Augustin, 2000).

2. Effects on heat stability

It is well known that the addition of soluble calcium salts reduces the heat stability of milk, whereas the addition of calcium complexing agents with the appropriate control of pH improves heat stability. Phosphates and citrates have often been used to increase the heat stability of concentrated milks (Augustin and Clarke, 1990; Pouliot and Boulet, 1991; Sweetsur and Muir, 1982a). A reduction in Ca^{2+} activity by the addition of these salts contributes to the improved heat stability of concentrated milks, but the effects of salts on the equilibrium of caseins between the serum and micellar phases of milk also affect heat stability.

Evidence for the importance of mineral-protein equilibria was seen by comparing the heat stability-pH profiles of concentrated milk with added EDTA or phosphates. Although EDTA caused a similar reduction of the Ca^{2+} activity in recombined concentrated milks compared to those with added phosphate, milks with added EDTA had reduced heat stability. This was attributed to the difference in the level of colloidal calcium phosphate in micelles which changes the partitioning of the caseins between the serum and colloidal phases (Augustin and Clarke, 1990).

The interest in mineral fortification of milk for the production of milks with higher nutritional value is a challenge. This is because the introduction of minerals upsets the mineral-protein equilibria in milk which will affect their stability. Philippe *et al.* (2004) showed that supplementation of skim milk with calcium gluconate, calcium lactate, or calcium chloride (up to 16 mmole added Ca/kg) decreased the heat stability. The addition of $MgCl_2$ or $FeCl_3$ (at a level of 8 mmole/kg) also reduced the heat stability of casein micelles (Philippe *et al.*, 2005). However, by manipulating the mineral equilibria of milk with the use of a combination of soluble calcium salts and orthophosphates, it is possible to produce milks (with up to 20 mmole added Ca/kg) that are stable to heating (Williams *et al.*, 2005). O'Kennedy *et al.* (2001) showed that denatured whey proteins could be used as a carrier for calcium phosphate and further that adequate heat stability at 130 °C of whey protein-calcium phosphate suspensions could be achieved by appropriate adjustment of pH.

3. Effects on surface properties

Superior foaming properties of milk have been obtained by addition of calcium complexing agents. Kelly and Burgess (1978) demonstrated that addition of sodium hexametaphosphate to milk protein concentrate solutions prepared by ultrafiltration improved foam volume and stability on whipping. The addition of EDTA to milk, which causes dissociation of the casein micelle, improved the foaming properties of milk (Ward *et al.*, 1997).

The increase in soluble casein for interaction with the interface during whipping accounts for the increase in the foaming capacity. It is possible that the increase in the viscosity of milks with increasing states of disaggregation of the casein micelle contributed to this stability.

Citrate salts have long been used in the processed cheese industry as ''emulsifying salts,'' and there is still interest in the mechanism of their action. Shirashoji *et al.* (2006) examined the effects of trisodium citrate on the properties of processed cheese. Increasing concentration of sodium citrate decreased the size droplets of the cheese. This effect is typical when emulsifying properties of a system are improved. This is expected as the complexation of calcium by citrate causes dissociation of the casein micelle, making the casein more available for emulsifying fat droplets. This possibly contributed to the reinforcement of the structure of the processed cheese.

D. Homogenization and shear

The main purpose of homogenization in the dairy industry is for the emulsification of fat. Homogenization results in the creation of smaller fat globules with altered interfaces. A more stable emulsion that is resistant to creaming is usually obtained on homogenization, and this has benefits for fluid milks and dairy products. Homogenization can also have other effects on the functionality of dairy products. For example, heat in combination with shear has been used for the microparticulation of globular proteins.

1. Effects on heat stability

Homogenization (up to 20.7 MPa) of whole milk decreases heat stability, with the effect being greater at increasing homogenization pressure. Homogenization of skim milk (up to 31 MPa) has only a negligible effect on skim milk (Sweetsur and Muir, 1983). The position of the homogenization process in the manufacture of concentrated milks in relation to the stage of addition of stabilizing salts (phosphates) influences heat stability. The stabilization of milk to heat by added phosphate was more effective when the phosphates were added prior to homogenization (Sweetsur and Muir, 1982b).

2. Effects on gelling properties

The effects of shear on the properties of gels are influenced by the presence of fat in the dairy systems. Homogenized fat droplets can act as active fillers in milk gels. Xiong *et al.* (1991) found that the addition of emulsified fat into skim milk increased the gelation rate and shear modulus of acid-induced milk gels and that decreasing the fat droplet size at the same fat content resulted in firmer gels. An increase in the fat content

of gels at the same solids nonfat:water ratio also increases the complex modulus of acid and heat-induced milk gels (Underwood and Augustin, 1997).

