

Dairy-based ingredients

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Dairy ingredients have long been used in the cereal-processing industry. They have mainly been added for their good functional properties, ease of production, and the nutritional fortification they provide to the final product. These ingredients also find applications in gluten-free products, where substitution of the structural protein complex of gluten is required in order to render the product suitable for consumption by people with celiac disease. An overview of the production of such dairy ingredients is provided here with their properties described, along with their applications and the problems encountered in the production of gluten-free bread.

Introduction

Over the years a number of dairy ingredients have been used in the food industry. Applications have been extensive and variable, especially for dairy proteins. For caseins and caseinates these applications include baked products, cheese and imitation cheese manufacture, coffee creamers, ice creams, pasta products, cultured milk products, whipped toppings, milk-type beverages, non-milk-type beverages, confectionary products, spreads, meat products, and others (Southward, 1989; Mulvihill, 1992; Damodaran, 1997a; Fox and McSweeney, 1998; Mulvihill and Ennis, 2003). For whey protein products, applications include beverages, confectionary, desserts and dressings, meat products, dairy products, and novel dairy products (De Wit, 1989; Mulvihill, 1992; Damodaran, 1997a; Mulvihill and Ennis, 2003). Lactose finds a number of applications in various dairy products, such as sweetened condensed milk, frozen milk products, milk and whey powders, confectionary products, baby foods, and also as flavor enhancer and an anti-caking agent (Morrissey, 1985; Fox and McSweeney, 1998).

Dairy ingredients are extensively used for their functionality, nutritional value, and ease of production. The most widely used dairy ingredients in gluten-free bread formulations are caseinates (Lazaridou *et al.*, 2007), skim milk powder (Moore *et al.*, 2004; McCarthy *et al.*, 2005), dry milk (Sanchez *et al.*, 2004), whey protein concentrate (Sanchez *et al.*, 2004), and milk protein isolate (Gallagher *et al.*, 2003a).

Production and properties of dairy ingredients: an overview

Production

Caseins

The starting material for the production of caseins, caseinates, and whey proteins is skim milk. The use of skim milk ensures that the fat content of the casein is low enough to minimize flavor defects arising from deterioration of lipids in the dried casein products. Following destabilization the insoluble casein is separated from the soluble whey proteins, lactose, and salts, washed to remove residual soluble solids, and then dried (Mulvihill, 1992; Maubois and Ollivier, 1997). Caseins can be produced in a number of ways, however industrially they are produced either by isoelectric precipitation or proteolytic coagulation. Detailed production methods have been reviewed (Mulvihill, 1989, 1992; Maubois and Ollivier, 1997; Fox and McSweeney, 1998; Mulvihill and Ennis, 2003) and are shown schematically in Figure 10.1. During isoelectric precipitation the pH of the skim milk is reduced to the isoelectric point of casein. The pH decrease can be achieved by addition of a culture of lactic acid bacteria (converting some of the lactose of the milk to lactic acid), or by addition of a dilute mineral or organic acid (producing lactic or acid casein respectively). The pH of skim milk can also be reduced to the isoelectric point of casein by mixing all or part of the milk with an ion-exchange resin at low temperature (Mulvihill, 1989; Maubois and Ollivier, 1997; Lucey and Singh, 2003; Mulvihill and Ennis, 2003).

In the manufacture of acid casein, precipitation is accomplished by under-pressure spraying dilute mineral acids (usually HCl) into preheated milk (25–30°C) flowing in the opposite direction until a pH of 4.6 is reached. Subsequently, steam is injected to heat the acidified milk to the precipitation temperature of about 50°C (Mulvihill, 1992; Maubois and Ollivier, 1997; Mulvihill and Ennis, 2003). During production of lactic casein, pasteurized skim milk is inoculated with one or more defined starters and incubated for 14–16 hours at 22–26°C. Under these conditions, the starters slowly ferment the lactose to lactic acid. As the pH of milk is falling towards the isoelectric point of casein, a casein gel network (the coagulum) is formed, with good water-holding capacity (Mulvihill, 1992; Fox and McSweeney, 1998). The coagulum is then pumped from the coagulation vats and cooked by direct steam injection.

The pH of skim milk can also be reduced to the isoelectric point of casein by mixing skim milk at low temperature (<10°C) with a cation-exchange resin in the hydrogen form in a reaction column. Cations in the milk are replaced by H⁺ to give a final pH of about 2.2. The deionized acidified milk is then mixed with untreated milk

