

Advances in Dairy Ingredients

Geoffrey W. Smithers and
Mary Ann Augustin

EDITORS



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Advances in Dairy Ingredients



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Edited by

Geoffrey W. Smithers

Mary Ann Augustin



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To

Bernard S. “Bernie” Horton (1937–2006)
Dairy colleague and friend

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Preface

Consumers are increasingly demanding more of their food without any compromise in safety. Consumer megatrends include health and nutrition, convenience, price, quality and taste, and reduced environmental impact associated with food processing operations. Dairy-derived ingredients provide today's food manufacturer with a vast array of possibilities when developing and formulating food products that meet many if not all the demands of the modern consumer. Dairy fluids (milk, whey, and colostrum) represent a rich source of components with valuable nutritional, functional, and biological characteristics. These components, including proteins, peptides, fats, carbohydrates, and minerals, form the foundation to a vast array of successful ingredients used universally in a range of formulated foods and beverages.

Commercial success has been built on a sound science understanding of these dairy components, their characteristics, and how they interact with other components in a complex food matrix. This underpinning science knowledge of dairy components and their food systems provides the modern food manufacturer, keen to meet marketplace demands, with the means to quickly develop new products with targeted traits. Moreover, underpinning knowledge allows dairy ingredient producers to provide food manufacturers with "tailored solutions" through sound scientific advice on incorporation of dairy ingredients into their formulations. Functional foods are emerging as key drivers of the global food economy, and dairy ingredients and technology are at the forefront in these developments.

Advances in Dairy Ingredients brings together food scientists, industry specialists, and marketers from around the world to provide a unique insight into the basis for the success of dairy ingredients in modern food products, and a glimpse into the future of new dairy ingredients and foods on the horizon. Market and manufacturing trends and opportunities are aligned with the latest science tools that provide the foundation to successfully and rapidly capture these opportunities. *Advances in Dairy Ingredients* provides an international perspective on recent developments in the area of dairy ingredients and dairy technology.

Advances in Dairy Ingredients should have wide appeal from academics through to industry personnel because of the mixture of content highlighting dairy science research and development linked to exploitation of this science and technology in the marketplace. The chapters comprising *Advances in Dairy Ingredients* provide the reader with insight into recent developments in respect to the major milk components, including high protein powders, lactose, specific biofunctional protein isolates, and milk fat globule membrane. The book also explores modern processing approaches for the manufacture of dairy ingredients such as separation technologies, nonthermal processing, and spray-dried emulsions. Unlike other books in the area, *Advances in Dairy Ingredients* provides the reader with recent developments in the use of dairy ingredients in the texturizing of foods and food microstructure, probiotics and prebiotics as ingredients, what consumers are demanding in

their dairy foods, as well as other foods containing dairy ingredients, and the no-compromise area of dairy safety. Finally, the reader is provided with a glimpse into the future of dairy ingredients and what will be the critical science developments that will underpin commercial success. We sincerely thank all the contributing authors.

We dedicate this book to a long time dairy colleague and friend—the late Bernard S. “Bernie” Horton. Bernie was a pioneer in the use of technology for the processing of dairy fluids, much of which is commonplace in modern dairy factories, and was a champion of dairy proteins as valuable nutritional and functional ingredients. Bernie helped shape today’s dairy industry and contributed to the success that dairy ingredients enjoy in the broader food industry of today.

Geoffrey W. Smithers
Mary Ann Augustin

Advances in Dairy Ingredients

1 Dairy Protein Powders

P. Schuck

1.1 INTRODUCTION

The purpose of the dehydration of milk and whey is to stabilize these products for their storage and later use. Dehydration by spray drying is a valuable technique for water evaporation. Milk and whey powders are used mostly in animal feeds. With changes in agricultural policies (such as the implementation of the quota system and the dissolution of the price support system in the European Union), the dairy industry has been forced to look for better uses for the dairy surplus and for the by-products of cheese (whey) produced from milk and buttermilk produced from cream. Studies on the use of protein fractions with nutritional qualities and functionality led us to believe that they could have several applications (Corredig, 2009; Thompson et al., 2009).

In the past 30 years, the dairy industry has developed new technological processes for extracting and purifying proteins (e.g., casein, caseinates, and whey proteins) (Kjaergaard et al., 1987; Maubois, 1991), such as milk protein concentrate (MPC), milk protein isolate (MPI), whey protein concentrate (WPC), whey protein isolate (WPI) (Goudédranche et al., 1980; Madsen and Bjerre, 1981; Maubois et al., 1987; Caron et al., 1997), micellar casein concentrates (MCC) and isolates (MCI) (Fauquant et al., 1988; Pierre et al., 1992; Schuck et al., 1994a,b) whey concentrates, and selectively demineralized whey concentrates (Jeantet et al., 1996), mainly because of the emergence of filtration technology (e.g., microfiltration [MF], ultrafiltration, nanofiltration, and reverse osmosis). This recent emergence of new membrane separation techniques and improvements in chromatographic resins now provide the dairy technologist with several types of techniques for the extraction and purification of almost all of the main milk proteins.

The most frequently used technique for the dehydration of dairy products is spray drying. It became popular in the dairy industry in the 1970s, but at that time, there were few scientific or technical studies on spray drying, and, in particular, none on the effects of spray drying parameters or on the effects of the physicochemical composition and microbiology of the concentrates on powder quality. Manufacturers acquired expertise in milk drying and eventually in whey drying processes through trial and error. Because of the variety and complexity of the mixes to be dried, more rigorous method based on physicochemical and thermodynamic properties have become necessary. Greater understanding

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of the biochemical properties of milk products before drying, water transfer during spray drying, the properties of powders, and influencing factors is now essential for the production of milk powder. The lack of technical and economic information and understanding of scientific methods prevents the manufacturer from optimizing his plant in terms of energy costs and powder quality. In view of the increasing development of filtration processes, the dairy industry requires greater understanding of the effects of spray drying on the quality of dairy protein powders.

A dairy powder is characterized not only by its composition (proteins, carbohydrates, fats, minerals, and water), but also by its microbiological and physical properties (bulk and particle density, instant characteristics, flowability, floodability, hygroscopicity, degree of caking, whey protein nitrogen index (WPNI), thermostability, insolubility index (ISI), dispersibility index, wettability index, sinkability index, “free fat,” occluded air, interstitial air, and particle size), which form the basic elements of quality specifications. There are well-defined test methods for the determination of powder characteristics according to international standards (Pisecky, 1986, 1990, 1997; American Dairy Products Institute, 1990; Master, 2002). These characteristics depend on drying parameters (e.g., type of tower spray drier, nozzles/wheels, pressure, agglomeration and thermodynamic conditions of the air, such as temperature, relative humidity, and velocity), the composition and physicochemical characteristics of the concentrate before spraying (e.g., viscosity, thermo-sensitivity and availability of water), and storage conditions. Several scientific papers have been published on the effects of technological parameters on these properties (Baldwin et al., 1980; Pisecky, 1980, 1981, 1986; De Vilder, 1986; Tuohy, 1989; Jeantet et al., 2008a; Master, 2002) (see Figure 1.1). Water content, water dynamics and water availability are among the most important properties for all these powder properties and powder characteristics.

The nutritional quality of dairy powders depends on the intensity of the thermal processing during the technological process. Thermal processing induces physicochemical changes that tend to decrease the availability of nutrients (e.g., loss of vitamins, reduction of available lysine content, and whey protein denaturation) or to produce nutritional compounds, such as lactulose (Straatsma et al., 1999a,b).

The aim of this chapter is to provide information on the extraction of milk proteins; the principles of spray drying, including equipment and energy consumption; the drying of high protein products, including the relationships between process and product; and the physical, functional, and biochemical properties of the powders. Following an in-depth introduction on dairy protein products, this chapter covers four major areas: the extraction of milk proteins, the principles of spray drying (equipment and energy consumption), the drying of dairy protein products and the properties of these powders.

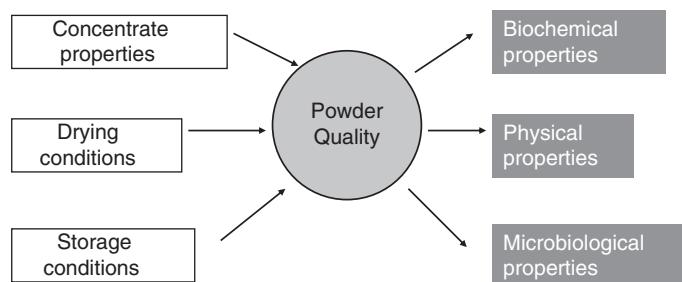


Figure 1.1 Properties and qualities of powders.

1.2 EXTRACTION OF MILK PROTEINS

1.2.1 Milk proteins

It would be impossible to develop the extraction of milk proteins without prior thorough knowledge of their biochemical and physicochemical properties. A brief description of the milk protein system is therefore important for understanding the principles used before discussing the recent developments in extraction procedures (Maubois and Ollivier, 1997).

Bovine milk contains several that are classically divided into two major groups, that is, caseins, proteins which are insoluble at pH 4.6 and 20°C, and whey proteins which remain in solution at pH 4.6. The protein content of normal milk is expressed as $N \times 6.38$. Milk contains 30–35 g protein/L. About 78% of these proteins are caseins, which consist of four principal components, α_{s1} , α_{s2} , β , and κ , in approximate ratios of 40:10:35:12. In milk, these caseins are organized in the form of micelles, which are large spherical complexes (diameters varying between 50 and 600 nm, average 120 nm) containing 92% proteins and 8% inorganic salts, principally calcium phosphate (Rollema, 1992; Swaisgood, 1992). The structure of the micelles has not yet been fully established, and there is still controversy between the supporters of the submicellar model and those of the coat-core model (Schmidt, 1982; Holt, 1992; Farrell et al., 2006; Horne, 2006). The casein micelles dissociate on removing colloidal calcium phosphate either by the addition of Ca chelating agents (e.g., phosphate, citrate, and EDTA) or by acidification. The casein micelles are partly responsible for the white color of milk. Their stability results from their zeta potential (approximately -20 mV) and from steric hindrance caused by the protruding (“hairy”) C-terminal segments of glycosylated κ -casein, which prevent the close approach of micelles. Removal of these protruding segments by chymosin, the main enzyme present in the neonate calf stomach, results in coagulation of the damaged casein micelles. The integrity of casein micelles is also affected by cooling. At temperatures lower than 4°C, β -casein and Ca phosphate are released into the serum phase of milk.

The whey protein fraction contains several proteins. The main components in bovine milk are β -lactoglobulin (β -Lg), α -lactalbumin (α -La), bovine serum albumin (BSA), and immunoglobulin (Ig), representing approximately 2.7, 1.2, 0.25, and 0.65 g/L, respectively (Alais, 1984). There are many other minor proteins, including lactoferrin (Lf), enzymes (lipoprotein lipase, acid and alkaline phosphatases, lysozyme, xanthine oxidase, lactoperoxidase, catalase, superoxide dismutase, α -amylase etc), growth factors, and hormones (Alais, 1984). The whey protein fraction of human milk is very different from that of bovine milk in that it contains no β -Lg and is very rich in α -La, Lf, lysozyme, and stimulatory factors (bifidus growth factor, epidermal growth factor, bombesin, insulin-like growth factors, etc.) (Fox and Flynn, 1992; Maubois and Ollivier, 1997).

1.2.2 Separation of proteins

Most of the dairy proteins, used as either nutritional or functional ingredients, are marketed in a dehydrated form (see Figure 1.2). The application of different processing steps allows the production of a wide range of different dried and stable intermediate dairy products. Many new uses for these constituents have emerged with the manufacture of formula products, substitutes, and adapted raw materials.

Figure 1.2 summarizes the procedures available for the separation of milk proteins. Before discussing the details of the processes shown in this diagram, it is necessary to set

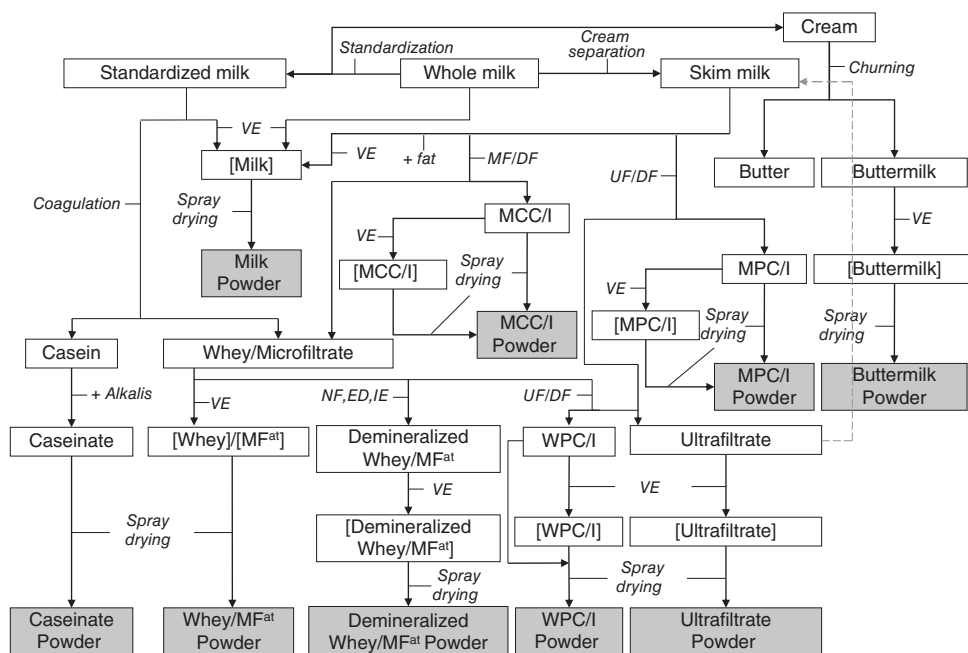


Figure 1.2 Fractionation of milk. [], Concentrate by vacuum evaporation; MCC/I, micellar casein concentrate/isolate; WPC/I, whey protein concentrate/isolate; MPC/I, milk protein concentrate/isolate; ED, electrodialysis; IE, ion exchange; VE, vacuum evaporation; MF, microfiltration; MF^{at}, microfiltrate; NF, nanofiltration; UF, ultrafiltration; DF, diafiltration.

out some general guidelines to food technologists who wish to develop strategies for extraction of a dairy protein or a group of dairy proteins.

At each stage of the extraction process, the preservation of desirable qualities (i.e., functional, biological, and nutritional) must be taken into account when choosing technologies and the physicochemical parameters to be used. Particular attention must be paid to successive heat treatments, the effects of which are cumulative. Like most liquid foods, milk and its derivatives are very favorable media for spoilage microorganisms. Consequently, pretreatments and temperature–time parameters and residence time temperature (Jeantet et al., 2008b) must be chosen in order to control microbial growth.

Each separation and fractionation step generates at least one coproduct; for environmental reasons, and because of their potential value, the coproducts must be considered not only as by-products, but also as value-added components of milk. Thus, all extraction procedures must be envisaged in an integrated technological concept that utilizes all of the products and effluents generated.

1.2.3 Pretreatment of milk

Milk collected by the dairy industry contains lipids (≈ 40 g/L) organized in fat globules. Most extraction procedures for milk proteins use skim milk as the starting material. Whole milk is therefore centrifuged in a separator comprising conical discs and running at 5000 rpm at around 50°C. The separated cream generally represents 10% of the volume of the entering whole milk. Fat separation is never perfect, and the skim milk thus obtained still

contains around 0.5 g/L fat, which can considerably influence the effectiveness of downstream processes and the resulting protein products.

In developed countries, milk is normally contaminated by common mesophilic and psychrotrophic microflora and rarely by pathogenic microorganisms. To minimize possible health hazards and to control bacterial growth during milk processing, moderate heat treatments (at 63°C for 30 minutes or pasteurization at 72°C for 15 seconds) are applied to skim milk before further processing. The consequences of this heat treatment are numerous and significant: it decreases the pH, shifts the delicate protein–calcium phosphate equilibrium, causes changes in the micellar structure of casein, which in turn affects its hydration and zeta potential (Fox, 1982), and initiates the Maillard reaction, which permanently modifies the functional and nutritional properties of the whey proteins (Maubois et al., 1995). An interesting recent development in the removal of microorganisms from skim milk is the use of membrane MF technology. Following the recommendations of Sandblom (1974) and Meersohn (1989), skim milk is microfiltered through a ceramic membrane (average pore size of 1.4 µm) at a temperature between 20 and 55°C. The average decimal reduction observed between the inlet milk and microfiltered milk is 2.6, corresponding to a reduction of the contaminating microorganisms of 99.5%.

1.2.3.1 *Isolation of whole proteins*

There are two major methods to produce dairy proteins: coprecipitation techniques and ultrafiltration technology. Coprecipitation involves the application of high heat during the precipitation process to cross-link the two major proteins (caseins and whey proteins), but this also results in denaturation of whey proteins. Calcium chloride or acid is also injected through a spray that is countercurrent to the direction of milk flow to provide full mixing. The mixture is transformed into curd in a holding tube (20–25 seconds). The curd is separated from the whey, and the coprecipitate is washed and pressed. At optimal process conditions, it is possible to recover 95–97% of the milk proteins.

There are three basic varieties of coprecipitates, each having different amounts of calcium: low calcium coprecipitate (0.1–0.5% Ca), medium calcium coprecipitate (1–1.5% Ca) and high calcium coprecipitate (2.5–3.5% Ca). These coprecipitates can then be dried and used as ingredients. The nutritional quality of coprecipitates is better than that of caseinates, but functionality of coprecipitates is limited to applications where denatured whey proteins are needed, such as in bakery products. To address this limitation, New Zealand Milk Products Inc. patented a process for the production of total MPIs in 1983 that has since been improved (Mistry, 2002). The process involves the coprecipitation of caseins and whey proteins using a series of pH adjustments to prevent the heat denaturation of whey proteins. A modification of this process that involves ultrafiltration and diafiltration has also been patented. An ultrafiltration process was developed for the production of high milk protein powders (MPC/MPI) for applications in cheese and yogurt making (Fox, 1982; Mistry, 2002). This process was developed using ultrafiltration and diafiltration of skim milk without pH adjustment to concentrate the proteins, followed by spray drying.

1.2.3.2 *Isolation of whole casein*

There are three principal ways available for the production of whole casein on an industrial scale, that is, isoelectric precipitation, rennet coagulation, and MF. Acid casein is produced

by the use of hydrochloric or sulfuric acid or by lactic acid produced by bacteria. Sufficient acid is added to reduce the pH of the milk to 4.6 and the mix is diluted with four parts water with thorough mixing. After a short holding period in a vat, the whey is drained off. The curd is washed twice with cold water, pressed and milled. Acid casein may also be produced by inoculating skim milk with acid-producing bacteria and incubating it at 20–30°C until the acidity reaches 0.64%. The curd is stirred and heated to 50–65°C. The whey is then drained and the curd washed twice with cold water and pressed for 10–15 hours. It is then milled and ready for drying. Rennet casein is made by adding sufficient rennet and calcium chloride to skim milk to cause it to clot in 20–30 minutes. Stirring is commenced 2–5 minutes after coagulation has started. The temperature is raised to 55–70°C, and the curd is cooked for 30 minutes. The whey is then drained off. Acid or rennet whey may be dried in a cabinet, tunnel, or spray dryer to a moisture content of 4%. The dried casein is milled and screened to an appropriate particle size. Casein is used in the manufacture of paint and plastics and for paper coating. It is also used in coffee/tea whiteners, whipping powders, and imitation milks (Maubois and Ollivier, 1997).

The most promising technology for the selective separation of casein micelles is undoubtedly membrane MF. When whole or skim milk is circulated through a MF membrane with a pore size diameter of 0.1–0.2 μm , a microfiltrate is obtained with a composition close to that of sweet whey. Moreover, it is crystal clear and can be sterile if the downstream equipment prevents recontamination. The retentate is an enriched solution of native and micellar casein, that is, MCC (Fauquant et al., 1988). Diafiltration against water allows its purification into micellar casein isolate (MCI), and it is easily concentrated again by MF and then spray-dried (Pierre et al., 1992; Schuck et al., 1994b). MCI has excellent rennet-coagulating abilities. The coagulation time of a 3% MCI solution is reduced by 53% compared with that of raw milk, and gel firmness at 30 minutes is increased by more than 50% (Pierre et al., 1992). MCI and its coproduct, the WPI obtained by submitting the microfiltrate to a subsequent ultrafiltration step, are excellent starting substrates for further fractionation and isolation of milk proteins.

1.2.3.3 *Fractionation of whole casein*

There is considerable interest in developing technologies for the fractionation of whole casein into individual components (α_{s1} , α_{s2} , β , and κ) on an industrial scale. These fractions can be potentially used in bovine milk-based infant formulas and for the preparation of biologically active peptides and specific additives. Most published studies have focused on isolation of β -casein, the main component of human casein, which contains numerous peptide sequences with physiological activities, such as the well-known β -casomorphin. The growing commercial interest in the production of functional peptide fragments of α_s - and κ -caseins will probably lead to future developments in the fractionation of these proteins, either from the coproduct resulting from fractionation of β -casein or from the native casein micelles dissociated by the combined action of pH, NaCl, and sodium citrate, as proposed by Pouliot et al. (1994).

1.2.3.4 *Whey protein separation*

There are several industrial methods suitable for the production of various WPCs and WPIs. The interest in whey processing is a result of two factors. One is a worldwide shortage of high-quality animal proteins that whey proteins may alleviate, and the other is the problem

with the disposal of whey. The high biological oxygen demand of whey makes this cheese by-product a pollutant so that it is more desirable to process it than to dump it.

In addition to traditional methods, such as evaporation and drying, modern methods used in industrial whey processing include ultrafiltration, MF, reverse osmosis (hyperfiltration) and demineralization (electrodialysis and ion exchange). The most commonly used membrane method in dairying is ultrafiltration. Its industrial application was aided by the introduction of cross-flow instead of dead-end filtration and the invention of asymmetric membranes (Carić, 1993). During the ultrafiltration of whey, low molecular weight compounds, such as lactose, minerals, nonprotein nitrogen, and vitamins, are separated in the permeate, whereas proteins are concentrated in the retentate. This permits a WPC to be obtained with 20–60% protein in total solids and low quantities of lactose and mineral matter. Permeate, a by-product of this processing, is used for producing lactose, alcohol, single-cell protein, yeast, galactose, glucose, cattle feed, and various pharmaceuticals.

Further increases in protein content (up to 98%) may be achieved by adding water to the feed. This procedure is called diafiltration. The best moment to start diafiltration is when the optimal total solids content has been reached and at a point where the ultrafiltration flux is still relatively high.

Sweet whey is first subjected to clarification (removal of casein fine particles, fat separation, and pasteurization). After pasteurization, the whey is cooled to 60–65°C and held at this temperature for 30–60 minutes before cooling to 50°C for ultrafiltration. This heat-and-hold treatment has the function of stabilizing the calcium phosphate complex, thus reducing the fouling of the membranes during ultrafiltration. Further reduction of other minerals in WPC is achieved by adjusting the pH of the whey to pH 5.7–6.0 with HCl. The solubility of calcium is increased with decreasing pH, thus resulting in a greater proportion of calcium in the permeate. After ultrafiltration, the retentate is pasteurized, evaporated (or not, depending on the viscosity and protein content), and dried.

1.2.3.5 Fractionation of whey proteins

The main proteins found in WPCs and isolates are: β -Lg, α -La, glycomacropeptides, BSA, Igs, Lf, and lactoperoxidase. Each of these proteins or groups of proteins has been proven or believed to have unique functional, nutritional, or nutraceutical properties. Some putative nutraceutical activities include digestive function (β -Lg and glycomacropeptide), anticarcinogenic properties (α -La), antimicrobial activity (Lf and lactoperoxidase) and passive immunity (Igs). There are also nutritional considerations. It is known that α -La binds minerals, specifically calcium, magnesium, zinc, and cobalt. By being bound to a protein, these minerals are more readily delivered for absorption in the human body. The lack of β -Lg in human milk suggests that bovine whey protein products rich in α -La and low in β -Lg would be more appropriate for infant formulae.

There is a considerable interest in developing technologies for the fractionation of whey proteins into α -La and β -Lg on an industrial scale (Maubois and Ollivier, 1997). A number of methods have been developed (Slack et al., 1986; Maubois et al., 1987; Pearce, 1987) with commercial scale potential to fractionate the major whey protein components, β -Lg and α -La, and to produce WPCs enriched in these fractions. These methods depend on either mild heat treatments of a whey concentrate or a clarified whey under controlled pH and ionic conditions, or on demineralization of whey concentrate under controlled pH conditions. These treatments are used to achieve selective reversible precipitation of α -La- or β -Lg-enriched fractions and the separation of the precipitate from β -Lg- or

α -La-enriched solutions. The precipitate is resolubilized by the addition of water and pH adjustment and then dried, while the soluble protein is further concentrated by ultrafiltration/diafiltration prior to drying.

1.3 DRYING PRINCIPLES

Drying is defined as the removal of a liquid, usually water, from a product by evaporation, leaving the solids in an essentially dry state. A number of different drying processes are in use in the dairy, food, chemical, and pharmaceutical industries, such as:

- spray drying;
- fluid bed drying;
- roller drying;
- freeze-drying;
- microwave drying; and
- superheated steam drying.

Due to considerations of drying economics and final product quality, the only processes of significance in milk protein powder manufacture are spray drying, fluid bed drying (the two most often in combination), and roller drying, although the latter is in only limited use nowadays. Only these three drying processes will be discussed here.

1.3.1 Roller drying

In roller drying, a preconcentrated product is applied as a thin film on the outer surface of an internally heated rotating metal drum (Refstrup, 2003). A vapor hood and exhaust system are placed above the drum. The milk film is scraped off the drum surface as a sheet of dry product by stationary knives located opposite the point of milk concentrate application. The product sheet or flakes fall into an auger trough, which partly disintegrates it and conveys it to a pneumatic cooling and conveying system, often with integrated milling, and thence to storage and packaging.

1.3.1.1 *Types of drum driers*

Several types of drum dryer exist. They can be characterized by the combination of the number of drums (single or double) or the method of product application (sump between two closely positioned drums, spray with nozzles, or immersed applicator roll system).

The main process parameters affecting the plant capacity and product properties are:

- Drum surface temperature: Saturated steam (at up to 0.5-MPa pressure, corresponding to about 150°C) is used as heating medium.
- Feed temperature: This may vary from about 10–80°C depending on the type of product. The higher the feed temperature, the greater is the plant capacity.
- Feed solids content: A total solids content of up to 45% is usually used. The higher the solids content, the faster is the product drying rate.
- Drum rotation speed: The time of exposure to the hot drum surface, and hence the final moisture content, is controlled by the rate of rotation of the drum. This is because, for

a given drum temperature, feed solids content, and viscosity, the drum rate of rotation also affects the thickness of product film.

- Distance between drums and/or applicator rollers: The gap between drums, which is usually $<100\mu\text{m}$, also controls the thickness of product film.
- Area of heat transfer surface: The plant capacity is proportional to the effective area of heat transfer.

Generally, drum drying has a number of serious disadvantages compared with spray drying. These include:

- Severe heat damage and protein denaturation during the slow drying and relatively long residence time on the hot drum (3–6 seconds), resulting in poor solubility and cooked or burned flavor.
- Relatively low evaporative capacity, with the greatest capacity of a single drum drying unit being about 1000 kg/h water evaporation.
- Inflexibility in relation to control of powder properties as there is no possibility of producing agglomerated or instant products with drum drying.

Despite these disadvantages, drum dryers are still in use in niche production where the special functional properties of drum-dried powders are desirable. For instance, the high “free fat” content of drum-dried whole milk is advantageous in the chocolate industry, and the high water-binding capacity of drum-dried skim milk is desirable in the meat-processing industry. Drum dryers are also used to dry off highly viscous cereal- or starch-based product blends that cannot easily be atomized (Refstrup, 2003).

1.3.2 Spray drying and fluid bed drying/cooling

The basic principle of spray drying is the exposure of a fine dispersion of droplets, created by means of atomization of pre-concentrated milk products on a hot air stream. Spray drying is an industrial process for the dehydration of a liquid by transforming the liquid into a spray of small droplets and exposing these droplets to a flow of hot air (Pisecky, 1997). The very large surface area of the spray droplets causes evaporation of the water to take place very quickly, converting the droplets into dry powder particles. The small droplet size created, and hence large total surface area, results in very rapid evaporation of water at a relatively low temperature, whereby heat damage to the product is minimized (Refstrup, 2003).

In fact, when a wet droplet is exposed to hot dry gas, variations in the temperature and the partial pressure of water vapor are spontaneously established between the droplet and the air. This results in heat transfer from the air to the droplet, which occurs under the temperature variation between the air and the droplet. Water transfer occurs in the opposite direction, and this is explained by variation in the partial pressure of water vapor between the air and the droplet surface.

Air is thus used both for fluid heating and as a carrier gas for the removal of water. The air enters the spray drier hot and dry and leaves wet and cool. Spray drying is a phenomenon of surface water evaporation maintained by the movement of capillary water from the interior to the surface of the droplet. As long as the average moisture is sufficient to feed the surface regularly, the evaporation rate is constant. If not, it decreases.

The drying kinetics is related to three factors:

- The evaporation surface created by the diameter of the particles. Spraying increases the exchange surface. For example, 1 L of liquid sprayed in particles of 100- μm diameter develops a surface area of 60 m², whereas the surface area for one sphere of the same volume is only approximately 5 dm².
- The difference in the partial pressure of water vapor between the particle and the drying air. A decrease in the absolute humidity of the air and/or an increase in the air temperature tend to increase the difference in the partial pressure of water vapor between the particle and the drying air.
- The rate of water migration from the center of the particle towards its surface. This parameter is essential for the quality of dairy powders. Indeed, it is important that there is always water on the surface of the product so that the powder surface remains at the wet bulb temperature for as long as possible. The rate of water migration depends on the water diffusion coefficient, which varies according to the biochemical composition, water content, and droplet temperature. This is why the calculation of this coefficient is complex, and the mathematical models suggested are not easily exploitable by the dairy industry.

1.3.2.1 Components of spray drying installations

To define the components of a spray drying installation, according to Masters (1991), Pisecky (1997) and Westergaard (2003), the main components of the spray drier shown in Figure 1.3 are as follows.

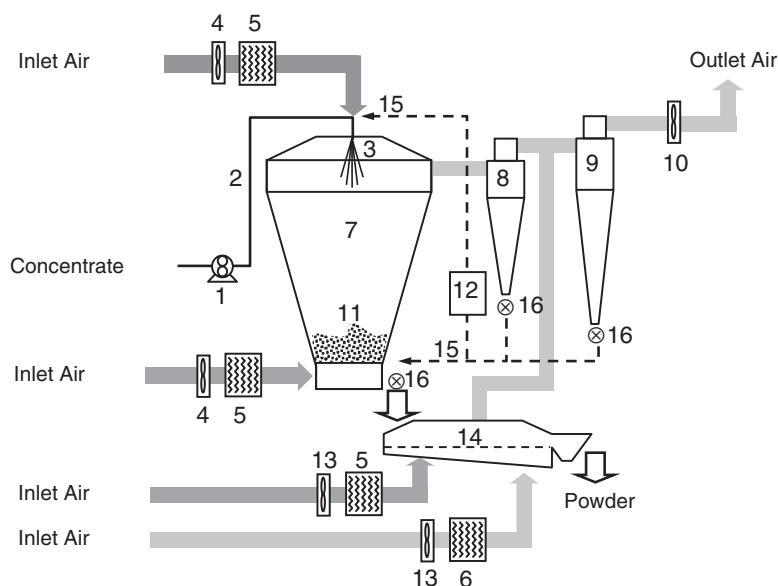


Figure 1.3 Multiple effect spray dryer. 1, Feed pump; 2, feed flow; 3, sprayer / air disperser; 4, inlet air fan; 5, air heater; 6, air cooler; 7, drying chamber; 8, primary cyclone; 9, secondary cyclone; 10, outlet air fan; 11, integrated fluid bed; 12, pressure conveyer system; 13, vibrofluidizer air fan; 14, vibrofluidizer; 15, reincorporating fines; 16, rotary valve.

Drying chamber (Figure 1.3)

Various designs of drying chambers are available on the market. The chamber can be horizontal (box drier), although in the dairy industry, the chamber design is generally vertical, with a conical or flat base. The most common is the cylindrical chamber with a cone of 40–60°, so that the powder can leave the chamber by gravity. This chamber may also have a flat bottom, in which case a scraper or suction device is needed to remove the powder from the chamber.

Air filtration

Until a few years ago, no special requirements were stipulated for the filtration of the process air for the spray drying process. Today, however, there are very strict requirements made by local authorities in order to ensure a cleaner operation. Common requirements for the different standards are:

- The air should be prefiltered and supplied by a separate fan to the fan/filter/heater room, and should be under pressure to avoid the entry of unfiltered air.
- The degree of filtration and filter position must depend on the final temperature of the process air as follows: for air to be heated above 120°C, only coarse filtration up to 90% is needed, and the filter should be placed on the pressure side of the fan; for air to be heated below 120°C or not heated at all, filtration must be 95% or above, and the filter must be placed after the heater/cooler. Some countries have even stricter requirements, demanding filtration of up to 99.995% (Westergaard, 2003).

Air heating system

The drying air can be heated in different ways: either indirectly by steam, oil, gas, or hot oil, or directly by gas or electricity. A steam heater is a simple radiator. The temperature to be obtained depends on the steam pressure available. The air heater consists of rows of finned tubes housed in an insulated metal case. In indirect oil and gas heaters, drying air and combustion gases have separate flow passages. The combustion gases pass through galvanized tubes that act as a heat transfer surface for the drying air. The combustion chamber is made of heat-resistant steel. Heaters of this type have an efficiency of about 85% in the range of 175–250°C. Direct gas heaters are used only when the combustion gas can be allowed to come into contact with the product. They are, therefore, uncommon in the dairy industry. The direct gas heater is cheap, it is highly efficient, and the temperature obtainable can be as high as 2000°C. When a plant is designed with an air heater with direct combustion, it is necessary to calculate the amount of vapor resulting from the combustion (44 mg/kg dry air/°C), as this will increase the humidity of the drying air. The outlet temperature must therefore be increased in order to compensate for this increase in humidity and to maintain the relative humidity. Electric air heaters are common in laboratory and pilot plant spray dryers. The heater has low investment costs, but is expensive to operate and therefore is not used in industrial-sized plants (Westergaard, 2003).

Air distribution

The system of air distribution is one of the most vital points in a spray dryer. There are various systems, depending on the plant design and the type of product to be produced. The most common system is where the air disperser is situated on top of the dryer ceiling

and the atomizing device is placed in the middle of the air disperser, thus ensuring optimal mixing of the air and the atomized droplets. The air flow chamber can be in cocurrent, counter-current, or mixed mode.

Atomization

An atomizing device with a feed supply system, such as feed tank, feed pump, water tank, concentrate heater, and atomizing device, is required. The aim of atomizing the concentrate is to provide a very large surface area from which the evaporation of water can take place. The smaller the droplets, the greater the surface area, and easier evaporation and better thermal efficiency of the dryer are obtained. The ideal situation from a drying point of view is a spray of droplets of the same size, where the drying time for all particles would be the same to obtain equal moisture content. As mentioned previously, air distribution and atomization are the key factors in successful utilization of the spray dryer. Atomization is directly responsible for many distinctive advantages offered by spray drying: first, the very short drying time of the particles; second, very short particle retention time in the hot atmosphere and low particle temperature (wet bulb temperature), and finally, transformation of the liquid feed into a powder with long storage stability ready for packing and transport.

In summary, the prime functions of atomization are:

- to produce a high surface to mass ratio, resulting in a high evaporation rate; and
- to produce particles of the desired shape, size, and density.

There are three types of atomizing devices: rotary atomizer (wheel or disc), nozzle atomizer (pressure, pneumatic, or sonic), and combined atomizer (rotary and pneumatic) (see Figure 1.3, no. 3).

The basic function of pressure nozzles is to convert the pressure energy supplied by the high-pressure pump into kinetic energy in the form of a thin film, the stability of which is determined by the properties of the liquid, such as viscosity, surface tension, density, and quantity per unit of time, and by the medium into which the liquid is sprayed.

The energy available for atomization in two-fluid atomizers is independent of liquid flow and pressure. The necessary energy (kinetic) is supplied by compressed air. Two-fluid atomization is the only successful nozzle method for producing very small particles, especially from highly viscous liquids. It is not normally used in the drying of milk products.

In rotary atomizers, the liquid is accelerated continuously to the wheel edge by centrifugal forces produced by the rotation of the wheel. The liquid is distributed centrally and then extends over the wheel surface in a thin sheet and discharged at high speed at the periphery of the wheel. The degree of atomization depends on peripheral speed, the properties of the liquid, and feed rate. According to Westergaard (2003), to select an optimal atomizer wheel, the liquid feed rate, peripheral speed, and viscosity of the liquid should be taken into consideration.

Powder recovery system

Separation of the dried product can be achieved by a primary discharge from the drying chamber followed by a secondary discharge from a particulate collector (using a cyclone,

bag filter, or electrostatic precipitation), followed by total discharge from the particulate collector, and finishing with final exhaust air cleaning in a wet scrubber and dry filter.

- *Cyclone.* The operating principle of the cyclone is based on a vortex motion in which the centrifugal force acts on each particle and causes the particle to move away from the cyclone axis towards the inner cyclone wall (Figure 2.3, nos. 8 and 9).
- *Wet scrubber.* The operating principle is based on the venturi scrubber principle. The droplet separator is designed according to cyclone principles, with a modified outlet, resulting in a minimum liquid level, thereby minimizing bacterial growth, and a design ensuring deaeration, thus avoiding the buildup of foam.
- *Bag filter.* This consists of numerous bags, installed so that each receives approximately equal quantities of air. The collection of bags is termed the baghouse. The simple principle of passing powder-laden air through a close woven fabric is one of the oldest methods of air cleaning, but it remains an effective means of powder separation, being able to remove particles below 10 μm . With the correct weave, 1- μm particle sizes can be collected. The performance of bag filters depends upon (1) the type of product handled, (2) the powder loading in air, (3) bag fabric, and (4) bag cleaning procedure. Modern bag filter designs have proven operational reliability, but do require regular maintenance and inspection to ensure no bag leakage. The slightest leak will quickly diminish the very high collection efficiency levels this equipment is capable of achieving (Master, 2002).

Authorities normally conclude that powder loss of 250 mg/m^3 (obtained classically by using only cyclones) is too high, and set a standard of 50 mg/m^3 (obtained by using cyclones plus bag filters or cyclones plus wet scrubber that requires a final cleaning of the air). Authorities demand reduced powder emission while powder producers demand lower energy consumption and reduced space requirements. For these reasons, a new powder recovery system has been developed, the cleanable-in-place (CIP-able) bag filter, which replaces the cyclones/bag filter (Westergaard, 2003).