Blends of whey protein isolate and denatured whey protein isolates were microparticulated using a microfluidizer prior to the formation of heat-set gels. Increasing the number of passes in the microfluidizer increased the hardness of the gels, an effect attributed in part to the more homogenous gelation of smaller aggregates (Sanchez *et al.*, 1999).

3. Effects on microparticulated whey proteins

A combination of heat and shear has been used to create whey protein particles with controlled particle size and properties. A well-known example of the use of microparticulation of thermally denatured whey protein is for the production of Simplesse® 100, a whey-based fat replacer (Lieske and Konrad, 1993). Shear can be used to modulate gel properties of whey protein isolate gels.

Spiegel and Huss (2002) controlled pH and calcium levels during heat treatment and shearing in a scraped surface heat exchanger to produce whey protein aggregates of between 0.5 and 10 μm, which give a smooth mouthfeel. Heating whey protein concentrate dispersions at 110 °C and a low pH (<5.5) produced the small aggregates.

Oestergaard (2005) obtained particles of 1–12 μm with creamy consistency by ultrafiltering whey to obtain a protein concentrate (60% protein) and then heating the concentrate under controlled shear rates in a scraped surface heat exchanger.

E. Dehydration

The conversion of liquid dairy streams into powders is an important processing operation in the dairy industry. The removal of water from a dairy stream during concentration and drying can influence the functional properties of the resultant powders. This depends on factors such as the composition of the stream to be dried, the type of driers used, and the conditions of drying. Both the physical characteristics of the powders (e.g., particle size, bulk density, occluded air) and their functional properties can be affected by drying (Tong, 2001).

Of all the major unit processes in conventional milk powder manufacture such as preheating, concentration, and drying, the preheating step has the major effect on milk powder functionality. In fact, skim milk powders are still classified on the basis of the heat treatment applied to the milk during powder manufacture (American Dairy Products Institute, 1990). The heat classification, based on the amount of undenatured whey protein in the milk powder, is still being used as a general guide to the physical functionality of milk powders and the selection of powders for

specific food applications. However, this guide should be used with caution as the initial content of whey protein and the nonprotein nitrogen content of the milk used in the powder production can affect this value.

The process for spray-drying of conventional skim and full-cream milk powders is routine in the dairy industry. However, there is still research on the drying of specialized and newer dairy powders (Kelly, 2006).

1. Milk powders for chocolate

A desirable property of milk powders intended for chocolate manufacture is a high level of unencapsulated fat (free-fat). The traditional method for manufacture of these powders has been roller drying as the method of drying gives rise to high levels of unencapsulated fat (>85% w/w of fat in powder). This contrasts with the low level of unencapsulated fat (<4% w/w of fat in powder) in milk powder (26% fat) made using the traditional processes of preheating, concentration, homogenization, and spray-drying.

Altering the composition of the milk, the process variables, and the order of unit operations during the manufacture of spray-dried powder can affect the level of unencapsulated fat. Increasing the content of total fat in powder (from 26% to 70% w/w) increases the level of unencapsulated fat in powders (Kelly *et al.*, 2002). Clarke and Augustin (2005) found that by separating full-cream milk into cream and skim milk fractions, pasteurizing the cream fraction, then cooling the cream and recombining it with a skim milk concentrate prior to drying increased ''free-fat'' in powder. Another method of increasing ''free-fat'' is homogenizing the cream fraction at high temperature and pressure prior to combining it with a skim milk concentrate. These methods enabled the production of whole milk powders (30% total fat in powder) with high levels of unencapsulated fat (up to 40% w/w of total fat). Another factor that influences the level of ''free-fat'' is the solid-fat content of the milk fat. Twomey *et al.* (2000) found that high-fat powders (56% total fat in powder) had increased the level of unencapsulated fat when there was an increase in the solid-fat content of milk fat.

The particle size of the powders, which is affected by milk composition and the solid-fat content of the milk fat, also affects the suitability of powders for chocolate manufacture (Keogh *et al.*, 2002). By increasing the spray nozzle size for the concentrate and increasing the air outlet temperature of the dryer, the particle size of milk powders can be increased (Keogh *et al.*, 2004). This makes milk powders more suitable for chocolate manufacture.

2. High-protein milk powders

Milk protein concentrate (MPC) powders (>50% protein) are typically made by ultrafiltration/diafiltration prior to drying. The drying of these high-protein concentrates is known to cause a loss of functionality. This is typically

exemplified by poor hydration properties, loss in solubility, and poor reconstitutability. Good solubility of powders is usually required to enable the functionality of the protein ingredient to be fully realized. This is because solubility is a prerequisite for many other functional properties of proteins such as their ability to build viscosity, gel, and stabilize foams and emulsions.