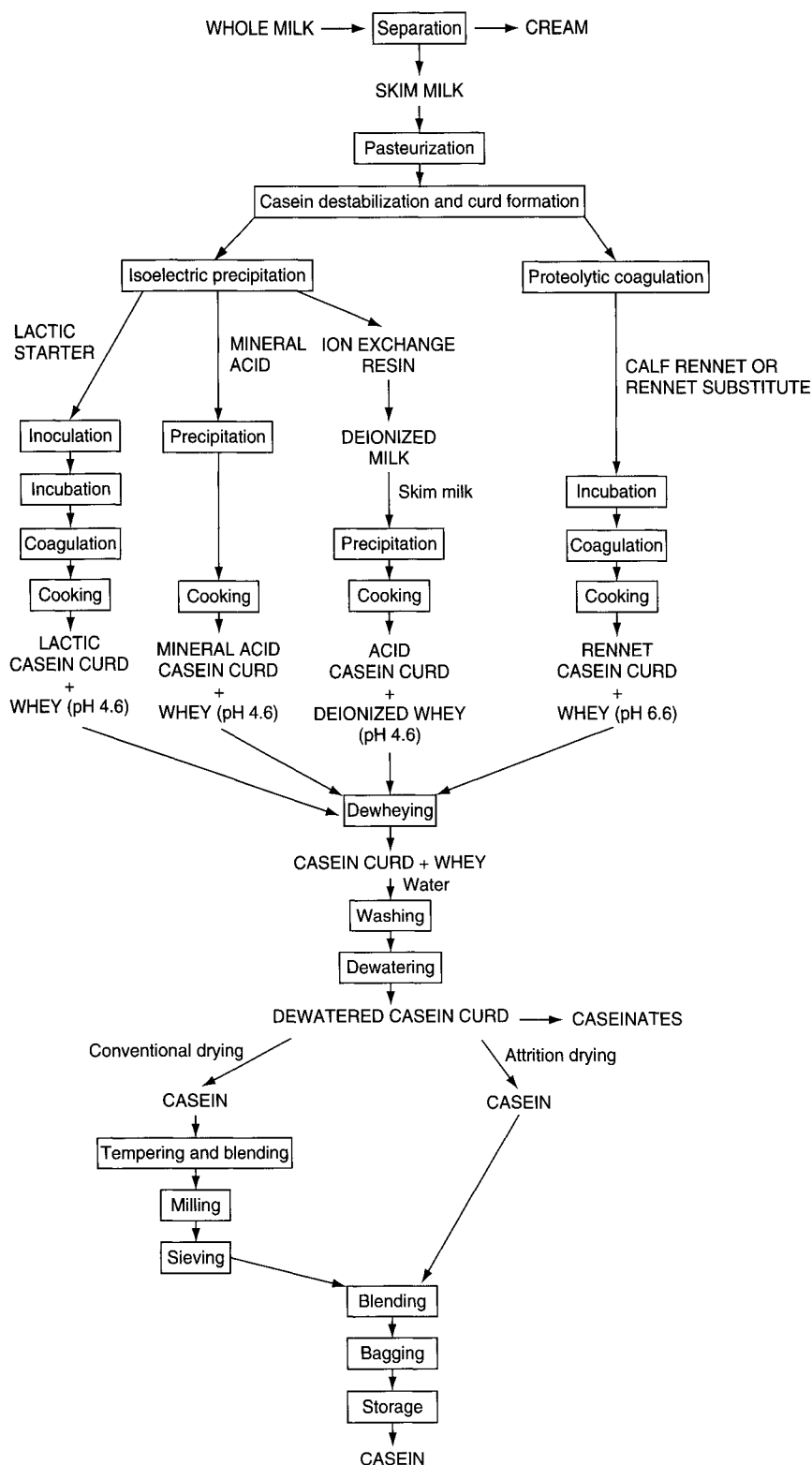


Figure 10.1 Schematic representation of casein production. Reproduced with permission from Mulvihill (1989).

to give the final desired precipitation pH of about 4.6. The mixture is finally heated to the coagulation temperature by direct steam injection (Mulvihill, 1989, 1992; Fox and McSweeney, 1998; Mulvihill and Ennis, 2003). This method is reported to increase the yield by up to 3.5%, giving resulting whey with a lower salt content. However, because of the difficulty of maintaining the ion exchanger under satisfactory bacterial conditions and because of the large volumes of effluents generated, this method is not widely used (Maubois and Ollivier, 1997). During proteolytic coagulation milk is treated with selected proteinases (rennets). The coagulated casein is recovered as rennet casein. However, during this procedure, the κ -casein is hydrolyzed and thus the properties of rennet casein differ fundamentally from those of acid casein (Fox and McSweeney, 1998).

Proteolytic coagulation is therefore described as a two-stage process: the first stage involves the specific hydrolysis of κ -casein to *para*- κ -casein and macropeptides; while the second stage involves coagulation of the rennet-altered casein micelles by Ca^{2+} at temperatures above 20°C. When such coagulum is produced from skim milk, it can be further processed to yield rennet casein (following similar steps as for the production of lactic casein) (Mulvihill, 1989, 1992; Hyslop, 2003; Mulvihill and Ennis, 2003).

Following the destabilization of the casein, the next steps in the casein production are dewheying, washing, dewatering, and drying (Figure 10.1). The efficiency of the dewheying step is of great importance in determining the whey volume recovered for further processing, the efficiency of the washing operation and the quality of the final casein produced (Mulvihill, 1989, 1992; Mulvihill and Ennis, 2003). The equipment used to achieve separation usually includes vibratory, moving, or stationary inclined screens made of nylon or fine mesh stainless steel, or inclined screens made of polyester fabric laid in a cascade-like profile which subjects the curd to turning and rolling as it travels down the slope (Mulvihill, 1989; Mulvihill and Ennis, 2003). Residual whey constituents (lactose, whey proteins, and salts) and free acids are removed from the dewheyed curd to a limited extent by washing the surface of the curd particles; and to a much larger extent by diffusion from within the curd particles. The rate of diffusion depends on the size and permeability of the curd particles, on the concentration gradient of the constituents between the interior of the particles and the washing water, and on the amount, temperature and movement of the washing water (Mulvihill, 1992). When washing is complete, casein curd is mechanically dewatered to minimize the quantity of water to be evaporated, and thus minimize the energy required during the subsequent thermal operation (Mulvihill, 1992; Mulvihill and Ennis, 2003).