1.3.2.2 *Types of drying installations*

According to Sougnez (1983), Masters (1991), and Pisecky (1997), the simplest types of installation are single-stage systems with a very short residence time (20–60 seconds). Thus, there is no real balance between the relative humidity of the air and the moisture content of the powder. The outlet temperature of the air must therefore be higher, and the thermal efficiency of the single-stage spray drier is then reduced. This type of drying chamber was the standard equipment for drying milk in the 1960s. Space requirements were small and building costs were low. Generally, installations without any posttreatment system are suitable only for nonagglomerated powders not requiring cooling. If necessary, a pneumatic conveying system could be added to cool the powder while transporting the chamber fraction and the cyclone fraction to a single discharge point.

The two-stage drying system consists of limiting the spray drying process to a process with a longer residence time (several minutes) to provide a better thermodynamic balance. This involves a considerable reduction in the outlet air temperature, and also an increase in the inlet air temperature. A second final drying stage is necessary to optimize the moisture content by using an integrated fluid bed (static) or an external fluid bed (vibrating), the air temperatures of which are 15–25°C lower than with a single-stage system to improve

and/or preserve the quality of the dairy powder (Figure 1.3, nos. 11 and 14). Consequently, the surrounding air temperature at the critical drying stage, and the particle temperature are also correspondingly lower, thus contributing to further improvement in economics. The integrated fluid bed can be either circular (e.g., multistage drier [MSD™] chamber, GEA-Process Engineering, Soeborg, Denmark) or annular (e.g., compact drier [CD] chamber). Two-stage drying has its limitations, but it can be applied to products such as skim milk, whole milk, precrystallized whey, caseinates, whey proteins, and derivatives. The moisture content of the powder leaving the first stage is limited by the thermoplasticity of the wet powder, that is, by its stickiness in relation to the water activity and the glass transition temperature (Roos, 2002). The moisture content leaving the first stage must be close to 7–8, 9–10, and 2–3% for skim/whole milk, caseinate/whey protein, and precrystallized whey powders, respectively. The two-stage drying techniques can be applied to the production of both nonagglomerated and agglomerated powders. However, this technique is very suitable for the production of agglomerated powders by separating the nonagglomerated particles from the agglomerates (i.e., collecting the cyclone fractions and reintroducing these fine fractions [called fines] into the wet zone around the atomizer of the chamber) (Figure 1.3, no. 15).

The three-stage drying systems, with an internal fluid bed as a second stage in combination with an external vibrating fluid bed as a third-stage drier, first appeared at the beginning of the 1980s, and were called compact drier instantization (CDI) or MSD. Today, they dominate the dairy powder industry (see Figure 1.3). Three-stage systems combine all the advantages of extended two-stage drying, using spray drying as the primary stage, fluid bed drying of a static fluid as the second drying stage and drying on an external vibrating fluid bed as the third drying stage. The final drying stage terminates with cooling to under the glass transition temperature. Evaporation performed at each stage can be optimized to achieve both gentle drying conditions and good thermal economy.

Compact drying is suitable for producing both nonagglomerated and agglomerated powders of practically any kind of dried dairy product. It can also cope successfully with whey powders, fat-filled milk, and whey products, as well as caseinates, both nonagglomerated and agglomerated. It has a fat content limit of about 50% fat in total solids. Powder quality and appearance are comparable with those of products from two-stage drying systems, but they have considerably better flowability, and the process is more economical. In comparison with CD, MSD can process an even wider range of products and can handle an even higher fat content. The main characteristic of a MSD powder is due to very good agglomeration and mechanical stability, low particle size fractions (below 125 μm), and very good flowability. Optimization of the process allows considerable improvement in drying efficiency and the quality of the product obtained is generally better. The various advantages are:

- Improved thermal efficiency—significant reduction in outlet air temperature, permitting an increase in inlet air temperature;
- Reduced material obstruction—the capacity in one volume is two or three times greater than for a traditional unit;
- Considerable reduction in powder emission to the atmosphere—reduction of the drying air flow and increase in powder moisture content decrease the loss of fine particles in the outlet air;
- Improved powder quality in relation to agglomeration level, solubility, dispersibility, wettability, particle size, density, and so on.

There are other examples of drying equipment such as the tall form drier, the Filtermat™ drier (GEA-Process Engineering), the Paraflash™ drier (SPX, Soeborg, Denmark), the Tixotherm™ drier (GEA-Process Engineering), and the Integrated Filter Dryer™ (IFD™) (GEA-Process Engineering). All these towers have characteristics related to the specific properties (e.g., high fat content) and type of the product (e.g., starch, maltodextrin, egg, and hygroscopic products) being dried.

In terms of energy balance, Westergaard (2004) showed that energy consumption varies according to the drying processes. Energy consumption is 1595, 1350, 1280, 1038, and 960 Kcal for a one-stage spray dryer, a two-stage spray dryer, a two stage-spray dryer with high inlet air temperature, CDI, and MSD, respectively, to produce 1 kg of powder from skim milk concentrate at 45% of total solids. This is explained by the increase in the number of drying stages simultaneously increasing the residence time, allowing increase in the inlet air temperature, concentrate flow rate, and finally energy yield while preserving/improving powder quality (Bimbenet et al., 2002).

1.4 DRYING OF DAIRY PROTEINS

1.4.1 Heat treatment

The native properties of milk components are mainly unaffected by moderate drying conditions. Depending on the preheating conditions, drier design, and temperature operation, the properties of the spray-dried powder may vary significantly. An evaporating milk droplet in a spray drier in cocurrent air flow initially does not appreciably exceed the unit bulb temperature and can be held effectively at temperatures below 60°C. As the falling temperature period is approached in the course of further evaporation, the temperature rises to a final value determined by the final temperature of the drying gas and the residence time in the drier. Under properly controlled spray drying conditions, the changes in milk protein structure and solubility are minor. Spray drying does not lead to significant denaturation of the whey protein, and the levels of denatured whey protein in dairy powders are more or less equal to those of condensed milk and heated milk fed into the dryer.

The WPNI has traditionally been used to classify milk powders. The WPNI expresses the amount of undenatured whey protein (milligrams of whey protein nitrogen per gram of powder) (Pisecky, 1997). It represents the sum of heat treatments to which the milk has been subjected prior to evaporation and spray drying. The heat treatment of a concentrate, and subsequently of a powder, has only a negligible effect on the WPNI. The main operation to adjust the required value is the pasteurization process, that is, time/temperature combination. However, there are many other factors that influence the WPNI, including the total amount of whey protein and the overall composition of the milk processed that can be influenced by animal breed and seasonal variations. The individual design of the processing equipment, that is, the pasteurizer and holding tubes, also has a significant influence. It is therefore difficult to predict the conditions for achieving the required WPNI on a general basis. Obviously, the primary purpose of heat pretreatment is to ensure the microbiological quality of dairy products. In milk powder production, the influence of the heat treatment on the denaturation of whey proteins to achieve the desired properties of the final products is just as important. Skim milk powder (SMP) for cheese manufacture should have as much undenatured protein as possible, that is, it should be *low heat* powder (WPNI > 6), whereas for bakeries, *high heat* powder with high denaturation is required

(WPNI < 1.5). For ice cream, chocolate, and confectionery, *medium heat* powder is required. According to Schuck et al. (1994a), the use of MF (pore diameter, 1.4 μm), coupled with low heat treatment during vacuum evaporation, allows the production of a “*very low heat*” SMP with a WPNI close to 9 mg of whey protein nitrogen/g of powder, a bacterial count <1000 coliform forming units (CFU)/g powder, a solubility index >99.5%, a dispersibility index >98.5%, and a wettability index <15 seconds. Such a powder has the same renneting time after water rehydration as the original raw milk, and can be used as a reference powder for either industrial or scientific purposes.

The stability of dairy protein powders during storage is critically affected by the moisture content and the storage temperature. More precisely, such stability is governed by the water activity (a_w) and the glass transition (T_g) temperature. The a_w should be close to 0.2 at 25°C for optimal preservation, with an ideal moisture content determined by using the sorption isotherm of some dairy powders. For example, the corresponding moisture content for skim milk, whey, and high protein powders must be close to 4, 2–3, and 6%, respectively. The optimal storage temperature must be below the T_g temperature, which is close to 40–50°C at 0.2 a_w for SMP (Schuck et al., 2007).

1.4.2 Water transfer

In relation to the drying of dairy concentrates of high protein content, reports by Schuck et al. (1994a,b) have shown that the increase in casein content of the concentrate decreases the water diffusion through the dried product, that is, the final residue obtained at the end of drying. Water transport is probably affected by the high micellar casein content of the sprayed droplet in the atomization tower, and, similarly, when the powder granule is dissolved in water.

Studies by Schuck et al. (1998; 1999) showed that sodium caseinate and potassium caseinate dried more easily than calcium caseinate. The limited water diffusion through the calcium caseinate may be explained by the structure of this colloidal dispersion. Whereas the casein subunits are more aggregated in calcium caseinate because of calcium binding, in sodium and potassium caseinates, the caseins are more soluble. The results showed that the water bound in a micellar casein structure was more strongly bound than that bound to the soluble caseins in sodium caseinate. The situation was intermediate (i.e., between that of micellar casein and sodium caseinate) for calcium caseinate. It was assumed that these differences in water transfer during drying could be explained by the casein structure. The decrease in water inside the dairy concentrate led to a decrease in the water concentration on the surface of the concentrate in the water activity meter, or on the surface of the droplet during spray drying, and decreased the drying kinetics. These results were confirmed by the desorption drying of two different types of protein with the same protein content (89% of total solids) and the same water content before desorption drying, but with different drying time. All these results show that the drying rate is dependent on the nature and structure of the casein. Water may be less available during the drying of a protein with a micellar structure than during the drying of a protein with a globular structure.

1.4.3 Energy consumption

The aims of this section are to evaluate water transfer during the spray drying of different dairy concentrates (WPCs, WPC35, WPC50, WPC70, and isolates WPI90), with or without heat denaturation, MCI, sodium caseinate (NaCas), and milk (with and without whey

protein enrichment), using thermodynamic and biochemical approaches. When the concentrate temperature, air flow rate, concentrate flow rate, total solids content of the concentrate, inlet air absolute humidity, inlet air temperature before and after heating, and outlet air temperature after drying are known, it is possible to determine the specific energy consumption (SEC), which is the ratio of the energy consumed in the evaporation of 1 kg of water (measured in kJ/kg water) (Bimbenet et al., 2002). Thus, if you spray dry only free water, the energy spent in terms of the SEC would be close to 2500 kJ/kg water. For example, if the concentrate has increasingly greater amounts of bound water to free water, the SEC increases to 10,000 kJ/kg water. The significance of very high SEC is related to the water, which becomes less and less available, limiting water transfer, and thus increasing the surface temperature of the droplet and consequently increasing the risk of protein denaturation of the powder.

1.4.3.1 Whey proteins

The results presented in Table 1.1 show that water transfer during spray drying decreased when the whey proteins were native proteins. For the same moisture, the SEC for drying was higher when (1) the native whey protein content increased in WPC and in milk and (2) the whey proteins were heat denatured in WPC35. However, the SEC was lower when the whey proteins were heat denatured in WPC50, WPC70, or WPI90. These results can be explained by the availability of the water (bound and unbound) in the concentrate in relation to the nature and the content of the whey proteins.

1.4.3.2 Caseins

The results presented in Table 1.1 show that water transfer during spray drying decreased when the micellar casein content increased. For the same moisture, the SEC for drying was higher when (1) the micellar casein content increased in MCI compared with skim milk, and (2) casein remained in a micellar state (as in MCI) rather than a soluble state (e.g., in NaCas). These results can be explained by the availability of the water in the concentrate in relation to the content and structure of the caseins. Water is more available when the caseins are soluble than when they are in a micellar state.

All these results also show that water transfer depends on the relationship between the water and the protein components, and that these components should be taken into account when optimizing spray drying parameters for proteins. Proteins have an important role in the mechanisms of water transfer during drying and rehydration. The residence time of the

Table 1.1. Specific energy consumption for the drying of dairy powders at 4% moisture.

		WPC				WPI		MCI	NaCas
Protein content (%)	35	50	70	90	90	90			
Heat treatment (72°C/4 min)	N	Y	N	Y	N	Y	N	N	N
SEC (±3%) (kJ/kg water)	5950	7700	6800	6550	7050	6600	7200	6500	6900

Y, heat treatment; N, no heat treatment; WPC, whey protein concentrate; WPI, whey protein isolate; MCI, micellar casein isolate; NaCas, sodium caseinate; SEC, specific energy consumption at 4% moisture content.

droplet and then the powder is so short that it is very difficult to study the mechanism of the structural changes in the protein without fundamental research into relationships between the process/product interactions.

1.5 POWDER PROPERTIES

Milk powders may be categorized by their physical, functional, biochemical, microbiological, and sensory properties (Carić, 2003). There is a significant interrelationship between them, which affects the final quality. The physical and functional properties of milk powders are especially important when the powders are intended for recombining and in the manufacture of various food products. When intended for use as a food ingredient, milk powders should be light in color, free of off-flavors, and easy to hydrate, disperse, and dissolve in water. The basic properties that determine the quality of milk powder, and where defects are most likely, include powder structure, solubility, water content, scorched particles, flowability, oxidative changes, flavor, color, and microorganism contamination.

1.5.1 Powder structure

The physical structure of milk powders can be defined as the way in which its chemical components are distributed and connected.

Powder structure is very strongly affected by the drying technique. Powder produced by roller drying has a compact structure of irregular shape with no occluded air. Roller-dried powder particles have a low bulk density (300–500 kg/m³) due to their irregular structure. The particles of spray-dried powder are spherical, with diameters in the range 10–250 µm. The particles contain occluded air and either large central vacuoles or smaller vacuoles, which are distributed through the interior of particles. The surface of spray-dried SMP particles is usually wrinkled but is smooth for high protein powders. The high inlet air temperature and large temperature differential between the hot air and the powder particles are the main causes of wrinkle formation, as is also the presence of lactose (Carić and Kaláb, 1987; Mistry et al., 1992; Aguilar and Ziegler, 1993).

1.5.2 Particle size distribution

According to Carić (2003), the particle size of powders, which affects its appearance, reconstitution, and flow characteristics, depends mainly on the atomization conditions and the viscosity of the concentrate. High atomizing pressure and low concentrate viscosity reduce particle size.

1.5.3 Powder density

Densities are classified into three groups: bulk (apparent) density, particle density, and the density of the dry milk solids. All three are very much interrelated.

1.5.3.1 Bulk density

Bulk density is regarded as the weight per unit volume and is expressed as kg/m³. It is a very important property, both from the point of view of cost and market requirements. Bulk

density is currently determined by measuring the volume of 100 g of powder in a 250-mL graduated glass cylinder. The bulk density of milk powders is a very complex property, being the result of many other properties and influenced by a number of factors, such as feed concentration, feed temperature, feed foamability, milk preheating, age thickening, feed composition, type of atomizer, particle temperature history, and particle size distribution (Pisecky, 1997). Bulk density depends also on particle density and occluded and interstitial air.

1.5.3.2 Particle density

Particle density corresponds to the mass of particles (in grams) having a total volume of 1 cm³. Particle density is influenced mainly by the amount of entrapped air. The processing factors that contribute significantly to particle density are viscosity and the incorporation of air into the concentrate prior to drying. The type of spray atomization affects air retention. Certain types of centrifugal spray-dried milk have more entrapped air than pressure spray products (Carić, 2003).

1.5.3.3 Occluded air

The occluded air content is defined as the difference between the volume of a given mass of particles and the volume of the same mass of air-free milk solids.

Many factors influence the occluded air content in powder particles, including incorporation of air into the feed, the system chosen for spray drying the concentrate, whipping action before and/or during atomization, properties of the feed, and the ability of the feed to form a stable foam. The content and state of proteins might markedly affect stable foam formation, while fat has the opposite effect. High fat concentrates are much less susceptible to foaming than skim milk. Undenatured whey proteins in skim milk have a greater tendency to foam, which can be reduced by heat treatment, which causes protein denaturation. Concentrates with a low total solids content foam more than highly concentrated content. A higher temperature reduces the tendency to form foam (Carić, 2003).

1.5.3.4 Interstitial air

Interstitial air is defined as the difference between the volume of a given mass of particles and the volume of the same mass of tapped powder. This property depends primarily on the particle size distribution and the degree of agglomeration (Carić, 2003).

1.5.4 Flowability

Flowability is the ability of a powder to flow freely, like sand, without forming lumps, clusters, or aggregates. Flowability can be measured as the time (in seconds) necessary for a given volume of powder to leave a rotary drum through given slits (Haugaard Sorensen et al., 1978) or by the method developed by Carr (1965). Flowability depends also on particle size and shape, density, and electrical charge. Large particles flow more easily than fines (particles with a diameter of <90 µm). Consequently, agglomeration is beneficial, as is uniformity of size. Moreover, according to Carić (2003), a wide variation in particle size permits fines to occupy spaces between the large particles, which results in closer packing.

1.5.5 Rehydration of dairy protein powders

Most food additives are prepared in powder form and need to be dissolved before use. Water interactions in dehydrated products and dissolution are thus important factors in food development and formulation (Hardy et al., 2002). Dissolution is an essential quality attribute of a dairy powder as a food ingredient (King, 1966). Many sensors and analytical methods, such as the ISI (International Dairy Federation, 1988; American Dairy Products Institute, 1990), nuclear magnetic resonance (NMR) spectroscopy (Davenel et al., 1997), turbidity, viscosity and particle size distribution (Gaiani et al., 2006) can now be used to study water transfer in dairy protein concentrates during rehydration. Using combinations of these methods, it is very easy to determine the different stages of the rehydration process (i.e., wettability, swellability, sinkability, dispersibility, and solubility).

1.5.5.1 Stages of the rehydration process

Wettability is the ability (expressed as time in seconds) necessary for a given amount of powder to penetrate the still surface of water. In other words, wettability is the ability of a powder to absorb water on the surface and get wet (Haugaard Sorensen et al., 1978). Generally, the wettability of powder particles depends on the surface activity of the particles, surface area, surface charge, particle size, density, porosity, and presence of moisture-absorbing substances.

Sinkability is the ability of powder particles to overcome the surface tension of water and sink into water after passing through the surface. Sinkability is expressed as milligrams of powder that sink per minute per centimeter square of surface area. This property of powder is influenced by the forces that tend to submerge a particle on the surface and depends on the density of the particles, that is, on the mass of the particles and the quantity of occluded air. Higher particle density and lower quantity of occluded air cause particles to sink (Carić, 2003).

Dispersibility reflects the ability of the wetted aggregates of powder particles to become uniformly dispersed when in contact with water. The effects of total heat treatment on casein during processing are of particular importance for good dispersibility. The dispersibility of milk powder can be improved by: (1) keeping the heat treatment on preheating to a minimum and (2) minimizing the holding time and temperature of the concentrate.

The ISI (in %), described by the IDF standard (International Dairy Federation, 1988) for skim milk, is the volume of sediment (for 50 mL) after rehydration (10 g of powder in 100 mL of distilled water, at 25°C), mixing (90 seconds, at 4000 rev/min) and centrifugation (300 seconds, at 160 g). With this method, the quantity of insoluble material (true and false not differentiated) can be determined.

1.5.5.2 Methods for assessing rehydration properties

NMR spectroscopy is a technique for determining the rate of solution, the time required for complete reconstitution of powders, and the transverse relaxation rate of reconstituted solutions (Davenel et al., 1997). With this method, it is possible to differentiate between the truly insoluble material and the falsely insoluble material. The falsely insoluble material can be explained by low water transfer during rehydration and not by denatured protein, which is truly insoluble (Schuck et al., 1994b).

For viscosity measurement, a rheometer can be used to obtain viscosity profiles. In our study, the blades were placed at right angles to each other to provide good homogenization. Industrial dissolution processes usually include stirring at a constant speed, and the experiments were therefore designed to provide a constant shear rate (100 per second). MCP was added to the rheometer cup manually. The aqueous phase used was distilled water at a volume of 18 mL. The powder was dispersed in the rheometer cup 50 seconds after starting the rheometer. Dissolution is highly dependent on temperature and concentration. The total nitrogen concentration employed to study these effects was about 5% (w/v), and the temperature was about 24°C (Gaiani et al., 2005, 2006).

The experiments to provide the turbidity profiles were carried out in a 2-L vessel equipped with a four-blade 45° impeller rotating at 400 rev/min. A double-walled jacket vessel maintained the temperature at 24°C. The turbidity sensor was placed 3 cm below the surface of the water and was positioned through the vessel wall to avoid disturbance during stirring. Turbidity changes accompanying powder rehydration were followed using a turbidity meter. The apparatus uses light in the near-infrared region (860 nm), the incident beam being reflected back at 180° by any particle in suspension in the fluid to a sensitive electronic receptor (Gaiani et al., 2005).

A laser light diffraction apparatus with a 5-mW He–Ne laser operating at a wavelength of 632.8 nm can be used to record particle size distributions. In the study of Gaiani et al. (2005, 2006), the particle size distribution of dried particles was determined using a dry powder feeder attachment, and the standard optical model presentation for particles dispersed in air was used. To measure the particle size distribution of micellar casein in concentrates, 0.5 mL of suspension was taken from the rheometer cup and introduced into 100 mL of prefiltered distilled water (membrane diameter, 0.22 µm) to reach the correct obscuration. The results obtained corresponded to average diameters calculated according to the Mie theory. The criterion selected was $d(50)$, meaning that 50% of the particles had diameters lower than this criterion (midpoint of cumulative volume distribution) (Gaiani et al., 2005, 2006).

Using this combination of three methods, it was possible to follow water transfer during rehydration and to obtain the wetting time, determined using the first peak of increased viscosity and turbidity, and the swelling time, determined using the second peak of viscosity in relation to the increase in particle size. The rehydration time was then determined according to stabilization of the viscosity, turbidity, and particle size values.

The results in Table 1.2 show that rehydration of MC powder occurs in various stages. First, there is wetting and swelling of the particles, followed by slow dispersion to reach a homogeneous fluid (Gaiani et al., 2005, 2006). Using an NMR method, Davenel et al. (1997) also demonstrated two stages during MC rehydration, attributed to water absorption by powder and solubilization of particles (i.e., swelling and dispersion stages). These authors estimated the water uptake by the powder at around 5 g water/g powder during the first 20 minutes of rehydration, but could not identify a wetting stage with this method.

1.5.5.3 *Rehydration properties of various dairy protein powders*

MC powders with a high ISI (14.5 mL) are generally considered to be poorly soluble powders in which rehydration of the micelle remains incomplete (Jost, 1993). On the other hand, the rehydration of whey powders in our study was totally different (Table 1.2). As the wettability of whey powders is poor, the turbidity instability at the beginning of the profile may be due to lump formation going past the sensor, as reported by Freudig et al.

Table 1.2. Reconstitution period, insolubility index, and rehydration time of dairy protein powders.

Powders	RP using NMR (minutes)	ISI using IDF standard (mL)	WT (minutes)	ST (minutes)	DT + SoIT (minutes)	RT (minutes)
MCP (G)	22	14.5	1	2	804	807
MCP (NG)	8	3.5	3	17	551	571
WPP (G)	5	<0.5	4	0	0	4
WPP (NG)	15	<0.5	17	0	0	17

MCP, micellar casein powder; G, granulated; NG, nongranulated; WPP, whey protein powder; RP, reconstitution period; ISI, insolubility index; WT, wetting time; ST, swelling time; DT, dispersibility time; SoIT, solubility time; RT, rehydration time = WT + ST + DT + SoIT.

(1999). For nongranulated (NG) WPI powder, the very long signal instability may be explained by a tendency for the lumps to stick together in a thick layer of wet particles, due to the small size of the particles. Powder swelling has not been reported for WPI powders, probably because globular protein powders bind less water than intact casein micelle powders. Many authors have also reported that whey powders have a lower water holding capacity than casein powders (De Moor and Huyghebaert, 1983).

As expected, granulation has been a positive effect on wetting. The wetting time has been systematically better for granulated particles. This phenomenon is well known, as fast wetting is enhanced, with large particles forming large pores, high porosity, and small contact angles between the powder surface and the penetrating water (Freudig et al., 1999). A surprising influence of granulation on the rehydration time was observed in our study (Gaiani et al., 2005). Depending on the nature of the protein, the granulation influence had opposite effects. WPI rehydration was enhanced for granulated particles, whereas the rehydration time was shorter for nongranulated particles of MCP. This was unexpected and could be explained by the rate-controlling stage. The controlling stage for whey proteins is wetting. As granulation improves the wetting stage, the rehydration of whey powders is enhanced for granulated particles. In contrast, in our study, the controlling stage for casein proteins was dispersion. In fact, even with a shorter wetting time, a granulated powder is slower to rehydrate than a nongranulated powder (Gaiani et al., 2005).

These results are not compatible with those of other studies, in which it was generally accepted that a single particle size around 200 μm (Neff and Morris, 1968) or 400 μm (Freudig et al., 1999) represented optimal dispersibility and sinkability. In fact, this optimal particle size depends on the composition of the dairy powder. As shown in Table 1.2, if the industry wishes to optimize powder rehydration, it seems to be better to rehydrate granulated powders when the protein is whey and to rehydrate nongranulated powders when the protein is casein.

A modification of the rehydration parameters recommended by the IDF method (1988) induced notable variations in the ISI of MCI powder. This index decreased to 6.2 mL if the casein content of the sample of MCI powder was close to the casein content of milk (25 g/L), if the duration of stirring increased from 90 to 900 seconds (ISI = 1.8 mL), if the rehydration temperature increased from 24 to 30°C (ISI = 7.2 mL), 40°C (ISI = 2.3 mL), 50°C (ISI = 0.9 mL%), or 60°C (ISI = 0.1 mL) or if the stirring velocity increased from 4000 to 10,000 rpm (ISI = 0.8 mL). The substitution of the rehydration water by a saline solution (NaCl at 0.1 mol/L), a microfiltrate, or an ultrafiltrate did not change the ISI for MCI powder (Schuck et al., 1994a,b). These results confirm that the ISI given by the IDF method (1988) results from a decrease in the water transfer toward the center of the particle

Table 1.3. Examples of biochemical and physical properties of some high protein powders.

	Unit	MCI	MPI	NaCas	WPI
a_w	–	0.24	0.22	0.20	0.19
Free moisture	g/kg	944.3	949.5	944.4	931.7
Total moisture	g/kg	932.5	938.2	938.2	924.8
Ashes _(550°C)	g/kg	88.9	75.8	36.9	44.5
TMN (TN \times 6.38)	g/kg	846.4	833.6	905.3	839.8
NS _{pH4.6} \times 6.38	g/kg	75.3	215.4	12.1	732.5
NPN	g/kg	5.5	2.5	2.2	29.2
Color L	–	69.6	73.5	73.2	73.1
Color a	–	–5.1	–5.5	–5.8	–6.0
Color b	–	12.0	9.3	10.0	13.1
Dispersibility	%	5.1	25.6	10.0	66.0
Solubility	%	64.6	59.5	99.8	99.8
Wetability	s	>120	>120	>120	>120
d (0.1)	μm	166	20	18	23
d (0.5)	μm	287	65	77	75
d (0.9)	μm	473	135	259	205
T_g onset	$^{\circ}\text{C}$	70	65	57	82
Flowability	–	71	47	46	56
Floodability	–	52	51	58	62
Bulk density	kg/m^3	242	292	303	318
Packed density	kg/m^3	290	354	570	507
Particle density	kg/m^3	1168	1205	1301	1224
Interstitial air	$\text{cm}^3.100\text{g}^{-1}$	68	60	155	117
Occluded air	$\text{cm}^3.100\text{g}^{-1}$	259	199	99	116
H at 39% RH	%	6.9	6.3	5.9	6.2
H at 75% RH	%	11.7	13.5	10.9	15.4

NaCas, sodium caseinate; MCI, micellar casein isolate; MPI, milk protein isolate; WPI, whey protein isolate; a_w , water activity; TMN, total nitrogen matter; TN, total nitrogen; NS, soluble nitrogen at pH 4.6; NPN, non protein nitrogen; d , diameter of the particle, (0. n), $n \times 100\%$ of particles which have a size below d ; T_g , glass transition temperature; H, hygroscopicity; RH, relative humidity.

and not from denaturation. Everything occurs as if the setting in contact with water created a high surface viscosity, slowing down the internal hydration of the MCI powder.

To summarize, Table 1.3 gives some examples of findings related to the physical and biochemical properties of various high protein powders.

1.6 CONCLUSION

Figure 1.4 shows that the biochemical, microbiological, and physical properties of a dairy powder and its recombined product depend on many parameters. For example: (1) the pretreatment process parameters, concentration by membrane filtration or by vacuum evaporation, crystallization, homogenization, spray drying, and fluidization, (2) the storage conditions (e.g., relative humidity, temperature, packaging) used to optimize stability over time, and (3) the rehydration conditions (e.g., stirring conditions, temperature, and concentration) used to improve water transfer to obtain the best quality recombined product from the corresponding powder.

Moreover, the biochemical composition (nature and content) and water availability interact in all the stages of production, stability, and rehydration. The quality of a dairy

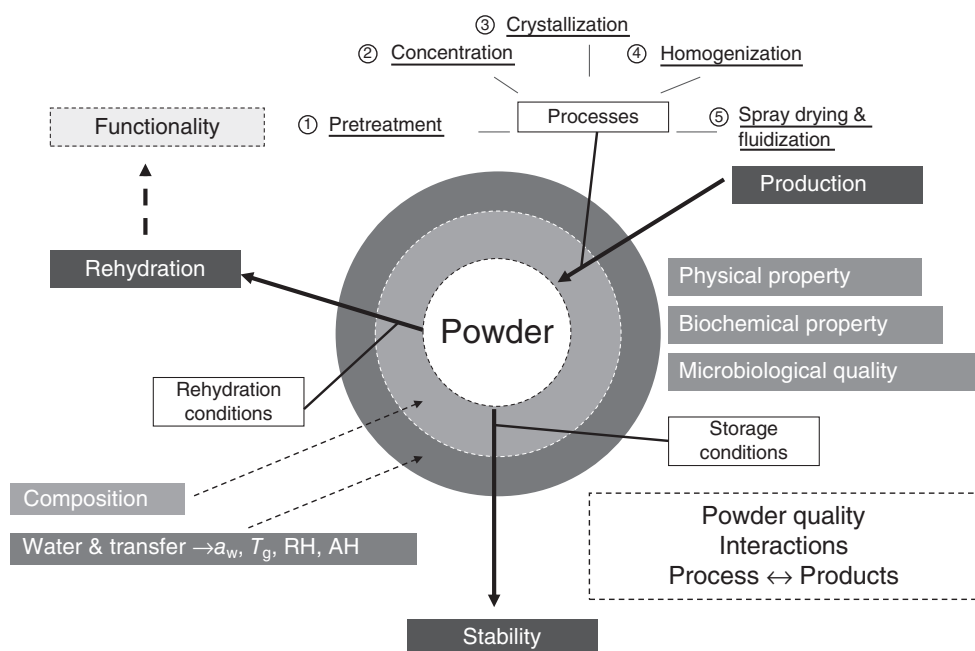


Figure 1.4 Properties of dairy powders in relation to production, storage, and rehydration.

powder can therefore only be improved if research is undertaken on the process–product interactions.

To conclude, this chapter explains certain processes for the extraction of milk protein and dehydration (spray drying) in order to clarify the effects of spray drying on the quality of protein powders during drying and rehydration. In this chapter, it was also demonstrated that the quality of these powders depends on the biochemical environment.

It is thus very important for the dairy industry to understand that enrichment of milk in micellar casein (by ultrafiltration or MF) decreases water transfer during the drying and rehydration processes. Insolubility (International Dairy Federation, 1988) is related to the lower water transfer required for rehydration and not to thermal denaturation, and reduction in water transfer is related to the micellar structure.

Moreover, it is essential for both dairy powder producers and dairy powder users to have a method to evaluate the rehydration behavior of dairy powders. The industry should take into account certain technological factors, such as granulation and the incorporation mode, and also the nature of the protein being rehydrated, in order to optimize the rehydration of dairy powders. In contrast to other studies, we found that improving the wetting stage by using granulated powders did not systematically improve total rehydration. Depending on the nature of the protein, it seems to be better to work with granulated powders for whey and nongranulated powders for micellar casein to obtain more rapid rehydration (Gaiani et al., 2007).

In conclusion, water transfer in dairy protein concentrates both during dehydration and during rehydration depends on the aqueous environment, the nature of the mineral salts, the structure of the dairy proteins, and powder properties (biochemical, microbiological, and physical).

REFERENCES

- Aguilar, C.A. and Ziegler, G.R. (1993) Lactose crystallization in spray-dried milk powders exposed to isobutanol. *Food Structure* **12**, 43–50.
- Alais, C. (1984) *Sciences du Lait. Principes des Techniques Laitières*, 4th ed. Paris: Sepsac.
- American Dairy Products Institute (1990) *Standards for Grades of Dry Milk Including Methods of Analysis*. Chicago: American Dairy Products Institute.
- Baldwin, A.J., Baucke, A.G., and Sanderson, W.B. (1980) The effect of concentrate viscosity on the properties of spray dried skim milk powder. *New Zealand Journal of Dairy Science and Technology* **15**, 289–297.
- Bimbenet, J.J., Schuck, P., Roignant, M., Brulé, G., and Méjean, S. (2002) Heat balance of a multistage spray-dryer: principles and example of application. *Le Lait* **82**, 541–551.
- Carić, M. (1993) Concentrated and dried dairy products. In: *Dairy Science and Technology Handbook, 2. Product Manufacturing*, edited by Y.H. Hui, pp. 257–300. New York: VCH Publishers Inc.
- Carić, M. (2003) Types and manufacture. In: *Encyclopedia of Dairy Sciences*, edited by H. Roginski, pp. 1869–1880. London: Academic Press.
- Carić, M. and Kaláb, M. (1987) Effects of drying techniques on milk powders quality and microstructure: a review. *Food Microstructure* **6**, 171–180.
- Caron, A., St-Gelais, D., and Pouliot, Y. (1997) Coagulation of milk enriched with ultrafiltered or diafiltered microfiltered milk retentate powders. *International Dairy Journal* **7**, 445–451.
- Carr, R.L. Jr. (1965) Evaluating flow properties of solids. *Chemical Engineering* **72**, 163–168.
- Corredig, M. (2009) *Dairy-Derived Ingredients*, 1st ed. Cambridge: Woodhead Publishing Limited.
- Davenel, A., Schuck, P., and Marchal, P. (1997) A NMR relaxometry method for determining the reconstitutability and the water-holding capacity of protein-rich milk powders. *Milchwissenschaft* **52**, 35–39.
- De Moor, H. and Huyghebaert, A. (1983) Functional properties of dehydrated protein-rich milk products. In *Physicochemical Aspects of Dehydrated Protein Rich Milk Products*, pp. 276–301. Proc. Int. Dairy Fed. Symp., Danish Govt. Res. Inst. Dairy Ind., Helsingor, Denmark.
- De Vilder, J. (1986) La fabrication de poudre de lait écrémé instantanée. I. Les caractéristiques physiques et chimiques. *Revue Agricole* **39**, 865–877.
- Farrell, H.M., Malin, E.L., Brown, E.M., and Qi, P.X. (2006) Casein micelle structure: what can be learned from milk synthesis and structural biology? *Current Opinion in Colloid and Interface Science* **11**, 135–147.
- Fauquant, J., Maubois, J.L., and Pierre, A. (1988) Microfiltration du lait sur membrane minérale. *Techniques Laitière* **1028**, 21–23.
- Fox, P.F. (1982) Heat-induced coagulation of milk, developments. In: *Dairy Chemistry, Vol I: Proteins*, edited by P.F. Fox, p. 189. London: Applied Science Publishers.
- Fox, P.F. and Flynn, A. (1992) Biological properties of milk proteins. In: *Advanced Dairy Chemistry, Vol 1: Proteins*, edited by P.F. Fox, p. 255. London: Elsevier.
- Freudig, B., Hogeckamp, S., and Schubert, H. (1999) Dispersion of powders in liquid in a stirred vessel. *Chemical Engineering and Processing* **38**, 525–532.
- Gaiani, C., Banon, S., Scher, J., Schuck, P., and Hardy, J. (2005) Use of a turbidity sensor to characterize casein powders rehydration: influence of some technological effects. *Journal of Dairy Science* **88**, 2700–2706.
- Gaiani, C., Scher, J., Schuck, P., Hardy, J., Desobry, S., and Banon, S. (2006) The dissolution behaviour of native phosphocaseinate as a function of concentration and temperature using a rheological approach. *International Dairy Journal* **16**, 1427–1434.
- Gaiani, C., Schuck, P., Scher, J., Hardy, J., Desobry, S., and Banon, S. (2007) Dairy powder rehydration: influence of proteins and some technological effects. *Journal of Dairy Science* **90**, 570–581.
- Goudédranche, H., Maubois, J.L., Ducruet, P., and Mahaut, M. (1980) Utilization of the new mineral UF membrane for making semi-hard cheeses. *Desalination* **35**, 243–258.
- Hardy, J., Scher, J., and Banon, S. (2002) Water activity and hydration of dairy powders. *Le Lait* **82**, 441–452.
- Haugaard Sorensen, I., Krag, J., Pisecky, J., and Westergaard, V. (1978) *Méthodes D'analyses Desproduits Laitiers Déshydratés*. Copenhagen: Niro A/S.
- Holt, C. (1992) Structure stability of bovine casein micelles. *Advances in Protein Chemistry* **43**, 63–151.