High-protein powders are generally more difficult to reconstitute compared to conventional skim milk powder (~34% protein), and the problems of reconstitution are worse with increasing protein content. The solubility of the MPC powders deteriorates further during storage. MPC powders with improved solubility are made by the addition of monovalent salts to the ultrafiltered retentate (Carr, 2002) or by the removal of calcium ions prior to drying (Bhaskar *et al*., 2003).

The effect of removing water beyond a critical level, as can happen during concentration and drying, can lead to aggregation and irreversible denaturation of protein species at an interface. The rate of water removal can induce changes in protein structure. The rate of dehydration is influenced by the mineral environment of dairy concentrates (Schuck *et al*., 1999). Any change in protein structure can potentially lead to an altered functionality of the powder on reconstitution.

The application of a preheat treatment (above 72 °C) to the milk protein retentate or increasing the inlet temperature from 200 to 250 °C impaired the hydration properties of MPC powders with high protein content (>70% protein) (De Castro-Morel and Harper, 2003). The insoluble particles obtained on reconstitution of high-protein MPC powders have been ascribed primarily to the hydrophobic association of casein micelles in the powders (Havea, 2006).

III. ENZYMATIC MODIFICATION PROCESSES

Enzymatic processes such as renneting, hydrolysis, and cross-linking with transglutaminase change the integrity of the casein micelles, resulting in physicochemical and functional changes to milk and milk-derived ingredients. The resultant properties of milk and milk-derived ingredients are largely dependent on the condition in which these enzymatic processes were carried out. An enzymatic route has the benefits of being less harsh compared to the modifications which use chemical agents.

A. Renneting

Renneting is the most used enzymatic process in the dairy industry. When milk is treated with rennet, a selective cleavage of the Phe(105)-Met(106) bond of κ-casein (hairy layer) occurs due to the action of chymosin. On cleavage, κ-casein is split into two polypeptides with very different

properties, a hydrophilic caseinomacropeptide containing residues (106–169) which diffuses into the serum and a hydrophobic para-κ-casein (para-casein; residues 1–105) which remains (Dalgleish, 1992). The cleavage of κ-casein removes the steric layer and part of the electrostatic repulsion, which stabilizes casein micelles. This results in a decrease in casein micellar size (10–14 nm decrease in diameter) and a decrease in zeta potential by ∼40%. Both these factors reduce micelle–micelle repulsion and promote the aggregation of the para-casein (Horne and Davidson, 1992; Walstra and Jenness, 1984; Walstra *et al.*, 1981). The para-casein can either be allowed to coagulate forming a cheese curd or precipitated to obtain rennet casein. This type of casein is commonly used as dairy-based cheese analogues.

1. Rennet gels

Many factors affect the renneting process. The gel development, the structural changes, and the final strength of the gel are influenced by several factors. Some of these factors include the amount of material capable of forming the gel, both the amount of protein and colloidal calcium phosphate (Casiraghi *et al.*, 1987; Green, 1987; McMahon *et al.*, 1993; Storry and Ford, 1982; Udabage *et al.*, 2001; Zoon *et al.*, 1988), the rate of gel formation, and the concentration of rennet used (Lomholt and Qvist, 1997; Okigbo *et al.*, 1985; Zoon *et al.*, 1988).

The rate of the enzymatic cleavage can proceed at temperatures as low as 4 °C, although increasing the temperature increases the rate of the reaction (Dalgleish, 1979). Another way of affecting the enzymatic process is to decrease the pH. The pH of maximum velocity is pH ∼6.0 (Van Hooydonk *et al.*, 1986b).

Differences in the coagulation rate of renneted micelles arise from the differences in the neutralization of negative charge within the micelles, a decrease in repulsion promoting the closest approach of micelles and allowing hydrophobic interactions (Dalgleish, 1992). Increasing the concentration of calcium at a fixed pH (Udabage *et al.*, 2001; Van Hooydonk *et al.*, 1986c), reducing the pH (Sharma *et al.*, 1994; Van Hooydonk *et al.*, 1986a), ultrafiltering milk (Sharma *et al.*, 1994), and increasing the temperature from 31 °C at pH 6.6 (Fox and Mulvihill, 1990) all promote the aggregation process.

Heat treatment of milk above 60 °C, which promotes whey protein denaturation and its complexation with κ-casein at normal milk pH (6.6), also affects renneting properties. An increase in rennet coagulation time and a decrease in gel firmness were observed with increased heat treatment of milk (Menard and Camier, 2005). Ultra-high temperature (UHT) treated milk failed to coagulate completely but the coagulation properties were restored by threefold concentration of the UHT milk (McMahon *et al.*, 1993).