To produce a stable and storable product that meets internationally recognized compositional standards for edible grade products, the casein curd is dried to <12% moisture. Traditionally the driers used were of the semi-fluidized vibrating type. In such systems, casein curd passes along vibrating perforated steel conveyors, while warm air is forced up through the perforations, partially fluidizing the curd as it dries. Currently, the most widely used drying technique involves using pneumatic ring driers (Kelly and O'Kennedy, 1986; Mulvihill and Ennis, 2003). These dryers are effectively large, stainless steel ducts (ring-shaped) through which high-velocity heated air and moist disintegrated casein curd are circulated continuously (Mulvihill,

1992; Mulvihill and Ennis, 2003). Dried casein is relatively hot as it emerges from the drier and the moisture content of individual particles varies. Therefore, it is necessary to temper and blend the dried product to achieve a final product of uniform moisture content (Mulvihill, 1989).

A drying process known as “attrition” drying is now widely used in casein manufacture. This process is based on the principle of grinding and drying in a single operation, and it allows the production of a casein product closely resembling spray-dried casein (Kelly and O’Kennedy, 1986; Mulvihill and Ennis, 2003). The drier consists of a fast-revolving, multi-chambered rotor and a stator with serrated surface. In the drier, turbulences, vortices, and cavitation effects result in a highly efficient drying, which produces very small particles with very large surface area. These particles are simultaneously dried in a hot air stream that passes through the drier concurrently with the curd. The dried casein is very fine with an overall average particle size of about 100 μm . The particles have good wettability and dispersability because they are irregular in shape and many contain cavities created by the rapid evaporative process (Kelly and O’Kennedy, 1986; Mulvihill, 1989, 1992; Mulvihill and Ennis, 2003).

Novel methods for casein production include cryoprecipitation, precipitation with ethanol, ultrafiltration, and high-speed centrifugation (Maubois and Ollivier, 1997; Fox and McSweeney, 1998; Mulvihill and Ennis, 2003). During cryoprecipitation, milk is frozen to -10°C . The ionic strength of the liquid phase increases with a concomitant increase in $[\text{Ca}^{2+}]$. The pH drops to approximately 5.8, due to the precipitation of calcium phosphates with the release of hydrogen ions. These changes destabilize the casein micelles which precipitate when the milk is thawed. In precipitation with ethanol, the casein in milk coagulates at pH 6.6 following addition of ethanol (to reach a final concentration of about 40%). Stability decreases sharply as the pH is reduced and only 15% ethanol is required at pH 6.

Caseinates

Acid caseins are insoluble in water but will dissolve in alkali under suitable conditions to yield water-soluble caseinates that may be spray- or roller-dried (Kelly and O’Kennedy, 1986; Mulvihill, 1992; Mulvihill and Ennis, 2003). Sodium caseinate, the water-soluble caseinate most commonly used in food, is usually prepared by solubilizing acid casein with NaOH. Towler (1976) has suggested the following protocol for the commercial production of sodium caseinate:

1. Mince casein curd from a dewatering device (about 45% solids) and then mix with water at 40°C to give a solid content of about 25%, before passing through a colloidal mill. The curd particle size must be reduced as much as possible.
2. Sodium caseinate should have a pH in the range of 6.6–6.8. Pumping NaOH into the casein slurry as it emerges from the mill at 45°C will result in the desired final caseinate pH. As the slurry has the consistency of toothpaste, it must be efficiently mixed with the NaOH with a mixer capable of coping with the high viscosity.
3. Transfer the mixture into a vat where solubilization occurs as the mixture is agitated and heated. The slurry is then re-circulated or pumped into a second vat where solubilization is completed as the temperature of the solution is raised to

about 75°C. Use an in-line pH meter to indicate whether the correct amount of NaOH has been added, or if any further regulation is required.

4. Pump the caseinate solution to a balance tank through a heat exchanger (increasing the temperature to about 95°C). Again, an in-line pH meter should be used to control further addition of NaOH, if required.
5. Pump the solution from the balance tank to the spray drier through an in-line viscometer that regulates addition of hot water (to control viscosity) and ensure sufficient atomization of the solution in the drier.

Other caseinates, such as calcium, ammonium, potassium, or citrated caseinates can also be produced. Calcium caseinate is produced by first passing “soft” casein curd through a mixer to get evenly sized particles. The particles are then mixed with water to give about 25% total solids. The mixture is passed through a colloidal mill and the temperature is adjusted to give milled slurry at 35–40°C. This is then mixed with a metered volume of $\text{Ca}(\text{OH})_2$ slurry to give the desired final pH. The mixture is agitated and re-circulated in a low-temperature conversion tank until conversion is complete (>10 minutes). Finally the dispersion is heated in a tubular heat exchanger to 70°C and is pumped directly to a spray drier (Roeper, 1977; Mulvihill, 1992; Mulvihill and Ennis, 2003). Ammonium caseinates can be prepared by a method similar to that used for the production of sodium caseinate, but NH_4OH or KOH is used instead of NaOH . Granular ammonium caseinate can be prepared by exposing dry acid casein to ammonia gas and then removing excess ammonia with a stream of air in a fluidized bed degassing system (Mulvihill, 1989). Citrated caseinates can also be produced in a similar fashion by using a mixture of trisodium citrate and tripotassium citrate instead of NaOH (Mulvihill, 1992; Mulvihill and Ennis, 2003).

Whey protein products

About 20% of bovine milk proteins belong to a group of proteins generally referred to as whey proteins. The whey proteins as a group are readily prepared from milk by any of the methods described for casein manufacture (Roberts, 1985; Fox and McSweeney, 1998). Whey and whey protein-enriched solutions are usually pasteurized using minimum temperature and holding times, and maintained at low temperature to minimize microbial and physico-chemical deterioration of the proteins and other whey constituents that would adversely alter functional and organoleptic properties of the resulting products (Mulvihill, 1992). The production of whey and whey protein-enriched fractions have been extensively reviewed (Huginin, 1985; Kelly and O’Kennedy, 1986; Morr, 1989; Mulvihill, 1992; Maubois and Ollivier, 1997; Mulvihill and Ennis, 2003).