- Horne, D.S. (2006) Casein micelle structure: models and muddles. *Current Opinion in Colloid and Interface Science* **11**, 148–153.
- International Dairy Federation (1988) *Dried Milk and Milk Products—Determination of Insolubility Index. IDF Standard 129A*. Brussels: International Dairy Federation.
- Jeantet, R., Schuck, P., Famelart, M.H., and Maubois, J.L. (1996) Intérêt de la nanofiltration dans la production de poudres de lactosérum déminéralisées. *Le Lait* **76**, 283–301.
- Jeantet, R., Croguennec, T., Mahaut, M., Schuck, P., and Brulé, G. (2008a) *Les Produits Laitiers*, 2nd ed. Technique et Documentation. Paris: Lavoisier.
- Jeantet, R., Ducept, F., Dolivet, A., Méjean, S., and Schuck, P. (2008b) Residence time distribution: a tool to improve spray-drying control. *Dairy Science and Technology* **88**, 31–43.
- Jost, R. (1993) Functional characteristics of dairy proteins. *Trends in Food Science and Technology* **4**, 283–288.
- King, N. (1966) Dispersibility and reconstitutability of dried milk. *Dairy Science Abstracts* **28**, 105–118.
- Kjaergaard, J.G., Ipsen, R.H., and Ilsoe, C. (1987) Functionality and application of dairy ingredients in dairy products. *Food Technology* **41**, 66–71.
- Madsen, R.F. and Bjerre, P. (1981) Production of cheese-base. *Nordeuropaeisk Mejeri Tidsskrift* **5**, 135–139.
- Master, K. (2002) *Spray Drying in Practice*. Charlottenlund, Denmark: SprayDryConsult International ApS.
- Masters, K. (1991) *Spray Drying*. Essex: Longman Scientific & Technical.
- Maubois, J.L. (1991) New applications of membrane technology in the dairy industry. *Australian Journal of Dairy Technology* **46**, 91–95.
- Maubois, J.L. and Ollivier, G. (1997) Extraction of milk proteins. In: *Food Proteins and Their Applications*, edited by S. Damodaran and A. Paraf, pp. 579–595. New York: Marcel Dekker.
- Maubois, J.L., Pierre, A., Fauquant, J., and Piot, M. (1987) Industrial fractionation of main whey proteins. *Bulletin of the International Dairy Federation* **212**, 154–159.
- Maubois, J.L., Léonil, J., Bouhallab, S., Mollé, D., and Pearce, J.R. (1995) Characterization by ionization mass spectrometry of a lactosyl- β -lactoglobulin conjugate occurring during milk heating of whey. *Journal of Dairy Science* **78**(suppl. 1), 133.
- Meersohn, M. (1989) Nitrate free cheese making with Bactocatch. *North European Food Dairy Journal* **55**, 108–113.
- Mistry, V.V. (2002) Manufacture and application of high milk protein powder. *Le Lait* **82**, 515–522.
- Mistry, V.V., Hassan, H.N., and Robison, D.J. (1992) Effect of lactose and protein on the microstructure of dried milk. *Food Structure* **11**, 73–82.
- Neff, E. and Morris, H.A. (1968) Agglomeration of milk powder and its influence on reconstitution properties. *Journal of Dairy Science* **51**, 330–338.
- Pearce, R.J. (1987) Fractionation of whey protein. In: *Trends in Whey Utilization*, edited by International Dairy Federation, International Dairy Federation Bulletin 212 pp. 150–153. Brussels: International Dairy Federation.
- Pierre, A., Fauquant, J., Le Graët, Y., Piot, M., and Maubois, J.L. (1992) Préparation de phosphocéinate natif par microfiltration sur membrane. *Le Lait* **72**, 461–474.
- Pisecky, J. (1980) Bulk density of milk powders. *Australian Journal of Dairy Technology* **35**, 106–111.
- Pisecky, J. (1981) Technology of skimmed milk drying. *Journal of the Society of Dairy Technology* **34**, 57–62.
- Pisecky, J. (1986) Standards, specifications and test methods for dry milk products. In: *Concentration and Drying of Food*, edited by D. MacCarthy, pp. 203–220. London: Elsevier.
- Pisecky, J. (1990) 20 years of instant whole milk powder. *Scandinavian Dairy Information* **4**, 74.
- Pisecky, J. (1997) *Handbook of Milk Powder Manufacture*. Copenhagen: Niro A/S.
- Pouliot, M., Pouliot, Y., Britten, M., Maubois, J.L., and Fauquant, J. (1994) Study of the dissociation of beta-casein from native phosphocaseinate. *Le Lait* **74**, 325–332.
- Refstrup, E. (2003) Drying of milk. In: *Encyclopedia of Dairy Sciences*, edited by H. Roginski, pp. 860–871. London: Academic Press.
- Rollema, H.S. (1992) Casein association and micelle formation. In *Advanced Dairy Chemistry, Vol I: Proteins*, edited by P.F. Fox, p. 111. London: Elsevier.
- Roos, Y.H. (2002) Importance of glass transition and water activity to spray drying and stability of dairy powders. *Le Lait* **82**, 478–484.
- Sandblom, R.M. (1974) Filtering process, Patent 8W 74 16257.

- Schmidt, D.G. (1982) Association of caseins and casein micelle structure. In: *Developments in Dairy Chemistry I. Proteins*, edited by P.F. Fox, pp. 61–86. London: Applied Science Publishers.
- Schuck, P., Piot, M., Méjean, S., Fauquant, J., Brulé, G., and Maubois, J.L. (1994a) Déshydratation des laits enrichis en caséine micellaire par microfiltration; comparaison des propriétés des poudres obtenues avec celles d'une poudre de lait ultra-propre. *Le Lait* **74**, 47–63.
- Schuck, P., Piot, M., Méjean, S., Le Graët, Y., Fauquant, J., Brulé, G., and Maubois, J.L. (1994b) Déshydratation par atomisation de phosphocaséinate natif obtenu par microfiltration sur membrane. *Le Lait* **74**, 375–388.
- Schuck, P., Roignant, M., Brulé, G., Davenel, A., Famelart, M.H., and Maubois, J.L. (1998) Simulation of water transfer in spray drying. *Drying Technology* **16**, 1371–1393.
- Schuck, P., Briard, V., Méjean, S., Piot, M., Famelart, M.H., and Maubois, J.L. (1999) Dehydration by desorption and by spray drying of dairy proteins: influence of the mineral environment. *Drying Technology* **17**, 1347–1357.
- Schuck, P., Méjean, S., Dolivet, A., Jenatet, R., and Bhandari, B. (2007) Keeping quality of dairy ingredients. *Le Lait* **87**, 481–488.
- Slack, A.W., Amundson, C.H., and Hill, C.G. (1986) Nitrogen solubilities of β -lactoglobulin and α -lactalbumin enriched fractions derived from ultrafiltered cheese whey retentate. *Journal of Food Processing and Preservation* **10**, 19–29.
- Sougnéz, M. (1983) L'évolution du séchage par atomisation. *Chimie Magazine* **1**, 1–4.
- Straatsma, J., Vanhouwelingen, G., Steenbergen, A.E., and Dejong, P. (1999a) Spray drying of food products. 1. Simulation model. *Journal of Food Engineering* **42**, 67–72.
- Straatsma, J., Vanhouwelingen, G., Steenbergen, A.E., and Dejong, P. (1999b) Spray drying of food products. 2. Prediction of insolubility index. *Journal of Food Engineering* **42**, 73–77.
- Swaigood, H.E. (1992) Chemistry of caseins. In: *Advanced Dairy Chemistry, Vol 1: Proteins*, edited by P.F. Fox, p. 63. London: Elsevier.
- Thompson, A., Boland, M., and Singh, H. (2009) *Milk Proteins: From Expression to Food*, 1st ed. San Diego: Elsevier.
- Tuohy, J.J. (1989) Some physical properties of milk powders. *Irish Journal of Food Science and Technology* **13**, 141–152.
- Westergaard, V. (2003) Dryer design. In: *Encyclopedia of Dairy Sciences*, edited by H. Roginski, pp. 871–889. London: Academic Press.
- Westergaard, V. (2004) *Milk Powder Technology Evaporation and Spray-Drying*. Copenhagen: Niro A/S.

2 Lactose: Chemistry, Processing, and Utilization

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and R.W. Sleight

2.1 INTRODUCTION

The industrial source of lactose is whey, the liquid by-product from the manufacture of cheese and casein. Each kilogram of cheese yields 7–9 kg of whey. Two-thirds of the dry weight of the generated whey is lactose. Minerals and proteins account for the bulk of the remaining third (Hobman, 1984). Traditionally, whey was disposed of as animal stockfeed or by irrigation and sewage treatment. This represented 30% of the worldwide production of whey in 2006 (Affertsholt-Allen, 2007). Dairy plants have now developed industrial processes for better returns such as spray drying into non-hygroscopic and demineralized whey powders and ultrafiltering to separate the protein fraction after which some of the remaining whey permeate is concentrated and the lactose is crystallized (Zall, 1992). Lactose is typically used in the food and pharmaceutical industries in a wide range of products from in-house cheese milk standardization to high end pharmaceutical applications such as dry powder inhalers (DPIs) (Lifran et al., 2000; Raghavan et al., 2000). In 2006, total world production of whey was 177 million tons, of which 70% was utilized in nonhygroscopic whey powder, lactose, whey protein concentrate (WPC), whey protein isolate (WPI), and specialty powder blends (Affertsholt-Allen, 2007). In addition, milk permeate from milk concentration contributes to the production of nonfat milk solids. Production of milk permeate is forecast to grow as economic pressures drive the expansion of ultrafiltration (UF) for standardization of retail and cheese milks. Therefore, increasingly large volumes of lactose-rich permeate streams will be available, representing a wealth of value-adding opportunities. However, the current disposal strategies for milk and whey permeates are wasteful and inefficient. Because of its high biological oxygen demand (BOD_5) (essentially due to lactose) and its salt content, the release of permeate into the environment is becoming less acceptable and more tightly regulated. Meanwhile, lactose still has a low value-added image for three reasons:

- The difficulties in controlling its often empirical manufacture so as to yield a consistent product;
- The perception that lactose intolerance among a majority of the world's population should lead to avoidance of lactose containing products, constraining its wider use as an ingredient; and

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- The limited and irregular return on investment because of the large cyclical variation in the price of lactose.

However, those three limiting factors can now be addressed by exploiting recent developments in the sciences related to the dairy field. As the discharge of dairy streams is increasingly restricted by regulatory constraints, dairy factories will move more and more toward zero discharge processing. Manufacturing high-quality, functional lactose and lactose derivatives should be an integral part of a new “zero discharge strategy.” Increasing the efficiency of the lactose manufacturing process will become paramount, and includes better understanding and control of the factors influencing the final crystallization step and the quality of the final lactose product. In addition to sustainable processing and value adding applications, a fresh approach to permeate utilization should include new nonfood high volume applications, such as deicing salts or ethanol production (Lifran et al., 2000). Many reviews in the literature provide background information on lactose. Particularly useful references are “Modern Approaches to Lactose production” (Durham, 2009) and “Lactose, Water, Salts and Minor Constituents” (McSweeney and Fox, 2009), as well as other reviews (Listiohadi et al., 2005a; Kirk et al., 2007; Gänzle et al., 2008). The nutritional aspects of lactose are covered in Hourigan (1984), Mustapha et al. (1997), Savaiano et al. (2006) and Schaafsma (2008). This chapter aims to review recent market developments and advances in the available knowledge base, which will help in building a sustainable and profitable strategy for lactose utilization as a food and pharmaceutical ingredient.

2.2 FORMS AND PROPERTIES OF LACTOSE

Lactose (4-O- β -D-galactopyranosyl-D-glucopyranose, $C_{12}H_{22}O_{11}$) is a disaccharide consisting of a D-glucose and a D-galactose molecule joined by a β -1,4-glycosidic linkage. It is the most abundant sugar in the milk of most mammals, and is also found in a few other sources, such as *Forsythia* and *Sapotacea* plants (Holsinger, 1988). Lactose is the major energy and carbon source in the diet of suckling mammals, with its components, glucose, providing the energy building blocks, and galactose being vital for brain development (Holsinger, 1988). It is present in varying concentrations depending on the species. The lactose level in human milk is around 7%, but it is 4.8% in the milk of cow, sheep, and buffalo, animals commonly used as the source of milk in the human diet (Nickerson, 1974). In addition to lactose, the lactose-based human milk oligosaccharides play a vital role in the development of the human infant (Kunz et al., 2000; Lifran et al., 2009). Lactose is a reducing sugar because of its aldehydic function on the glucose moiety of the molecule. Consequently, it undergoes oxidation, caramelization, and the Maillard reaction and forms glycosides. These and other chemical reactions of lactose have been reviewed (McSweeney and Fox, 2009). As shown in Table 2.1, lactose has four polymorphs: α -lactose monohydrate, α -lactose anhydrous (stable and unstable forms), and β -lactose anhydrous.

The α - and β -lactose forms differ by the position of the hydroxyl group on the carbon 1 of the glucose moiety. The α - and β -lactose anomers are able to transform from one to the other in solution, in a spontaneous process called mutarotation (Figure 2.1).

Polymorphism and mutarotation both contribute to the complex chemistry of lactose, conferring on each form different chemical and physical properties (Listiohadi et al., 2005a,b,c; Kirk et al., 2007).

Table 2.1. Currently known forms of lactose.

Crystalline	Monohydrate	α -Lactose
	Anhydrous	α -Lactose unstable
		α -Lactose stable
		β -Lactose
		Compound β/α lactose ^a
Amorphous		Mixture of α -lactose and β -lactose

^aLerk et al., 1984.

Source: Listioghadi et al., 2005a. Reproduced by permission of Dairy Industry Association of Australia.

Chemically pure lactose is rarely encountered. Edible grade lactose, the common product found in the dairy industry, is a mixture of lactose with small amounts of water, peptides and proteins, mineral salts, organic acids and their salts, and riboflavin. Pharmaceutical grades of α -lactose monohydrate generally remain contaminated with some lactose phosphate (Visser, 1980; Lifran et al., 2006, 2007). Commonly, the products obtained from laboratory suppliers are no better than pharmaceutical grade. Within the lactose fraction, it is not possible generally to obtain anomerically pure lactose. In research studies, precautions must be taken to characterize the nature of the lactose sample under study. The need for care is more acute when attempting to calibrate analytical methods against pure reference materials. The validity of research papers is limited, too frequently, by poor characterization of samples.

2.2.1 Types of lactose

2.2.1.1 α -Lactose monohydrate

The most common and stable form of lactose is crystalline α -lactose monohydrate. Each crystal of α -lactose monohydrate contains one molecule of water of crystallization for every lactose molecule. The water molecule is important in the structure and stabilization of the crystal lattice because it links together oxygen from four different lactose molecules (Clydesdale et al., 1997). As a result, α -lactose monohydrate is relatively nonhygroscopic. The hydrogen-bonding interaction within the α -lactose monohydrate crystal structure was elucidated recently by Smith et al. (2005) using X-ray diffraction (XRD). Three out of four lactose polymorphs: α -lactose monohydrate, α -lactose unstable anhydrous, and β -lactose, crystallize with monoclinic unit cells (Smith et al., 2005). The crystal structure of the stable anhydrous α -lactose is a more complex triclinic unit cell. Cooling batch crystallization of whey permeate is the common method of producing α -lactose monohydrate (see Section 2.3.1). It is available in edible and pharmaceutical grades, the latter being recrystallized from the former. Spray dried products rich in lactose include whey powder, whey permeate powder, and pharmaceutical grade spray dried lactose.

2.2.1.2 β -Lactose

β -Lactose exists in the solid state in an anhydrous crystalline form. Properties in which it differs from α -lactose monohydrate include its powder XRD pattern (unique reflection at

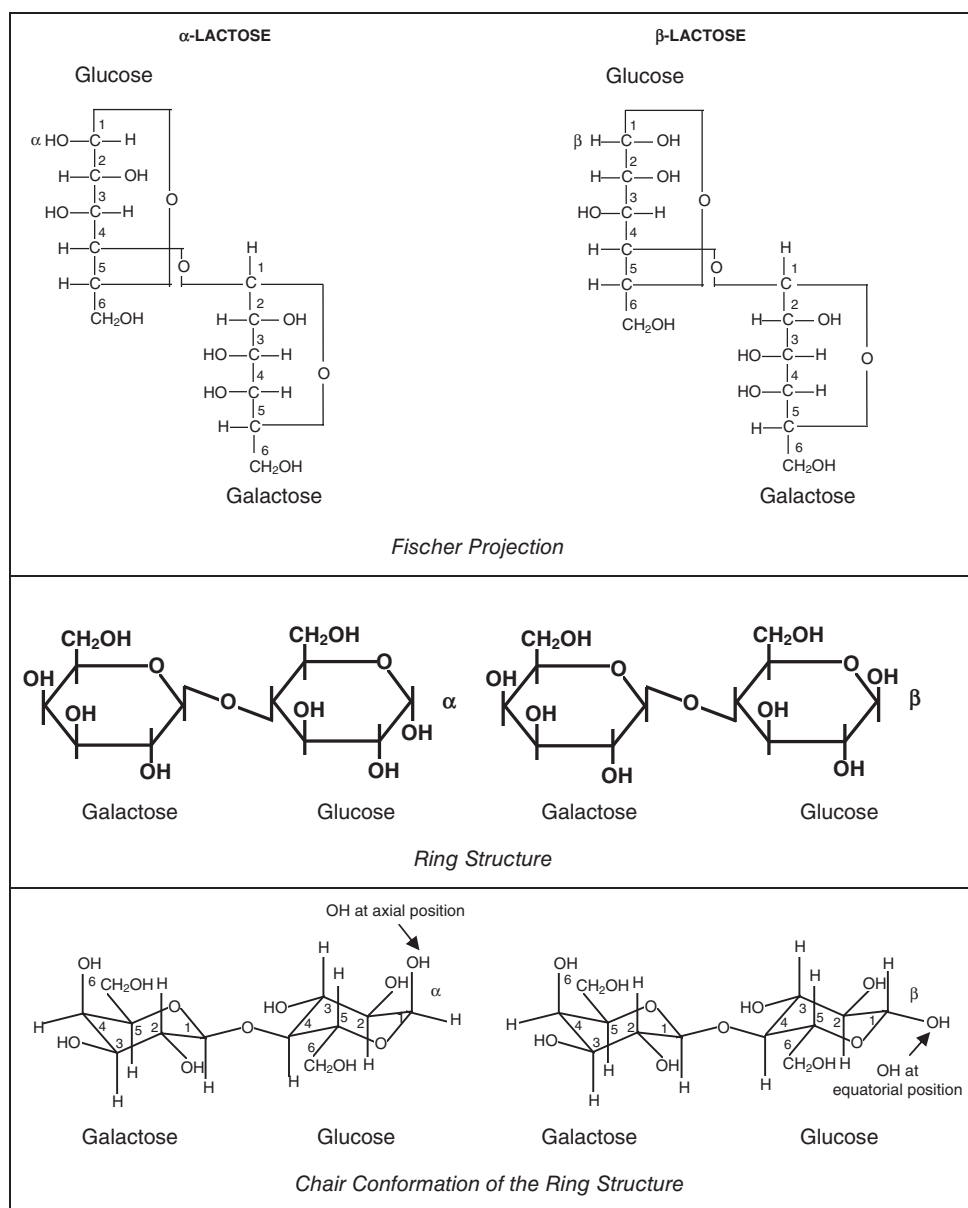


Figure 2.1 Molecular structures of α - and β -lactose by Fischer projection (top), ring structure (middle), and chair conformational structure (bottom) (Listiohadi et al., 2005a). Reproduced by permission of Dairy Industry Association of Australia.

10.5°, 2 θ , $d = 8.377 \text{ \AA}$) (Kirk et al., 2007), its higher critical relative humidity (RH), making it less hygroscopic, higher initial solubility and sweetness. β -Lactose anhydrous can be crystallized in the laboratory under a wide range of conditions all involving heat. A temperature of 93.5°C has been cited traditionally as the threshold temperature above which β -lactose can be crystallized from aqueous systems. Other factors affecting

crystallization include steam, pressure, organic solvents, such as ethanol, methanol, acetone, glycerol, and alkaline pH can also be involved (Hockett and Hudson, 1931; Buma and van der Veen, 1974; Olano, 1978; Parrish et al., 1980; Parrish and Brown, 1982; Singh and Shah, 1992; Lifran et al., 2001). The reported techniques to produce β -lactose in organic solvents, where the organic component acts as an antisolvent in the crystallization, use large amounts of solvent relative to the quantity of lactose. Under appropriate conditions of moisture, temperature, and residence time, β -lactose can be produced in an extruder (Asano et al., 1978; Van Leverink, 1981; Listiohadi, 2000). Commercial “anhydrous lactose” (high β) is usually made by roller-drying lactose solution above 93.5°C (Bell, 1930; Lerk, 1993). It is a mixture of β -lactose (70–80% of the total lactose) and anhydrous α -lactose. Production of β -lactose at a temperature lower than 93.5°C was reported by Vu et al. (2009).

2.2.1.3 *Stable and unstable forms of α -lactose anhydrous*

Anhydrous α -lactose exists in two forms: stable anhydrous α -lactose and unstable anhydrous α -lactose. They are produced when the molecule of water of crystallization is eliminated from the monohydrate; however, the crystals may be extensively fractured by heat and vacuum (Holsinger, 1988). Hockett and Hudson (1931) first produced stable anhydrous α -lactose by solvent extraction of the monohydrate (now called soft dehydration), with acidic methanol as the solvent. Later, methanol and other solvents, such as ethanol and toluene, were used (Buma and Wiegers, 1967; Kirk et al., 2007). Thermal or hard dehydration can produce both unstable and stable forms when α -lactose monohydrate crystals are heated at elevated temperatures (100–190°C) (Buma and Wiegers, 1967; Figura and Epple, 1995; Garnier et al., 2002). Using differential scanning calorimetry (DSC), Figura and Epple (1995) showed that unstable anhydrous α -lactose was a precursor of the stable anhydrous α -lactose when derived by thermal methods. At lower temperatures (100–130°C), unstable anhydrous α -lactose is first formed; but as the temperature rises (160–170°C), more thermal energy is available and a rearrangement occurs to form stable anhydrous α -lactose. The porous structure of the unstable anhydrous α -lactose crystal readily sorbs moisture from the atmosphere to recreate α -lactose monohydrate, in RH as low as 10% (Figura and Epple, 1995). In contrast, once stable anhydrous α -lactose is formed, α -lactose monohydrate will only be reformed at RH higher than 50% (Kirk et al., 2007) or upon dissolution and recrystallization of the anhydrous stable form in water. These contrasting behaviors in water sensitivity show the importance of understanding the differences between lactose polymorphs, which are all identical in chemical formula (Roos and Karel, 1992). This is of particular interest for the pharmaceutical applications of lactose, where it is believed the drying and milling processes could modify the original α -lactose monohydrate and hence have detrimental effects on drug delivery (Lerk et al., 1984; Vromans et al., 1987).

Crystallographic data are rare for the anhydrous α -lactose forms because it is difficult to produce these crystals. The difficulties include insolubility of lactose in nonaqueous solvents, rehydration of the anhydrous forms back to α -lactose monohydrate, and mutarotation leading to β -lactose. Kirk et al. (2007) established two characteristic reflections for stable anhydrous α -lactose by powder XRD, although their intensity is relatively weak (10.0–23.0°, 2 θ , $d = 4.933, 4.783$ Å). Conclusive measurements of the crystal lattice unit cell are still to be made.

2.2.1.4 Compound crystals of α - and β -lactose anhydrous

Compound crystals of α - and β -lactose where both isomers occur together in the same crystal lattice in different molar ratios have been reported, usually on the basis of XRD (Hockett and Hudson, 1931; Domovs and Freund, 1961; Bushill et al., 1965; Olano et al., 1977; Lerk et al., 1984; Kussendrager and Andreae, 1986; Drapier-Beche et al., 1997; Jouppila et al., 1997). Earl and Parrish (1983) used nuclear magnetic resonance (NMR) evidence to argue for the coexistence of α and β anomers in the crystal lattice of stable α -lactose anhydrous. However, Kirk et al. (2007) argued against the existence of these compound crystals based on their XRD data.

2.2.1.5 Amorphous lactose

Amorphous lactose, the most hygroscopic form of lactose (Buckton and Darcy, 1999), is noncrystalline, as the name suggests. The term amorphous refers to a viscous syrup of low moisture existing in a glassy state. The lactose molecules are not organized in a regular lattice, and their mobility is low enough for the lactose glass to appear solid (Hartel, 2001). Amorphous lactose is formed during drying (spray drying, roller drying, and freeze-drying) of a lactose solution when a rapid increase in viscosity restricts and even prevents crystallization (Holsinger, 1988).

Candidate methods for the determination of amorphous lactose, applicable to dairy products containing protein and salts, include gravimetric moisture sorption under conditions where all the amorphous lactose crystallizes as the monohydrate (Lehto et al., 2006) NIR, FTIR, and Raman spectroscopy. Lehto et al. (2006) compared seven methods for the determination of amorphous lactose in pure lactose made with amorphous lactose contents varying from zero to 100% by adjusting the ratio of ethanol (crystalline) to water (amorphous) in the feed solution. The authors recommend a combination of Raman spectroscopy and either solution calorimetry or gravimetric sorption methods for determination of the amorphous content of spray dried lactose, but these methods are not reliable for amorphous levels around 1% or less. Another Finnish group recommended XRD, Raman, and near infrared methods for determining the amorphous content of spray dried lactose (Savolainen et al., 2007).

Amorphous lactose in pharmaceutical products has been quantitated by inverse gas chromatography (GC) and can now be routinely measured by dynamic vapor sorption. Young et al. (2007) were able to measure as little as 0.05% amorphous content in pharmaceutical lactose by adsorption of octane in a dynamic vapor sorption instrument. Numerous authors (Buckton and Darcy, 1999; Young et al., 2007) have pointed out that the limit of detection of XRD for amorphous lactose is around 10% in largely crystalline matrices, yet many authors have used XRD to support a belief that a material was free of amorphous lactose. Small proportions of amorphous lactose are detectable by polarizing light microscopy. The reverse task of quantifying small quantities of crystalline material in a largely amorphous matrix is easier. Listiophadi et al. (2009) showed how TGA/SDTA could be used to determine amorphous content in lactose powders.

2.2.1.6 Characterization of lactose in the solid state

To explain the behavior and properties of powders containing lactose and water, there is a need to determine free water (moisture) and water of crystallization. Alternatively—but

less ideally—the determination of total water and water of crystallization has been used to indirectly determine free water. In addition, analysis is also needed to show whether the lactose is amorphous or crystalline, and to measure the β/α balance of the lactose. Moreover, it may be important to differentiate between anhydrous crystalline forms and α -lactose monohydrate. In dried milk and whey, these determinations must be performed in the presence of proteins and salts, thus limiting the range of available methods.

Listiohadi et al. (2005a) reviewed methods for the determination of the forms of lactose and their water content. In dairy science and technology, it is common to estimate the resistance to caking of dried whey and lactose by calculating the degree of crystallinity. This is the percentage of the total lactose present as α -lactose monohydrate (Anderson and Berlin, 1974; Pisecky, 1997). In the absence of simple methods for determining the water of crystallization in α -lactose monohydrate, it is assumed that the hydrated water can be estimated as the difference between the moisture (free or surface water) and the total water determined by “loss on heating” at low (2 hours at 80°C) and high (16 hours at 120°C) time/temperature profiles, respectively. In fact, neither condition gives an unequivocal estimate, and the former is close to the detection limit of the method and prone to error. The quantity of α -lactose monohydrate is calculated from the water of crystallization, assuming a 1 : 1 molar ratio between the lactose and water. The total lactose must be determined by an independent method, such as polarimetry (Roetman and van Schaik, 1975), colorimetry (Acton, 1977), or enzymic analysis (International Dairy Federation, 2001). Estimates of lactose “by difference” introduce errors into the calculation. Spray dried whey is expected to be stable to moisture during distribution and storage if it has a crystallinity of at least 75%. Such a product would not have more than 25% of its lactose in the amorphous state.

An alternative is to judge the resistance of lactose products to moisture-induced changes by determining the content of amorphous lactose independently and directly rather than by crystallinity. Listiohadi et al. (2009) showed how TGA/SDTA could be used to determine moisture (free water), water of crystallization, and amorphous content in lactose independently of each other. The method was capable of determining the moisture and water of crystallization in whey powder and skim milk powder, but the amorphous transition was obscured in these two dairy powders (Listiohadi et al., 2005c). The TGA/SDTA data could be used to determine the crystallinity of the whey powder. Listiohadi et al. (2009) also reaffirmed the validity of toluene (“Dean and Stark”) distillation and Karl Fischer titration for determination of total water. Toluene distillation is a known method for obtaining anhydrous lactose by dehydration (Buma and Wiegers, 1967). Loss-on-heating methods commonly used in the dairy industry for the determination of water in protein-containing dairy powders have been comprehensively reviewed and strongly criticized by Isengard (2001), who has proposed a new reference method for the determination of total water of dried dairy products by Karl Fischer titration (Isengard et al., 2006).

The importance of understanding and controlling the different forms of lactose is often overlooked by the dairy industry; however, it is paramount to the manufacturing processes of crystallization and spray drying, and to maintaining the quality of a wide range of lactose-containing products throughout storage. This can be illustrated by three examples. First, the control of the amorphous lactose level in milk powders is necessary in order to avoid caking while protecting the fat and the fat soluble vitamins from oxidation. The caking of the different polymorphs of lactose has been reported by Listiohadi et al. (2008). Second, the crystalline forms present in products, such as milk chocolate, for example, directly affect its texture and appearance upon storage. If the lactose incorporated into milk

chocolate is mostly in the amorphous state, exposure to heat and humidity will cause water uptake, crystallization, and release of the sorbed water thus imparting a gritty texture to milk chocolate. On the contrary, β -lactose has no negative effect (Novakova et al., 2002; Thomas et al., 2009). Third, the control of the form of lactose is also critical for high value-added pharmaceutical lactose products where excipient-drug interactions can be caused by polymorphic changes and their attendant moisture fluxes (Vromans et al., 1986, 1987).

King (1965) first summarized the relationships between the different forms of lactose in a diagram, and Walstra et al. (1999) modified King's chart. An updated diagram, including, among other missing information, the formation of amorphous lactose from α -lactose monohydrate by milling, and other mechanical processes, is provided in Figure 2.2. The current commercial products include crystalline α -lactose monohydrate, spray dried lactose containing up to 15% amorphous lactose, and anhydrous lactose (75–80% β content) (Lerk, 1993). If regularly updated, the diagram in Figure 2.2 can function as a useful summary of, and guideline to, lactose processing, along with Table 2.2, which provides the references used in the diagram.

2.2.2 Mutarotation

The two anomeric forms of lactose (α monohydrate and β anhydrous) are present in solution. Mutarotation is well researched, and the main findings were established last century (Troy and Sharp, 1930; Haase and Nickerson, 1966a; Buma and van der Veen, 1974; Roetman and Buma, 1974; Visser, 1982a; Morrissey, 1985). Walstra et al. (1999) and Holsinger (1997) provide good summaries on mutarotation kinetics and equilibrium. The β/α ratio of amorphous lactose will initially be that of the solution at the time of drying and will slowly change during storage. Amorphous lactose held at 25°C for 16 months had a β/α ratio of 1.25 (Roetman and van Schaik, 1975). Mutarotation is slowed but not stopped in the glassy state. Most researchers (Roetman, 1982) have used polarimetry to determine the ratio of β and α -lactose. Other methods, such as high-pressure liquid chromatography (HPLC) (Listiohadi et al., 2009) or GC (Paez et al., 1987), can be used.

2.2.3 Solubility and supersaturation

Lactose solubility is low compared with other sugars, such as sucrose (Herrington, 1948). Because lactose has two anomeric forms, the initial solubilities of the α - and β -lactose forms are different from the equilibrium solubility.

The equilibrium solubility of lactose in water has been researched extensively by Hudson (1904), Saillard (1919), Gillis (1920), Peter (1928), Hunziker and Nissen (1926), Herrington (1934c), Foremost Foods Co. (1970), and Visser (1982a). Table 2.3 compiles their findings.

The equilibrium lactose solubility increases with temperature and is determined by the mutarotation rate below 93.5°C. Above this temperature, the β -lactose solubility determines the overall solubility (Walstra and Jenness, 1984). Of the solid forms of lactose, α -lactose monohydrate is the most stable below this temperature, and β -lactose the most stable above it (Hudson, 1904). Therefore, two correlations, fitting the experimental data of Table 2.3, were compiled to describe the equilibrium solubility of lactose in water (Butler, 1998), depending on the temperature, as shown in Equations (2.1) and (2.2).

$$C_s = e^{2.389+0.028T} \quad T \leq 93.5^\circ\text{C}, \quad (2.1)$$

$$C_s = e^{3.569+0.015T} \quad T \geq 93.5^\circ\text{C}. \quad (2.2)$$

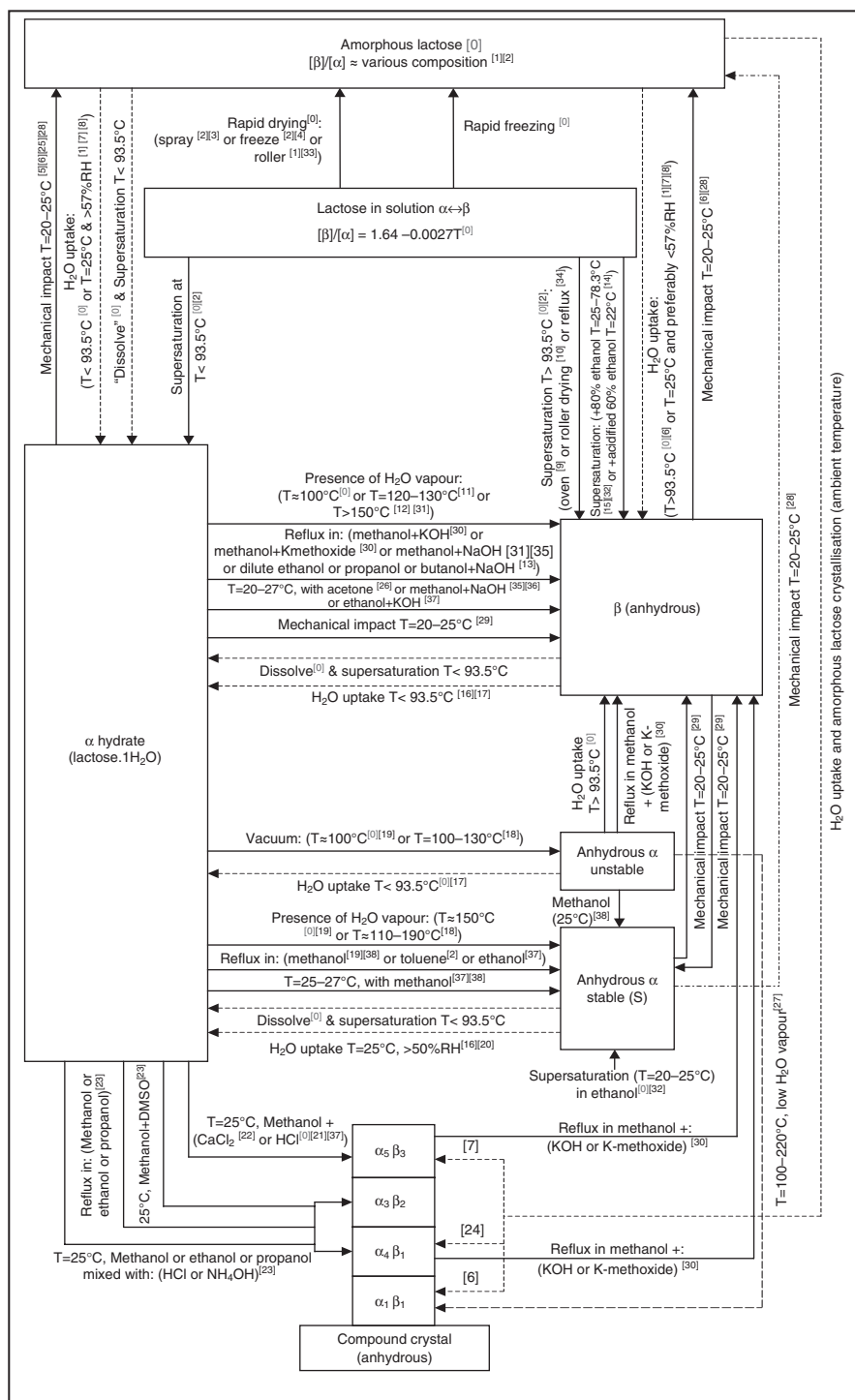


Figure 2.2 Lactose conversion diagram modified from Walstra et al. (1999). Refer to Table 2.2 for the list of citations.

Table 2.2. List of citations and references to the modified diagram of Walstra et al. (1999) in Figure 2.2.

Citation	Reference	Citation	Reference
0	Walstra et al. (1999)—original data	20	Figura and Epple (1995)
1	Listiohadi et al. (2005b)	21	Hockett and Hudson (1931)
2	Holsinger (1988)	22	Domovs and Freund (1961)
3	Sebhatu et al. (1994)	23	Olano et al. (1977)
4	Kedward et al. (2000)	24	Jouppila et al. (1997)
5	Newell et al. (2001a); Newell et al. (2001b)	25	Listiohadi et al. (2005c)
6	Lerk et al. (1984)	26	Garnier et al. (2002)
7	Bushill et al. (1965)	27	Kussendrager and Andrae (1986)
8	Wursch et al. (1984)	28	Otsuka et al. (1993)
9	Hudson (1908)	29	Otsuka et al. (1991)
10	Bell (1930)	30	Parrish et al. (1980)
11	Sharp (1934)	31	Olano et al. (1983)
12	Listiohadi (2000); Asano et al. (1978); Van Leverink (1981)	32	Madj and Nickerson (1976)
13	Olano (1978)	33	Angberg et al. (1991)
14	Singh and Shah (1992)	34	Itoh et al. (1978)
15	Lifran et al. (2001)	35	Olano and Rios (1978)
16	Listiohadi et al. (2008)	36	Drapier-Beche et al. (1997)
17	Cal et al. (1996)	37	Parrish and Brown (1982)
18	Sharp (1943)	38	Lim and Nickerson (1973)
19	Buma and Wiegers (1967)		

In Equations (2.1) and (2.2), C_s is the equilibrium lactose solubility concentration (g anhydrous lactose/100 g water), and T the temperature measured in °C. Below 93.5°C, lactose crystallizes as the α -lactose monohydrate form, which means that one water molecule is incorporated in the crystal lattice with every lactose molecule. Above 93.5°C, the equilibrium results in β -lactose crystallizing. Certain salts and sugars, alcohols, and whey components can, however, decrease the equilibrium solubility (Madj and Nickerson, 1976; Smart, 1988; Bhargava and Jelen, 1996). The initial solubility of α -lactose is the solubility of this anomer in the absence of the β -form and vice-versa. The initial solubilities of α and β -lactose in water are difficult to measure because of mutarotation. Visser (1982a) published the results of lactose solubility determination over a broad range of temperatures, and expressed, for the first time in the literature, the driving force for the crystallization of lactose monohydrate in a formula taking into account the depression of alpha lactose solubility by β -lactose. Visser (1982a) quantified the β -lactose depression of the α -lactose solubility by assuming β -lactose would have the same effect as sucrose. Using known data on lactose solubility in the presence of sucrose, Visser (1982a) calculated a factor F to give the degree of α -lactose solubility depression by other disaccharides. This factor was calculated for temperatures between 0 and 60°C, and can be expressed as a function of temperature (T) in Equation (2.3).

$$F = 0.159 + 0.00023T^{1.36}. \quad (2.3)$$

Visser (1982a) then proposed the following correlation to calculate the alpha lactose solubility ($C_{\alpha S}$):

$$C_{\alpha S} = \frac{C_s - FK(C - C_s)}{1 + K}. \quad (2.4)$$

Table 2.3. Equilibrium solubility of lactose in water.