2. Acid and rennet gels

Milk gels can be made by the combined action of rennet and acid. With the combined action of acid and rennet, gels can be made over a broader pH and temperature range than by acidification alone, with both the pH and rennet action influencing the resulting gel properties (Roefs *et al.*, 1990).

The properties of the mixed gels are different from rennet gels or gels made by acidification. Milk gels formed on acidification are relatively less viscous than rennet gels over timescales longer than 1 s (Van Vliet *et al.*, 1989). The aggregating species in milk renneted at pH 6.7 contains colloidal calcium phosphate, whereas those in acid gels are depleted in colloidal calcium phosphate.

B. Hydrolysis

Hydrolysis of proteins results in a cleavage of peptide bonds. This has the effect of reducing the molecular weight of the protein. The original structure and conformation of the protein are lost. Depending on the sites cleaved by enzymes, a range of peptides with altered ratios of hydrophobic to hydrophilic groups are obtained. All these changes will have significant effects on the functionality of the protein. The digestibility of protein is altered and allergenicity of the protein can be reduced. Physical functional properties that are affected by hydrolysis include heat stability, gelling properties, foaming, and emulsification. Hydrolysis is now commonly used to make physiologically functional dairy ingredients.

Most of the current interest in hydrolysis of milk proteins is directed at the production of bioactive peptides. This aspect is not covered here but reviews provide an update of these interests (Korhonen and Pihlanto, 2006). Hydrolysis of proteins for modification of functionality has been also covered by reviews (Chobert, 2003; Foegeding *et al.*, 2002; Kilara and Panyam, 2003).

1. Effects on surface properties

As hydrolyzed proteins are smaller than unhdyrolyed proteins, they can move to an interface and stabilize it more rapidly than intact proteins. However, in comparison to intact proteins, the smaller peptides in hydrolysates form a less cohesive film at the interface and this can affect the stability of the emulsions and foams.

The effects of hydrolysis on surface properties depend on the type of milk protein and the conditions of hydrolysis. Hydrolysis of globular proteins results in the exposure of buried hydrophobic groups. This enhances surface hydrophobicity that improves surface properties. The degree of hydrolysis needs to be optimized for good surface properties. This is governed by the type of protein used, the extent of hydrolysis, and the enzymes used.

Foaming properties are affected by hydrolysis of proteins. Limited hydrolysis (4–10%) of whey protein concentrate (WPC80) by a protease from *Bacillus licheniformis* results in improved foaming properties (Chen, 2003). Partial hydrolysis (up to 6.5%) of whey protein concentrate (WPC35) by pepsin also improved foaming properties but increasing hydrolysis (>6.5%) impaired foaming and emulsification properties. This was considered to be due to the destabilizing effects of small peptides (Konrad *et al.*, 2005). Giardina *et al.* (2004) showed that hydrolysis of caseinate diminished its ability to foam. The differences may be related to the different structure of the intact proteins, differences in enzymes used, and conditions of the reaction.

Hydrolysis of proteins has marked effects on their emulsifying properties. Hydrolyzed whey protein with a degree of hydrolysis of between 10% and 20% had good emulsifying properties (Dalgleish and Singh, 1998). Euston *et al.* (2001) found that whey protein concentrates with low degree of hydrolysis (4–10%) impaired the emulsifying capacity of whey protein concentrate but increasing the degree of hydrolysis to 10–27% improved emulsifying capacity. However, further increases in the degree of hydrolysis reduced emulsion stability and heat stability of emulsions.

In analyzing the observations, factors that control surface activity and stability of foams and emulsions should be considered. Analysis of sequences of peptides and their properties from enzymatic digests may provide a more rational approach to the development of protein hydrolysates with superior emulsifying properties (Panyam and Kilara, 2004). A peptide must be surface-active for it to lower the surface tension. For it to contribute to stability, it should be able to form cohesive films. Both these properties are required for the formation of foams and emulsions. Rahali *et al.* (2000) suggested that an alternative distribution of hydrophobic and hydrophilic sites on the peptides is necessary for emulsification. van der Ven *et al.* (2001) suggested that the emulsifying capacity appears to be unrelated to molecular weight or degree of hydrolysis but that emulsion stabilization properties were related to molecular weight, with peptides of >2 kDa being required to impart stability. In the case of foam formation, hydrolysates with few large hydrophobic peptides were required for rapid diffusion to the interface and for stabilization of the bubble (Rahali and Gueguen, 2000). For foam stability, whey protein hydrolysates required a sufficient amount of >3 kDa peptides while for casein hydrolysates peptides of >7 kDa were desirable (van der Ven *et al.*, 2002).