On a commercial scale, whey protein-rich products are prepared by (Fox and McSweeney, 1998):

1. Ultrafiltration/diafiltration of acid or rennet whey to remove varying amounts of lactose, and spray-drying to produce whey protein concentrates (30–80% protein).
2. Ion-exchange chromatography: proteins are adsorbed on an ion exchanger, washed free of lactose and salts, and then eluted by pH adjustment. The eluate is freed

of salts by ultrafiltration, and spray-dried to yield whey protein isolate (about 95% protein).

3. Demineralization by electrodialysis and/or ion exchange, thermal evaporation of water and crystallization of lactose.
4. Thermal denaturation, recovery of precipitated protein by filtration/centrifugation and spray-drying to yield lactalbumin, which has very low solubility and limited functionality.

Coprecipitates

Following precipitation of caseins from milk by acidification or renneting, the whey proteins remain soluble in the whey. However, these can be coprecipitated in combination with the casein by first heating milk to temperatures that denature the whey proteins, thus inducing their complexation with casein. Thereafter, the milk protein complexes are precipitated by acidification to pH 4.6 or by a combination of added CaCl_2 and acidification (Mulvihill, 1989, 1992; Fox and McSweeney, 1998; Mulvihill and Ennis, 2003). Products thus obtained are referred to as casein–whey coprecipitates and yields obtained can reach 92–95% of total milk protein, compared with <80% for rennet or acid caseins (Mulvihill, 1992; Mulvihill and Ennis, 2003).

Milk protein concentrate

Skim milk may also be processed directly by ultrafiltration/diafiltration to yield milk protein concentrates that contain a range of protein contents around 80% (Vetter, 1985; Maubois and Ollivier, 1997; Kelly *et al.*, 2003), and in which the casein is in a similar micellar form to that found in milk, while the whey proteins are also reported to be in their native form. These products have a relatively high ash content since protein-bound minerals are retained (Mulvihill, 1992; Kelly *et al.*, 2003).

Properties of dairy ingredients

Dairy proteins are available to food chemists in a wide variety of products. These products are readily available in dry, liquid, or condensed form, depending on the user's needs and handling capabilities. Usually, the selected dairy protein product will reflect both functionality considerations and cost efficiency (Stahel, 1983; Tow, 1985; Mannie, 1999).

Solubility

A typical solubility/pH profile of casein shows that close to its isoelectric pH the acid form of casein is completely insoluble (Roberts, 1985; Fox and McSweeney, 1998; Mulvihill and Ennis, 2003), whereas at pH values >5.5, the casein is converted to a cationic salt (Na, K, or NH_3) and is completely soluble (Mulvihill, 1992). Insolubility in the isoelectric point is clearly advantageous in the production of acid casein and is exploited in the production of two major families of dairy products (i.e. fermented milks and fresh cheeses) (Fox and McSweeney, 1998). Sodium and potassium caseinates exhibit improved solubility and functionality

compared with calcium caseinate. This is probably due to larger sized and more strongly interacting calcium caseinate aggregates due to cross-linking by the divalent cations. Sodium and potassium caseinates are completely soluble in water at pH value above 5.5, while calcium caseinate forms stable colloidal dispersions rather than solutions (Kelly and O'Kennedy, 1986). It is well known that the solubility of whey proteins is a function of both pH and ionic strength (De Wit, 1989; Cayot and Lorient, 1997). Due to their native conformation, whey proteins are soluble at low ionic strength over the entire pH range required for food applications (Claypool, 1985). In the undenatured form, whey proteins exhibit little water-binding capacity (Huginin, 1985; Cayot and Lorient, 1997). However, being globular proteins, salting out at high salt concentrations decreases their solubility (Mulvihill, 1992). The solubility of whey proteins is impaired by heat treatment above 70°C when the pH is between 4.0 and 6.5, and this has serious consequences for the foaming and emulsifying abilities of whey proteins (De Wit, 1989; Cayot and Lorient, 1997).

Solubility of coprecipitates can vary markedly under the influence of pH, agitation, mixing time and power, temperature, particle size and concentration of the casein product as well as the presence of other species (e.g. dissolved salts). The solubility of coprecipitates has generally been considered in the pH range from 6 to 10. However, it has been demonstrated that coprecipitates can also be dissolved in acids at pH 2–3 (Southward and Goldman, 1975). Generally, all grades of coprecipitates are less soluble than sodium caseinate at pH 7, the insoluble fraction consisting mainly of denatured whey protein, and representing from 4 to 15% of the coprecipitates (Southward and Goldman, 1975; Kelly and O'Kennedy, 1986).

Heat stability

Sodium, potassium, and ammonium caseinates are very heat stable. As Mulvihill (1992) states, a 3% (w/v) sodium caseinate solution at pH 7.0 can be heated at 140°C for 60 minutes without coagulation. Calcium caseinate, however, has much lower heat stability and even 1% (w/v) dispersions gel on heating at 50–60°C. Whey proteins are susceptible to denaturation at temperatures higher than 70°C. The susceptibility of whey proteins to heat denaturation is influenced by factors such as pH, Ca^{2+} , protein concentration and the presence of sugars (Mulvihill and Fox, 1989; Mulvihill, 1992; Fox and McSweeney, 1998; Singh and Havea, 2003). When heated, the bonds creating the tertiary structure of the protein globules are destroyed, unfolding of the protein molecules occur and new protein–protein interactions result. Loss of solubility is one functional change which occurs following protein denaturation (Huginin, 1985; Cayot and Lorient, 1997).