Author	Hudson (1904)	Herrington (1934c)	Visser (1982a)	Others ^a
Temperature (°C)	Equilibrium solubility (g anhydrous lactose/100 g water)			
0	11.92	11.9		
10		15.1		
15	17.01		16.86	
20		19.2	19.1	19.2
21.5				20
25	21.70		21.82	21.7, 21.6
28				24
30		24.8	24.81	24.8
33			26.95	
38				30.7
40			32.75	32.6, 31.8
48			41.09	42.1
50		43.7	43.46	44.1, 43.7
55			50.19	
57				56.0
60			58.4	58.7, 59.5
65			67.72	
70			78.26	78.2
73.5				84.5
75			90.4	
79.1				98.4
80			104.6	
85				123.5
87.2				122.5
88.2				127.3
90		143.9		
92				135.3
93.5				143.9

^aCompiled from the results of Saillard (1919), Gillis (1920), Peter (1928), Foremost Foods (1970), and Hunziker and Nissen (1926).

In Equation (2.4), C_s is the equilibrium lactose solubility in g anhydrous lactose/100 g water as described above by Equations (2.1) and (2.2), F is the factor accounting for β -lactose depression of the α -lactose solubility (Equation 2.3), C is the total lactose concentration, and K is the equilibrium constant, describing the equilibrium ratio of β and α -lactose.

The equilibrium constant K is dependent on temperature, and the correlation of Roetman and Buma (1974) should be used, as it is based on the most reliable data (Visser, 1982a; Butler, 1998). Equation (2.5) shows the linear relationship between the equilibrium constant (K) and the temperature (T).

$$K = 1.644 - 0.0026T. \quad (2.5)$$

The absolute α -lactose supersaturation (S_α) should be chosen as the crystallization driving force, since it is the form of lactose that crystallizes under the temperature range industrially investigated. The α -lactose supersaturation is defined as the difference between

α -lactose concentration and solubility: $(C_\alpha - C_{as})$. As $C_\alpha = \alpha_f C$, this supersaturation can be estimated using Equation (2.6).

$$S_\alpha = C_\alpha - C_{as} = \alpha_f C - \frac{C_s - FK(C - C_s)}{1 + K} \quad (2.6)$$

In Equation (2.6), α_f is the alpha lactose fraction of the lactose solution at a given temperature, C is the total lactose concentration, F is the factor accounting for β -lactose depression of α -lactose solubility, estimated from Equation (2.3), K is the equilibrium constant calculated from Equation (2.5), and C_s is the lactose solubility calculated from Equations (2.1) or (2.2).

The α -lactose fraction of the lactose solution at a given temperature (α_f) can be estimated by the following correlation (Equation 2.7), taken from the work of Buma and van der Veen (1974):

$$\alpha_f = 0.0004T + 0.3782 \quad (2.7)$$

Lactose solutions can become highly supersaturated before spontaneous crystallization occurs. Supersaturation is reached when a solution contains more lactose molecules than is thermodynamically allowed (Hartel and Shastry, 1991), that is to say, when its concentration is higher than the lactose equilibrium solubility. Above the lactose solubility, three regions of supersaturation exist, as shown on Figure 2.3. At high supersaturation levels, the labile zone is the area where spontaneous nucleation can occur. Below this region, in a narrow intermediate zone, nucleation will only occur when seed crystals are present. This is called secondary nucleation. At lower supersaturations, the metastable zone presents a region where nucleation will not occur but added seed crystals will grow. The concentration boundaries for the metastable zone are the lactose solubility and secondary nucleation threshold (SNT) concentration (C_{snt}). The concentration boundaries of the metastable zone can vary with changes in agitation rate and the presence of additives (Hartel and Shastry, 1991). However, Butler et al. (1997) derived the following correlation for the SNT concentration (Equation 2.8).

$$C_{snt} = e^{2.992 + 0.0196T} \quad (2.8)$$

Equations (2.1) and (2.8) were used to calculate the equilibrium lactose solubility and SNT concentration, respectively, to build Figure 2.3.

While using chemical potentials would allow a true understanding of the mechanism of crystallization, the measured lactose concentrations are sufficient when comparing crystallization kinetics under various conditions (Hartel, 2001). The most accurate method to calculate supersaturation based on concentration to date was developed by Visser (1982a).

2.2.4 Properties of lactose crystals

α -Lactose monohydrate crystallizes easily below 93.5°C, and is the most common form of lactose. Water of crystallization makes up 5% of the formula weight. It is not widely understood that commercial samples of α -lactose monohydrate usually contain up to 5% of the total lactose in the β rather than the α form (Paez et al., 1987; Angberg, 1995; Liothadi et al., 2005a; Kirk et al., 2007). The product presents as hard particles, it is not hygroscopic, and may have different shapes depending on the supersaturation level, from a prism to a tomahawk, because of the difference in the growth rates of the individual faces and the changes in these relative growth rates (Herrington, 1934b; Van Kreveld and

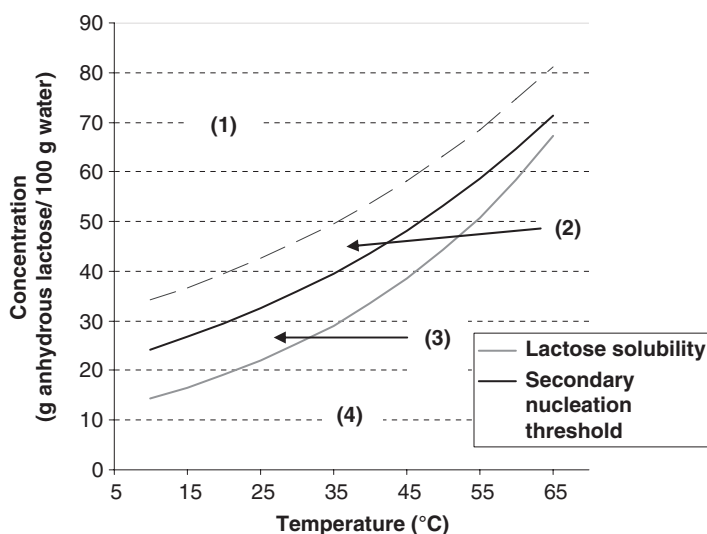


Figure 2.3 Metastable and solubility regions for lactose. Supersaturated zones include: (1) labile zone, (2) intermediate zone, (3) metastable zone, and (4) undersaturated zone.

Michaels, 1965; Raghavan et al., 2000). Under a strong driving force, crystallization is less influenced by adsorption of the β anomer on the growing crystal surface and tends to deliver prisms and needles. The dominant shape of the crystal changes to the tomahawk as crystallization proceeds, the supersaturation declines, and the relative effect of the β anomer increases. The most common shape found under industrial lactose crystallization resembles a tomahawk. Milling of the product to achieve a standard particle size means that scanning electron microscope (SEM) pictures show a mixture of fragments and intact tomahawk crystals. The temperature and the presence and type of impurities also affect the final crystal habit (its morphology) (Hunziker and Nissen, 1927; Herrington, 1934b; Van Kreveld and Michaels, 1965; Jelen and Coulter, 1973b). This complexity, typical of crystallization in dairy products, complicates predictability and control (Nickerson, 1974). The physical properties (density melting point, refractive index) of lactose crystals can be found in Hudson (1904) and McDonald and Turcotte (1948).

Most of the growth on α -lactose crystals occurs from the apex of the crystals, on the (010) and (110) faces along the b - and a -axis respectively (Van Kreveld and Michaels, 1965). The apex is the site of the original nucleus. Jelen and Coulter (1973a) undertook experiments where the top of the tomahawk was removed and did not grow again. The base-only growth of the lactose crystals accounts for its slower growth rate compared with the multidirectional growth of other sugars under the same conditions. The slow, base-only growth of α -lactose may be explained by the selective inhibition of two of its crystal faces by β -lactose (Raghavan et al., 2000).

2.3 MANUFACTURE OF LACTOSE

Global production of lactose of all types was 870,000t in 2006, up from 724,000t in 2002 (Affertsholt-Allen, 2007). The alliance between Fonterra and Friesland-Campina-DMV

Table 2.4. Composition of different permeates from ultrafiltration.

Component	UF milk permeate	Sweet whey UF permeate	Acid whey UF permeate
Total solids (%)	5.8	5.8	5.8
Lactose (%)	4.9	4.9	4.1
Crude protein (%)	0.3	0.3	0.4
Ash (%)	0.5	0.5	0.7
Lactic acid (%)	–	0.2	0.5
Calcium (ppm)	280	300	950
Phosphorus (ppm)	330	400	550

gives them a large share of the pharmaceutical grade lactose market and the dominant position in lactose production ahead of Leprino Foods. Lactose prices are highly volatile, attaining fourfold increases and peaking at their highest level between June 2006 and June 2007 before decreasing again.

2.3.1 Industrial processes for α -lactose monohydrate

The raw material for lactose manufacture is usually permeate from the UF of whey or milk. There are three main types of permeate with different compositions, as presented in Table 2.4.

Milk permeate is generated from UF of skim milk for the standardization of milk for cheese and casein, or for the manufacture of milk protein concentrate. Sweet whey permeate comes from the UF of renneted whey for the manufacture of WPC. Acid whey permeate is generated from UF of lactic, sulfuric, or hydrochloric casein whey for the manufacture of WPC. The differences in composition will heavily impact on the performance of the lactose manufacturing process. For example, the higher calcium and phosphorus load of acid whey will exacerbate the problem of calcium phosphate fouling the membranes and the evaporators. In addition, the higher level of acid in acid whey permeates impacts on the ease of drying and separation of lactose from minerals. Milk permeate offers manufacturers greater flexibility in use than whey permeate. Production of permeate powder uses permeate as a whole, whereas recovery of lactose and sialyllactose fractionates the permeate, leaving behind the minerals and other components. The further one travels down the fractionation pathway, the greater is the ingenuity required to devise simple, inexpensive, and safe separation processes that operate effectively at the industrial scale. It is also more difficult to utilize the residual material. Thus, commercially viable, high margin uses for mother liquor, the effluent stream remaining after lactose manufacture, are yet to be developed.

In a sense, edible lactose can be regarded as demineralized permeate. A range of technologies is available to remove some or most of the minerals prior to crystallization. These pretreatments are designed to improve the color, ash, particle size and size distribution and yield of the final lactose product. Current technology for manufacture of edible grade lactose commonly includes demineralization of permeate by nanofiltration, as advocated by Guu and Zall (1992), with recent work directed at efficiency improvements in membrane operation (Cuartas et al., 2004). In milk permeates and whey streams, the stabilizing effect of casein is absent, and the calcium phosphate system is very prone to precipitation. This manifests as fouling and limits what can be achieved in evaporators and membrane

processing. Cheese whey permeate notionally contains about 350 ppm calcium and 400 ppm phosphorus. At these concentrations, whey and permeates are supersaturated with calcium and phosphate. Consequently, whey permeate is thermodynamically unstable and precipitation of some form of calcium phosphate will take place sooner or later (Schmidt and Both, 1987). Calcium phosphate precipitation is favored by increased concentration, alkaline pH, and elevated temperatures (its solubility decreases with temperature). Rapid removal of calcium and phosphate prior to heating and concentration is the preferred option for lactose processing. In effect, the aim is to accelerate the inevitable slow deposition and conduct it under controlled conditions.

Rosamaninho and Melo (2006a) took a different course after reviewing work on calcium phosphate deposition from a wide range of fields. They approached the problem as an exercise in how to control crystallization. Nucleation precedes crystal growth, and the driving force for crystal growth is the degree of supersaturation, which will be influenced by concentration and temperature. Because the local supersaturation adjacent to a heating surface in an evaporator, or in a concentration polarization layer adjacent to a membrane, may be much higher than that operating in the bulk solution, local deposition (i.e., fouling) may result. This theoretical framework also allowed the authors to explain the modulation of the fouling by agents, such as citrate and peptides, in terms of adsorption of the modulators on the growing crystal face, leading in turn to inhibition or promotion of crystal growth. Rosamaninho and Melo (2006a,b, 2008) explained their fouling results in simulated milk ultrafiltrate through the crystallization of calcium phosphates, as did Rice et al. (2009) working with UF permeate. However, an aspect well known to industry practitioners but neglected in the research literature is that permeate composition varies from process to process (milk permeate, cheddar permeate, mozzarella permeate, and lactic whey permeate), and from day to day and plant to plant.

The GEA Westphalia dicalciumphosphate (DCP) process (GEA, 2008) is one version of the precipitation process for the stabilization of protein-free dairy streams by the removal of “calcium phosphates.” In this process, whey permeate is adjusted to pH 6.7 by the addition of NaOH and heated to 75–80°C for 20–30 minutes. The resulting calcium precipitate is stabilized by a short holding time then separated by cross-flow UF with diafiltration. The slurry is then spray dried into a fine powder. There are some variations whereby the calcium precipitate can be separated in a decanter washer and then dried; however, calcium driers require special care as the calcium precipitate is highly abrasive. Being a natural milk product (Harju, 2001), the milk calcium can be sold as a dairy ingredient rather than a food additive.

After the whey permeate demineralization steps, concentration by falling film evaporation takes the total solids to ~60%. In the absence of decalcification, lactose evaporators typically have operational cycles as low as 4–6 hours with frequent CIP (clean-in-place) cleaning to remove calcium precipitates, which foul the heating surfaces. The industrial crystallization of lactose is usually performed by a slow batch cooling process, yielding edible grade lactose containing a significant level of nonlactose components. Purification to pharmaceutical-grade lactose requires activated carbon treatment and a second crystallization step (Hartel and Shastry, 1991). The traditional processes to make edible and pharmaceutical grade lactose are described in Holsinger (1997), Paterson (2009), and Durham (2009). The specifications of the different products can be found in the various food and pharmaceutical standards, such as Codex Alimentarius (2001), the Food Chemicals Codex (1996), or the U.S., Japanese, and British Pharmacopeia (2001) (see Table 2.5).

Table 2.5. Data included in the specifications for the composition of α -lactose monohydrate and anhydrous lactose products.

Analysis	USP (1995)	British Pharmacopeia (2001)	Codex Alimentarius (2001)	Food Chemicals Codex (1996)
α-Lactose monohydrate				
Lactose content (anhydrous basis) ^a	99.8%	99.8%	>99.0%	98.0–100.5%
Ash (sulfated, anhydrous basis)	<0.1%	<0.1%	<0.3%	<0.3% ^b
Water content ("as is" basis)				
• Loss on drying at 120°C for 16 hours	-	-	<6.0%	4.5–5.5%
• Loss on drying at 80°C for 2 hours	<0.5%	<0.5%	-	-
• Karl Fischer	4.5–5.5% ^c	4.5–5.5% ^c	-	-
Anhydrous lactose				
Lactose content (anhydrous basis) ^a	>99.8%	>99.8%	-	98.0–100.5%
Ash (sulfated, anhydrous basis)	<0.1%	<0.1%	-	<0.3% ^b
Water content ("as is" basis)				
• Loss on drying at 120°C for 16 hours	-	-	-	<1.0%
• Loss on drying at 80°C for 2 hours	<0.1%	<0.1%	-	-
• Karl Fischer	<1.0%	<1.0%	-	-

^aNo lactose polymorphism is described. Lactose content is calculated using its molecular weight (for monohydrate and for anhydrous).

^bReported as "Residue on Ignition."

^cFormamide is used as the solvent.

-, not specified.

The current processes have many difficulties and give poor returns (Durham et al., 1997b). As mentioned above, the fouling of membrane surfaces and evaporators occurs regularly because of the high calcium concentration in the whey permeate, leading to short runs, unnecessary wastes, and high cleaning costs. Second, the crystallizers are overloaded with materials that will contaminate the lactose crystals, including minerals, proteins, organic acids, and riboflavin. These contaminants greatly affect the growth kinetics of the lactose crystals. Their removal by washing the crystals requires large volumes of precious wash water, which also dissolves some of the crystals, while some of the contaminants are still included in the final lactose product. The yields are generally low (65–75%), and the crystallization process leaves large quantities of mother liquors to be disposed of.

Chromatographic purification of lactose can overcome these difficulties. Harju and Heikkilä (1990), Harju, (2007), and Hramtsov et al. (1990) first developed chromatographic processes to separate the mother liquor in order to increase lactose recovery. In 1997, an improved chromatographic process was designed and optimized by Durham et al. (1997a). The underlying principle of the process is to produce ultra-pure lactose syrup before the final crystallization step. The process was developed based on three steps: ion exchange decalcification, recycling whey salts to regenerate the ion exchange resin, and purification of the lactose by ion exclusion chromatography. This patent has been licensed by Groupe Novasep (Theobald, 2007), and can be applied to the pretreatment of permeate,

mother liquor, or whey prior to secondary purification by chromatography, ion exchange, or other means. The resultant purified lactose stream is suitable for direct crystallization into pharmaceutical grade lactose or conversion into lactose derivatives (Durham and Hourigan, 2007). Among the ionic compounds removed is lactose phosphate, therefore producing a purified product comparable with the “nonionic” lactose produced in the laboratory by Visser (Visser, 1982b; Lifran et al., 2004, 2007). In effect, this process captures the lactose (up to one-third of the feed) that is normally lost in the mother liquor and recovers it as a high-grade product while achieving an environmental footprint much lower than the traditional technology. The manufacturers’ operational flexibility is increased because the high purity lactose needs far less downstream purification such as the steps usually needed for the removal of colored by-products. Similarly, efficiencies and yields are increased when the lactose is to be converted to lactulose, galacto-oligosaccharides (GOS), and other high margin derivatives.

2.3.2 Creation of amorphous lactose during the α -lactose monohydrate manufacturing process

Amorphous lactose destabilizes α -lactose monohydrate when exposed to water vapor. Ahlneck and Zografi (1990), from theoretical calculations, postulated that levels of moisture as low as 0.1% in sucrose containing 0.5% in the amorphous form could have the same impact as 20% moisture in completely amorphous sucrose and decrease the T_g from 52 to 9°C. The sorbed water tended to concentrate at the amorphous sites. Buckton and Darcy (1999) developed a similar argument and supported it with thermogravimetric data for lactose. They described the amorphous sites as reactive “hot spots” that “amplify” the influence of sorbed water. These are the sites where water will be concentrated, where the amorphous lactose will crystallize on exposure to humid environments, and from which the water released by crystallization will diffuse to initiate the crystallization at the next amorphous site. In effect, the ratio of the water to the amorphous content is more relevant than the water content per se.

The industrial process for the manufacture of α -lactose monohydrate usually includes the crystallization of large crystals, which are then separated from the mother liquor, washed, dried, milled to the required particle size, and bagged before storage. As a result of grinding and milling, the crystalline α -lactose monohydrate partly converts to amorphous lactose, which is then able to agglomerate under certain conditions of RH and temperature (Morita et al., 1984). This can in turn lead to caking of stored or shipped products. Another way in which amorphous lactose may form in α -lactose monohydrate powder is suggested by the findings of Shastry and Hartel (1996). In an analogous process, they found that films of amorphous sucrose can form on the surface of rapidly dried sucrose crystals. In a similar fashion, Bronlund and Paterson (1997) argued that the surface of α -lactose monohydrate could be coated with amorphous lactose during rapid drying of the wet crystals harvested from the centrifuge. In 2004, they found 9.1% amorphous lactose in a commercial pharmaceutical grade α -lactose monohydrate that sorbed ~25% more moisture at a_w 0.2 and 20°C than the dried amorphous-free material exposed to the same a_w , and showed that small quantities of amorphous lactose in crystalline lactose powders had a significant effect on the moisture sorption isotherm (Bronlund and Paterson, 2004).

Many studies have confirmed the formation of amorphous lactose during milling (Otsuka et al., 1991, 1993; Listioghadi et al., 2005d). Newell et al. (2001a,b) used inverse GC to show that even levels of milling-induced amorphous lactose as low as 1% can produce

behavior similar to a completely amorphous sample possibly because the amorphous lactose is predominantly collected on the surface of the crystal. Price and Young (2005) grew a large crystal of α -lactose monohydrate on a stainless steel disc, subjected it to grinding by ceramic spheres, then examined the surface of the crystal by SEM and “tapping mode” atomic force microscopy (AFM). Both techniques revealed clear damage, such as the formation of pits and craters, to the crystal structure after milling for as little as 1 minute. The damage was described as an increase in surface roughness. The observations were visual, and no attempt was made to measure the formation of amorphous lactose. Das et al. (2010) used IGC to measure damage to the surface of coarse and micronized inhaler grade α -lactose monohydrate. They were unable to detect amorphous lactose by DVS in the coarse lactose or in the micronized sample either before or after micronizing. But the surface energy values were higher in the micronized sample, and it agglomerated after storage at 75%. It may be that minor damage to the surface of an α -lactose monohydrate crystal—less than that required to form amorphous lactose—may predispose the damaged crystal to agglomerate.

There is clear experimental evidence that amorphous lactose is formed by milling and grinding, but poor understanding of how this happens. The amorphous form is disordered and in a higher energy state than the crystalline form. It is possible that there is sufficient heat and moisture at the point of impact of the ceramic ball on the surface in a ball mill to dissolve some of the crystal, which rapidly cools and dries, forming amorphous lactose. This will be very difficult to verify experimentally. Industrial processes for the manufacture of α -lactose monohydrate result in products contaminated with low levels of amorphous lactose, which will be sufficient to permit moisture induced crystallization if the product is stored at inappropriate temperature and RH and caking may result. In pharmaceutical applications, the behavior in granulation, tableting, and DPIs may be affected.

2.3.3 Crystallization theory and research trends

Despite technological advances in whey permeate purification, crystallization is still used to both separate lactose from other components in whey permeate and to form particles of the desired shape and a size suitable for harvest in the centrifuges. Industrial lactose crystallization is complex (Nickerson, 1974), can be unpredictable, and has proven difficult to model. However, a sound knowledge of nucleation and crystallization kinetics enables modeling and prediction of the industrial crystallization final performance in terms of yield, crystal median size, and crystal particle size distribution.

Nucleation is the formation of a solid crystalline phase from a supersaturated solution (Randolph and Larson, 1988). Primary nucleation occurs in the labile zone and refers to the formation of nuclei requiring energy, in the absence of other particles. Secondary nucleation can occur in the metastable region if existing particles are already present to provide the surface for generation of nuclei (Mullin, 2001). Lactose exists in the metastable zone under a large range of temperatures (Herrington, 1934a) because of mutarotation effects. Since the metastable zone is larger for lactose than for other sugars, it is possible to minimize unwanted nucleation during cooling batch crystallization (Vu et al., 2006a).

Different methods, such as polarimetry, refractometry, gravimetry, water activity, or video microscopy, have been devised to study lactose crystallization kinetics (Haase and Nickerson, 1966b; Twieg and Nickerson, 1968; Nickerson and Moore, 1973, 1974a; Arel-lano et al., 2004; Mimouni et al., 2005; Vu et al., 2006a; Bhandari and Burel, 2007). The growth kinetics of lactose crystals were studied under various conditions, such as

in stagnant or agitated solutions, in batch or for single seeds. In these publications, the experimental systems included most of the impurities commonly present in whey permeate during the industrial lactose manufacture, while research on pure lactose has been reported by Visser (1982b), Vu et al. (2006a,b), and Lifran et al. (2004). The effects of supersaturation and temperature on lactose crystal growth have been extensively studied both qualitatively and quantitatively since they have the biggest impacts on the process. Other parameters, such as agitation, pH, presence, and quality of nuclei and crystal content of the crystallizing slurry, also have an influence (Hartel, 2001). Lactose crystal growth rate increased strongly with increasing supersaturation (Van Kreveld and Michaels, 1965; Visser, 1982b), with the exponent of the supersaturation varying within the range of 2–4, depending on the temperature and other conditions (Jelen and Coulter, 1973a; Shi et al., 1989, 1990). The growth dependence on supersaturation is also related to temperature. Increasing temperature increased the crystal growth rate (Whittier and Gould, 1931; Haase and Nickerson, 1966b; Jelen and Coulter, 1973a). Jelen and Coulter (1973a) demonstrated that the choice of expression of the supersaturation affects the temperature dependence of the crystallization kinetics. When a supersaturation ratio was used, growth linearly increased, with temperature between 30 and 70°C. When a concentration difference was used, growth increased from 30 to 50°C with no further increases for higher temperatures. Jelen and Coulter (1973a) found different supersaturation dependence with increasing temperature. At 30°C, the order of the reaction was equal to 2.5, while at higher temperature, the dependence decreased to a second order reaction. The pH influences the mutarotation equilibrium, which may affect the rate of crystallization. However, Visser (1980) showed that the effect of pH on the growth rate was related to the presence of lactose phosphate, while pure lactose had only a small dependence of growth on pH. In addition, in the pH range of 4–7 generally found in the lactose industrial manufacture, pH does not significantly affect lactose growth rate (Jelen and Coulter, 1973a). Broken, milled, or washed crystals increased the growth rate (Van Kreveld and Michaels, 1965; Jelen and Coulter, 1973a; Nickerson and Moore, 1974a; Bhargava and Jelen, 1996). The smoother the crystal surface, the slower the crystal growth so that the manner in which a crystal was formed may affect its growth. For example, lactose crystals formed under contact nucleation show very slow growth (Shi et al., 1989, 1990). This is of importance if seeding is used to initiate the crystallization. A high number of crystals in the crystallizing slurry can decrease the growth rate due to mass transfer effects because of the competition for the available excess concentration. This was observed for sucrose crystals by Zagrodski (1968), and resulted in smaller final crystals for the same reason.

Academic research has focused on the relatively simple case of batch crystallization at constant (isothermal) temperature.

However, industrially, batch crystallization is conducted while cooling. Temperature has complex effects on aspects such as supersaturation, nucleation, and mutarotation (Hartel and Shastry, 1991). Therefore, it is important to obtain an optimal cooling strategy from a mathematical model of batch cooling crystallization. Paterson (2009) also stressed the importance of selecting an appropriate cooling strategy. Lactose crystallization using ultra-pure lactose syrup and lactose crystals as seeds can be predicted and modeled in terms of yield, crystal median size, and crystal particle size distribution. Vu et al. (2006a) developed a new approach, solving a dynamic optimization problem to find the model and the optimal cooling temperature profile for seeded batch crystallizations in a laboratory and pilot plant scale. They applied the developed model in the simulations of continuous, semi-batch and evaporative batch cooling crystallizations. They found that batch cooling, although less

efficient, is generally used in factories due to the lower capital and maintenance costs. In contrast, evaporative batch operation is much faster, but implementation is more complicated, and nucleation is difficult to control. Edible lactose is made in batches by cooling the impure lactose feed (supersaturated to more than 60°Brix) from 70°C to ambient temperature. Auto-nucleated batch crystallization is still commonly practiced in many lactose factories due to a higher production rate without the cost of making seed crystals. A self-nucleated operation is very sensitive to temperature and allows little margin for error. To achieve adequate control of large batches in the manufacturing plant, it is essential to have a cooling strategy for the crystallization of “impure” (i.e., containing permeate components in addition to lactose) and supersaturated lactose feed. The strategy should be adaptable to cases where seeding is used, and where it is not, and be able to demonstrate the pros and cons of each case (Vu et al., 2006b).

Novel crystallization technologies have been recently introduced in the literature. Although the production of tomahawk-shaped crystals of α -lactose monohydrate is the dominant industrial process, research examples show how different types and shapes of crystals can be achieved by varying the supersaturation of lactose. The lactose supersaturation is increased when water-miscible organic antisolvents are added to the crystallizing solution. Variation of the solvent composition and temperature results in alterations to the yield, purity, β/α composition, shape, and size of the lactose crystals (Olano et al., 1983). For example, crystals with up to 90% β content were obtained in the laboratory when a 40% aqueous lactose solution was refluxed with alkaline methanol (Olano et al., 1983). Lifran et al. (2001) described alcoholic demineralization and crystallization to produce unique rosette crystals with high value-added pharmaceutical applications. Zeng et al. (2000a) were able to crystallize α -lactose monohydrate with different shapes and surface smoothness by varying the temperature of crystallization and adding small quantities of methanol, ethanol, or glycerol to the crystallizing solution. Larhrib et al. (2003) obtained crystals with equal β and α contents from systems containing 90% acetone. In an attempt to control crystal size distribution (CSD) and growth rate dispersion, the growing lactose crystals can be constrained in small droplets dispersed in an immiscible medium, like Carbopol gels (Zeng et al., 2000b) or kerosene (Dombrowski et al., 2007). Vu et al. (2009) confirmed the lactose/water/ethanol phase diagram of Machado et al. (2006) and extended it to the lactose/water/ethanol/glycerol system. By replacing some of the ethanol in the lactose/water/ethanol phase with glycerol, they reduced the volatility of the solvent system, altered the shape, size and CSD of the crystals, and could produce β contents up to 90% at temperatures of 70°C and less. Moreover, glycerol was compatible with food and pharmaceutical use. Vu et al. (2004) and Bund and Pandit (2007) described sonocrystallization for faster crystallization with higher recovery. Shi et al. (2006) have patented a crystallization technique to control growth rate dispersion and produce large crystals.

Farber et al. (2003) studied the formation of granules on a microscope slide by the evaporation of saturated solutions of lactose at 23°C and 63% RH. Pharmaceutical-grade lactose from commercial suppliers was used: lactose monohydrate from three suppliers, a spray-dried lactose, and “anhydrous lactose” (high β content). Crystallization was monitored by optical microscopy and XRD. Nucleation centers formed within minutes at the periphery of the microscope slides, but a long period (up to weeks) was required to completely cover the slide with crystals. XRD revealed that the crystals first formed were α -lactose monohydrate, and that β -lactose dominated in the later stages. The XRD scans were supported by SEM micrographs. There seemed to be an initial phase of nucleation perhaps associated with heightened evaporation at the edge of the droplet, but the propagation phase

seemed to be much slower, perhaps reflecting slower crystallization rates for β -lactose, a finding reminiscent of Angberg's (1995). Seeding of the droplets with the starting material did not increase the rate of crystallization. The authors related the results to the agglomeration process in pharmaceutical manufacture.

2.3.4 Effect of impurities on lactose crystal growth

The components present in whey have been extensively studied alone or in combination in order to determine their effect on the lactose crystallization kinetics. Among components naturally occurring in whey, β -lactose, various soluble salts, riboflavin, and lactose phosphate have been identified as having a negative impact on lactose growth. Herrington (1934b) first suggested that β -lactose inhibited the growth of α -lactose by adsorbing on the fast-growing faces of the crystals. It is now accepted that β -lactose selectively blocks the growth of individual faces of α -lactose, resulting in low growth rates and the typical tomahawk shape (Van Krevelde, 1969; Visser and Bennema, 1983; Dincer et al., 1999; Raghavan et al., 2000). Needle-like crystals were obtained when low amounts of β -lactose were present. Industrial lactose crystallization takes place in the presence of large amounts of β -lactose tending to equilibrate with α -lactose by mutarotation. This is particularly relevant to the later stages of cooling crystallization where the temperature is falling below 30°C and the rate of mutarotation relative to crystallization has slowed.

Additives, such as alcohols (Herrington, 1934a; Singh and Shah, 1992), sucrose, glucose and maltose (Herrington, 1934a; Van Krevelde and Michaels, 1965; Michaels and Van Krevelde, 1966; Nickerson and Moore, 1974b; Smart, 1988), and several acids and bases (Michaels and Van Krevelde, 1966; Twieg and Nickerson, 1968; Jelen and Coulter, 1973b; Nickerson and Moore, 1974b), were also investigated. Molecules other than α -lactose may alter the solubility and hence the supersaturation of lactose; the rate of mutarotation and hence the β/α ratio in the solution; and the viscosity and hence the rate of diffusion of lactose molecules in solution.

Some impurities adsorb on the growing crystal surface. Visser (1980, 1984, 1988) demonstrated how commercial pharmaceutical-grade lactose was contaminated by lactose phosphate mixtures that retarded the growth of single lactose crystals by preferential incorporation in the crystal lattice on all the faces, but especially the strongest growing (010) and (110). The practical importance of this contamination is not limited to growth rate retardation. Nieuwmeyer et al. (2008) demonstrated that the lactose phosphate contaminant exacerbated the degradation of a model steroid drug in a granulated dosage form. Granules stored at 40°C and 75% RH lost 65% of the initial steroid content over 6 months. Reliable delivery of the stated dose is all important in pharmaceutical science.

Although Visser demonstrated that pharmaceutical lactose is routinely contaminated with lactose phosphate, researchers have continued to use it as the benchmark for pure lactose in crystallization studies, when in fact Visser's "nonionic" lactose, made by removing lactose phosphate, should have been the reference lactose. Lifran et al. (2006, 2007) confirmed Visser's findings under conditions relevant to the industrial batch cooling crystallization. Other components have recently been studied, such as escin (a compound with applications in the pharmaceutical and cosmetic industries [Grases and March, 1991; Grases et al., 1994]), and other sugars structurally related to lactose (Garnier et al., 2002) and proteins (Wang et al., 2001; Mimouni et al., 2005). These studies used pharmaceutical grade lactose in their experiments without further purification or analysis of the lactose phosphate content. Finally, the effect of combined impurities, such as in whey permeate,

can also be explained in term of their interactions with lactose phosphate. Several studies found that the growth rate of lactose crystals in permeate was faster than in water (Haase and Nickerson, 1966b; Kapil et al., 1991; Butler, 1998). It is likely that the observed enhancement of the growth rate by the presence of minerals was in fact due to the reduced growth inhibition of lactose phosphate when the ionic strength was high. In the presence of salts, above a certain ionic strength, the effect of lactose phosphate is reduced, because the cations from the added salts tend to neutralize the lactose phosphate anions, causing them to desorb, resulting in faster growth rates. Despite this “ruling out” of lactose phosphate inhibition by salts, lactose phosphate still gets integrated in the crystals (Visser, 1988). It appears that working at very low pH or adding salt would be sufficient to counteract the action of lactose phosphate in a commercial situation; however, these solutions are not practical. Adding hydrochloric acid to lower the pH down to 1 would incur extra costs, the stainless steel used would have to be of higher quality, and a large volume of water would be required to wash away the acid so that the final product fits the specifications for acidity. Similarly, adding salts would increase raw material and waste treatment costs, and washing decreases yields. In addition, these solutions using large volumes of water would no longer be environmentally acceptable. The removal of lactose phosphate before the crystallization step, along with the other impurities present in whey permeate, appears to be a better alternative.

Other sugar-based molecules also have a growth retarding effect on lactose crystals. Citing the work of Wood (1969), Nickerson (1970) reported that galactooligosaccharides (GOS) slowed the crystallization rate of α -lactose monohydrate and changed the shape of the crystal. The shape varied between the 1 : 3, 1 : 4, and 1 : 6 linked galactosides and the effect of the galactosides was attributed to adsorption on the growing crystal face. Smart and Smith (1991) showed that small additions of a mixture of two 1 : 4 linked galactosyl lactoses (1 g/100 g crystallizing solution) decreased the crystallization rate of lactose by one-third during isothermal batch crystallization at 35°C. Needle-shaped crystals rather than the usual tomahawks were prominent. The GOS, according to Wood and Nickerson, resulted from the action of β -galactosidase originating in the starter culture and acting on lactose both during curd formation and in whey concentration and processing. Anecdotal reports from industry about the difficulty of crystallizing lactose in Mozzarella whey and spray drying it are common. Thermophilic starters, such as those containing *Streptococcus thermophilus*, are used in the production of Mozzarella cheese. The β -galactosidase of *Streptococcus thermophilus* is capable of converting up to 40% of lactose to oligosaccharides in milk and whey (preferably concentrated) at temperatures up to 55°C (Greenberg and Mahoney, 1983; Smart, 1991; Pivarnik et al., 1995; Mahoney, 1998). Such temperatures can occur during the manufacture of Mozzarella cheese and the concentration and crystallization of its whey. Rao et al. (2004) found levels of galactose and lactic acid/lactates to be higher in Mozzarella whey than cheddar whey, as did Gernigon et al. (2009, 2010), and ascribed the stickiness and spray drying difficulties of Mozzarella whey to excessive levels of the hygroscopic compounds, galactose, and lactic acid. Neither group examined their whey for the presence of GOS. The results of Smart and Smith (1991) suggest that when crystallizing lactose from Mozzarella whey permeate, the crystallization may be slow, the yield may not be achieved in the expected time, and needles may not be completely harvested by the centrifuges designed to harvest larger tomahawks. In the spray drying of Mozzarella whey, it may be more important than usual to achieve a high degree of crystallinity in the lactose to avoid stickiness during drying and storage. Galactooligosaccharides also exist with lactose in high concentrations in commercial GOS syrups. It may be that the galactooligosaccharides impede the crystallization of lactose in these

syrops. The literature does not contain reports of thorough investigations of these points. The inhibition of lactose crystallization due to galactosyl lactose reported by Smart and Smith (1991) is marked and comparable with that of lactose phosphate reported by Lifran et al. (2007). Tjuradi and Hartel (1995) reported that oligosaccharide fractions from glucose syrups inhibited the crystallization of sucrose from solution.

2.4 EFFECT OF MOISTURE ON LACTOSE IN THE SOLID STATE

Flowability of lactose materials is an important technological property underlying sticking to the walls of spray driers, pipes, and pneumatic conveyors in the dairy industry, caking of shipped or stored products, and blockages of hoppers, fillers, or tabletters in the pharmaceutical industry. On the plus side, instantizing and granulation depend on the same science. The complex relationships between the forms of lactose (amorphous and crystalline) and the storage conditions must be understood. Technologists and engineers must be able to predict the rate and extent of sticking and caking, its changing nature over time, its hardness, how to prevent it, and how to recover caked products. Because of its commercial importance in both the food and the pharmaceutical industry, the field of lactose crystallization in the solid state has been a very active area of research. The tendency to cake is related to the contact area of the particles, which in turn is inversely related to particle size. Caking and stickiness have been reviewed by Purutyan et al. (2005), Adhikari et al. (2001) and Aguilera et al. (1995), among others. Listiohadi et al. (2005a) reviewed the properties of lactose and its caking behavior. Flow of powders has been measured via angle of repose, discharge rate from funnels, the torque on impellers, fluidized behavior, and blow tests. Shear and tensile strength have been measured by Jenicke cell. The resistance to flow of plugs or tablets has been related to density and hardness as measured by penetrometers or texture analyzers. Methods should be simple, accurate, reliable, reproducible across many laboratories, and suitable for industrial application. New experimental techniques and insights from the developing field of molecular mobility have been applied in the last 30 years. A number of physical and chemical changes in solid and semisolid foods have been attributed to molecular mobility. During sorption, water plasticizes molecules in the amorphous state, decreasing their viscosity and increasing the mobility of molecules. These changes at the molecular level are revealed by an increase in water activity, a_w , and a decrease in the glass transition temperature (T_g). The concept of molecular mobility has been reviewed by Reid and Fennema (2008) and Simatos et al. (2009). Moisture-induced crystallization and its consequences have been correlated with molecular mobility as measured by NMR (Lai and Schmidt, 1990; Lloyd et al., 1996). Many authors have measured T_g by differential scanning calorimetry (DSC). To improve the understanding of the mechanisms controlling the flowability of lactose materials, sticking, agglomerating, and caking behavior should be monitored by methods that measure the underlying molecular changes concurrently but independently of the changes to flow. These methods should include chemical analysis, spectroscopy, thermal analysis, and sorption studies.