2. Effects on gelling properties

Hydrolysis of proteins can be used to manipulate gel properties of whey proteins (Foegeding *et al.*, 2002). The hydrolysis of whey proteins (>18%) can lead to gel formation (Doucet *et al.*, 2001). Gelation was attributed to

the small molecular weight peptides that were held together by noncovalent interactions (Doucet *et al.*, 2003).

The treatment of whey proteins with a protease from *B. licheniformis* has been shown to induce gelation of both unheated and heated whey proteins. Increasing the degree of hydrolysis resulted in earlier gelation and increases in gel firmness (Ju *et al.*, 1997). As with intact whey protein gels, the properties of gels made from whey protein hydrolysates varied with pH, but this was dependent on whether the whey proteins were denatured. Gels made with hydrolysates were also sensitive to the presence of salts, with increasing salt concentration leading to more coagulum-like gels (Otte *et al.*, 1999).

C. Transglutamination

Transglutaminase (EC2.3.2.13) catalyzes an acyl-transfer reaction and this results in the formation of cross-links between glutamine and lysine residues. The introduction of new cross-links has important consequences for the functionality of proteins. Many aspects have been covered in a review on the use of transglutaminase in milks and dairy products, which show that it can be used to improve various functional properties (Jaros *et al.*, 2006). Selected aspects are highlighted below.

1. Effects on heat stability

Transglutaminase treatment of milk offers a novel way to improve the heat stability of milks without the use of chemical additives. Transglutaminase-treated milk had markedly improved heat stability at pH > 6.5 compared to untreated milk. This may be related to the effect of intramolecular cross-links formed in transglutaminase-treated milk, which prevents the dissociation of caseins from the micelles under conditions where it would have otherwise occurred (e.g., when colloidal calcium phosphate is removed). This was considered to be the mechanism by which the enzyme-treated milk was stabilized to heat treatment (O'Sullivan *et al.*, 2002a,b).

The treatment of micellar casein dispersions altered pH-heat stability profiles. At a pH up to 6.45, there was a negligible effect on heat stability, but stability to heat treatment at 140 °C was markedly improved when pH was increased to 7.1. In these systems, there was minimal intermolecular cross-linking between micelles (Mounsey *et al.*, 2005).

2. Effects on water-binding and gelling properties

Transglutaminase-treated cross-linked milk that was subsequently acidified with glucono-δ-lactone formed significantly firmer gels, which had a finer protein network than untreated acid milk gels (Faergemand and Qvist, 1997). Treatment of casein micelles with transglutaminase by

addition of the enzyme to milk containing glucono-δ-lactone results in firmer gels with superior water-binding properties compared to acid gels made from untreated micellar casein. Different gel structures in transglutaminase-treated micellar dispersions can be manipulated by controlling the extent of intra- and intermolecular cross-linking of micelles and the degree of disaggregation of the micelles (Schorsch *et al.*, 2000a,b).

The use of transglutaminase increases the strength of acidified whey gels. This was achieved by first cross-linking whey proteins at high pH (7–8) using transglutaminase, followed by cold-set acidification with glucono-δ-lactone to low pH (∼4). Although the cold-set gels made with enzyme-treated whey proteins were less homogenous than those made with untreated whey proteins, the enzyme-treated gels were much firmer. This was attributed to the formation of additional cross-links between enzyme-treated whey proteins (Eissa and Khan, 2005; Eissa *et al.*, 2004).

IV. CHEMICAL MODIFICATION PROCESSES

It is well established that chemical modification of proteins, such as acylation, succinylation, esterification, chemical hydrolysis, and phosphorylation, cause changes in the physical properties of proteins and their digestibility. Chemical agents have generally been used for the synthesis of chemical-modified proteins. However, there are opportunities to use the Maillard reaction, a natural reaction that occurs on heat treatment of food, for covalently attaching sugars and polysaccharides with reducing sugar groups to a protein.

A. Use of chemical agents

Most chemical agents used for studying the chemical modification of proteins are not suitable for food applications. These studies nevertheless demonstrate how changes in amino acid side chains, and both the structure and conformation of proteins can impact on functionality. A comprehensive review of chemical modification of milk proteins has been carried out (Chobert, 2003). Only some highlights and more recent work on modification with chemical agents are covered here.

1. Acylation

Acylation (e.g., acetylation and succinylation) modifies the charge of proteins. When succinylation is carried out, positive amino groups are replaced by negative succinyl groups, inducing a greater increase in negative charge compared to acetylation where the amino groups are replaced by neutral acetyl groups.