Gelation and coagulation

Milk proteins can undergo gelation, and, in most cases, casein is the component involved (Mulvihill and Fox, 1989; Mulvihill, 1992). Gelation or coagulation occurs when milk is subjected to limited proteolysis by acid proteinases, which hydrolyze the micelle-stabilizing κ -casein, producing *para*- κ -casein-containing micelles which coagulate at the concentration of Ca^{2+} in the milk serum (Mulvihill, 1992; Lucey and

Singh, 2003). Acid gelation is exploited during manufacturing of fermented milks, acid cheeses, and acid caseins. Concentrated calcium caseinate dispersions (>15% protein) gel on heating at 50–60°C. Gelation temperature increases with increased protein concentration (to 20%) and when the pH is in the range 5.2–6.0. The gel liquefies slowly on cooling but reforms on heating. Calcium caseinate is the only milk protein system reported to have reversible thermal gelation properties (Mulvihill and Fox, 1989; Mulvihill, 1992), and κ -casein appears to be the component principally responsible. Hydrophobic bondings are considered to be involved (Mulvihill and Fox, 1989). The caseins are remarkably heat stable and do not undergo thermally induced gelation except under extremely severe conditions.

Thermal sensitivity is undesirable when a soluble whey protein-enriched product is prepared. However, this property can be exploited in the production of thermal gels from whey proteins, which have excellent thermal gelling properties (Mulvihill and Fox, 1989; Mulvihill, 1992; Singh and Havea, 2003). β -Lactoglobulin is considered to be the most important whey protein from a thermal gelation viewpoint, although bovine serum albumin and the immunoglobulins are also known to form stable gels on heating (De Wit, 1989; Mulvihill and Fox, 1989; Cayot and Lorient, 1997; Singh and Havea, 2003). Whey protein concentrates and isolates with a range of gelling properties can be produced by a selection of whey types or variations in processing conditions during manufacturing (Mulvihill and Fox, 1989; Mulvihill, 1992; Carr *et al.*, 2003). Gelation temperature ranges from 50 to 90°C, although it has been shown that it is possible to produce whey protein concentrates which gel at 20°C (Kelly and O’Kennedy, 1986).

Hydration

Many of the functional food applications of dairy proteins depend on their ability to hydrate, and thus bind or entrap water. Under the general property “hydration” one might include solubility, dispersibility, wettability, water absorption, swelling, thickening, gelling, rheological behavior, water-holding capacity, syneresis, and dough formation (Mulvihill and Fox, 1989). The ability to bind and hold water without syneresis is critical in many foods. Although the caseins are relatively hydrophobic, they contain regions of high, medium, or low hydrophobicity and of high negative charge, high positive charge, or low net charge (Carr *et al.*, 2003), and they bind about 2 g water/g, which is typical of proteins (Fox and McSweeney, 1998). The level of hydration of proteins is strongly influenced by the level of available water and it is common to relate the degree of hydration to the relative humidity of the environment to which the protein is exposed (Mulvihill, 1992). Hydration increases with increasing pH and is relatively independent of NaCl concentration, which is especially important in the efficacy of casein in meat-based applications (Fox and McSweeney, 1998). A plot of bound water as a function of relative humidity yields a water sorption isotherm which gives useful information on the water-binding or hydration characteristics of the proteins. Isotherms for sodium caseinate, acid casein, and micellar casein show that the hydration of the acid casein is higher than that of micellar casein, the differences being small for $a_w < 0.6$ and much greater when $a_w > 0.6$. High hydration values for sodium caseinate at high a_w indicate swelling and

solubilization (Mulvihill, 1992). The water-holding capacity of sodium caseinate is higher than that of both calcium caseinate and micellar casein (Fox and McSweeney, 1998). The influence of dispersed particles on small and large deformation properties of a concentrated sodium caseinate composite has recently been examined, and the amphiphilic properties of the sodium caseinate were demonstrated (Manski *et al.*, 2007). The water absorption of coprecipitates in flour dough mixtures has been studied and generally insoluble coprecipitates have lower water absorption values than soluble ones (Southward and Goldman, 1975).

Viscosity

Because of hydration, swelling, and polymer–polymer interactions, caseinates form highly viscous solutions at concentrations higher than 15%, and even at high temperatures the viscosity of solutions containing more than 20% protein is so high that they are difficult to process (Mulvihill and Fox, 1989; Mulvihill, 1992). The effects of solution conditions on the viscosity of caseins/caseinates are crucial. The viscosity of sodium caseinate is strongly dependent on pH, with a minimum at pH 7.0 (Mulvihill, 1992). Ammonium caseinate is more viscous than sodium caseinate between pH 6 and 8.5 (Mulvihill and Fox, 1989). When compared with sodium caseinate, calcium caseinate has lower viscosity at the same concentration and pH. The reason lies in the differences between the structure of the calcium caseinate particles in the calcium caseinate dispersions and the sodium caseinate aggregates in the sodium caseinate solutions (Carr *et al.*, 2003). The various manufacturing conditions also affect the viscosity of casein/caseinates. Excessive heating of milk prior to casein manufacture, or of casein curd during drying, leads to increased viscosity of the caseinates prepared from such caseins. Precipitation at lower than normal pH values (e.g. 3.8) and especially at higher pH values (e.g. 5.05) also increases the viscosity of caseinates, while even the viscosity of roller-dried caseinate is higher than that of spray-dried caseinate (Mulvihill and Fox, 1989; Mulvihill, 1992; Carr *et al.*, 2003). Solubilized conventional coprecipitates are more viscous than sodium caseinate and their viscosity increases with increasing calcium concentration. Low calcium coprecipitates were found to have a viscosity similar to that of acid casein, while the viscosity of medium- and high-calcium coprecipitates was relatively high when the pH was above 7 (Southward and Goldman, 1975). Solutions of total milk protein have viscosities between those of sodium caseinate and conventional coprecipitates (Mulvihill, 1992). The viscosity of caseinates can be markedly reduced by treatment with disulfide-reducing and/or sulfhydryl-blocking agents. However, because of the reagents used, those caseinates would be of little interest for the food industry (Mulvihill and Fox, 1989).