2.4.1 Moisture-induced crystallization of amorphous lactose

Roos and colleagues studied the sorption of amorphous lactose and milk powders and determined adsorption isotherms at ambient temperatures. They examined the sorbed materials by DSC (Roos and Karel, 1992) and XRD (Jouppila et al., 1997; Haque and Roos,

2005). Roos (2002) summarized the decline of T_g with increasing water content in amorphous lactose undergoing sorption. In the dry state, the T_g is 101°C, but it drops to 24°C when the water content is 6.8 g/100 g, corresponding to a water activity of 0.37. These values are known as the critical water content and water activity, respectively: the water content and water activity at which amorphous lactose will crystallize at 24°C. In dried skim milk, the corresponding water content is 7.6 g/100 g, indicating that more water must be sorbed in dried skim milk to achieve the same degree of change to the lactose (Roos, 2002).

Lloyd et al. (1996) heated previously humidified amorphous lactose in aluminum cylinders at temperatures between 30 and 100°C for 3 hours. As the temperature was increased, both the plug density and the hardness of the lactose initially stayed constant before increasing sharply at a temperature that corresponded to the T_g (identified by DSC and NMR). The NMR data provided a measure of molecular mobility. The rise in density and hardness was explained by increased viscous flow at temperatures that exceeded the T_g . Scanning electron micrographs showed that the starting material was amorphous, but the heated plugs were crystalline. The crystallized heated plugs shrank dramatically. The shrinkage became more pronounced the more the temperature exceeded the T_g . The variation of T_g with water activity was fitted to a polynomial that was consistent with the results of Roos and Karel (1990). Vuataz (2002) found a similar polynomial applied to spray-dried skim and whole milk commercial products, but within the restricted water activity range of 0.12–0.65, the T_g declined linearly with water activity for both SMP and WMP from 60.6 to –15.5°C. Thus, it can be predicted that a sample of dried milk with an a_w of 0.60 would have T_g of –8.4°C, while one with a_w of 0.20 would have T_g of 49.1°C. In line with the experimental results of many authors, the likelihood of caking during storage and use would be much less for the latter.

Paterson and colleagues have completed a number of studies into the sticking, cohesiveness and caking of lactose itself and lactose containing dairy powders (Paterson, 2009). They developed methods for measuring stickiness by blow tests and particle gun, and have shown that while T_g must be exceeded by 25°C for spray-dried amorphous lactose to be instantaneously sticky, exceeding the T_g by only a few degrees will be sufficient to allow caking on storage (Paterson et al., 2005). The amorphous lactose examined in this study was shown to be free of crystals by polarizing light microscopy.

Five steps in the moisture-induced crystallization of amorphous lactose can be discerned. (1) Initially amorphous lactose adsorbs water on its surface, leading to a transition from a glassy to a rubbery state at the glass transition temperature (T_g) (Roos and Karel, 1992). At 25°C, this will occur after sorbing water equivalent to about 5% of its dry mass. (2) In the second step, absorption of water continues in the bulk after passing through the T_g . The sorptive capacity of amorphous solids is attributed to their large void volume. As sorption continues, viscosity declines and the material becomes sticky. Water activity increases. The value of the T_g declines as water is sorbed, eventually allowing it to drop below the storage temperature of the material (Roos, 2002; Vuataz, 2002). These changes are indicative of increased molecular mobility (Lai and Schmidt, 1990). Bulk absorption is likely to continue until the amorphous lactose has sorbed water equivalent to around 10% of its mass. (3) Step 3 is mutarotation, which is facilitated by the increased molecular mobility. Mutarotation will allow the replenishment of α anomer lost through crystallization (Listiohadi et al., 2005b). Mutarotation will overlap the preceding and subsequent stages. (4) Step 4 is nucleation. Once sufficient water has been sorbed in bulk absorption, nucleation is triggered. There is some evidence that the crystallization rate of amorphous

lactose can be accelerated by seeds (Schmitt et al., 1999), but at the high supersaturation levels operating here, self-nucleation cannot be ruled out. Nucleation becomes autocatalytic (Burnett et al., 2004, 2006). This point marks the initiation of the release of the sorbed water held in the voids of the amorphous lactose. (5) Crystal growth and liberation of sorbed water occurs in Step 5. The released water will diffuse away from the site of crystallization, carrying with it dissolved/plasticized lactose and other solutes, and will deliver this lactose to fresh nuclei where further crystallization can occur (Buckton and Darcy, 1999; Burnett et al., 2004, 2006). Crystal growth is triggered when the mobility of water molecules is high enough. This in turn is related to the sorption of sufficient water from the environment. The results of many authors, including the state diagrams of Vuataz (2002) and Roos (2002), indicate that this threshold is in the vicinity of 15–20% water content. Conditions of 75% RH and temperatures of 25°C or above can give a rapid “quantitative” conversion of amorphous lactose to α -lactose monohydrate (Listiohadi et al., 2005b). At the lower values of RH encountered during storage and distribution in industry, amorphous lactose is likely to crystallize as one of the anhydrous forms (Bushill et al., 1965; Jouppila et al., 1998; Berggren and Alderborn, 2004; Listiohadi et al., 2005b). Fine needle-like crystals are common in powders where amorphous lactose has crystallized following sorption of moisture. These may be immature crystals of α -lactose monohydrate or crystals of the anhydrous forms. These crystals may fuse together. At its fullest development, moisture-induced crystallization of amorphous lactose will generate a fused, hard, crystalline, dense mass (Briggner et al., 1994). If exposed as plugs, the plugs will display shrinkage (Lloyd et al., 1996; Listiohadi et al., 2005a). The liberated water can facilitate physical and chemical changes, including Maillard browning, loss of protein solubility, and enzymic inactivation (Burin et al., 2004; Buera et al., 2005; Listiohadi et al., 2005c). It is conceivable that at some point, there may be too much water: the supersaturation of lactose may decline, and the rate of crystallization may decrease. Alternatively, crystallization may proceed until all the amorphous lactose is crystallized. The overall rate of moisture induced crystallization has been related to the value of $(T - T_g)$, where T is the product storage temperature (Roos and Karel, 1990). The greater the value of $(T - T_g)$, the sooner the lactose will crystallize.

While many researchers have examined the moisture-induced crystallization of lactose at ambient temperatures by varying the RH, others have crystallized the amorphous lactose by raising the temperature in humid environments. Rises in RH and temperature have a reinforcing effect on moisture induced crystallization of amorphous lactose while a rise in RH will counteract a drop in temperature and vice versa. These results have practical application in spray drying (Langrish, 2008; Islam and Langrish, 2010), extrusion, and chocolate manufacture. In these studies, it is common to find that the resulting crystals are dominated by β -lactose (Schmitt et al., 1999; Ibach and Kind, 2007; Nijdam et al., 2007), as the temperature is raised above the ambient temperature.

The concept of molecular mobility provides an explanation of the delay in the moisture-induced crystallization of lactose in the presence of proteins, microcrystalline cellulose, and other compounds. The water loosely bound to these macromolecules will be less available to facilitate the crystallization of lactose. Some authors have referred to this action as an inhibition of the crystallization. This is unlikely since known inhibitors of the crystallization of lactose operate competitively and follow Langmuir's isotherm. These include β -lactose (Dincer et al., 1999), and, at trace level, lactose phosphate (Visser, 1988; Lifran et al., 2007). It is accepted that competitive inhibitors have less effect on crystallizations operating under the strong driving force of high supersaturation. With the exception of

β -lactose, competitive inhibition would not be expected to play a significant role in moisture-induced crystallization. Substances, such as protein, microcrystalline cellulose, and polyvinylpyrrolidone, are more likely to exert an influence on the moisture-induced crystallization of lactose by forestalling increases in the mobility of the water rather than by competitive inhibition of crystallization.

In products where the presence of amorphous lactose cannot be avoided, the a_w should be kept around 0.2 or below at ambient temperatures to ensure the temperature of the material is well below its T_g at that moisture level. Hence, the driving force for crystallization, $(T - T_g)$, will be negative, and the rate of crystallization will be imperceptibly low. The viscosity will remain in the glassy range. Temperature gradients and condensation in products during packing, storage, and distribution should be avoided. Compounds that compete with amorphous lactose for water, including casein, whey proteins, milk salts, microcrystalline cellulose, and maltodextrins may delay the onset of moisture-induced crystallization. An alternative approach is “preemptive” crystallization, where crystallization is induced in a deliberate and controlled fashion. Examples include spray-dried pharmaceutical grade lactose, nonhygroscopic whey powder, and permeate powder. Agglomerated/instantized milk powder is partly crystallized during manufacture. Sweetened condensed milk is notable for interactions between the crystallizing lactose and sucrose systems and for the use of seeding to maintain the size of the lactose crystals below the level of sensory perception (Tan, 2009).

2.4.2 Effect of moisture on the crystalline forms of lactose

Apart from α -lactose monohydrate, two products are also commercially available: roller-dried (high β) “anhydrous lactose” with 75–80% of the lactose in the β form and spray-dried lactose with its amorphous content reduced to 10–12% as a result of precrystallization prior to drying. The sorption of these products at ambient temperatures has been studied and compared with that of the anhydrous α forms (Angberg, 1995; Cal et al., 1996; Listiophadi et al., 2008).

The most stable crystalline form of lactose, the form into which all the anhydrous forms will convert if the RH is high enough, is α -lactose monohydrate. In contrast to the limited adsorption at lower humidities, Listiophadi et al. (2008) found that both anhydrous α forms sorbed water at 75% RH and rapidly converted to the monohydrate, whereas exposure of the roller dried product at 75% RH converted around 25% of its β content to anhydrous α -lactose in 90 days. Angberg (1995) showed that conversion approached completion in 100 days at RH of 94%. With β -lactose, the hydration was slower than with the anhydrous α forms and occurred in two steps. The rate-determining step was mutarotation, which proceeded rapidly at 94% RH but more slowly at lower RH. The second step was hydration of the anhydrous α form to α -lactose monohydrate. When microcrystalline cellulose was mixed with the anhydrous lactose, the water was preferentially sorbed by the cellulose, and the lactose was protected. Cal et al. (1996) found that the roller-dried (high β) product held at 100% RH at ambient temperatures gained 5% in mass and lost β content as α -lactose monohydrate formed. Listiophadi et al. (2008) found that the spray-dried and roller-dried (high β) products formed cakes rated as “hard” in the texture analyzer after 90 days at 75% RH, but this did not happen to these products at lower humidities. While the anhydrous α forms sorbed considerable amounts of water—twice as much as the roller dried and four times that of the spray dried product at 75% RH—neither formed hard cakes. These results strongly suggest that the water release associated with the crystallization of amorphous

lactose is integral to caking. With α anhydrous samples, the water is sorbed but never released, as it is taken into the crystal lattice to form the monohydrate. It is the released water that increases molecular mobility and allows the crystallization front to propagate through the sample.

Paterson and Bronlund (2009) suggested that commercial crystalline α -lactose monohydrate (devoid of amorphous lactose) subjected to temperature fluctuations during packing or distribution can undergo capillary condensation, leading to caking. They recommended packing at a_w of 0.3 or below to avoid capillary condensation. Packing at a lower a_w (below 0.2) would be necessary if the product was contaminated by amorphous lactose— as is the case for most commercial lactose monohydrate products (see previous page).

2.4.3 Effect of moisture and amorphous lactose content in lactose-rich dairy powders

Commercially available spray dried lactose-rich powders, such as whey powder, whey permeate powder, and pharmaceutical-grade spray-dried lactose usually contain 10–15% amorphous lactose. The manufacture of these products generally include a “precrystallization” step prior to spray drying. This ensures that a high proportion (typically 75% or higher) of the lactose in the spray-dried product is in the nonhygroscopic α -lactose monohydrate form (Vromans et al., 1987; Darcy and Buckton, 1998). In other spray-dried dairy powders, such as whole milk, skim milk powders (SMP), and infant formula, the lactose is largely amorphous (Roetman, 1979). Instantized SMP has improved wetting and dispersability. This is also achieved by partial crystallization and agglomeration in the drier. About half (48%) of the lactose in the final powder will be crystallized, leaving the other half in the unchanged amorphous state (Roetman, 1979). Interestingly, while some commercial permeate powders (containing 85% lactose) are available with most of the lactose in the amorphous form, equipment manufacturers are advocating the use of continuous crystallizer/driers to produce nonhygroscopic forms with elevated proportions of lactose crystallinity (Andersen, 2005; Pisecky, 2005). Lower degrees of crystallinity can be tolerated in temperate climates, but not in tropical climates, as the amorphous lactose has a significant effect on the moisture sorption and caking characteristics of the α -lactose monohydrate present in the powder (Listiophadi et al., 2005b).

In milk powders, scanning electron microscopy shows that amorphous lactose is the continuous phase that encases the protein and fat. Crystallization of this amorphous lactose cracks the smooth surfaces of the particles, decreasing the solubility of the protein, and, in whole milk powder, increasing the proportion of fat that is exposed on the surface (free fat). The free fat (including fat soluble vitamins contained therein) is susceptible to autooxidative flavor defects (Warburton and Pixton, 1978; Roetman, 1979; Caric and Kalab, 1987). In a thorough study, Roetman (1979) prepared a number of spray dried milk and whey powders, with varying levels of crystalline lactose and examined them by SEM and polarimetric β/α analysis. He found that while it was possible for amorphous lactose to cover the lactose crystals, thus obscuring them from view by SEM, they could still be seen under polarized light. In whey with 33% of the lactose precrystallized, tomahawks of α -lactose monohydrate could be seen. In contrast, samples in which the lactose underwent moisture-induced crystallization contained fine needle-shaped crystals. Spray-dried skim milk had the expected smooth surfaced particles but, when the skim milk was seeded before drying, the resulting powder contained tiny lactose crystals enclosed in the powder particles. These crystals were only revealed when the powder was examined under polarized light. In fact,

27% of the lactose in this product was in crystalline form. Lai and Schmidt (1990) examined the sorption of SMP at 20°C and 12 different a_w values between 0.01 and 0.94. Sorption was followed by weight gain, SEM, and NMR. They concluded that crystallization of the lactose was evident at a_w of 0.54 and above. At this a_w , the sorption peak occurred at 2 days and corresponded to a sharp rise in mobility of water molecules as measured by NMR transverse relaxation rate. The mobility of the water molecules decreased after the sorption peak as the lactose crystallized. Small lactose crystals were evident on the surface of the milk particles above a_w 0.54. Particles were not examined by polarizing light microscopy so the possible presence of crystals buried in the interior of the particles could not be ruled out. The sorption peak appeared earlier in samples exposed at higher values of a_w . This is one of the few studies to measure the mobility of water molecules and to find that mobility increased just prior to crystallization and declined after it. Other authors have examined spray-dried SMP and amorphous lactose by SEM, most recently Haque and Roos (2006). Vuataz (2002) examined spray-dried skim and whole milk powders (WMP)—most of them commercial products. He examined the increase in RH in the headspace of a sealed sample of WMP held at 65°C and the sorption of SMP for 8 hours and 75% RH at 25°C. The changes in RH and mass, respectively, were correlated with NIR peaks for water and lactose and T_g determined by DSC. The sorption isotherm, the variation of T_g with total solids and water activity, and the state diagram for lactose in milk powders determined by Vuataz were very similar to those described by Roos (2002) for pure lactose, indicating the lack of influence (other than delay in sorption) of nonlactose milk solids.

2.5 LACTOSE APPLICATIONS

Lactose has many applications, from feed for fermentations, building block for plastics, chemicals, and drugs, to an ingredient in foods and high value excipient (filler) in pharmaceuticals. Traditional major food outlets include infant formula, milk chocolate, and other confectionery, baked goods, as a carrier base for dry food ingredient mixes (soup and sauces), in salad dressing, brine, meat, and sausages, and as a filler in artificial sweeteners (Lifran et al., 2000).

Throughout the first half of the twentieth century, the milk in milk chocolate came from whole milk powder or chocolate crumb, both roller dried. Compared with spray-dried milk, the roller-dried powder had a lower bulk density and higher degrees of whey protein denaturation, nonenzymic browning, lactose crystallinity, and free fat (Augustin, 2001). The roller process ended with a milling step to break up the sheets as they were removed from the roller. While the spray-dried particles were smooth with vacuoles containing occluded air, the roller-dried particles were rough and more porous (Caric and Kalab, 1987). It had long been recognized (King, 1965) that crystallization of lactose cracked the surface of dried particles and allowed occluded fat to reach the surface of the dried particle. Roller-dried whole milk powder (WMP) was well suited to the manufacture of milk chocolate. Chocolate crumb was made by drying (often by roller drying) a mixture of whole milk and sucrose (similar to sweetened condensed milk) with cacao solids and milling the dried material. It had a longer storage life than WMP because the polyphenols in cacao protected the dairy fat from oxidative rancidity, and it had a high level of free fat, which was desirable for chocolate manufacture. In the second half of last century, spray driers replaced roller driers, but the attributes of spray dried milk for chocolate making have not matched those of roller-dried products. There is a considerable body of research directed to

perfecting spray-dried milks that would be well suited to the manufacture of chocolate (Haylock and Dodds, 2009). Lactose can constitute around 10% of the mass of milk chocolate. Since the moisture of chocolate must be kept below 1% to maintain microbial safety and quality, it is desirable that the sugars (sucrose and lactose) added to the conche should be in the crystalline form rather than the sponge-like amorphous form. One of the functions of conching is to remove moisture by crystallizing amorphous sugars at elevated temperatures (60–70°C), and it is well known that the water thus released can cause agglomeration (“grit” and “clusters”) in the chocolate mass if too much amorphous lactose is allowed to enter the conche (Beckett, 2009). Twin screw extrusion of milk-based materials delivers drier chocolate ingredients with high levels of free fat and high proportions of crystallinity in the lactose fraction (i.e., lactose with a low amorphous content). Koc et al. (2003) increased the proportion of free fat in whole milk powder from 10% to within the range 82–96% by twin screw extrusion at temperatures between 54 and 71°C and found the lactose crystallized predominantly in the β form at 71°C. Francke and Heinzelmann (2008) subjected WMP and an SMP/anhydrous milk fat (AMF) mixture to twin screw extrusion at 75°C. The amorphous lactose peak on a moisture sorption curve was obliterated by the extrusion, indicating that the lactose was crystallized. Extrusion produced favorable outcomes for particle density and free fat, and the extruded materials made acceptable chocolate with lower viscosity and yield value. Several groups have established the value of twin screw extrusion for drying and interconversion of lactose forms (Asano et al., 1978; van Leverink, 1981; Listiohadi, 2000), but not with a particular focus on use in chocolate. A better understanding of the interactions between water, amorphous, and crystalline lactose forms present in milk chocolate can directly lead to better control of the texture and appearance of lactose-containing chocolate products upon storage.

In addition, since the turn of this century, lactose has been widely adopted for the standardization of retail and powdered milk (Rattray and Jelen, 1996). A recent economic analysis on various scenarios for utilization of whey and related products showed that large-scale processing, such as plants capable of taking 2–3 million liters of milk per day for cheesemaking, was necessary to gain economic advantage (Peters, 2005). Even so, Peters reasoned that the gains from WPC and WPI products were modest and subject to competitive pressures. He concluded that more attention should be paid to marketing the lactose streams, including lactose derivatives, such as GOS, for their “unique biological and functional properties.” Commercial GOS products have been reviewed recently by Playne and Crittenden (2009). The market for GOS is related to their ability to deliver, albeit to a limited extent, the diverse physiological functions of human milk oligosaccharides (e.g., Kunz et al., 2000). According to market research analyses (Affertsholt-Allen, 2007), two major trends have emerged in lactose utilization: sales in inhalation-grade lactose will be boosted by the demand for new inhalation products, and lactose derivatives, such as lactulose, GOS, lactosucrose, and sialyllactose, will grow, provided new sustainable and profitable processes are developed. Other lactose derivatives include lactitol, lactobionic acid, tagatose, alcohol, and lactic acid (both obtained by fermentation). *In situ* generation of GOS and lactobionic acid provides opportunities for product innovation. GOS are formed during yoghurt fermentation (e.g., Toba et al., 1983; Mahoney, 1998). In 1965, Rand demonstrated that milk could be “set” by the formation of either gluconic acid or lactobionic acid through the action of glucose oxidase or hexose oxidase, respectively (Rand, 1965, 1972; Rand and Hourigan, 1975). Lactobionic acid’s sweet taste distinguishes it from other food acids. *In situ* generation of lactobionic acid from lactose by hexose oxidase could have application in yoghurt and cheese. Hexose oxidase has been the subject

of some patent activity (e.g., Lynglev, 2006). Lactic acid produced from lactose can be polymerized into polylactic acid, as a rigid, brittle, and transparent material suited for rigid packaging (Pennings, 2006). More information on lactose derivatives can be found in Gänzle et al. (2008) and Lifran et al. (2009). As the demand for lactose-reduced/lactose-free products is increasing, research into the enzymic hydrolysis of lactose is still active to develop cost-efficient processes (Pivarnik et al., 1995; Temiz et al., 2003; Thomet and Rehberger, 2004). Cost and difficulty of storage both limit the production and use of hydrolyzed lactose syrup. Finally, the highest value pharmaceutical-grade lactose, with the most exacting physical specification, is inhalation or dry powder inhaler (DPI) lactose, a carrier for drugs used for the treatment of asthma and chronic obstructive pulmonary disease, administered locally to the lungs and airways by inhalation. Annual production is only of the order of 500 t under preferred vendor agreements with global pharmaceutical manufacturers. The DPI lactose price is an order of magnitude greater than normal pharmaceutical grade. Research and development in this area is growing rapidly, and delivering exciting advances in the scientific knowledge of lactose (Kaerger et al., 2006; Chow et al., 2007).

2.6 SUMMARY

Increasing the size of the markets for lactose will remain a challenge. There is not one single solution to the whey permeate utilization problem. However, rather than focus only on proteins and lactose-free products, dairy manufacturers would benefit from developing new marketing strategies and new technologies based on sound science for lactose and its derivatives. Strong legislative constraints and environmental pressures are already discouraging the simple discharge of excess whey and milk permeates. Future strategies based on the scientific advances described in this review should develop further the potential of lactose as both an important ingredient for the formulation of food and pharmaceutical products and as raw material for high volume markets in nonfood applications.

REFERENCES

- Acton, G.H. (1977) The determination of lactose in cheese. *Australian Journal of Dairy Technology* **32**, 111–112.
- Adhikari, B., Howes, T., Bhandari, B.R., and Truong, V. (2001) Stickiness in foods: a review of mechanisms and test methods. *International Journal of Food Properties* **4**(1), 1–33.
- Affertsholt-Allen, T. (2007) Market developments and industry challenges for lactose and lactose derivatives. *IDF Lactose Symposium*, May 14–16, Moscow, Russia.
- Aguilera, J.M., Del Valle, J.M., and Karel, M. (1995) Caking phenomena in amorphous food powders. *Trends in Food Science and Technology* **6**, 149–155.
- Ahlneck, C. and Zografi, G. (1990) The molecular basis of moisture effects on the physical and chemical stability of drugs in the solid state. *International Journal of Pharmaceutics* **62**, 87–95.
- Andersen, G. (2005) The Anhydro Paraflash process for production of whey and permeate powder. *Innovations in Food Technology* **27**, 20 and 22–23.
- Anderson, B.A. and Berlin, E. (1974) Moisture analysis and estimation of crystalline α -lactose in whey powders. *Journal of Dairy Science* **57**(7), 786–792.
- Angberg, M. (1995) Lactose and thermal analysis with special emphasis on microcalorimetry. *Thermochimica Acta* **248**, 161–176.
- Angberg, M., Nystrom, C., and Castensson, S. (1991) Evaluation of heat-conduction microcalorimetry in pharmaceutical stability studies. III. Crystallographic changes due to water vapour uptake in anhydrous lactose powder. *International Journal of Pharmaceutics* **73**, 209–220.

- Arellano, M.P., Aguilera, J.M., and Bouchon, P. (2004) Development of a digital video-microscopy technique to study lactose crystallisation kinetics in situ. *Carbohydrate Research* **339**, 2721–2730.
- Asano, Y., Aoki, Y., and Yamazaki, N. (1978) Method of producing β -lactose. U.S. Patent, No. 4,083,733.
- Augustin, M.A. (2001) Dairy ingredients in chocolate—chemistry and ingredient interactions. *Food Australia* **53**(9), 389–391.
- Beckett, S.T. (2009) Conching. In: *Industrial Chocolate Manufacture and Use*, 4th ed. edited by S.T. Beckett, pp. 192–223. Chichester, UK: Wiley-Blackwell.
- Bell, R.W. (1930) Some methods of preparing quickly soluble lactose. *Industrial and Engineering Chemistry* **22**(1), 51–54.
- Berggren, J. and Alderborn, G. (2004) Long-term stabilisation potential of poly(vinylpyrrolidone) for amorphous lactose in spray-dried composites. *European Journal of Pharmaceutical Sciences* **21**, 209–215.
- Bhandari, B. and Burel, B. (2007) Prediction of lactose crystals present in supersaturated lactose and whey solutions by measuring the water activity. *International Journal of Food Properties* **10**, 163–171.
- Bhargava, A. and Jelen, P. (1996) Lactose solubility and crystal growth as affected by mineral impurities. *Journal of Food Science* **61**, 180–184.
- Briggnier, L.-E., Buckton, G., Bystrom, K., and Darcy, P. (1994) The use of isothermal microcalorimetry in the study of changes in crystallinity induced during the processing of powders. *International Journal of Pharmaceutics* **105**, 125–135.
- British Pharmacopeia (2001) *Volume 1: Medicinal and Pharmaceutical Substances*, The Stationery Office, London.
- Bronlund, J. and Paterson, T. (2004) Moisture sorption isotherms for crystalline, amorphous and predominantly crystalline lactose powders. *International Dairy Journal* **14**, 247–254.
- Bronlund, J.E. and Paterson, A.H.J. (1997) The role of amorphous sugar in the caking of freshly milled food powders. In: *Proceedings of CHEMECA, 25th Australian and New Zealand Chemical Engineering Conference*, Rotorua, New Zealand.
- Buckton, G. and Darcy, P. (1999) Assessment of disorder in crystalline powders—a review of analytical techniques and their application. *International Journal of Pharmaceutics* **179**, 141–158.
- Buera, P., Schebor, C., and Elizalde, B. (2005) Effects of carbohydrate crystallisation on stability of dehydrated foods and ingredient formulations. *Journal of Food Engineering* **67**, 157–165.
- Buma, T.J. and van der Veen, H.K.C. (1974) Accurate specific optical rotations of lactose and their dependence on temperature. *Netherlands Milk and Dairy Journal* **34**, 129–132.
- Buma, T.J. and Wiegers, G.A. (1967) X-ray powder patterns of lactose and unit cell dimensions of β -lactose. *Netherlands Milk and Dairy Journal* **21**, 208–213.
- Bund, R.K. and Pandit, A.B. (2007) Sonocrystallization: effect on lactose recovery and crystal habit. *Ultrasonics Sonochemistry* **14**, 143–152.
- Burin, L., Jouppila, K., Roos, Y., Kansikas, J., and Buera, M.P. (2004) Retention of β -galactosidase activity as related to Maillard reaction, lactose crystallisation, collapse and glass transition in low moisture whey systems. *International Dairy Journal* **14**, 517–525.
- Burnett, D.J., Thielmann, F., and Booth, J. (2004) Determining the critical relative humidity for moisture-induced phase transitions. *International Journal of Pharmaceutics* **287**, 123–133.
- Burnett, D.J., Thielmann, F., Sokoloski, T., and Brum, J. (2006) Investigating the moisture-induced crystallisation kinetics of spray-dried lactose. *International Journal of Pharmaceutics* **313**, 23–28.
- Bushill, J.H., Wright, W.B., Fuller, C.H.F., and Bell, A.V. (1965) The crystallisation of lactose with particular reference to its occurrence in milk powder. *Journal of the Science of Food and Agriculture* **16**, 622–628.
- Butler, B. (1998) *Modelling Industrial Lactose Crystallisation*. PhD Thesis, Department of Chemical Engineering of the University of Queensland, Brisbane.
- Butler, B.K., Zhang, H., Johns, M.R., Mackintosh, D.L., and White, E.T. (1997) The influence of growth rate dispersion in crystallisation. In: *Proceedings of CHEMECA 97, 25th Australian and New Zealand Chemical Engineer's Conference and Exhibition*, September 29–October 1, Rotorua, New Zealand.
- Cal, S., Iglesias, G., Souto, C., Concheiro, A., Gomez-Amoza, J.L., and Martinez-Pacheco, R. (1996) Effects of hydration on the properties of a roller-dried β -lactose for direct compression. *International Journal of Pharmaceutics* **129**, 253–261.
- Caric, M. and Kalab, M. (1987) Effects of drying techniques on milk powders' quality and microstructure: a review. *Food Microstructure* **6**, 171–180.
- Chow, A.H.L., Tong, H.H.Y., and Chattopadhyay, P. (2007) Particle engineering for pulmonary drug delivery. *Pharmaceutical Research* **24**(3), 411–437.

- Clydesdale, G., Roberts, K.J., Telfer, G.B., and Grant, D.J.W. (1997) Modeling the crystal morphology of α -lactose monohydrate. *Journal of Pharmaceutical Sciences* **86**(1), 135–141.
- Codex Alimentarius (2001) *Codex Standard for Sugars*, Codex STAN 212-1999 (Amd. 1-2001), Joint FAO/WHO Food Standards Programme, pp. 1–5.
- Cuertas, B., Alcaina, M.I., and Soriano, E. (2004) Separation of mineral salts and lactose solutions through nanofiltration membranes. *Food Science and Technology International* **10**(4), 255–262.
- Darcy, P. and Buckton, G. (1998) Crystallisation of bulk samples of partially amorphous spray-dried lactose. *Pharmaceutical Development and Technology* **3**(4), 503–507.
- Das, S., Larson, I., Young, P., and Stewart, P. (2010) Understanding lactose behaviour during storage by monitoring surface energy change using inverse gas chromatography. *Dairy Science and Technology* **90**(2–3), 271–285. doi, 10.1051/dst/2009051.
- Dincer, T.D., Parkinson, G.M., Rohl, A.L., and Ogden, M.I. (1999) Crystallisation of α -lactose monohydrate from dimethyl sulfoxide (DMSO) solutions: influence of β -lactose. *Journal of Crystal Growth* **205**, 368–374.
- Dombrowski, R.D., Litster, J.D., Wagner, W.J., and He, Y. (2007) Crystallisation of α -lactose monohydrate in a drop-based microfluidic crystalliser. *Chemical Engineering Science* **62**, 4802–4810.
- Domovs, K.B. and Freund, E.H. (1961) Methanol-soluble complexes of lactose and of other carbohydrates. *Journal of Dairy Science* **43**, 1216–1223.
- Drapier-Beche, N., Fanni, J., Parmentier, M., and Vilasi, M. (1997) Evaluation of lactose crystalline forms by nondestructive analysis. *Journal of Dairy Science* **80**(3), 457–463.
- Durham, R.J. (2009) Modern approaches to lactose production. In: *Dairy Derived Ingredients*, edited by M. Corredig, Chapter 5, Cambridge: Woodhead. Woodhead Food Series No. 175.
- Durham, R.J. and Hourigan, J.A. (2007) Waste management and co-product recovery in dairy processing. In: *Handbook of Waste Management and Co-Product Recovery in Food Processing*, vol. 1, edited by K. Waldron, pp. 332–387. Cambridge: Woodhead.
- Durham, R.J., Hourigan, J.A., Sleight, R.W., and Johnson, R.L. (1997a) Process for the purification of nutrients from food process streams. Patent No. WO99/04903, U.S. Patent No. 6,475,390, European Patent No. 1023121.
- Durham, R.J., Hourigan, J.A., Sleight, R.W., and Johnson, R.L. (1997b) Whey fractionation: wheying up the consequences. *Food Australia* **49**(10), 460–465.
- Earl, W.L. and Parrish, F.W. (1983) A cross-polarization-magic-angle sample spinning NMR study of several crystal forms of lactose. *Carbohydrate Research* **115**, 23–32.
- Farber, L., Tardos, G.I., and Michaels, J.N. (2003) Evolution and structure of drying material bridges of pharmaceutical excipients: studies on a microscopic slide. *Chemical Engineering Science* **58**, 4515–4525.
- Figura, L.O. and Epple, M. (1995) Anhydrous α -lactose: a study with DSC and TXRD. *Journal of Thermal Analysis* **44**, 45–53.
- Food Chemicals Codex (1996) *FCC IV / Monograph Specifications*, Committee on Food Chemicals Codex: Food and Nutrition Board Institute of Medicine National Academy of Sciences, 4th ed. Washington, DC.: National Academy Press.
- Foremost Foods Co. (1970) *Lactose: chemical applications, Lactose pharmaceutical applications, Trade Literature*, Foremost Foods, 111 Pine St., San Francisco, CA 94111, USA.
- Francke, K. and Heinzelmann, K. (2008) Structure improvement of milk powder for chocolate processing. *International Dairy Journal* **18**, 928–931.
- Gänzle, M.G., Haase, G., and Jelen, P. (2008) Lactose: crystallisation, hydrolysis and value-added derivatives. *International Dairy Journal* **18**, 685–694.
- Garnier, S., Petit, S., and Coquerel, G. (2002) Dehydration mechanism and crystallisation behaviour of lactose. *Journal of Thermal Analysis and Calorimetry* **68**, 489–502.
- GEA (2008) *De-calcification of UF permeate*, GEA Process Engineering Inc. http://www.geafiltration.com/filtration_library/decalcification_UF_permeate.pdf. Accessed April 11, 2008.
- Gernigon, G., Piot, M., Beaucher, E., Jeantet, R., and Schuck, P. (2009) Physicochemical characterisation of Mozzarella cheese wheys and stretchwaters in comparison with several other sweet wheys. *Journal of Dairy Science* **92**(11), 5371–5377.
- Gernigon, G., Schuck, P., and Jeantet, R. (2010) Processing of Mozzarella cheese wheys and stretchwaters: a preliminary view. *Dairy Science and Technology* **90**(1), 27–46.
- Gillis, J. (1920) *Recueil des Travaux Chimiques des Pays-Bas et de la Belgique* **39**, 88, as cited by Roetman and Buma (1974).

- Grases, F. and March, J.G. (1991) Adsorption processes during crystalline growth: an analytical tool. *Trends in Analytical Chemistry* **10**(6), 190–195.
- Grases, F., Garcia-Ferragut, L., Costa-Bauza, A., Prieto, R., and March, J.G. (1994) Determination of escin based on its inhibitory action on lactose crystallisation. *Analytica Chimica Acta* **288**, 265–269.
- Greenberg, N.A. and Mahoney, R.R. (1983) Formation of oligosaccharides by β -galactosidase from *Streptococcus thermophilus*. *Food Chemistry* **10**, 195–204.
- Guu, M.Y.K. and Zall, R.R. (1992) Nanofiltration concentration: effect on the efficacy of lactose crystallisation. *Journal of Food Science* **57**(3), 735–739.
- Haase, G. and Nickerson, T.A. (1966a) Kinetic reactions of alpha and beta lactose. I: mutarotation. *Journal of Dairy Science* **49**, 127–132.
- Haase, G. and Nickerson, T.A. (1966b) Kinetic reactions of alpha and beta lactose. II: crystallisation. *Journal of Dairy Science* **49**, 757–761.
- Haque, M.D. and Roos, Y.H. (2006) Differences in the physical state and thermal behaviour of spray-dried and freeze-dried lactose and lactose/protein mixtures. *Innovative Food Science and Emerging Technologies* **7**, 62–73.
- Haque, M.K. and Roos, Y.H. (2005) Crystallisation and X-ray diffraction of spray-dried and freeze-dried amorphous lactose. *Carbohydrate Research* **340**, 293–301.
- Harju, M. (2001) Milk sugars and minerals as ingredients. *International Journal of Dairy Technology* **54**(2), 61–63.
- Harju, M. (2007) Chromatographic separation of lactose and its applications in the dairy industry. *IDF Symposium on Lactose and Its Derivatives*, Moscow, May 14–16, 2007.
- Harju, M.E. and Heikkilä, H.O. (1990) A process of recovering lactose from whey. U.S. Patent, No. 4,955,363.
- Hartel, R.W. (2001) *Crystallisation in Foods*, Chapter 6—Crystal Growth, pp. 192–229. Gaithersburg, MD: Aspen Publishers.
- Hartel, R.W. and Shastry, A.V. (1991) Sugar crystallisation in food products. *Critical Reviews in Food Science and Nutrition* **1**(1), 49–112.
- Haylock, S.J. and Dodds, T.M. (2009) Ingredients from Milk. In: *Industrial Chocolate Manufacture and Use*, 4th ed. edited by S.T. Beckett, pp. 76–100. Chichester, UK: Wiley-Blackwell.
- Herrington, B.L. (1934a) Some physico-chemical properties of lactose. I. The spontaneous crystallisation of supersaturated solutions of lactose. *Journal of Dairy Science* **17**, 501–518.
- Herrington, B.L. (1934b) Some physico-chemical properties of lactose. II. Factors influencing the crystalline habit of lactose. *Journal of Dairy Science* **17**, 533–542.
- Herrington, B.L. (1934c) Some physico-chemical properties of lactose. IV. The solubility of lactose in salt solutions, the isolation of a compound of lactose and calcium chloride. *Journal of Dairy Science* **17**, 805–814.
- Herrington, B.L. (1948) Lactose and the carbohydrates. In: *Milk and Milk processing*, Chapter 7, edited by B.L. Herrington, pp. 79–91. New York: McGraw-Hill.
- Hobman, P.G. (1984) Review of processes and products for utilization of lactose in deproteinated milk serum. *Journal of Dairy Science* **67**, 2630–2653.
- Hockett, R.C. and Hudson, C.S. (1931) A novel modification of lactose. *Journal of the American Chemical Society* **53**, 4455–4456.
- Holsinger, V.H. (1988) Lactose. In: *Fundamentals of Dairy Chemistry*, 3rd ed. edited by N.P. Wong, R. Jenness, M. Keeney, and E.H. Marth, pp. 279–342. New York: van Nostrand Reinhold.
- Holsinger, V.H. (1997) Physical and chemical properties of lactose. In: *Advanced Dairy Chemistry, Volume 3: Lactose, Water, Salts and Vitamins*, 2nd ed. edited by P.F. Fox, pp. 1–38. London: Chapman and Hall.
- Hourigan, J.A. (1984) Nutritional implications of lactose. *Australian Journal of Dairy Technology* **39**(3), 114–120.
- Hramtsov, A.G., Rokhmistrov, V.V., Evdokimov, I.A., Kostina, V.V., Abdulina, E.R., and Pavlov, V.A. (1990) Milk sugar making by membrane technology. In: *Brief Communication and Poster Abstract IDF, Vol. II, 23rd International Dairy Congress*, pp. 393, Montreal, Canada.
- Hudson, C.S. (1904) The hydration of milk sugar in solution. *Journal of the American Chemical Society* **26**(9), 1065–1082.
- Hudson, C.S. (1908) Further studies on the forms of milk sugar. *Journal of the American Chemical Society* **30**(11), 1767–1783.
- Hunziker, O.F. and Nissen, B.H. (1926) Lactose solubility and lactose crystal formation. I. Lactose solubility. *Journal of Dairy Science* **4**, 517–537.