The use of the modified proteins has shown the importance of electrostatic interactions in the formation of milk protein-based gels. Succinylation of milk affects the rennet coagulation time and the rate of firming of the coagulum. While some have ascribed the effects to the impaired rate of the primary stage of renneting process, as reflected in the slower release of the caseinomacropeptide (Lieske *et al*., 2000), others have considered that succinylation affected the secondary stage of aggregation by increasing electrostatic repulsions (Vidal *et al*., 1998).

The pH at which cold-set acid whey gels are formed decreases with succinylation. Unmodified β-lactoglobulin starts to aggregate and gel at a pH of 5.1 (near its pI), while succinylated forms of the protein only gel at pH 2.5. This contrasts with the effects of methylation of carboxylic acid groups which removes negative charge, resulting in gelation at alkaline pH (Alting *et al*., 2002).

Acylation affects the casein micelles of milk. The main effects are increased dissolution of the calcium and phosphate from the micelle and increased solubilization of caseins as a consequence of acylation (Vidal *et al*., 2002). As the equilibria of caseins between the micellar and serum phases are known to affect a number of functional properties (e.g., gelation, emulsification), it may be expected that acylation will affect functionality.

2. Esterification with alcohols

As natural milk proteins have acidic isoelectric points, they have low solubility at acid pH and this compromises many of their functional properties in acidic environments. Esterification of proteins increases the net negative charge and raises the isoelectric point of proteins, making them more functional at acidic pHs.

Esterification of milk proteins (β-lactoglobulin, α-lactalbumin, β-casein) with methanol, ethanol, or propanol improves their solubility in the pH range 3–6 and emulsifying activity and stability at low pH (3–5). The extent of improvement was dependent on the degree of esterification, the type of ester group attached, and the nature of the milk protein (Sitohy *et al*., 2001a). Esterification also resulted in reduced digestibility of the proteins by trypsin (Sitohy *et al*., 2001b).

3. Phosphorylation

Caseins have natural ester-bound phosphate and this gives caseins some of their unique properties. Whey proteins do not naturally contain phosphate ester groups. Studies have shown that the phosphorylation of caseins and whey proteins creates novel functionality in these proteins.

Van Hekken and Strange (1997) phosphorylated whole caseins using $POCl_3$. Solutions (0.2–0.7% protein) containing the superphosphorylated caseins, with higher amounts of bound phosphorus (9- to 12.5-mmole

bound P/mmole casein) compared to unmodified caseins (5.6-mmole bound P/mmole casein), were more resistant to thickening in high Ca^{2+} solutions at low protein concentrations. At higher protein concentrations (1–4% protein), they formed gels at high Ca^{2+} concentrations (20–30 mM).

Li *et al*. (2005) phosphorylated whey protein isolate by dry heating in the presence of pyrophosphate. Phosphorylation improved the stability of the whey protein to heat at pH 7. Gels made with phosphorylated proteins were firmer, more resilient, and had better water-holding capacity compared to untreated whey protein isolate gels.

B. Maillard reaction

The Maillard reaction is a complex series of reactions that begins with the interaction of an amino group with a reducing sugar group. The reaction is well known for its effects on the physical properties of food, particularly the color and flavor of foods (Fayle and Gerrard, 2002; Nursten, 2005). The Maillard reaction can occur naturally as in the production or storage of milk powders (Guyomarc'h *et al*., 2000). Under these conditions, the extent of the Maillard reaction is not controlled and is considered to have detrimental effects on powder quality. However, under controlled conditions, it has potential to be used for production of tailored dairy ingredients. The changes in the structure of the protein on conjugation of sugars or polysaccharides under controlled conditions give rise to the development of differentiated functionalities, which are useful when the modified protein is used in ingredient applications (Kato, 2002; Oliver *et al*., 2006a).

There has been much recent interest in the use of glycation for modification of proteins as it is a naturally occurring reaction in foods. It is viewed as an attractive alternative to modification of proteins compared to the use of chemical agents.

The type of carbohydrates and proteins used and the conditions of the reaction have to be controlled to optimize functionality while minimizing excessive browning and formation of other undesirable products which can be obtained in the final stages of the Maillard reaction. Various researchers have examined the effects of type of protein and sugar or carbohydrate, amounts of reactants, and conditions of reaction on a range of functional properties of milk proteins (Chevalier *et al*., 2001a,b).