Due to their compact globular shapes (Carr *et al.*, 2003), solutions of undenatured whey proteins are much less viscous than caseinate solutions. They exhibit minimum viscosity around the isoelectric point (pH 4.5) and relative to water, their viscosity decreases between 30 and 65°C, but increases thereafter owing to protein denaturation (Mulvihill and Fox, 1989; Mulvihill, 1992; Carr *et al.*, 2003). Above 85°C a further increase in viscosity was observed as a consequence of protein aggregation (De Wit, 1989). Heat-denatured whey proteins, although retaining most of their secondary

structure, are linked together and, depending on the environment during denaturation and during measurement, can have a perceived hydration of over 10 g of water/g protein, compared with 0.2 g water/g protein for whey proteins that are in their native globular state (Carr *et al.*, 2003). The viscosity of whey concentrates in the range from 25 to 40% total solids depends strongly on the composition and pre-heat treatment of the whey. Additionally, in a food process, protein solutions are frequently subjected to high degrees of shear and extremes of temperature (Carr *et al.*, 2003). Shear exerted on dispersions of denatured whey proteins may break up large aggregates and this can result in a decrease in viscosity (De Wit, 1989).

Emulsifying—foaming

Soluble caseinates have greater emulsifying capacities than the more aggregated caseins, and aggregated caseins give emulsions with higher viscosity (Mulvihill and Fox, 1989; Dalgleish, 1997). In general, caseinates have superior emulsification properties than whey protein concentrates, presumably due to a more favorable balance between exposed hydrophobic and hydrophilic region which impart surfactant like properties to them (Kelly and O’Kennedy, 1986; Kelly *et al.*, 2003). Caseinates behave quite differently from whey protein concentrate during emulsification. During the formation of emulsions in caseinate protein continues to be absorbed from the bulk phase as new surface is formed, whereas with whey protein concentrate proteins already adsorbed are spread or unfolded over the newly formed surface in preference to further adsorption from the bulk solution (Mulvihill and Fox, 1989; Dalgleish, 1997). The most relevant factors affecting the emulsifying properties of whey proteins are protein concentration, protein solubility, pH, salts, presence of other solutes and temperature (De Wit, 1989; Cayot and Lorient, 1997). Homogenization of oil in whey protein concentrate systems resulted in decreasing droplet size as the whey protein concentration increased 10-fold. The ability of whey proteins to stabilize oil/water emulsions seems to be particularly affected by the ionic strength and the pH of the aqueous phase. Obviously, electrostatic interactions around the isoelectric point are responsible for protein aggregation and, as a consequence, the proteins are less flexible and therefore less prone to form a cohesive interfacial film (De Wit, 1989; Mulvihill and Fox, 1989; Cayot and Lorient, 1997). The presence of salts during the emulsification process (outside the pI) may also affect the emulsion activity of whey proteins by influencing their conformation and solubility. Temperature is another factor that affects the emulsifying properties of whey proteins. In particular, the rate of diffusion to the newly formed interface as well as the rates of adsorption and unfolding increase with increasing temperature (De Wit, 1989; Cayot and Lorient, 1997).

Foams can be defined as colloidal systems in which air bubbles are dispersed into an aqueous continuous phase (Damodaran, 1997b). Essential for the formation of protein-based foams is a rapid diffusion of protein to the air–water interface to reduce surface tension, followed by partial unfolding of the protein (De Wit, 1989). The most important foaming characteristics of proteins are foam volume (% overrun) and foam stability. For optimum protein concentrations and whipping times, both sodium and calcium caseinates gave a higher overrun than whey protein concentrate (Mulvihill and Fox, 1989). Southward and Goldman (1978) reported that sodium caseinate gave

slightly lower overrun values than egg albumin but the sodium caseinate foams were less stable. Stability increased with the addition of sugar. The whipping properties of industrially prepared whey protein products are affected by several factors. The most relevant of those are: concentration and state of the whey proteins, pH, ionic environment, (pre-) heat treatment and the effect of lipids. As the whey protein concentration is increased the foam becomes denser, with more uniform air bubbles of a finer texture. Generally overrun (foam volume minus initial liquid volume) increases with protein concentration to a maximum value after which it decreases again (De Wit, 1989). In practice, while the caseins are very good emulsifiers and foam readily, the resulting foams are not very stable (Mulvihill, 1992; Fox and McSweeney, 1998). Two macroscopic processes in foams affect the stability of protein stabilized foams, the rate of liquid drainage from the lamellae and the film rupture. The rates of these two processes are dependent on the physical properties of the protein film and the physics of the lamella itself (Damodaran, 1997b). Cayot and Lorient (1997) also suggest that improvement in foam stability is related to the protein's ability to form a cohesive film. Conversely, the flexibility of the protein's polypeptide chain, although essential for foam creation, is detrimental to foam stability. Indeed, for a protein to foam well and stabilize the foam, it should display a proper balance of flexibility and rigidity at the air/water interface (Damodaran, 1997b).