- Hunziker, O.F. and Nissen, B.H. (1927) Lactose solubility and lactose crystal formation. II. Lactose crystal formation. *Journal of Dairy Science* **10**, 139–154.
- Ibach, A. and Kind, M. (2007) Crystallisation kinetics of amorphous lactose, whey-permeate and whey powders. *Carbohydrate Research* **342**, 1357–1365.
- International Dairy Federation (2001) *Dried milk, dried ice-mixes and processed cheese—determination of lactose content*. Part 1, ISO/FDIS 5765/IDF 79(1) (E) and Part 2, ISO/FDIS 5765/IDF 79(2) (E), Brussels.
- Isengard, H.-D. (2001) Water content, one of the most important properties of food. *Food Control* **12**, 395–400.
- Isengard, H.-D., Kling, R., and Reh, C.T. (2006) Proposal of a new reference method to determine the water content of dried dairy products. *Food Chemistry* **96**, 418–422.
- Islam, M.I.U. and Langrish, T.A.G. (2010) An investigation into lactose crystallisation under high temperature conditions during spray drying. *Food Research International (Ottawa, Ont.)* **43**, 46–56.
- Itoh, T., Katoh, M., and Adachi, S. (1978) An improved method for the preparation of crystalline β -lactose and observations on the melting point. *Journal of Dairy Research* **45**, 363–371.
- Jelen, P. and Coulter, S.T. (1973a) Effects of supersaturation and temperature on the growth of lactose crystals. *Journal of Food Science* **38**, 1182–1185.
- Jelen, P. and Coulter, S.T. (1973b) Effects of certain salts and other whey substances on the growth of lactose crystals. *Journal of Food Science* **38**, 1186–1189.
- Jouppila, K., Kansikas, J., and Roos, Y.H. (1997) Glass transition, water plasticization, and lactose crystallization in skim milk powder. *Journal of Dairy Science* **80**(12), 3152–3160.
- Jouppila, K., Kansikas, J., and Roos, Y.H. (1998) Crystallization and X-ray diffraction of crystals formed in water-plasticized amorphous lactose. *Biotechnological Progress* **14**, 347–350.
- Kaerger, J.S., Price, R., Young, P.M., Edge, S., and Tobyn, M.J. (2006) Carriers for DPIs: formulation and regulatory challenges. *Pharmaceutical Technology Europe* **18**(10), 25–30.
- Kapil, V., Dodeja, A.K., and Sarma, S.C. (1991) Lactose manufacture—a review. *Indian Food Packer* **45**, 52–62.
- Kedward, C.J., MacNaughtan, W. and Mitchell, J.R. (2000) Crystallization kinetics of amorphous lactose as a function of moisture content using isothermal differential scanning calorimetry. *Journal of Food Science* **65**(2), 324–328.
- King, N. (1965) The physical structure of dried milk. *Dairy Science Abstracts* **27**, 91–104.
- Kirk, J.H., Dann, S.E., and Blatchford, C.G. (2007) Lactose: a definitive guide to polymorph determination. *International Journal of Pharmaceutics* **334**, 103–114.
- Koc, A.B., Heinemann, P.H., and Ziegler, G.R. (2003) A process for increasing the free fat content of spray-dried whole milk powder. *Journal of Food Science* **68**(1), 210–216.
- Kunz, C., Rudloff, S., Baier, W., Klein, N., and Strobel, S. (2000) Oligosaccharides in human milk: structural, functional and metabolic aspects. *Annual Review of Nutrition* **20**, 699–722.
- Kussendrager, K.D. and Andreae, A.C. (1986) Process for preparing lactose products. U.S. Patent, No. 4,594,110.
- Lai, H.M. and Schmidt, S.J. (1990) Lactose crystallization in skim milk powder observed by hydrodynamic equilibria, scanning electron microscopy and ^2H nuclear magnetic resonance. *Journal of Food Science* **55**(4), 994–999.
- Langrish, T.A.G. (2008) Assessing the rate of solid-phase crystallisation for lactose: the effect of the difference between material and glass transition temperatures. *Food Research International (Ottawa, Ont.)* **41**, 630–636.
- Larhrib, H., Martin, G.P., Prime, D., and Marriott, C. (2003) Characterisation and deposition studies of engineered lactose crystals with potential for use as a carrier for aerolised salbutamol sulphate from dry powder inhalers. *European Journal of Pharmaceutical Sciences* **19**, 211–221.
- Lehto, V.-P., Tenho, M., Vaha-Heikkilä, K., Harjunen, P., Paalysaho, M., Valisaari, J., Niemela, P., and Jarvinen, K. (2006) The comparison of seven different methods to quantify the amorphous content of spray dried lactose. *Powder Technology* **167**, 85–93.
- Lerk, C.F. (1993) Consolidation and compaction of lactose. *Drug Development and Industrial Pharmacy* **19**, 2359–2398.
- Lerk, C.F., Andreae, A.C., De Boer, A.H., De Hoog, P., Kussendrager, K., and van Leverink, J. (1984) Transitions of lactoses by mechanical and thermal treatment. *Journal of Pharmaceutical Sciences* **73**(6), 857–859.
- Lifran, E.V., Hourigan, J.A., Sleigh, R.W., and Johnson, R.L. (2000) New wheys for lactose. *Food Australia* **53**(4), 120–126.

- Lifran, E.V., Sleigh, R.W., Hourigan, J.A., Johnson, R.L., Dalziel, S., and Steele, R.J. (2001) Method for purification of lactose. PCT Patent No. WO 02/50089 A1, NZ No. 526,975, U.S. No. 7,754,876.
- Lifran, E.V., Vu, T.T.L., Durham, R.J., Hourigan, J.A., and Sleigh, R.W. (2004) Crystallisation kinetics of lactose in the absence of lactose phosphate, paper no. 67, In: *Proceedings of CHEMECA, Sustainable Processes—32nd Australasian Chemical Engineering Conference*, September 26–29, Sydney, Australia.
- Lifran, E.V., Vu, T.T.L., Durham, R.J., Hourigan, J.A., and Sleigh, R.W. (2006) Lactose phosphate and its origin in cheese-making: a case study. *Australian Journal of Dairy Technology* **61**(2), 163–166.
- Lifran, E.V., Vu, T.T.L., Durham, R.J., Hourigan, J.A., and Sleigh, R.W. (2007) Crystallisation kinetics of lactose in the presence of lactose phosphate. *Powder Technology* **179**, 43–54.
- Lifran, E.V., Hourigan, J.A., and Sleigh, R.W. (2009) Lactose derivatives: turning waste into functional foods. *Australian Journal of Dairy Technology* **64**(1), 89–93.
- Lim, S.G. and Nickerson, T.A. (1973) Effect of methanol on the various forms of lactose. *Journal of Dairy Science* **56**(7), 843–848.
- Listiohadi, Y.D. (2000) *Modification of sugars by extrusion*. MSc (Honours) Thesis, University of Western Sydney.
- Listiohadi, Y.D., Hourigan, J.A., Sleigh, R.W., and Steele, R.J. (2005a) Properties of lactose and its caking behaviour. *Australian Journal of Dairy Technology* **60**, 33–52.
- Listiohadi, Y.D., Hourigan, J.A., Sleigh, R.W., and Steele, R.J. (2005b) Role of amorphous lactose in the caking of α -lactose monohydrate powders. *Australian Journal of Dairy Technology* **60**, 19–32.
- Listiohadi, Y.D., Hourigan, J.A., Sleigh, R.W., and Steele, R.J. (2005c) An exploration of the caking of lactose in whey and skim milk powders. *Australian Journal of Dairy Technology* **60**, 207–213.
- Listiohadi, Y.D., Hourigan, J.A., Sleigh, R.W., and Steele, R.J. (2005d) Effect of milling on the caking behaviour of lactose. *Australian Journal of Dairy Technology* **60**, 214–224.
- Listiohadi, Y.D., Hourigan, J.A., Sleigh, R.W., and Steele, R.J. (2008) Moisture sorption, compressibility and caking of lactose polymorphs. *International Journal of Pharmaceutics* **359**, 123–134.
- Listiohadi, Y.D., Hourigan, J.A., Sleigh, R.W., and Steele, R.J. (2009) Thermal analysis of amorphous lactose and α -lactose monohydrate. *Dairy Science and Technology* **89**, 43–67.
- Lloyd, R.J., Chen, X.D., and Hargreaves, J.B. (1996) Glass transition and caking of spray-dried lactose. *International Journal of Food Science and Technology* **31**, 305–311.
- Lynglev, G.B. (2006) Method for producing a fermented dairy product. U.S. Patent, No. 7,090,876.
- Machado, J.J.B., Coutinho, J.A., and Macedo, E.A. (2006) Solid-liquid equilibrium of α -lactose in ethanol/water. *Fluid Phase Equilibria* **173**, 121–134.
- Madj, F. and Nickerson, T.A. (1976) Effect of alcohols on lactose solubility. *Journal of Dairy Science* **59**, 1025–1032.
- Mahoney, R.R. (1998) Galactosyl-oligosaccharide formation during lactose hydrolysis: a review. *Food Chemistry* **63**(2), 147–154.
- McDonald, E.J. and Turcotte, A.L. (1948) Density and refractive indices of lactose solutions. *Journal of Research of the National Bureau of Standards* **41**, 63–68.
- McSweeney, P.L.H. and Fox, P.F. eds. (2009) *Advanced Dairy Chemistry, Volume 3: Lactose Water Salts and Minor Components*, 3rd ed. New York: Springer.
- Michaels, A.S. and Van Kreveland, A. (1966) Influences of additives on growth rates in lactose crystals. *Netherlands Milk and Dairy Journal* **20**, 163–181.
- Mimouni, A., Schuck, P., and Bouhallab, S. (2005) Kinetics of lactose crystallisation and crystal size as monitored by refractometry and laser light scattering: effect of proteins. *Le Lait* **85**, 253–260.
- Morita, M., Nakai, Y., Fukuoka, E., and Nakajima, S.-I. (1984) Physicochemical properties of crystalline lactose. II. Effect of crystallinity on mechanical and structural properties. *Chemical and Pharmaceutical Bulletin* **32**(10), 4076–4083.
- Morrissey, P.A. (1985) Lactose: chemical and physico-chemical properties. In: *Developments in Dairy Chemistry—3, Lactose and Minor Constituents*, edited by P.F. Fox, Chapter 1, New York: Elsevier Applied Science.
- Mullin, J.W. (2001) *Crystallisation*, 4th ed. Oxford: Butterworth-Heinemann.
- Mustapha, A., Hertzler, S.R., and Savaiano, D.A. (1997) Lactose: nutritional significance. In: *Advanced Dairy Chemistry, Volume 3: Lactose, Water, Salts and Vitamins*, 2nd ed. edited by P.F. Fox, London: Chapman and Hall.
- Newell, H.E., Buckton, G., Butler, D.A., Thielmann, F., and Williams, D.R. (2001a) The use of inverse phase gas chromatography to study the change of surface energy of amorphous lactose as a function of relative humidity and the processes of collapse and crystallisation. *International Journal of Pharmaceutics* **217**, 45–56.

- Newell, H.E., Buckton, G., Butler, D.A., Thielmann, F., and Williams, D.R. (2001b) The use of inverse phase gas chromatography to measure the surface energy of crystalline, amorphous, and recently milled lactose. *Pharmaceutical Research* **18**(5), 662–666.
- Nickerson, T.A. (1970) Lactose. In: *By-Products from Milk*, 2nd ed. edited by B.H. Webb and E.O. Whittier, Chapter 12, Westport, CT: AVI.
- Nickerson, T.A. (1974) Lactose: occurrence. In: *Fundamentals of Dairy Chemistry*, 2nd ed. edited by B.H. Webb, A.H. Johnson, and J.A. Alford, Chapter 6, pp. 273–319. Westport, CT: The AVI Publishing Company.
- Nickerson, T.A. and Moore, E.E. (1973) Factors influencing lactose crystallisation. *Journal of Dairy Science* **57**, 1315–1319.
- Nickerson, T.A. and Moore, E.E. (1974a) Alpha lactose and crystallisation rate. *Journal of Dairy Science* **57**, 160–164.
- Nickerson, T.A. and Moore, E.E. (1974b) Factors influencing lactose crystallisation. *Journal of Dairy Science* **57**, 1315–1319.
- Nieuwmeyer, F., Maarschalk, K., and Vromans, H. (2008) Lactose contaminant as steroid degradation enhancer. *Pharmaceutical Research* **25**(11), 2666–2673.
- Nijdam, J., Ibach, A., Eichorn, K., and Kind, M. (2007) An X-ray diffraction analysis of crystallised whey and whey permeate powders. *Carbohydrate Research* **342**, 2354–2364.
- Novakova, H., Copikova, J., Maixner, J., and Maryska, M. (2002) The production of clusters in milk chocolate. *International Journal of Food Science and Technology* **37**, 485–495.
- Olano, A. (1978) Treatment of forms of lactose with dilute alcoholic solutions of sodium hydroxide. *Journal of Dairy Science* **61**, 1622–1623.
- Olano, A., Bernhard, R.A., and Nickerson, T.A. (1977) Alteration in the ratio of α - to β -lactose co-crystallized from organic solvents. *Journal of Food Science* **42**(4), 1066–1068.
- Olano, A., Corzo, N., and Martinez-Castro, I. (1983) Studies on β -lactose crystallization. *Milchwissenschaft* **38**(8), 471–474.
- Olano, A., and Rios, J.J. (1978) Treatment of lactose with alkaline methanolic solutions: production of beta-lactose from alpha-lactose hydrate. *Journal of Dairy Science* **61**, 300–302.
- Otsuka, M., Ohtani, H., Kaneniwa, N., and Higuchi, S. (1991) Isomerization of lactose in solid-state by mechanical stress during grinding. *The Journal of Pharmacy and Pharmacology* **43**, 148–153.
- Otsuka, M., Ohtani, H., Otsuka, K., and Kaneniwa, N. (1993) Effect of humidity on solid-state isomerization of various kinds of lactose during grinding. *The Journal of Pharmacy and Pharmacology* **45**(1), 2–5.
- Paez, M., Martinez-Castro, I., Sanz, J., Olano, A., Garcia-Raso, A., and Saura-Calixto, F. (1987) Identification of the components of aldoses in a tautomeric equilibrium mixture as their trimethylsilyl ethers by capillary gas chromatography. *Chromatographia* **23**(1), 43–46.
- Parrish, F.W. and Brown, M.L. (1982) Solid state transformations of α -lactose monohydrate in alcoholic media. *Journal of Dairy Science* **65**, 1688–1691.
- Parrish, F.W., Ross, K.D., and Valentine, K.M. (1980) Formation of β -lactose from the stable forms of anhydrous α -lactose. *Journal of Food Science* **45**, 68–70.
- Paterson, A.H.J. (2009) Production and uses of lactose. In: *Advanced Dairy Chemistry, Volume 3: Lactose, Water, Salts and Minor Constituents*, 3rd ed. edited by P.L.H. McSweeney and P.F. Fox, pp. 105–120. New York: Springer.
- Paterson, A.H.J. and Bronlund, J.E. (2009) The practical implications of temperature induced moisture migration in bulk lactose. *Journal of Food Engineering* **91**(1), 85–90.
- Paterson, A.H.J., Brooks, G.F., Bronlund, J.E., and Foster, K.D. (2005) Development of stickiness in amorphous lactose at constant T-T_g levels. *International Dairy Journal* **15**, 513–519.
- Pennings (2006) *Hycail BV*. <http://www.hycail.com/pages/engels/hycailen.html>.
- Peter, P.N. (1928) Solubility relationships of lactose-sucrose solutions. I. Lactose-sucrose solubilities at low temperatures. *The Journal of Physical Chemistry* **32**(12), 1856–1864.
- Peters, R.H. (2005) Economic aspects of cheese making as influenced by whey processing options. *International Dairy Journal* **15**, 537–545.
- Pisecky, J. (1997) *Handbook of Milk Powder Manufacture*. Denmark: Niro A/S.
- Pisecky, J. (2005) Spray drying in the cheese industry. *International Dairy Journal* **15**, 531–536.
- Pivarnik, L.F., Senecal, A.G., and Rand, A.G. (1995) Hydrolytic and transgalactosylic activities of commercial β -galactosidase (lactase) in food processing. *Advances in Food and Nutrition Research* **38**, 1–102.

- Playne, M.J. and Crittenden, R.G. (2009) Galacto-oligosaccharides and other products derived from lactose. In: *Advanced Dairy Chemistry, Volume 3: Lactose, Water, Salts and Minor Constituents*, 3rd ed. edited by P.L.H. McSweeney and P.F. Fox, pp. 121–201. New York: Springer.
- Price, R. and Young, P.M. (2005) On the physical transformations of processed pharmaceutical solids. *Micron (Oxford, England)* **36**, 519–524.
- Purutyan, H., Pittenger, B.H., and Tardos, G.I. (2005) Preventing caking during solids handling. *Chemical Engineering Progress* **101**(5), 22–28.
- Raghavan, S.L., Ristic, R.I., Sheen, D.B., Sherwood, J.N., Trowbridge, L., and York, P. (2000) Morphology of crystals of α -lactose hydrate grown from aqueous solution. *Journal of Physical Chemistry B* **104**, 12256–12262.
- Rand, A.G. (1965) Enzymatic formation of acid in milk. *Journal of Dairy Science* **48**, 1556.
- Rand, A.G. (1972) Direct enzymatic conversion of lactose to acid: glucose oxidase and hexose oxidase. *Journal of Food Science* **37**(5), 698–701.
- Rand, A.G. and Hourigan, J.A. (1975) Direct enzymatic conversion of lactose in milk to acid. *Journal of Dairy Science* **58**, 1144–1150.
- Randolph, A.D. and Larson, M.A. (1988) *Theory of Particulate Processes, Analysis and Techniques of Continuous Crystallisation*, 2nd ed. San Diego: Academic Press.
- Rao, R.D., Wendorff, W.L., and Smith, K. (2004) Changes in galactose and lactic acid content of sweet whey during storage. *Journal of Food Protection* **67**(2), 403–406.
- Ratnay, W. and Jelen, P. (1996) Protein standardization of milk and dairy products. *Trends in Food science and Technology* **7**(7), 227–234.
- Reid, D.S. and Fennema, O.R. (2008) Water and ice. In: *Fennema's Food Chemistry*, 4th ed. edited by S. Damodaran, K.L. Parkin, and O.R. Fennema, pp. 18–82. Boca Raton, FL: CRC Press.
- Rice, G., Barber, A., O'Connor, A., Stevens, G., and Kentish, S. (2009) Fouling of NF membranes by dairy ultrafiltration permeates. *Journal of Membrane Science* **330**, 117–126.
- Roetman, K. (1979) Crystalline lactose and the structure of spray-dried milk products as observed by scanning electron microscopy. *Netherlands Milk and Dairy Journal* **33**, 1–11.
- Roetman, K. (1982) Methods for the quantitative determination of crystalline lactose in milk products. *Netherlands Milk and Dairy Journal* **36**(1), 1–52.
- Roetman, K. and Buma, T.J. (1974) Temperature dependence of the equilibrium β/α ratio of lactose in aqueous solution. *Netherlands Milk and Dairy Journal* **28**, 1555–1165.
- Roetman, K. and van Schaik, M. (1975) The β/α ratio of lactose in the amorphous state. *Netherlands Milk and Dairy Journal* **29**, 225–237.
- Roos, Y. and Karel, M. (1990) Differential scanning calorimetry study of phase transitions affecting the quality of dehydrated materials. *Biotechnology Progress* **6**, 159–163.
- Roos, Y. and Karel, M. (1992) Crystallization of amorphous lactose. *Journal of Food Science* **57**(3), 775–777.
- Roos, Y.H. (2002) Importance of glass transition and water activity to spray drying and stability of dairy powders. *Le Lait* **82**, 475–484.
- Rosamaninho, R. and Melo, L.F. (2006a) The effect of citrate on calcium phosphate deposition from simulated milk ultrafiltrate (SMUF) solution. *Journal of Food Engineering* **73**, 379–387.
- Rosamaninho, R. and Melo, L.F. (2006b) Calcium phosphate deposition from simulated milk ultrafiltrate on different stainless steel-based surfaces. *International Dairy Journal* **16**(1), 81–87.
- Rosamaninho, R. and Melo, L.F. (2008) Protein-calcium phosphate interactions in fouling of modified stainless steel-based surfaces by simulated milk. *International Dairy Journal* **18**(1), 72–80.
- Saillard, E. (1919) The solubility of lactose in water. *Chimie et Industrie* **2**, 1035.
- Savaiano, D.A., Boushey, C.J., and McCabe, G.P. (2006) Lactose intolerance symptoms assessed by meta-analysis: a grain of truth that leads to exaggeration. *The Journal of Nutrition* **136**(4), 1107–1113.
- Savolainen, M., Jouppila, K., Pajamo, O., Christiansen, L., Strachan, C., Karjalainen, M., and Rantanen, J. (2007) Determination of amorphous content in the pharmaceutical process environment. *The Journal of Pharmacy and Pharmacology* **59**, 161–170.
- Schaafsma, G. (2008) Lactose and lactose derivatives as bioactive ingredients in human nutrition. *International Dairy Journal* **18**, 458–465.
- Schmidt, D.G. and Both, P. (1987) Studies on the precipitation of calcium phosphate. I. Experiments in the pH range 5.3 to 6.8 at 25°C and 50°C in the absence of additives. *Netherlands Milk and Dairy Journal* **41**, 105–120.

- Schmitt, E.A., Law, D., and Zhang, G.G.Z. (1999) Nucleation and crystallisation kinetics of hydrated amorphous lactose above the glass transition temperature. *Journal of Pharmaceutical Sciences* **88**(3), 291–296.
- Sebhatu, T., Elamin, A.A., and Ahlneck, C. (1994) Effect of moisture sorption on tableting characteristics of spray dried (15% amorphous) lactose. *Pharmaceutical Research* **11**(9), 1233–1238.
- Sharp, P.F. (1934) Method of preparing lactose. U.S. Patent, No. 1,956,811.
- Sharp, P.F. (1943) Stable crystalline anhydrous alpha lactose product and process. U.S. Patent, No. 2,319,562.
- Shastri, A.V. and Hartel, R.W. (1996) Crystallisation during drying of thin sucrose films. *Journal of Food Engineering* **30**(1–2), 75–94.
- Shi, Y., Hartel, R.W., and Liang, B. (1989) Formation and growth phenomena of lactose nuclei under contact nucleation conditions. *Journal of Dairy Science* **72**, 2906–2915.
- Shi, Y., Liang, B., and Hartel, R.W. (1990) Crystallisation kinetics of alpha lactose monohydrate in a continuous cooling crystalliser. *Journal of Food Science* **55**(3), 817–820.
- Shi, Y., Liang, B., and Hartel, R.W. (2006) Crystal refining technologies by controlled crystallisation. U.S. Patent Application, No. 0128953A1.
- Simatos, D., Champion, D., Lorient, D., Loupiac, C., and Roudaut, G. (2009) Water in dairy products. In: *Advanced Dairy Chemistry, Volume 3: Lactose, Water, Salts and Minor Constituents*, 3rd ed. edited by P.L.H. McSweeney and P.F. Fox, pp. 457–526. New York: Springer.
- Singh, R.K. and Shah, B.B. (1992) Characterisation of lactose crystallised in acidified aqueous ethanol. *Journal of the Science of Food and Agriculture* **58**, 161–164.
- Smart, J.B. (1988) Effect of whey components on the rate of crystallisation and solubility of α -lactose monohydrate. *New Zealand Journal of Dairy Science and Technology* **23**(4), 275–289.
- Smart, J.B. (1991) Transferase reactions of the β -galactosidase from *Streptococcus thermophilus*. *Applied Microbiology and Biotechnology* **34**, 495–501.
- Smart, J.B. and Smith, J.M. (1991) Effect of selected compounds on the rate of α -lactose monohydrate crystallisation, crystal yield and quality. *International Dairy Journal* **1**, 41–53.
- Smith, J.H., Dann, S.E., Elsegood, M.R.J., Dale, S.H., and Blatchford, C.G. (2005) α -Lactose monohydrate: a redetermination at 150 K. *Acta Crystallographica. Section E, Structure Reports Online* **61**(8), o2499–o2501.
- Tan, R. (2009) Manufacture of sweetened condensed milk and the significance of lactose therein. In: *Advanced Dairy Chemistry, Volume 3: Lactose, Water, Salts and Minor Constituents*, 3rd ed. edited by P.L.H. McSweeney and P.F. Fox, pp. 36–57. New York: Springer.
- Temiz, A., Yilmaz, R., Saglam, N., and Ulger, C. (2003) High fructose syrup production from whey lactose using microbial β -galactosidase and glucose isomerase. *Milchwissenschaft* **58**(3,4), 121–124.
- Thomas, N.R., Shumway, L.S., and Hansen, L.D. (2009) Quantitative X-ray diffraction determination of α -lactose monohydrate and β -lactose in chocolate. *Journal of Food Science* **74**(7), C513–C518.
- Theobald, J. (2007) Purification processes for lactose. *IDF Lactose Symposium*, May 14–16, Moscow, Russia, Proceedings. International Dairy Federation, Brussels.
- Thomet, A. and Rehberger, B. (2004) Sugar syrup from dairies for the food industry. Agroscope Liebefeld-Posieux. *European Dairy Magazine* **16**(2), 30–33.
- Tjuradi, P. and Hartel, R.W. (1995) Corn syrup oligosaccharide effects on sucrose crystallisation. *Journal of Food Science* **60**(6), 1353–1356.
- Toba, T., Watanabe, A., and Adachi, S. (1983) Quantitative changes in sugars, especially oligosaccharides, during fermentation and storage of yoghurt. *Journal of Dairy Science* **66**(1), 17–20.
- Troy, H.C. and Sharp, P.F. (1930) Alpha and beta lactose in some milk products. *Journal of Dairy Science* **13**, 140–157.
- Twieg, W.C. and Nickerson, T.A. (1968) Kinetics of lactose crystallisation. *Journal of Dairy Science* **51**, 1720–1724.
- U.S. Pharmacopoeia (USP) (1995) *The National Formulary: USP 23, NF 18*. United States Pharmacopoeial Convention Inc., Washington, DC.
- Van Krevelde, A. (1969) Growth rates of lactose crystals in solutions of stable anhydrous alpha-lactose. *Netherlands Milk and Dairy Journal* **23**(4), 258.
- Van Krevelde, A. and Michaels, A.S. (1965) Measurements of crystal growth of α -lactose. *Journal of Dairy Science* **48**, 259–264.
- Van Leverink, J. (1981) Extrusion process for the preparation of anhydrous stable lactose. U.S. Patent, No. 4,280,997.

- Visser, R.A. (1980) A natural crystal growth retarder in lactose. *Netherlands Milk and Dairy Journal* **34**, 255–275.
- Visser, R.A. (1982a) Supersaturation of α -lactose in aqueous solutions in mutarotation equilibrium. *Netherlands Milk and Dairy Journal* **36**, 89–101.
- Visser, R.A. (1982b) Growth of non-ionic lactose at various temperatures and supersaturations. *Netherlands Milk and Dairy Journal* **36**, 167–193.
- Visser, R.A. (1984) Experiments for tracing growth retarders in lactose. *Netherlands Milk and Dairy Journal* **38**, 107–133.
- Visser, R.A. (1988) Crystal growth retarding of α -lactose hydrate by sugar phosphates, a continued study. *Netherlands Milk and Dairy Journal* **42**, 449–468.
- Visser, R.A. and Bennema, P. (1983) Interpretation of the morphology of α -lactose hydrate. *Netherlands Milk and Dairy Journal* **37**, 109–137.
- Vromans, H., Bolhuis, G.K., Lerk, C.F., Kussendrager, K.D., and Bosch, H. (1986) Studies on tableting properties of lactose: VI. Consolidation and compaction of spray dried amorphous lactose. *Acta Pharmaceutica Suecica* **23**, 231–240.
- Vromans, H., Bolhuis, G.K., Lerk, C.F., Van De Biggelaar, H., and Bosch, H. (1987) Studies on tableting properties of lactose. VII. The effect of variations in primary particle size and percentage of amorphous lactose in spray dried lactose products. *International Journal of Pharmaceutics* **35**, 29–37.
- Vu, T.T.L., Durham, R.J., Hourigan, J.A., and Sleight, R.W. (2004) The uses of ultrasonic power and solvents in lactose cooling crystallisation, Paper No. 86. In: *Proceedings of CHEMECA, Sustainable Processes—32nd Australasian Chemical Engineering Conference*, September 26–29, Sydney, Australia.
- Vu, T.T.L., Durham, R.J., Hourigan, J.A., and Sleight, R.W. (2006a) Dynamic modelling optimisation and control of lactose crystallisations: comparison of process alternatives. *Separation and Purification Technology* **48**, 159–166.
- Vu, T.T.L., Durham, R.J., Hourigan, J.A., and Sleight, R.W. (2006b) Temperature control strategies for batch crystallisation: dynamic optimisation and experimental approaches, Paper No. 254. In: *Proceedings of CHEMECA, Knowledge and Innovation—34th Australasian Chemical Engineering Conference*, September 18–21, Auckland, New Zealand.
- Vu, T.T.L., Huynh, L., and Hourigan, J.A. (2009) Effects of solvents on characteristics of crystalline lactose extracted in ternary and quaternary systems. *Advanced Powder Technology* **20**, 251–256.
- Vuataz, G. (2002) The phase diagram of milk: a new tool for optimising the drying process. *Le Lait* **82**, 485–500.
- Walstra, P. and Jenness, R. (1984) *Dairy Chemistry and Physics*, Chapter 3, pp. 27–36. New York: John Wiley and Sons.
- Walstra, P., Geurts, T.J., Noomen, A., Jellema, A., and Van Boekel, M.A.J.S. (1999) *Dairy Technology: Principles of Milk Properties and Processes*. New York: Marcel Dekker.
- Wang, H.C., Kurimoto, M., Kahr, B., and Chmielewski, J. (2001) α -Lactose monohydrate single crystals as hosts for matrix isolation of guest biopolymers. *Bioorganic and Medicinal Chemistry* **9**, 2279–2283.
- Warburton, S. and Pixton, S.W. (1978) The significance of moisture in dried milk. *Dairy Industries International* **43**, 23, and 26–27.
- Whittier, E.O. and Gould, S.P. (1931) Speed of crystallisation of lactose, galactose, glucose and sucrose from pure solution. *Industrial Engineering and Chemistry* **23**(6), 670–679.
- Wood, J.M. (1969) *Personal communication*, Kaponga, New Zealand, cited in Nickerson (1970).
- Wursch, P., Rosset, J., Kollreutter, B., and Klein, A. (1984) Crystallization of β -Lactose under elevated storage temperature in spray-dried milk powder. *Milchwissenschaft* **39**(10), 579–582.
- Young, P.M., Chiou, H., Tee, T., Traini, D., and Chan, H.-K. (2007) The use of organic vapour sorption to determine low levels of amorphous content in processed pharmaceutical powders. *Drug Development and Industrial Pharmacy* **33**, 91–97.
- Zagrodski, S. (1968) Influence of the amount of sugar crystals present in a solution on the crystallisation rate. *Zucker* **21**, 658.
- Zall, Y.K. (1992) Sources and composition of whey and permeate. In: *Whey and Lactose Processing*, edited by J.G. Zadow, Chapter 1, pp. 1–72. London: Elsevier Applied Sciences.
- Zeng, X.M., Martin, G.P., Marriott, C., and Pritchard, J. (2000a) The influence of crystallisation conditions on the morphology of lactose intended for use as a carrier for dry powder aerosols. *The Journal of Pharmacy and Pharmacology* **52**, 633–643.
- Zeng, X.M., Martin, G.P., Marriott, C., and Pritchard, J. (2000b) Crystallisation of lactose from Carbopol gels. *Pharmaceutical Research* **17**(7), 879–886.

3 Dairy Ingredients Containing Milk Fat Globule Membrane: Description, Composition, and Industrial Potential

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3.1 INTRODUCTION

Cell membranes are still considered a subject of study in many disciplines and are considered the holders of many secrets in biology. One membrane in particular, the membrane that covers every globule of fat, the milk fat globule membrane (MFGM), is avidly studied by many groups of scientists around the world, and is yielding very important new information. This chapter gives a current view on the relevant discoveries that place the MFGM in a unique position for food and dairy product applications. In fact, the manufacture of butter and cheese generate by-products that have not found their place in the market, or are underutilized commercially, whereas they have high nutritional and functional potential. In this category are buttermilk and butter serum, with unprecedented nutritional and functional properties that could be exploited when incorporated in novel food products. The presence of the MFGM in these food or dairy products promise to deliver more than nutritional benefits by including nutraceuticals for health and well-being; however, there is still a lot to know, test, prove, and develop about this component of milk.

With consumers becoming more health conscious, the demand for food products that deliver nutritional and functional benefits has increased. In the dairy industry, the interest in the MFGM is no exception. This milk component was initially described in the 1970s and 1980s as the membrane that surrounds fat globules in milk, preventing coalescence and rancidity of lipids (Keenan et al., 1971; Mather and Keenan, 1975, 1998; Jarasch et al., 1977; Patton, 2004; Singh, 2006; Dewettinck et al., 2008). In the last two decades, the nutraceutical properties of the MFGM have been explored (Spitsberg, 2005; Argov et al., 2008a). In fact, anticancer and antihypercholesterolemic activities, antimicrobial and antiviral properties, such as inhibition of the ulcer-forming bacterium *Helicobacter pylori* and rotavirus, and suppression of diseases, such as multiple sclerosis, have been ascribed to the MFGM. In addition, in clinical studies, complementing infant food with MFGM and micronutrients leads to more copper and vitamin B12 uptake by infants (Lonnerdal et al., 2006). Also, MFGM complementation has reduced the incidence and prevalence of diarrhea in infants (Zavaleta et al., 2006). One contemporary example of the research and innovation in MFGM has been in the pharmaceutical field, where liposome formation for

the delivery of drugs, vitamins, and nutrients is being designed and tested (Thompson and Singh, 2006; Bezelgues et al., 2009).

Buttermilk originates during cream churning, where the fat globules are destabilized with subsequent agglomeration and coalescence, displacing the MFGM and water-soluble components in cream into the aqueous phase. This by-product, known as sweet buttermilk, is the main commercial source of MFGM, which has a low cost but currently very limited market (Jiménez-Flores and Brisson, 2008; Jiménez-Flores and Higuera-Ciapara, 2009; Lopez et al., 2010). A second source is butter serum, which is popular in countries where butter is made from very high fat content cream (plastic cream), and there is very little traditional buttermilk production. Currently, in the U.S. industry, sweet buttermilk is mainly used in food products, such as ice cream and bakery, with very limited use as surface-active ingredient (Jinjarak et al., 2006; Sodini et al., 2006; Dewettinck et al., 2008). In fact, in 2007, the use of buttermilk in the United States was mainly for baking to improve flavor and texture, representing 79.9% of the 49.8 million pounds of dry buttermilk sold. The dairy industry also utilized buttermilk (13.9%) in products, such as ice cream, recombined milk, and cheese, and about 3.4% was used for prepared dry mixes (American Dairy Products Institute, 2007). In 2008, 1644 billion pounds of butter were produced in the United States, generating approximately the same amount of buttermilk (U.S. Department of Agriculture) (Morin et al., 2007a,b). We could be using this product to complement food and obtain the health benefits ascribed to MFGM constituents, such as proteins and phospholipids (PL), which are present in concentrations of 32.9 and 2.03 g per 100 g of dry buttermilk, respectively (Jiménez-Flores and Brisson, 2008).

The genesis of PL characterization in milk started in the 1960s (Sprecher et al., 1965; Webb et al., 1974). More recently, with the use of novel technologies, their physicochemical properties have been exploited, highlighting the emulsification and antifoaming properties of MFGM. In addition, these lipids have been associated with enhanced health, especially those phospholipids present in specific animal tissues, such as sphingomyelin and phosphatidylcholine, cerebroside, and gangliosides (considered important glycolipids). In fact, PL and their metabolites have important biological roles, such as cell and membrane communication, myelination in the central nervous system, and as membrane receptors. Sources with high concentrations of PL, such as the MFGM, should be considered of high value by food technologists, and efforts need to be made to include them in food products that not only to deliver essential nutrients but functional compounds. Besides sweet buttermilk, other sources, such as sour and whey buttermilk, have been characterized as seen in Table 3.1. Among them, butter serum offers the highest concentration of phospholipids on a dry basis and has promise to become a potential source of MFGM (Rombaut et al., 2006a,b). However, in any of these sources, other milk proteins “dilute” the concentration of MFGM components. Thus, much research has been undertaken to adapt filtration techniques, such as microfiltration and ultrafiltration, to concentrate or purify MFGM without the use of chemical solvents. At the same time, the effects of homogenization and heat treatment on the native structure of the MFGM and the potential implications these may impart on product functionality and nutrition need to be evaluated.

Despite all the health benefits offered by the milk fat globule membrane, little effort has been given by the technological community to incorporate MFGM constituents into food products, and the MFGM still remains the least understood component of nature’s most nutritious food: milk (Argov et al., 2008b). With the latest techniques in biology, chemistry, and physics, such as confocal microscopy, proteomics, genomics, glycomics (Tao et al., 2008), and other reliably objective physical and chemical tools (such as laser

Table 3.1. Phospholipid and protein composition in dairy products (adapted from Morin et al., 2006; Fong et al., 2007; Dewettinck et al., 2008; Jiménez-Flores and Higuera-Ciapara, 2009).