1. Conditions for preparation of Maillard conjugates

The Maillard reaction can occur under wet conditions in solutions or in the powdered state in humidified atmospheres [typically 60–80% relative humidity (RH)]. Studies have shown that when the Maillard reaction is carried out in the powdered state in humidified atmospheres (''dry'' reaction, 65% RH, 50 °C, 2–48 hour), the structure of the whey proteins

are not significantly altered, whereas there are significant structural changes in proteins when a reaction is carried out in aqueous systems (60 °C, 6–130 hour, pH 7.2) (Morgan *et al.*, 1999).

2. Effects on solubility and heat stability

The covalent attachment of a sugar or a carbohydrate with a reducing sugar end to the free amino groups of a protein causes a loss in positive charge. This results in a change in the solubility profile of the protein as a function of pH and heat treatment.

Improved solubility at low pH was obtained on conjugation with casein with maltodextrin under ''dry'' conditions (Shepherd *et al.*, 2000). Compared to unreacted protein, β-lactoglobulin that was glycated with sugars (arabinose, galactose, glucose, lactose, rhamose, or lactose) at 60 °C (aqueous systems, pH 7.2, 72 hour, anaerobic conditions) was more soluble at acidic pH and more stable to heating at pH 5 (Chevalier *et al.*, 2001a). These studies demonstrate the usefulness of the Maillard reaction for enabling dairy proteins to have differentiated properties compared to the unmodified proteins.

3. Effects on surface properties

There has been a significant interest in the use of the Maillard reaction for improving the emulsifying properties of proteins. The introduction of a sugar or polysaccharide group changes the charge on the protein. This has an impact on its emulsifying capacity and solubility. When a polysaccharide is used, it has the added advantage of imparting increased stability. This is because of the coupling of the steric stabilizing influence of the polysaccharide to the surface-activity of the proteins.

Shepherd *et al.* (2000) showed that conjugation of caseins with maltodextrins improved the emulsifying capacity and stability of caseins at low pH. Darewicz and Dziuba (2001) observed improved emulsifying capacity and stability in glycated β-casein in aqueous systems (37 °C, pH 7.4, 24 hour). This was related to better solubility of the glycated protein and to its ability to form thicker layers around the oil droplets. However, there was no change in emulsifying properties when β-casein was glycated in aqueous systems at lower pH and temperature for longer times (60 °C, pH 6.5, 72 hour) (Groubet *et al.*, 1999).

Chevalier *et al.* (2001a) glycated β-lactoglobulin in aqueous systems and obtained improvement in the foaming and emulsifying properties, but the improvement obtained depended on the type of sugar used. This was attributed to the differences in the site of glycation with the different sugars used (Chevalier *et al.*, 2001b).

The conjugation of protein with polysaccharides has been examined. The emulsifying properties of whey protein isolate that was conjugated to low methoxy pectins under ''dry'' heat conditions had superior emulsion

stabilization properties at pH 5.5. This modification allows whey protein isolates to be used in acidic conditions (Neirynck *et al.*, 2004). Others have compared the effects of type of milk protein and pectin. Einhorn-Stoll *et al.* (2005) formed milk protein (casein or whey protein)–pectin (low or high methoxy pectin) conjugates under ''dry'' reaction conditions (50–60 °C, 65–80% RH, pH 5.8–7.0, up to 15 days). In the systems they examined, the conjugate made with whey protein isolate and high methoxy pectin was the best emulsifier. Caseinate was found to be an unsuitable substrate for effective conjugation and it was suggested that the thermodynamic incompatibility between caseins and pectins contributed to poor conjugate formation.

Maillard conjugates made by interaction of milk proteins with sugars have been shown to enhance the delivery of omega-3 oils. In this application, the good emulsifying properties of the Maillard conjugate and the inbuilt antioxidant activity of the Maillard products enable the production of high-fat tuna oil powders (50% fat) with improved shelf life stability (Augustin *et al.*, 2006).

4. Effects on viscosity and gelation

Another consequence of the Maillard reaction on functionality is a modification of viscosity. Oliver *et al.* (2006b) found that glycoconjugates of casein with inulin and reducing sugars had higher viscosity compared to unmodified casein. Although high viscosity was obtained when reducing sugars (ribose or glucose) were conjugated with casein, this was accompanied by excessive browning. With the use of inulin in combination with fructose, it was possible to markedly increase viscosity without either gelation or excessive browning.

As the Maillard reaction progresses, there can be cross-linking between protein species and the formation of polymeric species. The formation of these species is expected to further modify the viscosity and gelation properties of the proteins.

V. EMERGING PROCESSES

There are emerging food processing technologies that have the potential for altering the functionality of milk and dairy products. These include static high-pressure processing, dynamic high-pressure processing, ultrasound, pulsed-electric field, and microwave heating.