Southward and Goldman (1978) found that soluble high- and medium-calcium coprecipitates both exhibited good emulsion-stabilizing properties. Soluble acid coprecipitates had the lowest stabilizing capacity of those coprecipitates examined, yet it still compared favorably with commercial sodium caseinate. In addition, all coprecipitates when whipped alone or with sugar exhibited greater foam volume and stability than the corresponding sodium caseinate whips (Southward and Goldman, 1978).

Application of dairy ingredients in gluten-free food

The replacement of gluten presents a major technological challenge, as gluten is an essential structure-building protein, contributing to the appearance and crumb structure of many baked products (Gallagher *et al.*, 2004; Lazaridou *et al.*, 2007). A large number of investigative techniques have been used in many studies for understanding the fundamental mechanical/rheological properties of gluten, including small and large deformation tests, temperature and frequency sweeps, bubble inflation, microscopy, and more (Schofield *et al.*, 1984; Weegels *et al.*, 1994; Dobraszczyk and Roberts, 1994; Guerrieri *et al.*, 1996; Janssen *et al.*, 1996; Stathopoulos *et al.*, 2000, 2001; Toufeili *et al.*, 2002; Dobraszczyk and Morgenstern, 2003; Dobraszczyk, 2004; Li *et al.*, 2004; Stathopoulos *et al.*, 2006, 2007). Studies on the rheology of gluten-free baked products have taken place recently (Gallagher *et al.*, 2003a, 2003b, 2004; Schober *et al.*, 2003; Moore *et al.*, 2004, 2006; Sanchez *et al.*, 2004; McCarthy *et al.*, 2005; Lazaridou *et al.*, 2007), and this field is developing rapidly. Over the years a lot of projects have been undertaken in the area of fortification/supplementation of wheat flour with dairy ingredients and products rather than gluten substitution (Stahel, 1983;

Harper and Zadow, 1984; Dubois and Dreese, 1985; Tow, 1985; Gelinas *et al.*, 1995; Erdogdu *et al.*, 1995a, 1995b; Erdogdu-Arnoczky *et al.*, 1996; Mann, 1996; Mannie and Asp, 1999; Kenny *et al.*, 2000, 2001; O'Brien *et al.*, 2000; Crowley *et al.*, 2002; Singh *et al.*, 2003; Gallagher *et al.*, 2005; Esteller *et al.*, 2006). Other ingredients (reviewed in other chapters of this book) have been used in gluten replacement, including starches and gums or hydrocolloids, as well as dietary fiber.

A few years ago it was not believed that, because of their properties, milk proteins can be used to replace gluten in bakery products. However, their use as a nutritional supplement and their functional effects had been accepted (Mulvihill, 1992; Hambræus and Lonnerdal, 2003). Nutritional benefits include increasing calcium and protein content, as well as supplying essential amino acids (i.e. lysine, methionine, and tryptophan) (Kenny *et al.*, 2000). Recently, the addition of dairy products in gluten-free bread formulas is common practice, for increasing water absorption and therefore enhancing the handling properties of the batter (Gallagher *et al.*, 2004). In addition to the nutritional benefits and the increased water absorption, reduced staling rate and increased crust color are some of the advantages of dairy ingredients in breadmaking (Stahel, 1983; Harper and Zadow, 1984; Dubois and Dreese, 1985; Tow, 1985; Cocup and Sanderson, 1987; Gelinas *et al.*, 1995; Mann, 1996; Mannie and Asp, 1999; Kenny *et al.*, 2000; O'Brien *et al.*, 2000; Crowley *et al.*, 2002; Esteller *et al.*, 2006).

Gallagher *et al.* (2003b) applied seven dairy powders to a gluten-free bread formulation. In general, addition of powders with high protein/low lactose content (i.e. sodium caseinate and milk protein isolate) resulted in breads with an improved overall shape and volume, as well as a firmer crumb texture. The breads also had a better appearance (white crumb and dark crust) and organoleptically they scored well. Depending on the powder and level of addition, differences in loaf volume were observed. Inclusions of dairy powders reduced the loaf volume by about 6%, confirming previous data (Gelinas *et al.*, 1995; Erdogdu-Arnoczky *et al.*, 1996), however, increasing the level of inclusion of sweet whey, sodium caseinate, and milk protein isolate allowed recovery of the loaf volume. The opposite effect was observed when using demineralized whey, fresh milk solids, and skim milk powder. Overall, this work has proved that without a detrimental effect to the loaf volume, application of dairy powders can give products that are more appealing to the panelists than the control formulations (Gallagher *et al.*, 2003a).

Response surface methodology (RSM) has recently been used in order to optimize dry milk and whey protein concentrate fortification of a gluten-free bread formulation. Addition of 7.5% soy flour and 7.8% dry milk to a previously developed formulation increased the protein content from 1 to 7.3% and modified, to a small extent, the sensory quality of the resulting bread (Sanchez *et al.*, 2004). RSM has also been used to optimize water and hydroxypropylmethylcellulose (HPMC) content in a gluten-free bread formulation containing rice flour, potato starch, and skim milk powder (McCarthy *et al.*, 2005).