Product	Phospholipids (g/100 g)		Protein percentage ^a in	
	Wet basis	Dry basis	Product	Dry
Raw milk	0.035	0.28	3.3	–
Skim milk	0.02	0.28	3.41	7.55
Cream	0.19	0.40	3.16	–
Isolated MFGM	0.65	6.5 ^b	1.8	60 ^b
Butter	0.19	0.23	0.85	–
Sweet Buttermilk	0.16	2.03	3.31	32.95 ^b
Butterserum	1.25	11.54	–	–
Fresh acid buttermilk ^c	0.31	1.86	3.31	–
Acid buttermilk whey	0.10	1.84	0.99	84.7 ^b
Whey buttermilk powder (WBP) ^b	–	14.81 ^b	–	47.39 ^b
SPE-WBP ^{bd}		60.59 ^b		72.69 ^b
Cheddar cheese	0.15	0.25	–	24.89
Cheddar cheese whey	0.02	0.26	3.49	35.2

^aAverage from several sources and laboratory data.^bNumbers from corresponding author's laboratory data.^cSour buttermilk.^dSolid phase extracted (SPE) whey buttermilk powder (WBP).

tweezers and atomic force microscopy), the complex biological structure and interaction of fat globules and MFGM can be characterized *in situ* or with very little destructive manipulation, and thereby provide knowledge of the native components that benefit mammalian neonates.

This chapter highlights the chemical, biochemical, and nutritional characteristics of the milk fat globule membrane, as well as its dairy sources. It attempts to give an overview of the origin, composition, structure, health benefits, and technical aspects of the MFGM that have been identified for this novel ingredient as a potential nutraceutical. Methodologies for further investigation are presented. These facts are offered as a basic platform from which we hope readers can find ideas and inspiration for furthering the knowledge and application of this milk component and resource.

3.2 ORIGIN AND FUNCTION OF THE MFGM

Milk lipids are mainly composed of triacylglycerols that originate within the bilayer of the rough endoplasmic reticulum (rER) of epithelial cells of the mammary glands where they start their journey towards the apical membrane (Nielsen et al., 1999; Robenek et al., 2006a,b). However, freeze-fracture electron microscopy has shown that lipid droplets developed alongside the rER membrane. Regardless of the lipid droplet biogenesis, a membrane known as the milk fat globule membrane (MFGM) surrounds the lipids. This trilayer membrane acts as an emulsifier and prevents coalescence of fat droplets and lipase activity (Dewettinck et al., 2008). The fat globule size depends on many factors, such as cow breed and lactation stage. For example, fat globules from Jersey cows are larger than those from Friesian cows, with average diameters of 4.5 and 3.5 μm , respectively (Singh, 2006).

The triacylglycerols are first assembled into microdroplets, which range in diameter from less than 0.5 μm to more than 4 μm , at the basal membrane of the secretory mammary cells (Keenan et al., 1971). A monolayer membrane consisting of polar phospholipids and proteins surrounds the assembled triacylglycerols, which do not form part of this monolayer (Wooding and Kemp, 1975). As the microdroplets migrate through the cytoplasm, they coalesce to form bigger droplets called cytoplasmic lipid droplets (CLD); a formation that is promoted by calcium and by ganglioside mediation (Mather and Keenan, 1998; Keenan and Mather, 2006; Jiménez-Flores and Brisson, 2008). It has been hypothesized that migration is assisted by microtubules; however, research is inconclusive to support such a mechanism (Keenan and Mather, 2006). The CLD then approach the apical surface and are gradually enveloped in the membrane and bud out of the secretory cells (Mather, 2000; Keenan and Mather, 2006; Dewettinck et al., 2008). During the enveloping process, a distance of 10–20 nm is kept between the outer surface of the droplet and the cytoplasmic face of the plasma membrane, which appears to be filled with electron-dense material when observed in micrographs. The origin of such material is still unknown (Keenan and Mather, 2006; Dewettinck et al., 2008). The formation of secretory vesicles that pick up microdroplets has also been suggested for milk fat globule (MFG) excretion, but cytochemical evidence favors the plasma membrane envelopment mechanism (Mather and Keenan, 1998; Keenan and Mather, 2006). In fact, butyrophilin (BTN), an integral MFG protein, plays a role in globule secretion because it is highly expressed on the apical membrane and concentrated at budding locations (Mather and Keenan, 1998; Keenan and Mather, 2006; Dewettinck et al., 2008). In the secreted milk, lipid globule diameter ranges from 0.15 μm to more than 15 μm with slight variation in their fatty acid composition between small (1–3 μm) and large (6 μm) globules (Fauquant et al., 2005; Michalski et al., 2006); however, small MFG, ranging between 2.3 and 8.0 μm , have no significant difference in their phospholipid and sterol content (Fauquant et al., 2007).

Argov et al. (2008b) speculated that the distribution in diameter might imply a different role than just fat delivery to neonates (Kemsley, 2008). In fact, membrane vesicles consisting of phospholipids and proteins, with 30–100 nm and 100–1000 nm diameters, respectively known as exosomes and microvesicles, have been observed in milk without association of triglycerides and cholesterol (Silanikove et al., 2006; Argov et al., 2008b; Admyre et al., 2007). Their genesis and functions are unknown, but they have been associated with immunoregulation and antitumoral activities (Silanikove et al., 2006). Admyre et al. (2007) identified these vesicles as exosomes that have the capacity to influence immune responses in human breast milk. Argov et al. (2008a) have designated them as lactosomes in human milk, whereas Silanikove and Shapiro (2007) called them whey membrane particles (WMP) in bovine milk.

In approximately 1% of bovine fat globules, some cytoplasm will be entrapped between the bilayer membrane and the fat droplet surface, which forms a crescent or “signet.” Keenan and Mather (2006) support an original suggestion by Patton (2004) that crescents might be a pathway for the introduction of hormones or other bioactive molecules in milk. Knowledge about their molecular formation may help us decipher the mechanism that controls milk fat secretion, which is still an area of speculation. Also, there is a lot of emphasis on postsecretion modification of the MFGM, which may be the origin of membrane fragments seen in skim milk; however, the extent of this event is uncertain (Mather and Keenan, 1998; Mather, 2000; Keenan and Mather, 2006; Singh, 2006). In fact, mechanical treatments like heating, homogenization, aeration, and agitation may enhance MFGM release into the serum phase (Evers, 2004; Rombaut et al., 2006b).

3.3 COMPOSITION AND STRUCTURE OF THE MFGM

The MFGM is a complex structure. It is 10- to 50 nm thick and contains phospholipids, sphingolipids, and specific membrane proteins, as seen in Figure 3.1. Phospholipids and proteins account for over 90% of the membrane's dry weight (Singh, 2006). Some of the proteins are an integral part of the membrane, and others are peripheral or loosely attached within the trilayer membrane (Dewettinck et al., 2008; Lopez et al., 2008). The first membrane, derived from the rER, is a monolayer containing phospholipids and proteins. The second membrane is a bilayer containing glycosylated and nonglycosylated proteins, glycerophospholipids and sphingolipids, enzymes, cholesterol, and other minor components. Between the inner membrane and the bilayer, there is an electron-dense coat rich in proteins (Rombaut et al., 2006b; Lopez et al., 2008). A glycocalyx forms a fourth layer that acts as a source of specific bacterial and viral ligands and varies during lactation (Spitsberg and Gorewit, 1997; Evers, 2004; Wilson et al., 2008). A recent and detailed description of milk fat synthesis during lactation is given by Bionaz and Loor (2008).

The gross composition of the MFGM, reported in literature, differs as a result of isolation, purification, and techniques used in analysis (Evers, 2004; Keenan and Mather, 2006; Dewettinck et al., 2008). Furthermore, the composition can be altered by physiological, chemical/enzymatic, and physical/mechanical factors (Evers, 2004). The latter includes cooling, drying, separation, agitation, heating, and homogenization (Michalski and Januel, 2006; Dewettinck et al., 2008; Jiménez-Flores and Brisson, 2008; Lopez et al., 2008). With

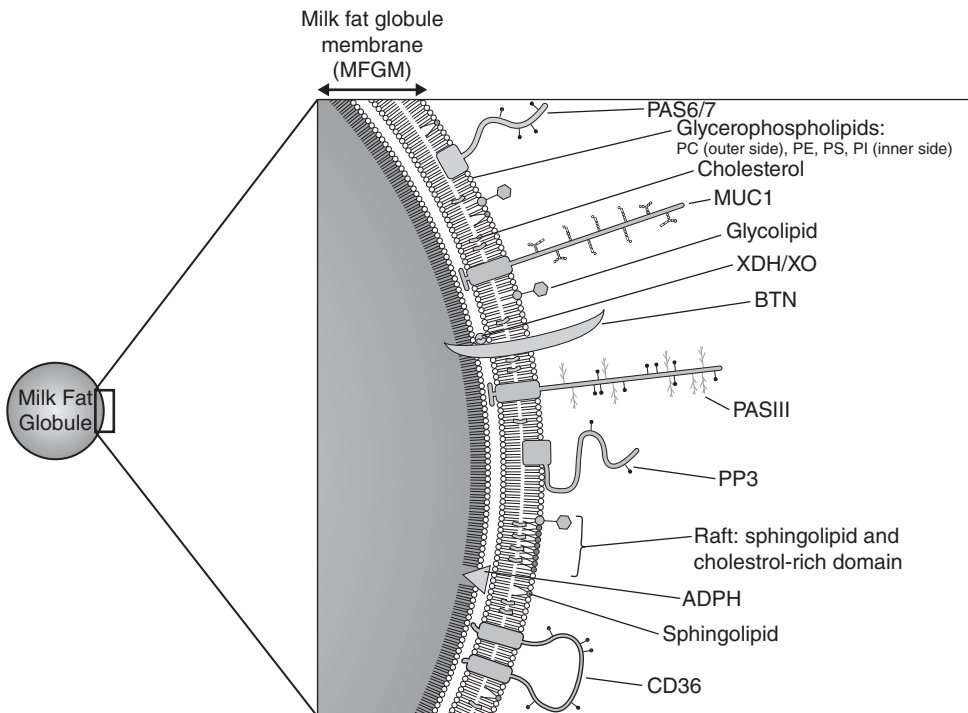


Figure 3.1 Schematic representation of the structure of the milk fat globule membrane (adapted from Lopez et al., 2008; Dewettinck et al., 2008; Bionaz and Loor, 2008).

respect to physiological factors, Lopez et al. (2008) observed large differences in the fatty acid (FA) composition of the phospholipids in the milk from cows fed a regular diet and a diet rich in polyunsaturated FA. The latter resulted in a significant decrease in saturated fatty acid content in milk, which enhances milk's nutrition quality, especially for people with coronary heart disease risks (Lopez et al., 2008; Jensen, 2002).

3.3.1 Lipids of the milk fat globule membrane

Polar lipids, such as glycerophospholipids and sphingolipids, constitute less than 1% of the MFG total lipids; nevertheless, they function as intracellular signaling molecules, provide a framework structure, and have nutritional and functional properties, such as emulsification (Jiménez-Flores and Brisson, 2008; Lopez et al., 2008). Indeed, research has also found a relationship between polar lipid consumption and enhanced health (Spitsberg, 2005). Polar lipids are amphiphilic molecules comprised of a hydrophobic tail and a hydrophilic head. Their morphology is influenced by the presence of water (Fedotova and Lencki, 2008). On the MFGM, they are distributed heterogeneously and present in different percentages (Dewettinck et al., 2008; Jiménez-Flores and Brisson, 2008; Lopez et al., 2008). These phospholipids contain high levels of long-chain fatty acids (FA) such as palmitic, stearic, and tricosanoic acids (Singh, 2006; Sanchez-Juanes et al., 2009). No difference has been observed in the FA composition of MFGM phospholipids regardless of season (Fauquant et al., 2005). Other lipids, such as triglycerides (TG), diglycerides, monoglycerides, sterols, and sterol esters, are mainly present in the milk fat globule core, as seen in Table 3.2. Among these, triglycerides represent about 60% of the neutral lipids in the milk fat globules, and cholesterol accounts for 90% of the total sterols in milk fat globules (Mather, 2000; Keenan and Mather, 2006; Rombaut et al., 2006a; Dewettinck et al., 2008).

Table 3.2. Composition of milk lipids and their distribution between the milk fat globule (MFG) core, the milk fat globule membrane (MFGM), and the skim milk (adapted from Jensen, 2002; Michalski and Januel, 2006; Fauquant et al., 2007; Jiménez-Flores and Brisson, 2008).

Lipid class	Content in total fat (g/kg)	Fraction in %		
		Globule core	MFGM	Skim phase
Neutral glycerides				
Triacylglycerol	958–983	100		
Diacylglycerol	2.8–22.5	≈90	≈10	?
Mono-acylglycerol	0.3–3.8	Traces	Traces	Traces
Free fatty acids	1.0–4.4	60	≈10	30
Phospholipids ^a	2.0–11.1	–	65	35
Small globule (3 μm) ^b			28.4 ^c	
Large globule (6 μm)			36.9 ^c	
Cerebrosides	1.0	–	70	30
Gangliosides	0.1	–	≈70	≈30
Sterols		80	10	10
Cholesterol	3.0–4.6			
Cholesteryl ester	≤0.2			
Carotenoids + Vit. A	0.02	≈95	≈5	Traces

^aPL in the MFGM pellet.

^bSmall globule tend to contain more sphingomyelin.

^cPL% in total lipids of the MFGM from microfiltrated globules.

Small globules contain more medium-chain fatty acids and less stearic acid within the triglyceride core (Fauquant et al., 2005). Other constituents are hexoses, hexosamines, and sialic acids, which are associated exclusively with proteins and glycosphingolipids (Keenan and Mather, 2006).

3.3.1.1 *Phospholipids*

Glycerophospholipids have a glycerol backbone with two fatty acids attached on position sn-1 and sn-2. The third hydroxyl group has a phosphate group esterified to an organic compound, such as choline (Rombaut et al., 2006a). The latter, phosphatidylcholine (PC), represents up to 35% of the total phospholipids, and is a major constituent of cell membranes that provides structure and maintains the permeability barrier (Dewettinck et al., 2008; Jiménez-Flores and Higuera-Ciapara, 2009). Depending on the water concentration and temperature, PC can take on up to four morphologies, including lamellar crystalline (L_c), lamellar β' ($L_{\beta'}$), lamellar α (L_α), and oblique β' ($P_{\beta'}$) (Fedotova and Lencki, 2008). Phosphatidylethanolamine (PE) is highly unsaturated and makes up to 30% of the total phospholipids in MFGM. It is mainly found in nerve tissue and brain, accounting for approximately half of the phospholipid content in the latter. Its fatty acid composition differs from PE present in whole milk, presenting more C18:1 and C18:2 and less C14:0 and C16:0 (Sanchez-Juanes et al., 2009). Another phospholipid, phosphatidylinositol (PI), acts as a substrate for several enzymes involved in cell signaling and can be phosphorylated to form phosphatidylinositol phosphate (PIP). Phosphatidylserine (PS) is a negatively charged phospholipid component of cell and blood platelets that assist in blood coagulation. In addition, PS may reduce the risk of dementia and cognitive dysfunction in the elderly as presented in Table 3.3 (Dewettinck et al., 2008). The distribution of these phospholipids is not homogenous throughout the MFGM. Concentrated in the inner surface are mostly PE, PS, and PI, while PC, sphingomyelin (SM), and glycosphingolipids, such as cerebroside and gangliosides, are observed in the bilayer membrane (Lopez et al., 2008).

In the market, sour and sweet buttermilk and quark-skimmed cheese are the highest polar lipid-containing products (Jiménez-Flores and Brisson, 2008; Jiménez-Flores and Higuera-Ciapara, 2009). With all the health benefits and biological roles that phospholipids and sphingolipids have, it will be beneficial to fortify food with these low-price products; however, it is important for the food technologist to know the structure of these molecules and understand their chemistry. Phospholipids are good emulsifiers but differ in properties. Indeed, PC and PE tend to stabilize water-in-oil emulsions due to their low hydrophilic-lipophilic balance (HLB) value, while SM, PI, and PS tend to form oil-in-water emulsions (Fedotova and Lencki, 2008, 2010). As Jiménez-Flores and Brisson (2008) stated, it is essential to understand the biological mechanism of nutritional delivery to incorporate MFGM constituents into foods, so food scientists can formulate products that preserve the optimal structure of these lipids and deliver the proper nutrition and functionality.

3.3.1.2 *Sphingolipids*

Sphingolipids are highly bioactive molecules that constitute up to one-third of the MFGM polar lipid fraction (Dewettinck et al., 2008; Jiménez-Flores and Brisson, 2008). They are characterized by a sphingoid base, which is a long-chain aliphatic amine (12–22 carbon atoms) (Rombaut et al., 2006a). Attachment of a fatty acid via a hydroxyl group forms a

Table 3.3. Nutritional aspects of polar lipids of the MFGM (adapted from Rombaut et al., 2006a; Dewettinck et al., 2008; Jiménez-Flores and Brisson, 2008; Wat et al., 2009).

Component	PL % ^a	Nutritional aspects
Sphingolipids and metabolites	18.0–34.1 ^b	Reduction of the number of aberrant crypt foci and adenocarcinomas Shift in tumor type (malignant \Rightarrow benign) Anticholesterolemic Protection of the liver from fat- and cholesterol-induced steatosis Suppression of gastrointestinal pathogens Neonatal gut maturation Myelination of the developing central nervous system Endogenous modulators of vascular function Associated with age-related diseases and the development of Alzheimer
Sphingosine 1-phosphate		Mitogenic
Phosphatidylcholine (PC)	19.2–37.3	Support liver recovery from toxic chemical attack or viral damage Protects the human GI mucosa against toxic attack Reduction of necrotizing enterocolitis Alleviates orotic acid-induced fatty liver
Lysophosphatidylcholine (lysoPC)	2	Bacteriostatic and bactericidal capacity Strong gastroprotective role in the duodenal mucosa
Phosphatidylethanolamine (PE)	19.8–42.0	Maintains hemostasis
Phosphatidylinositol (PI)	5–11%	Substrate in cell signaling Promotes plasma cholesterol transport and metabolism
Phosphatidylserine (PS)	1.9–10.5	Restore normal memory on a variety of tasks Positive effects on Alzheimer patients Improve exercise capacity of exercising humans

^aRelative phospholipid content (g per 100g of polar lipid including sphingomyelin).^bSphingomyelin content.

ceramide that is the basic unit for the formation of a sphingophospholipid, such as sphingomyelin (SM). The latter has a high degree of saturation that facilitates complex formation with cholesterol, known as a lipid raft. These are rigid domains implicated in cellular processes, like signal transduction, endocytosis, and cholesterol trafficking (Dewettinck et al., 2008; Lopez et al., 2008, 2010). Sphingomyelin is primarily located in the outer membrane of the MFGM, and is present in higher percentages in milk derived from cows fed with unsaturated fatty acids due to the presence of smaller globules and consequently more surface area (Lopez et al., 2008). An analysis of sphingolipids in the most commonly consumed dairy products found similar sphingolipid concentrations in nonfat dry milk and fermented products, indicating that starter cultures do not contribute to SM in dairy products (Jiménez-Flores and Higuera-Ciapara, 2009); however, we can manipulate the concentration of this essential polar lipid by changing the diet of cows (Lopez et al., 2008). Furthermore, SM has been shown to reduce aberrant crypt foci and the appearance of colon adenocarcinoma, to influence neonatal gut maturation, and to contribute to myelination of the developing rat central nervous system, among other functions (Dewettinck et al., 2008; Jiménez-Flores and Higuera-Ciapara, 2009). Sphingomyelin metabolites also provide health benefits, as shown in Table 3.3.

3.3.2 Milk fat globule membrane proteins

MFGM proteins constitute 1–2% of the total bovine milk protein, and depending on the milk source and how it is processed, 25–70% of the MFGM may be polypeptides, ranging in molecular weight (MW) from 15,000 to 240,000 Da (Ye et al., 2002; Riccio, 2004; Dewettinck et al., 2008; Jiménez-Flores and Higuera-Ciapara, 2009). Indeed, Murgiano et al. (2009) detected differences between Chianina and Holstein cattle in the amount of proteins associated with mammary gland development, lipid droplets formation, and host defense mechanisms. A detailed description and suggested nomenclature of the known and major MFGM proteins are presented by Mather (2000). Using proteomic techniques, Reinhardt and Lippolis (2006) identified 120 proteins, which have been grouped into seven categories, as seen in Table 3.4. One-fourth of the identified proteins still have unknown functions. Among the proteins, there are about 28 enzymes, including some protein kinases, oxidases, 5'-nucleotidase, adenosine triphosphatase, and phosphodiesterase (Keenan and Mather, 2006; Reinhardt and Lippolis, 2006). Half of these enzymes belong to the hydrolase class, and the most abundant enzymes are alkaline phosphatase and xanthine oxidase (Singh, 2006). The biological role of the MFGM-associated enzymes has been focused on their damaging effects, which makes their presence undesirable in dairy products because they might cause off-flavors or affect processing properties (Keenan and Mather, 2006). The purpose of some enzymes is still unclear. It has been hypothesized that they might originate from the cytoplasmic crescents, making them transient MFGM proteins (Mather and Keenan, 1998; Keenan and Mather, 2006).

The protein content also varies throughout the cow's lactation period (Ye et al., 2002). Indeed, two of the major proteins in the MFGM, xanthine oxidoreductase (XOR) and the mammary gland-specific BTN, fluctuate during the 300-day lactation period. They are high during the early and late stages compared with the mid-lactation. Despite the change in concentration, the fat globule size was not influenced and the molar ratio (4:1, BTN:XOR) between these proteins remained the same throughout, which suggests a close interaction (Ye et al., 2002; Keenan and Mather, 2006; Singh, 2006). Some of the most important proteins are discussed below, and the protein composition of the MFGM can be visually examined using two-dimensional gels as seen in the example of a 2D PAGE in Figure 3.2.

3.3.2.1 Butyrophilin

Butyrophilin is a glycosylated protein that is mainly found in the apical side of epithelial cells, in budding fat droplets, and in the inner coat of the MFGM (Franke et al., 1981). It

Table 3.4. MFGM Protein classification using proteomics techniques (adapted from Reinhardt and Lippolis, 2006).

Protein functionality	Percentage
Membrane/protein trafficking	23
Cell signaling	23
Fat transport and/or metabolism	11
Transport	9
Protein synthesis, binding and/or folding	7
Immune functions	4
Milk proteins: β - and κ -casein	2
Unknown function	21

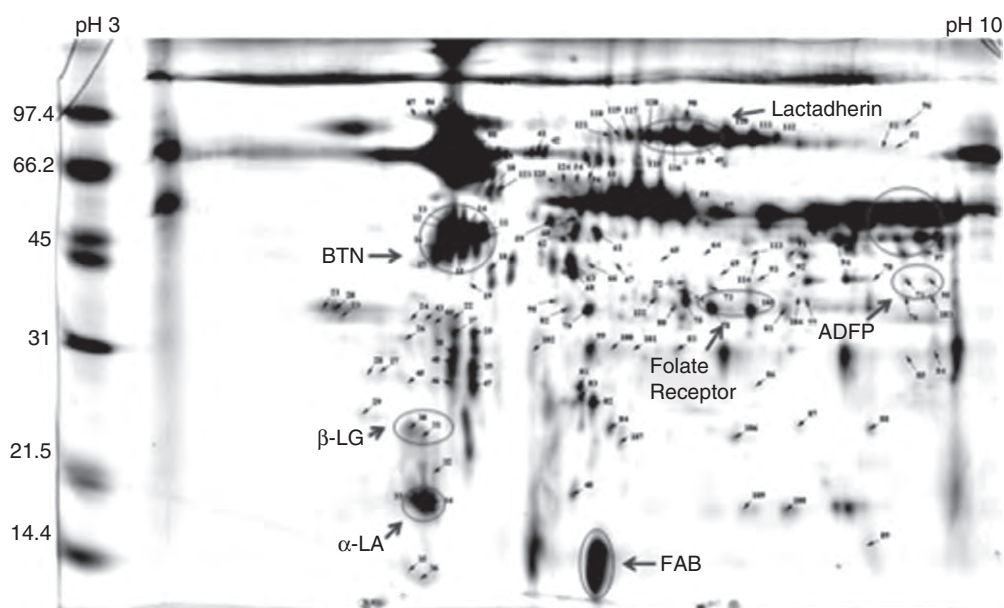


Figure 3.2 Two-dimensional gel contrasting the difference between fresh milk MFGM proteins and those in HTST-pasteurized milk. Comparison was made using Delta2D program. Left lane is the MW standard, small dots are the proteins identified, and the circled regions indicate proteins with major changes from fresh to pasteurized milk.

represents the most abundant protein of the MFGM, constituting about 40% by weight of the total MFGM proteins in milk from Holstein cows and approximately 20% from Jersey milk (Singh, 2006, Jiménez-Flores and Higuera-Ciapara, 2009). Its apparent molecular weight is 66–67 kDa, with a 5% carbohydrate content (Singh, 2006). Fatty acids are also covalently bound to BTN, which merits its name from the Greek *butyros* and *philos*, signifying “affinity for butter” (Keenan and Mather, 2006); palmitic, stearic, and oleic acid are the predominant protein-bound fatty acids in both BTN and XOR (Singh, 2006). BTN is part of the immunoglobulin (Ig) superfamily of adhesive and receptor proteins. Indeed, its N-terminal has two Ig-like folds, and the C-terminal comprises a B30.2 domain, which is predicted to have two Ig folds that function as a protein-binding domain (Mather and Jacks, 1993; Mather, 2000; Keenan and Mather, 2006; Jeong et al., 2009).

BTN is highly expressed during lactation and forms complexes with XO to envelop the nascent fat globule via adipophilin (Keenan and Mather, 2006). However, freeze-fracture localization studies have shown that milk fat secretion is controlled by interactions between BTN present in plasma membrane and in the secretory granule phospholipid monolayer (Robenek et al., 2006a). Jeong et al. (2009) provides evidence that the cytoplasmic domain of BTN binds to xanthine reductase as it was originally hypothesized. In addition, it has been suggested that BTN transfers phosphorus groups to tyrosine residues in proteins, activating them and playing a critical role in controlling the physiological activity of proteins in the secretory cells and within the MFGM. The genes controlling the expression of the BTN gene (BTN1A1) is described in detail in Bionaz and Looor (2008).

To date, the consumption of BTN continues to be a controversial topic due to its implicated association with multiple sclerosis (MS), an autoimmune-mediated inflammatory disease that affects the central nervous system (Mather and Linington, 1999; Rutter, 2006;

Dewettinck et al., 2008). Some research suggests that dairy products exacerbate symptoms or even produce the disease because BTN resembles a polypeptide named myelin oligodendrocyte glycoprotein (MOG), a putative autoantigen that induces experimental autoimmune encephalomyelitis (EAE). The latter disease has clinical symptoms similar to human MS (Riccio, 2004; Dewettinck et al., 2008); however, in other experiments, mice treated with BTN, either before or after MOG injections, did not show clinical manifestation of EAE (Dewettinck et al., 2008). Mather and Linington (1999) have modified BTN to suppress an autoimmune response, which can also be used to screen for MS susceptibility. BTN has also been associated with autism due its resemblance with neurospecific antibodies; however, its etiopathogenesis is unknown (Riccio, 2004). Until the role of BTN with respect to MS and autism is elucidated, dairy products should be included in our daily diet to receive its health benefit, as the etiology of these diseases is a combination of genetic susceptibility and environmental factors (Rutter, 2006). The health benefits attributed to other MFGM proteins is presented in Table 3.5.

3.3.2.2 Xanthine oxidoreductase

Xanthine oxydoreductase (XOR) is a member of the moldyl hydroxylase family, which is widely expressed in tissues but has higher activity in bovine milk as compared with goat, sheep, and human milk (Silanikove and Shapiro, 2007). Moreover, XOR is highly expressed during pregnancy and parturition (Keenan and Mather, 2006). It can be present as xanthine

Table 3.5. Health benefits of MFGM components (adapted from Riccio, 2004; Spitsberg, 2005; Michalski and Januel, 2006; Pan et al., 2006; Fong et al., 2007; Dewettinck et al., 2008; Jiménez-Flores and Brisson, 2008).

Proteins	MW (kg/mol)	Reported heath benefits/functions
Mucin 1 (MUC1) ^a	160	Antiviral action/antitrotavirus, especially in neonates
Mucin 15 (MUC15 or PAS III) ^a	94–100	Antiviral action
Butyrophilin (BTN) ^a	66	Suppression of multiple sclerosis
Xanthine oxidase (XO)	150–155	Bactericidal agent
Cluster of differentiation (CD36 or PAS IV) ^a	78	Glycoproteins that act as receptors due to high sugar content
Fatty acid binding protein (FABP)	15	Cell growth inhibitor
BRCA1 and BRCA2	210	Anticancer factor (FABP as selenium carrier)
Lactadherin (PAS 6/7) ^a	43–59	Inhibition of breast cancer
		Role of epithelialization, cell polarization, cell movement and rearrangement, neurite outgrowth, synaptic activity in the central nervous system, and protection against viral infection in the gut
Adipophilin (ADPH)	52	Milk synthesis
Other components ^b		
β-Glucuronidase inhibitor		Inhibition of colon cancer
<i>Helicobacter pylori</i> inhibitor		Prevention of gastric diseases
Cholesterolemia-lowering factor		Anticholesterol activity
Vitamin E and carotenoids		Antioxidants
Phosphoproteins		Source of organic phosphorus and Ca-phosphate

^aGlycosylated protein.

^bPhospholipids described in Table 3.3.

dehydrogenase (XD) and converted to xanthine oxidase (XO) by proteolysis or formation of disulphide bonds (Spitsberg and Gorewit, 1998; Mather, 2000; Silanikove and Shapiro, 2007). It has been shown that plasmin activity is responsible for this interconversion, and the low oxygen and low enzyme environment of the inner membrane decreases the conversion to XO, resulting in a mixed population. However, XD is more predominant in the inner membrane with a nonenzymatic role in fat globule secretion (Silanikove and Shapiro, 2007). XD is not very stable in solution and reverts slowly to XO at pH 7.5 (Spitsberg and Gorewit, 1998). The active molecule is a homodimer with monomeric molecular masses of 150 kDa, which accounts for up to 20% of the coat proteins, where XOR binds with high affinity to the carboxyl end of BTN. Each subunit contains one molybdenum, one flavin adenine dinucleotide, and two nonidentical iron-sulfur redox centers (Mather, 2000; Martin et al., 2004; Keenan and Mather, 2006). During MFGM isolation, XOR disperses in the supernatant but remains tightly bound to the membrane, and can be isolated using high salt and nonionic detergents (Mather, 2000; Keenan and Mather, 2006). Keenan and Mather (2006) have proposed that the XDH/BTN complex interacts with other proteins to mediate interactions between the lipid core and the membrane. Besides purine catabolism, XOR generates reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as nitric oxide (NO) and peroxynitrite, that act as antimicrobial agents in the neonatal gut. Silanikove and Shapiro (2007) concluded that XD is a source of freely available extramembrane protein that plays an essential role in the development of innate immunity (Martin et al., 2004; Jiménez-Flores and Higuera-Ciapara, 2009). Specifically, 33% of the free XD is found in the outer surface of the MFGM, 46.7% in solution, and 20.5% in the outer surface of exosomes. This form may also affect the physicochemical oxidative properties of milk, especially during storage (Silanikove and Shapiro, 2007).

3.3.2.3 *Adipophilin*

Adipophilin is also known as adipocyte differentiation-related protein (ADRP), which is ubiquitously expressed in the presence of long-chain free fatty acids, especially in the mammary glands and in differentiating preadipocytes (Chang and Chan, 2007). Clusters of ADRP have been observed in the cytoplasmic leaflet of the rough endoplasmic reticulum, where lipid droplet biogenesis might take place as proposed by Robenek et al. (2006b). The apparent molecular weight of adipophilin is 52 kDa, but calculations from the cDNA result in a lower molecular mass, suggesting posttranslational modification, especially acylation (Nielsen et al., 1999). During preadipocyte differentiation, adipophilin is highly expressed, and the protein embeds itself in the periphery of the intracellular lipid droplets. As adipocytes mature, ADRP is replaced by proteins known as perilipins; however, this mechanism is devoid in the epithelial cells of the mammary glands (Nielsen et al., 1999; Dewettinck et al., 2008). In the fat globules, adipophilin associates with the XOR/BTN complex and facilitates the envelopment of the globules with plasma membrane during milk fat droplet secretion (Nielsen et al., 1999; Heid and Keenan, 2005; Keenan and Mather, 2006; Jeong et al., 2009). When the membrane is disrupted, adipophilin remains attached in the MFGM-associated coat material (Keenan and Mather, 2006). Now, it is considered to be one of the major MFGM proteins.

3.3.2.4 *Glycoprotein PAS6 and PAS7*

PAS6 and PAS7 are glycoproteins of the MFGM that have been identified by staining with periodic acid Schiff glycoprotein (PAS). The respective molecular masses are 50 and

47 kDa, and their polypeptide core is identical, suggesting that the difference in molecular weight is due to the N-linked carbohydrate moieties. Three glycosylation sites have been detected in PAS6, while PAS7 only includes two (Hvarregaard et al., 1996). These molecules are loosely attached to the MFGM and can be removed with salt treatment, such as $MgCl_2$ (Mather, 2000; Singh, 2006). The human homologue, known as lactadherin, has been shown to bind to rotavirus and protect the gastrointestinal tract of newborn infants from infection (Keenan and Mather, 2006). More research is needed to define the exact role that these proteins contribute to the bovine milk fat globule membrane.

3.4 HEALTH BENEFITS OF THE MILK FAT GLOBULE MEMBRANE

Progress in the knowledge of the composition and role of milk fat globule membrane components has led to the realization that some possess biological properties beyond their nutritional significance. Spitsberg (2005), in his review, refers to the MFGM as a nutraceutical with nutritional and pharmaceutical potential. In fact, the MFGM has antimicrobial proteins, illness suppressors, and micronutrient-binding proteins that bind compounds, such as iron, zinc, copper, folate, and vitamin B1, and other components, with anticarcinogenic properties, as seen in Table 3.5. Indeed, Lonnerdal et al. (2006) complemented infant food with bovine MFGM and micronutrients to evaluate its effect on children's growth, nutrition, and morbidity. In a double-blind study with 6- to 11-month-old infants ($n = 550$), fortification of food was beneficial in the copper and vitamin B12 status among the subjects, which enhance growth and normal function of the brain and nervous system, respectively (Lonnerdal et al., 2006). Wat et al. (2009) supplemented bovine PL to mice fed a high-fat diet resulting in a reduction in liver weight, total liver lipid, and serum lipid levels, which might be of therapeutic benefit in humans with nonalcoholic fatty liver disease, especially obese and diabetic patients.

3.4.1 Anticancer properties of MFGM

Long-chain nonesterified free fatty acids are the building blocks for triglyceride synthesis. They are increasingly being recognized as intracellular mediators of gene expression. Mutations in the breast-ovarian cancer susceptibility (BRCA1 and BRCA2) gene may be responsible for a significant number of breast and ovarian cancers. This gene produces BRCA1 and BRCA2 proteins that suppress the regulation of tumor growth. The proteins expressed by the BRCA1 and BRCA2 have been identified in the MFGM of human and bovine milk by Western blot and immunoprecipitation (Spitsberg and Gorewit, 1999; Vissac et al., 2002). They inhibit the growth of various cell types, including bovine mammary and human breast cancer cells, and influence cellular proliferation and differentiation. Fatty acid binding proteins isolated from MFGM have been found to inhibit the growth of some breast cancer cell lines *in vitro* at very low concentrations.

In addition, several lipids found in the MFGM can act as intracellular signaling molecules. Sphingomyelin has been shown to possess anticancer properties (Table 3.3). Its metabolites, sphingosine and ceramide, mediate numerous imperative cell functions, including regulating cell growth, development, and differentiation. Studies have shown sphingomyelin to be effective in suppressing colon tumors. Recently, it was shown that an

ingredient rich in bovine PL from the MFGM prevented intestinal cancer in rats (Snow et al., 2010)

3.4.2 Antimicrobial and antiviral properties of the MFGM

Milk and colostrum contain several antimicrobial factors, which are transferred from the mother to the neonate and contribute to the protection against infectious diseases (Affolter et al., 2009). For many species, the milk-derived antimicrobial system is crucial for survival of the neonate. The components of the MFGM are involved with anti-infection or antiadhesion, and hence with protection of the neonate from various viral and bacterial infections (Lopez et al., 2008). Currently, there is evidence that the protective effect of milk on viral infections is a complex mechanism of MFGM components. Zavaleta et al. (2006) tested the antimicrobial and antiviral properties of bovine MFGM on the incidence and duration of diarrhea in infants. In a double-blind study with 6–11 month old infants, MFGM fortification reduced diarrhea incidence and prevalence.

The antimicrobial and antiviral activities of proteins and lipids are currently receiving increasing interest, especially in the context of the preparation of infant formula and medical food. A review by Pan et al. (2006) summarized the knowledge on the protein lactoferrin, most comprehensively studied for its beneficial immunological, antimicrobial, and anticarcinogenic properties. This review examined the potential mechanism of lactoferrin as a powerful inhibitor of viral infection (Pan et al., 2006). One interesting result, bovine lactoferrin was found to be more effective against viral infections than human lactoferrin. Isaacs et al. (1995) conducted an *in vitro* study in which fatty acids and monoglycerides were added to infant formula. They found that fatty acids and monoglycerides with chain lengths varying from 8 to 12 carbons were more antiviral and antibacterial than long-chain monoglycerides (Isaacs et al., 1995).