The technology that has attracted the most interest in the dairy industry to date is static high-pressure processing. Many studies have examined the use of high pressure processing for inactivation of microflora. However, it has the potential to alter the physical and technological properties of milk, making it an alternative to other processing methods

for altering the functionality of milk proteins. Much fundamental research has been carried out to show that high pressure (100–600 MPa) causes significant changes to the milk. Notable among these, which can impact on milk functionality, are the disruption of casein micelles, the denaturation of whey proteins, and pressure-induced changes to the mineral equilibria of milk. Some of the consequences of the high-pressure treatment include a reduced rennet coagulation time, an increased cheese yield, and an increase in the firmness of yoghurts (Huppertz *et al.*, 2002, 2006; López-Faňdino, 2006).

Dynamic high pressure, at pressures higher than those used in conventional dairy processing, has also been examined. The use of higher pressure homogenizers (e.g., Microfluidizer or Emulsiflex equipment) in place of conventional homogenizers results in a smaller size distribution of fat globules and changes to the organization of milk protein components (Dalgleish *et al.*, 1996; Paquin, 1999). Recent work has shown that high homogenization pressures (41–186 MPa) cause changes to the structural properties of casein micelles. There was a decrease in micelle size and an increase in the amount of nonsedimentable caseins in the serum (Sandra and Dalgleish, 2005). The structural changes in the casein micelle are expected to have consequences for some of the functional properties of milks. Further work is needed to ascertain the nature and extent of these effects.

Hardham *et al.* (2000) showed that UHT milk treated by microfluidization has adequate heat stability and further that creaming of the milk on storage was reduced. Whiteley and Muir (1996) found that microfluidization was effective for reducing particle size of concentrated milks. Surprisingly, the heat stability of the microfluidized milk was also markedly improved. Further work is required to understand the effects of microfluidization on heat stability. The use of microfluidization as a means to improve the heat stability of whey proteins has been investigated (Iordache and Jelen, 2003). These authors found that microfluidization (150 MPa) of heated whey protein concentrate suspensions disintegrated the insoluble particles in these solutions to nonsedimenting particles. However, these particles that were resolubilized by microfluidization were still sensitive to secondary heat-induced coagulation.

Ultrasound may be used for disruption of fat globules in milk and is an alternative to homogenization for this purpose. It also has the potential to alter the functionality of milk, as demonstrated by its effects on the properties of yoghurt (Vercet *et al.* 2002). These authors showed that the simultaneous application of heat and ultrasound (12 s at 20 kHz) under moderate pressure (2-kg pressure) improved the textural properties of yoghurts.

The effects of high pressure and ultrasound as well as other emerging processing technologies such as pulse-electric field and microwave heating

on the properties of milk and their impact on the functional properties of milk need to be examined in more detail. All these processing treatments result in a stress being applied to the milk system, which will no doubt result in changes to functionality. More research is required to examine the extent to which these processing technologies can be used as an alternative to or in combination with traditional processes (e.g., heating, homogenization, and acidification) to alter functionality of milk and dairy ingredients.

VI. CONCLUSION

Milk and dairy ingredients are valued ingredients in the market place. The functionality of the protein components in milk makes them useful in a range of food applications. Understanding the inherent properties of the individual milk proteins, their interactions with other milk components and also with other components in the final food matrix during processing, is essential for the design of ingredients.

The need to deliver nutrients while maintaining physical functionality represents an added challenge to the design of dairy systems for the delivery of bioactives. The new functionalities that can be achieved by various processing treatments will need to be reexamined when dairy foods are used as delivery vehicles for bioactives. This is because the balance of the components in the system is changed, and this adds a layer of complexity to both formulation and processing. The ability to expand and combine the traditional processes and emerging technologies is an opportunity to meet the demands of providing dairy foods that have sensory appeal and also deliver the health benefits of the added nutrients.

Continued research into the fundamental aspects of milk proteins and the responses to various stresses is necessary for further differentiation of milk products and the delivery of ingredients with consistent quality for target applications. New approaches are required to meet the challenges of designing fitness-for-purpose dairy ingredients. A more complex science approach to complement the traditional reductionist approach to dairy ingredient development may provide further insights into the interactions of ingredients in food formulations and processing environments. A multidisciplinary approach, bringing together traditional dairy and food scientists with scientists from other disciplines such as materials science, molecular science, nanotechnology, and the science of complex systems, is desirable. Methods that enable an examination of dairy and food systems in real time and on different length scales are expected to provide new information about the relationship between functionality and organization of food components into supramolecular and higher hierarchical structures. This information may then be used as the basis for

new processing and formulation strategies to engineer step-changes in the development of new dairy ingredients.

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