Another important benefit from using dairy ingredients in bakery and gluten-free products is the extension of shelf-life (Mannie and Asp, 1999; Kenny *et al.*, 2000). Gallagher *et al.* (2003b) assessed the effect of dairy powder addition to the

intermediate and long-term shelf-life of gluten-free breads stored under a modified atmosphere by examining the staling profile of the formulations. They found that addition of milk protein isolate resulted in loaves with increased volume and better appearance and acceptability, ultimately there were no changes in the staling rate. Recently, textural studies have been conducted by Moore *et al.* (2004) with two gluten-free bread recipes, one containing 37.5% (dry weight) skim milk powder. Results were compared with those obtained using commercially available gluten-free flour and regular wheat bread. Baking tests showed that wheat bread and the bread made from the commercially available mix yielded significantly higher loaf volumes, while all the gluten-free breads were brittle after 2 days of storage. However, these changes were less pronounced when skim milk powder was present, indicating a positive influence of the dairy product addition. Using confocal laser-scanning microscopy it was shown that the dairy-based gluten-free bread contained network-like structures resembling the gluten network of wheat bread crumb.

The effect of sodium caseinate in combination with different hydrocolloids has been recently investigated (Lazaridou *et al.*, 2007). The type and extent of influence on bread quality was dependent on the specific hydrocolloids used and their supplementation levels.

Problems associated with the incorporation of dairy ingredients in gluten-free cereal products

Currently, many gluten-free breads available in the market are of poor quality and flavor, and many exhibit a dry, crumbly texture (Gallagher *et al.*, 2003a, 2004; McCarthy *et al.*, 2005). Gluten is considered the “structural” protein of breadmaking, and its absence often results in a liquid batter rather than a dough pre-baking. Many gluten-free baked breads exhibit a crumbly texture, poor color, and other quality defects post-baking (Gallagher *et al.*, 2004; McCarthy *et al.*, 2005). Gluten slows the movement of water in the dough by forming an extensible protein network, thus keeping the crumb structure softer (Gan *et al.*, 1995; Gallagher *et al.*, 2003a). In gluten-free batters, the absence of gluten may allow the increase of water movement from crumb to crust, thereby resulting in a firmer crumb and a softer crust (Gallagher *et al.*, 2003a). A further problem of gluten-free bread is the lighter color of the crust (Gallagher and Gormley, 2002). Recently, it was shown that inclusion of dairy powders results in a darkening of the crust, probably due to Maillard browning and caramelization reactions (Gallagher *et al.*, 2003a). However, almost every milk fraction has been described as loaf-volume depressing (Harper and Zadow, 1984; Erdogdu-Arnoczky *et al.*, 1996; Kenny *et al.*, 2000; Singh *et al.*, 2003; Esteller *et al.*, 2006), thus it is important to clearly determine the proper content of dairy ingredients which will allow an increase in color without leading to a reduced volume of the final bread.

An important aspect that has to be considered when developing gluten-free formulations based on dairy products is the lactose-content of the powders. People with celiac disease have been reported to be lactose-intolerant, so products containing high

lactose concentration are not suitable for them, because of the absence of the lactase enzyme which is produced by the villi (Ortolani and Pastorello, 1997; Gallagher *et al.*, 2004; Moore *et al.*, 2004). Another problem associated with gluten-free formulations is the selected starch source. Regardless of the type of dairy ingredient involved, the starches used in gluten-free recipes are often wheat starches. Those should, in principle, be free of gluten and gliadins. However, it is very difficult to completely remove the gliadins, therefore trace amounts of the allergenic proteins might be present (McCarthy *et al.*, 2005). Methods for detecting gluten-containing cereals in gluten-free applications have been developed (Olexova *et al.*, 2006), and should be used to investigate the gluten-free status of the selected starch.

Future trends

It is currently believed that, despite the obstacles encountered, it will be possible to completely replace gluten with (one or more) functional dairy ingredients, without negatively affecting the rheological or the organoleptic properties of the gluten-free bread. Presently mixtures of gums, hydrocolloids, and dairy protein products are the most popular approach (McCarthy *et al.*, 2005; Lazaridou *et al.*, 2007). Mixtures of gluten-free flours, soy protein isolate, in combination with gums (locust bean, guar, konjak, xanthan, HPMC) and dietary fiber, are expected to be present in the next generation of gluten-free breads, along with functional dairy ingredients.

Current research aims to completely substitute gluten with a functional casein-based ingredient. The principle behind this approach is that by increasing the calcium concentration to an optimum level in the casein/caseinate ingredient it will be possible, under the correct pH and ionic strength conditions, to replace the highly functional (covalent) S-S bonds in a wheat dough with calcium (coordination) links (Stathopoulos and O'Kennedy, 2007).

Sources of further information and advice

There are a number of resources available regarding celiac disease, e.g. publications, recipe books, and websites. Interested individuals should have no problem locating a wealth of information, while most Western countries have celiac disease societies providing information and support. On the subject of dairy ingredients, their production, and their applications in the food and baking industry, there are a number of reviews available (Mulvihill, 1989, 1992; De Wit, 1989; Southward, 1989; Dalgleish, 1997; Morr, 1989; Mulvihill and Fox, 1989; Maubois and Ollivier, 1997; Cayot and Lorient, 1997; Damodaran, 1997a; Fox and McSweeney, 1998; Fox, 2003; Swaisgood, 2003; De Kruif and Holt, 2003; O'Connell and Fox, 2003; Nieuwenhuijse and van Boekel, 2003; Kelly *et al.*, 2003; Mulvihill and Ennis, 2003; Dickinson, 2003; Singh and Havea, 2003; Carr *et al.*, 2003) and the reader is advised to seek some of those for information in greater detail than provided in this chapter.

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