3.4.3 MFGM and lactic acid bacteria binding

It is important to study the interaction between lactic acid bacteria, intestinal cells, and MFGM to aid in the design and formulation of dairy foods containing probiotic strains. In order to understand how lactic acid bacteria transfer from the dairy product to the intestinal lining, the manner and mechanisms of the binding must be understood. Research has suggested that proteins on the bacterial cell surface (S-layer proteins) play a major role in the bacteria's ability to bind by interacting with specific markers (Kirjavainen et al., 1998). To expand on this theory, researchers have discovered that in some *Lactobacillus* strains, upon removal of the S-layer, the binding ability of the bacterium is greatly compromised (Sillanpää et al., 2000; Roos and Jonsson, 2002; Frece et al., 2005). In the attempt to further understand binding properties, studies isolating binding proteins from bacteria that have the ability to bind to mucus and its components are being conducted in order to characterize and classify the various methods of binding (Ryan et al., 2001). While many studies are focused on the protein-binding properties, our group is developing an assay that gives a quantitative measurement of lactic acid bacteria's affinity to bind to various lipids found in dairy foods. Preliminary results showed strain-specific binding to phospholipids, where regardless of composition, each strain showed specific binding affinity (Elizondo-Bacherio et al., 2007). An increased and nonspecific binding of the phospholipids was associated with decreased levels of triglycerides. Looking at the dairy product, Jiménez-Flores and Brisson (2008) show us that caution has to be taken in the processing, as the specificity of



Figure 3.3 Confocal laser scanning image showing interactions between *Lactobacillus reuteri* T-1 strain with milk fat globules in raw cream. The raw cream was stained with phosphatidylethanolamine-lissamine rhodamine B (in red) and the bacteria with acridine orange (in green) (scale bar: 10 μ m).

binding is directly impacted. There are many similarities between the components found on the MFGM and intestinal cells (carbohydrate chains, proteins, glycoproteins, enzymes, and phospholipids) that need to be researched to examine the various component roles in the success of probiotics in dairy. Figure 3.3 illustrates the tight binding between lactic acid bacteria and milk fat globules.

3.5 TECHNICAL ASPECTS AND FOODS BASED ON MFGM

3.5.1 Emulsifying and stabilizing properties of MFGM

MFGM is the native emulsifier in milk. The food industry recognizes the amphiphilic nature of the components in MFGM, phospholipids, and glycoproteins, all of which are found in buttermilk powder and are excellent emulsifying agents (Kanno et al., 1991; Corredig and Dalgleish, 1997a,b). The water distribution properties of the MFGM (either purified or in dairy ingredients) aid in thickening formulations and foaming of processed foods. However, the liability is lipid oxidation, causing the powder to go rancid in approximately 6 months. The major advantages of using buttermilk solids in functional food systems pertain to their significant antioxidant activity and buffering capacity (Wong and Kitts, 2003) and to their high phospholipid content, and their suppressive effect on certain pathogens (Evers, 2004; Jiménez-Flores and Higuera-Ciupara, 2009).

MFGM components have also been used to stabilize emulsions and enhance the absorption of fat-soluble nutrients, such as vitamin D₃ and vitamin A. It has been speculated that MFGM-stabilized microdispersions interact with bile salts and enzymes (Bezelgues et al., 2009). For example, Bezelgues et al. (2009) utilized micelles (monolayers) stabilized with MFGM components to evaluate the capacity of liposoluble compounds to be incorporated

into mixed micelles during digestion. Emulsions stabilized with MFGM had a twofold accumulation of tocopherol and lycopene when compared with emulsions stabilized with milk proteins, suggesting a nutritional application as absorption promoters of liposoluble nutrients in low-fat food products. Many researchers are starting to see the value of buttermilk beyond emulsification to include the benefits of the MFGM components.

3.5.2 Potential delivery systems derived from MFGM

The rich content of phospholipids (PL) in the MFGM, and their unique chemical composition, facilitates the use of its constituents in the formation of liposomes, which are used in the pharmaceutical and cosmetic industries (Singh, 2006; Thompson and Singh, 2006). These are vesicles formed through the self-assembly of amphiphilic molecules, such as PL, creating bilayers with an aqueous core. During their formation, hydrophobic molecules can be incorporated into the bilayer, while hydrophilic molecules comprise the core (Singh, 2006). In the food industry, liposomes can be utilized to protect sensitive ingredients or to increase the efficacy of additives (Thompson and Singh, 2006); however, the popular methodologies to make liposomes are not feasible on a large scale due to kinetics and the use of detergents and solvents. Furthermore, the current PL sources, soy and egg, have high purification costs and a different composition to those PL from milk. Thus, utilization of low-cost sources, such as buttermilk or butter serum, could alleviate the restrictions of liposome production. Thompson and Singh (2006) have successfully used microfluidization, which uses high pressure to force fluid into microchannels to overcome the limitations of other techniques (Thompson and Singh, 2006). This method can generate a large volume of liposomes in a reproducible manner without promoting oxidation, and the inclusion of MFGM phospholipids increases liposome dispersion and stability (Thompson and Singh, 2006).

3.5.3 MFGM components as part of food systems

The use of condensed sweet cream buttermilk at low levels of supplementation improved the yield of pizza cheese without affecting compositional, rheological, or sensory properties. Also, buttermilk has been used in other dairy products, such as yogurt, ice cream, and cheese, where the final yield was increased due to the water-holding capacity of phospholipids (Sodini et al., 2006). Fedotova and Lencki (2008) fortified butter with MFGM phospholipids, which stabilized crystal morphology; however, at elevated levels, their addition caused a coalescence of the aqueous phase, creating water pockets that may affect sensory properties. A subsequent study by the same group showed that the addition of PL increases butter hardness and reduces the tendency to oil-off at elevated temperatures. The optimal butter product contained a blend of 60–80% globule fat (Fedotova and Lencki, 2010).

The milk produced during the first week of lactation, known as colostrum, is free of casein and rich in immunoglobulins and MFGM proteins (Affolter et al., 2009). One of the main proteins present is lactoferrin, which is correlated with beneficial immunological, antimicrobial, and anticarcinogenic properties. Some current nutraceutical formulations utilize lactoferrin for the treatment of various ailments, including cardiovascular disease (Mao et al., 2007). Methods have been developed for the processing of colostrum to diminish the microbial load and obtain a stable and available product, which may be added to a variety of foods (Scammell, 2001). In addition, milk oligosaccharides from human milk

are linked to intestinal protection from pathogens (Wilson et al., 2008), and their potential use in infant formula is being considered, especially for premature infants who are susceptible to necrotizing enterocolitis (Kemsley, 2008).

3.5.4 Isolation of the MFGM

The yield and composition of the MFGM, as mentioned previously, depend on the technique utilized for its isolation from cream, buttermilk, or whole milk. Loosely bound proteins, and especially the amount of skim milk proteins, vary in the final MFGM fraction (Keenan and Mather, 2006; Singh, 2006). Also, the concentration of triglyceride differs due to cross-contamination from the globule core (Mather, 2000; Fauquant et al., 2005; Keenan and Mather, 2006). On a microscale, MFGM is purified using a common four-step procedure (Mather, 2000; Dewettinck et al., 2008). First, the fat globules are isolated out of the milk by centrifugation with a physiological buffer layer that also washes the fat globules, eliminating the second step and increasing the yield of MFGM material (Patton and Huston, 1986). Alternatively, upon centrifugation, the globules can be washed two or three times with buffers (Keenan and Mather, 2006; Singh, 2006). In addition, a milk salt buffer or sucrose is usually added to increase the density of the serum and minimize MFGM loss (Singh, 2006). Then, the globules are disrupted by either churning (agitation), cycles of freezing and thawing, treatment with detergents, or by suspension in polar and aprotic solvents (Keenan and Mather, 2006). This step may lead to loss of proteins, such as XOR or BTN, due to their dispersion in the salt/detergent phase. Once the membrane is disrupted, it can be collected by ultracentrifugation, protein precipitation at low pH, or induction of membrane aggregation with ammonium sulfate followed by centrifugation (Keenan and Mather, 2006; Singh, 2006). The pathways used to isolate MFGM in a laboratory setting or in a pilot plant are detailed in Figure 3.4, while on a commercial scale, MFGM isolation is more suitable from buttermilk or other sources discussed later. Michalski et al. (2006) patented a microfiltration method to isolate fat globules with different diameters. This technique results in large globules (6 μ m) that can be churned easily for buttermaking (Fauquant et al., 2007).

The composition of MFGM is altered by heat treatment and homogenization due to protein denaturation and interactions with serum proteins (Lee and Sherbon, 2002). Upon heat treatment, a layer of whey protein, especially β -lactoglobulin and α -lactalbumin, deposits on the MFGM, possibly through intermolecular disulfide bonding (Kim and Jimenez-Flores, 1995; Michalski and Januel, 2006; Singh, 2006). If milk is heated after homogenization, then β - and α -lactoglobulins absorb to the casein micelles already incorporated onto the membrane (Ye et al., 2004a,b). The exact mechanism of protein interaction has not been elucidated; however, it has been speculated that it could be via sulfhydryl-disulfide interchange or by displacement of the original MFGM material (Michalski and Januel, 2006; Singh, 2006). Its association with β -lactoglobulin starts at 60–65°C and increases with heating time until a plateau level of about 1.0 mg/g of fat is reached; this accounts for 1% of the β -lactoglobulin found in milk (Singh, 2006). Association of α -lactalbumin displays a similar mechanism, but less protein is absorbed onto the membrane. Also, small amounts of κ -casein seem to interact with the MFGM when milk is heated above 80°C; such interaction may be bridged by the already absorbed β -lactoglobulin. Higher temperature treatments (140°C for 16 seconds) cause less casein absorption (Kim and Jimenez-Flores, 1995). In addition, heating promotes the migration of PAS7 into the serum phase (Lee and Sherbon, 2002; Singh, 2006). The total MFGM protein isolated from

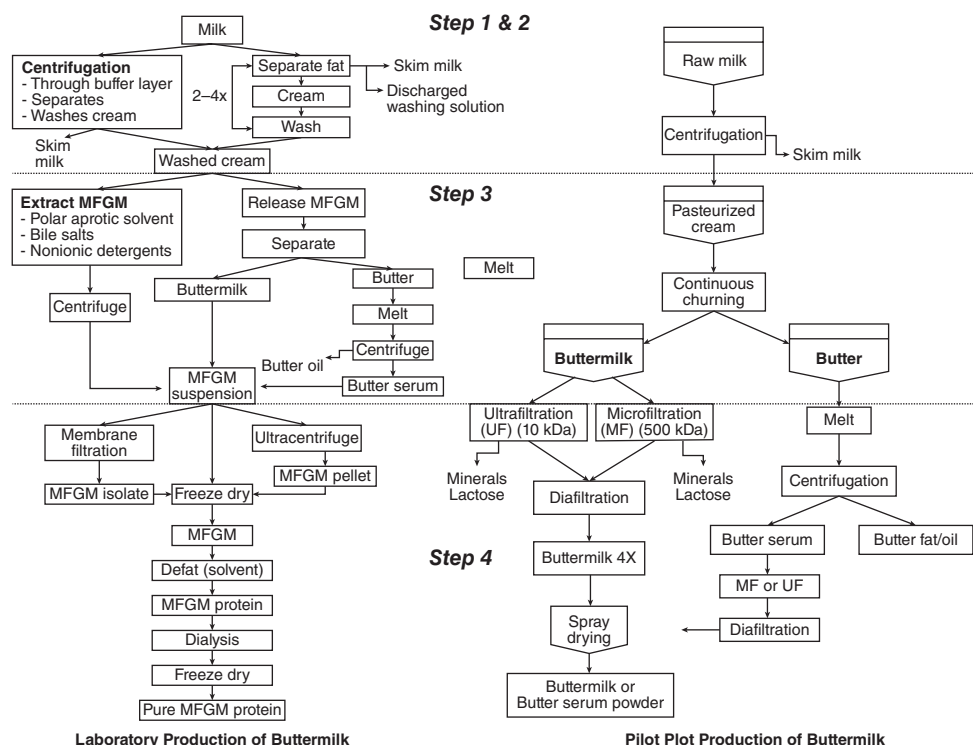


Figure 3.4 Milk fat globule isolation in a laboratory and pilot plant setting. MFGM can be purified using a four-step procedure: step 1: cream (globule) isolation; step 2: washing of globules; step 3: disruption of globules; and step 4: membrane collection (Dewettinck et al., 2008).

the cream depends on the heat treatment. For example, cream processed at 50°C for 10 minutes results in approximately 50% MFGM protein loss and formation of aggregates via intermolecular disulfide bonds in the absence of serum proteins (Ye et al., 2002). In regard to phospholipids, cream pasteurization results in buttermilk with higher lipid content; however, spray drying of buttermilk decreases its phospholipid content (Morin et al., 2007b).

3.6 MFGM: A NOVEL PRODUCT FROM DAIRY PRODUCTS

The industrial and potential health benefit properties of the MFGM components have led to its isolation and concentration from dairy products. The commercial source is sweet buttermilk, which is the aqueous phase resulting from butter churning that contains proteins, lactose, MFGM, and other minor constituents (Rombaut et al., 2006b; Sodini et al., 2006). Compared with skim milk and whole milk, buttermilk has seven times more phospholipids on a dry basis (0.28 g per 100 g vs. 2.03 g per 100 g, respectively) (Sodini et al., 2006). Despite its high content of MFGM components and low cost, buttermilk has very limited commercial value (Singh, 2006; Jiménez-Flores and Brisson, 2008; Jiménez-Flores and Higuera-Ciajara, 2009). There are also other sources of buttermilk, such as cultured (sour) and whey buttermilk, derived from churning of cultured cream and whey cream;

however, they do not have high demand in the industry. Sodini et al. (2006) analyzed buttermilk's functional properties, including protein solubility, viscosity, emulsification, and foaming, and compared them to skim milk and whey. The fat content was higher in buttermilk, but the presence of MFGM components imparted greater emulsification and lower foaming capacity to sweet, sour, and especially whey buttermilk as compared with skim milk and whey. The functional properties of the buttermilk were pH dependent with the exception of whey buttermilk, whose functionality makes it ideal for low pH foods (Sodini et al., 2006).

Another source of MFGM is butter serum, a by-product of anhydrous milk fat production. It can be made by either separation of butter upon melting and centrifugation, or homogenization of 75% cream until phase inversion is induced creating butter serum (Rombaut et al., 2006b). The phospholipid content in this fraction is five times higher than that of buttermilk (11.54 g per 100 g on dry basis, see Table 3.1). Two more MFGM-enriched milk fractions are whey protein concentrate (WPC) and buttermilk protein concentrate (BMP), which have been characterized in terms of their protein composition (Affolter et al., 2009). Affolter et al. (2009) found 244 proteins in WPC and 133 in BMP, from which 28 in these two fractions can be accounted for in the research performed by Reinhardt and Lippolis (2006). Nevertheless, the main problem with MFGM purification is the presence of caseins and whey proteins, which has triggered the quest for novel purification techniques involving membranes.

Microfiltration features a pressure-driven membrane filtration, with pressure less than one bar and membrane pore size between 10^{-1} and $10^1 \mu\text{m}$, which is commonly used for the removal of bacteria and separation of macro-molecules (Corredig et al., 2003; Morin et al., 2006). In the dairy industry, this is employed to remove lipids from whey for production of whey protein concentrate (WPC). With regard to MFGM purification, casein makes microfiltration difficult even after the use of casein micelle dissociating agents, such as citrate. Corredig et al. (2003) used sodium citrate to dissociate casein micelles, resulting in an MFGM concentrate of approximately 60% (w/w) protein and 35% (w/w) lipid. However, citrate dissociation requires the use of diafiltration to wash out minor constituents, and results in high permeate losses (Rombaut et al., 2006b). Morin et al. (2006) fractionated buttermilk and whey buttermilk using microfiltration, resulting in twofold and 50% increases in phospholipid concentration, respectively. Another study by the same group used washed cream to remove some of the caseins and whey proteins that disturb milk fractionation. A twofold increase in the permeation flux was observed, as well as a 66% increase in the phospholipid content compared with regular cream buttermilk (Morin et al., 2007a). Rombaut et al. (2006b) suggested coagulation of casein by acid or rennet addition, followed by MFGM purification using ultrafiltration or microfiltration techniques. Furthermore, finding effective ways to utilize the by-products of microfiltration, such as the citrate-containing permeate and the precipitated caseins, should be an active area of research to find relevant applications in foods (Singh, 2006).

Ultrafiltration is utilized to concentrate large and macromolecules, such as the proteins in milk and whey. It uses pressure of 1–10 bar and membrane pore size between 10^{-2} and $10^{-1} \mu\text{m}$. Despite its functionality, this technique has not been fully used to purify MFGM components. Our group currently explores ultrafiltration to obtain whey buttermilk powder in conjunction with supercritical fluid extraction (SFE). The use of SFE as a solvent to extract nonpolar lipids has revolutionized the solvent-extraction methods because it does not leave solvent residues in the final product. This technique concentrates the phospholipids but does not separate out proteins from them (Singh, 2006). Spence et al. (2009)

utilized microfiltration and SFE to remove triglycerides and concentrate the phospholipid content in buttermilk powder, generating powders with a 70% decrease in total lipids (Spence et al., 2009).

3.7 METHODOLOGY TO MONITOR THE BIOLOGICAL ACTIVITY OF THE MFGM BEFORE AND AFTER PROCESSING

Given some of the advances in the scientific and technological fields, there are a variety of new tools to monitor the biological activity of MFGM in foods, which will increase our knowledge of the membrane structure, especially before processing. This allows us to find methods to preserve the native structure of the MFGM, and sets a standard for generating a product similar to what the neonate receives.

3.7.1 Atomic force microscopy

Atomic force microscopy (AFM) is the ideal technique to provide a means of visualizing, mapping, and measuring monolayer domain formation binding events, and other membrane–membrane interactions. An AFM operates by measuring attractive or repulsive forces between a tip and the sample. In its repulsive “contact” mode, the instrument lightly touches a tip at the end of a leaf spring or “cantilever” to the sample. A laser beam is reflected from the back of the cantilever and detected by a split photodiode. As the tip raster scans over the sample, the vertical deflection of the cantilever, and thus the repulsive force, is measured by the change in direction of the reflected laser beam. In “constant force” mode, the signal from the photodiode is used to adjust the height of the cantilever, allowing the AFM tip to move over the surface while applying a constant force. Thus, in contact mode, the AFM can be used to produce a height of topography image of the surface, as well as measure forces (Jiménez-Flores and Brisson, 2008).

The use of AFM is a powerful tool for the study of raft structure and properties. For example, in milk lipids isolated from buttermilk, confirmation of domains detected in monolayers was performed using AFM technology (Figure 3.5) (Gallier et al., 2009). A Langmuir film balance mounted on an epifluorescence microscope was used to analyze the physical behavior of the monolayer films and the coexistence of different phases, which allows analysis of the formation and fluidity of microdomains, such as lipid rafts.

3.7.2 Confocal laser scanning microscopy

The precise location of the main components of the MFGM remains hypothetical due to the lack of methodologies allowing its characterization. Evers (2004) published an extensive review about the structure and molecular organization of the MFGM (isolated using physical or chemical techniques), which were obtained using morphological or biochemical techniques. The characterization of the organization of the components requires the use of microscopy. Using confocal laser scanning microscopy (CLSM), Lopez et al. (2008) developed a protocol allowing the labeling of phospholipids in milk and dairy products. In addition Lopez et al. (2010) utilized confocal microscopy to reveal the organization of the MFGM, especially the lateral structure of polar lipids, glycolipids, and glycoproteins (Lopez et al., 2010). Evers et al. (2008) used lipophilic probes and fluorescent lectin wheat

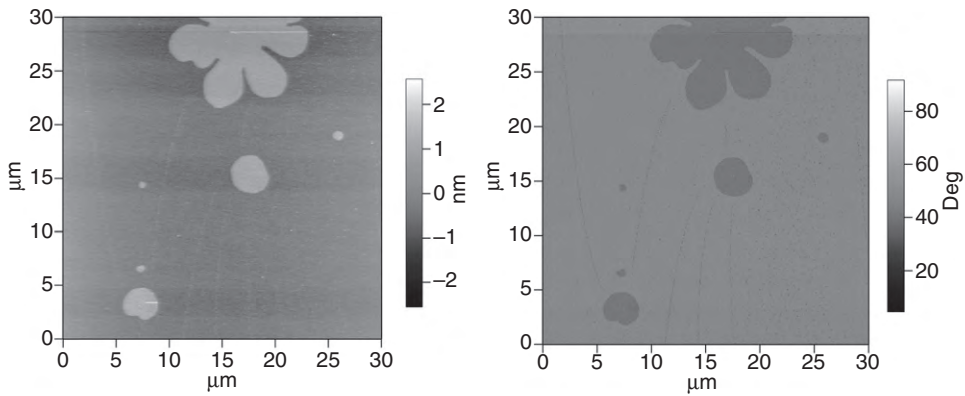


Figure 3.5 AFM of domains from monolayer studies of MFGM. Mojonier/solid-phase extraction (SPE-extracted BMP, deposited at 50 mN/m and 15°C). Height image (left) and phase image (right).

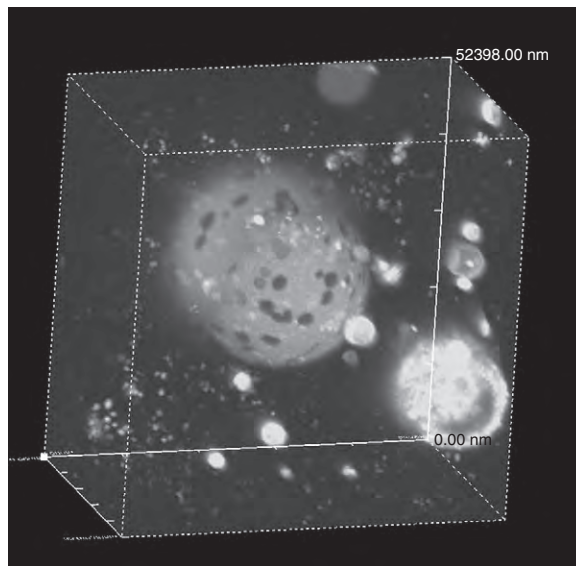


Figure 3.6 Confocal laser scanning image in 3D of a large milk fat globule from raw cream stained with phosphatidylethanolamine-lissamine rhodamine B (original picture in red). (Scale bar: 5 μm.)

germ agglutinin (WGA) to investigate the organization of the MFGM in milks from animal species (Lopez et al., 2010). The MFGM surface, especially the phospholipid monolayer, was characterized by microscopic techniques at our facility (Figure 3.6) (Gallier et al., 2009).

3.7.3 Laser tweezers and the MFGM

Laser tweezers, sometimes called optical tweezers, are used for a technique built upon the principle that small particles/objects can be trapped in the waist of a strongly focused laser beam. The optical trap results from the fact that the objects that are caught in the focus of

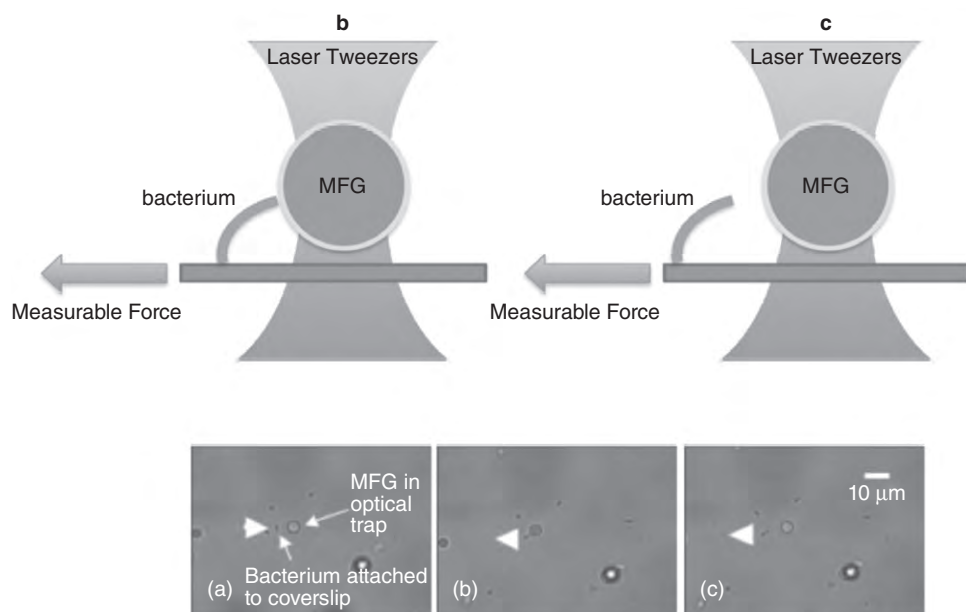


Figure 3.7 Principle of laser tweezers operation. In panel a, an MFG is held in the optical trap, while a bacterium (SD2112), which is attached to the coverslip, is made to approach the MFG by moving the microscope stage. The thick arrow shows the direction of stage travel. In panel b, attachment has occurred and the bacterium is being pulled from the MFG. In panel c, the bacterium has detached. The diagram presents only events on panels b and c.

the laser beam experience a restoring force if they try to leave the high intensity volume. The optical tweezer system has been used for direct manipulation of a variety of micrometer-sized objects and for force measurement in the piconewton (pN) region (Jiménez-Flores and Brisson, 2008). Indeed, Argov et al. (2008b) utilized laser tweezers Raman spectroscopy to characterize submicron-sized particles in human milk. They observed a significant difference in the triglyceride composition of human fat globules depending on their diameter, especially in nanoparticles (less than 1 μm), implying that these components have other functions besides fat delivery. Such findings open the door to nanoscience, which can help us reveal the association between food structure and functionality (Argov et al., 2008a). Furthermore, our research group uses laser tweezers to objectively measure binding force between lactic acid bacteria and the MFGM (Figure 3.7) (Brisson et al., 2010). Through a systematic examination of the role of each of the MFGM components in binding through membrane–membrane interactions under a variety of conditions, we hope to determine the necessary combination of components involved in efficient binding and action of the MFGM, thus generating a better delivery system.

3.8 THE FUTURE OF MFGM AND ITS COMPONENTS

Based on the number of patents and scientific publications on the description, characterization, isolation, and use of the MFGM or any of its components, it seems very likely that formulated foods containing high concentrations of milk-derived phospholipids,

glycolipids, glycoproteins, or other specific components from the MFGM, which are proven to provide significant health and functional advantages, will find their way into the market in the near future. There are two very good reasons to anticipate the development of such products. One is the general consumer's expectation of health-oriented and nutritious foods that fit their ideas and lifestyle. The second is that these valuable MFGM components in buttermilk, cream, and whey are mostly in the category of phospholipids and gangliosides, and are not being utilized commercially (mostly considered by-products of cheese, whey, or butter), thus are economically attractive. We hope that in the near future, more studies will reveal the complete detailed functional, biological and nutritional aspects of the MFGM. In turn, this knowledge should further expand our understanding of the fundamentals that will allow us the design of optimal processes to maintain the function of the MFGM throughout the line of production and absorption of nutrients in the human intestine.

For example, emulsion-based functional foods are a broad class of products finding new application in the marketplace. The amphiphilic nature of the components in MFGM, phospholipids, and glycoproteins, gives them excellent emulsifying properties (Kanno, 1990; Corredig and Dalgleish, 1997a, 1998). Therefore, Yano et al. (1994) have taken advantage of the extraordinary emulsifying ability of the MFGM and developed a method to protect and deliver numerous bioactive compounds, using it as their basic substrate (Yano et al., 1994). It has also been shown that in some instances, the phospholipids (PL) derived from milk yield more stable emulsions and liposomes than those derived from plant sources (Singh, 2006). Lysophospholipids released from the MFGM by phospholipase treatment of milk significantly help in the emulsification of water and fat during the processing of mozzarella cheese (Lilbæk et al., 2006). Mora-Gutiérrez and Gurin (2007) have developed a very complex set of combinations for various nutraceuticals in emulsion-based systems (Mora-Gutiérrez and Gurin, 2007). The emulsification properties of the MFGM have been used to prepare stable compositions of hydrophobic bioactive compounds based on specific grain size fractions containing a high density of lipid microspheres with high stability. Also, the use of nanotechnological procedures will provide a novel means of incorporating nutraceuticals into functional foods. Nanoparticles made with biopolymers can, for instance, become an effective way to deliver specific bioactive compounds to specific sites where they will exert their beneficial properties. Shefer and Shefer (2005) have developed a novel controlled-release system based on solid nanospheres of hydrophobic nature encapsulated in moisture-sensitive microspheres with enhanced stability and delivery control (Shefer and Shefer, 2005).

All of the above arguments point to a bright future for the incorporation of MFGM components into functional foods containing dairy components, but various obstacles will have to be resolved before the benefits of the research are seen in the market. Among these obstacles are the complex regulatory aspects surrounding functional foods in general, as well as the multiple technological limitations posed by the physical and chemical environment necessary for the desirable bioactive ingredients to maintain their properties. Few studies have been performed on this specific topic, but recently, the processing and storage stability of five candidate ingredients for milk fortification has been analyzed. Isoflavones and chondroitin sulfate were stable during the processing and storage conditions used in the experiments, whereas glucosamine, lactoferrin, and creatine exhibited very unstable behavior, thus losing a significant part of their desirable bioactive properties (Uzzan et al., 2007). Studies of this type will have to be performed with MFGM components in potential functional dairy products in order to determine conditions for maintaining optimum organoleptic properties, as well as to provide full assurance to consumers that the health benefits

of the added compounds will remain present when the food or beverage vehicles are finally consumed.

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REFERENCES

- Admyre, C., Johansson, S.M., Qazi, K.R., Filen, J.-J., Lahesmaa, R., Norman, M., Etienne, P.A., Neve, A.S., and Gabrielsson, S. (2007) Exosomes with immune modulatory features are present in human breast milk. *Journal of Immunology (Baltimore, Md.: 1950)* **179**(3), 1969–1978.
- Affolter, M., Grass, L., Vanrobaeys, F., Casado, B., and Kussmann, M. (2009) Qualitative and quantitative profiling of the bovine milk fat globule membrane proteome. *Journal of Proteomics* **73**(6), 1079–1088.
- American Dairy Products Institute (2007) <http://www.adpi.org/NewsStatistics/StatisticalInformation/statistics2007>
- Argov, N., Lemay, D.G., and German, J.B. (2008a) Milk fat globule structure and function: nanoscience comes to milk production. *Trends in Food Science & Technology* **19**(12), 617–623.
- Argov, N., Wachsmann-Hogiu, S., Freeman, S.L., Huser, T., Lebrilla, C.B., and German, J.B. (2008b) Size-dependent lipid content in human milk fat globules. *Journal of Agricultural and Food Chemistry* **56**(16), 7446–7450.
- Bezelgues, J.-B., Morgan, F., Palomo, G., Crosset-Perrotin, L., and Ducret, P. (2009) Short communication: milk fat globule membrane as a potential delivery system for liposoluble nutrients. *Journal of Dairy Science* **92**(6), 2524–2528.
- Bionaz, M. and Loor, J.J. (2008) Gene networks driving bovine milk fat synthesis during the lactation cycle. *BMC Genomics* **9**, 366–387.
- Brisson, G., Payken, H.F., Sharpe, J.P., and Jimenez-Flores, R. (2010) Characterization of *Lactobacillus reuteri* interaction with milk fat globule membrane components in dairy products. TBD.
- Chang, B.H.-J. and Chan, L. (2007) Regulation of triglyceride metabolism. III. Emerging role of lipid droplet protein ADFP in health and disease. *American Journal of Physiology. Gastrointestinal and Liver Physiology* **292**(6), G1465–G1468.
- Corredig, M. and Dalgleish, D.G. (1997a) Isolates from industrial buttermilk: emulsifying properties of materials derived from the milk fat globule membrane. *Journal of Agricultural and Food Chemistry* **45**(12), 4595–4600.
- Corredig, M. and Dalgleish, D.G. (1997b) Studies on the susceptibility of membrane-derived proteins to proteolysis as related to changes in their emulsifying properties. *Food Research International (Ottawa, Ont.)* **30**, 689–697.
- Corredig, M. and Dalgleish, D.G. (1998) Effect of heating of cream on the properties of milk fat globule membrane isolates. *Journal of Agricultural and Food Chemistry* **46**(7), 2533–2540.
- Corredig, M., Roesch, R.R., and Dalgleish, D.G. (2003) Production of a novel ingredient from buttermilk. *Journal of Dairy Science* **86**(9), 2744–2750.
- Dewettinck, K., Rombaut, R., Thienpont, N., Le, T.T., Messens, K., and Van Camp, J. (2008) Nutritional and technological aspects of milk fat globule membrane material. *International Dairy Journal* **18**(5), 436–457.
- Elizondo-Bacherio, D.T., Uson, S. III, and Jimenez-Flores, R. (2007) Lipid binding characterization of lactic acid bacteria in dairy products. Paper presented at the *Proceedings of the annual American Society for Microbiology*, Toronto, Canada.

- Evers, J.M. (2004) The milkfat globule membrane—compositional and structural changes post secretion by the mammary secretory cell. *International Dairy Journal* **14**(8), 661–674.
- Evers, J.M., Haverkamp, R.G., Holroyd, S.E., Jameson, G.B., Mackensie, D.D.S., McCarthy, O.J. (2008) Heterogeneity of milk fat globule membrane structure and composition as observed using fluorescence microscopy techniques. *International Dairy Journal* **18**(12), 1081–1089.
- Fauquant, C., Briard-Bion, V., Leconte, N., and Michalski, M.-C. (2005) Differently sized native milk fat globules separated by microfiltration: fatty acid composition of the milk fat globule membrane and triglyceride core. *European Journal of Lipid Science and Technology* **107**(2), 80–86.
- Fauquant, C., Briard-Bion, V., Leconte, N., Guichardant, M., and Michalski, M.-C. (2007) Membrane phospholipids and sterols in microfiltered milk fat globules. *European Journal of Lipid Science and Technology* **109**(12), 1167–1173.
- Fedotova, Y. and Lencki, R. (2008) The Effect of phospholipids on milkfat crystallization behavior. *Journal of the American Oil Chemists' Society* **85**(3), 205–212.
- Fedotova, Y. and Lencki, R. (2010) The effect of phospholipids on butter physical and sensory properties. *Journal of the American Oil Chemists' Society* **87**(1), 75–82.
- Fong, B.Y., Norris, C.S., and MacGibbon, A.K.H. (2007) Protein and lipid composition of bovine milk-fat-globule membrane. *International Dairy Journal* **17**(4), 275–288.
- Franke, W., Heid, H., Grund, C., Winter, S., Freudenstein, C., Schmid, E., Jarasch, E.D., and Keenan, T.W. (1981) Antibodies to the major insoluble milk fat globule membrane-associated protein: specific location in apical regions of lactating epithelial cells. *The Journal of Cell Biology* **89**(3), 485–494.
- Frece, J., Kos, B., Svetec, I.K., Zgaga, Z., Mrsa, V., and Suskovic, J. (2005) Importance of S-layer proteins in probiotic activity of *Lactobacillus acidophilus* M92. *Journal of Applied Microbiology* **98**, 285–292.
- Gallier, S., Gragson, D., Everett, D.W., and Jimenez-Flores, R. (2009) Surface Characterization of Bovine Milk Phospholipid Monolayers and Native Milk Fat Globule Membrane by Microscopic Techniques. California Polytechnic State University San Luis Obispo.
- Heid, H.W. and Keenan, T.W. (2005) Intracellular origin and secretion of milk fat globules. *European Journal of Cell Biology* **84**(2–3), 245–258.
- Hvarregaard, J., Andersen, M.H., Berglund, L., Rasmussen, J.T., and Petersen, T.E. (1996) Characterization of glycoprotein PAS-6/7 from membranes of bovine milk fat globules. *European Journal of Biochemistry* **240**(3), 628–636.
- Isaacs, C.E., Litov, R.E., and Thormar, H. (1995) Antimicrobial activity of lipids added to human milk, infant formula, and bovine milk. *The Journal of Nutritional Biochemistry* **6**(7), 362–366.
- Jarasch, E.-D., Bruder, G., Keenan, T.W., and Franke, W.W. (1977) Redox constituents in milk fat globule membranes and rough endoplasmic reticulum from lactating mammary gland. *The Journal of Cell Biology* **73**(1), 223–241.
- Jensen, R.G. (2002) The composition of bovine milk lipids: January 1995 to December 2000. *Journal of Dairy Science* **85**(2), 295–350.
- Jeong, J., Rao, A.U., Xu, J.L., Ogg, S.L., Hathout, Y., Fenselau, C., and Mather, I.H. (2009) The PRY/SPRY/B30.2 domain of butyrophilin 1A1 (BTN1A1) binds to xanthine oxidoreductase implications for the function of BTN1A1 in the mammary gland and other tissues. *The Journal of Biological Chemistry* **284**(33), 22444–22456.
- Jiménez-Flores, R. and Brisson, G. (2008) The milk fat globule membrane as an ingredient: why, how, when? [Proceedings Paper]. *Dairy Science & Technology* **88**(1), 5–18.
- Jiménez-Flores, R. and Higuera-Ciajara, I. (2009) Beverages based on milk fat globule membrane (MFGM) and other novel concepts for dairy-based functional beverages. In: *Functional and Speciality Beverage Technology*, edited by P. Paquin, pp. 281–296. Boca Raton, FL; New York; Washington, DC: CRC Press, Woodhead Publishing Limited.
- Jinjarak, S., Olabi, A., Jimenez-Flores, R., Sodini, I., and Walker, J.H. (2006) Sensory evaluation of whey and sweet cream buttermilk. *Journal of Dairy Science* **89**(7), 2441–2450.
- Kanno, C. (1990) Secretory membranes of the lactating mammary-gland. *Protoplasma* **159**(2–3), 184–208.
- Kanno, C., Shimomura, Y., and Takano, E. (1991) Physicochemical properties of milk-fat emulsions stabilized with bovine-milk fat globule-membrane. *Journal of Food Science* **56**, 1219–1223.
- Keenan, T.W. and Mather, I.H. (2006) Intracellular origin of milk fat globules and the nature of the milk fat globule membrane. In: *Advanced Dairy Chemistry: Lipids 2*, edited by P.F. Fox and P.L.H. McSweeney, pp. 137–171. New York: Springer.
- Keenan, T.W., Olson, D.E., and Mollenhauer, H.H. (1971) Origin of the milk fat globule membrane. *Journal of Dairy Science* **54**(3), 295–299.

- Kemsley, J. (2008) Unraveling breast milk. *C&EN: Chemical & Engineering News*, September 29, 13–17.
- Kim, H.-H.Y. and Jimenez-Flores, R. (1995) Heat-induced interactions between the proteins of milk fat globule membrane and skim milk. *Journal of Dairy Science* **78**(1), 24–35.
- Kirjavainen, P.V., Ouwehand, A.C., Isolauri, E., and Salminen, S.J. (1998) The ability of probiotic bacteria to bind to human intestinal mucus. *FEMS Microbiology Letters* **167**, 185–189.
- Lee, S.J. and Sherbon, J.W. (2002) Chemical changes in bovine milk fat globule membrane caused by heat treatment and homogenization of whole milk. *The Journal of Dairy Research* **69**(4), 555–567.
- Lilbæk, M.L., Høier, E., Fatum, T.M., Ipsen, R., and Sorensen, N.K. (2006) Improving the yield of Mozzarella cheese by phospholipase treatment of milk. *Journal of Dairy Science* **89**, 4114–4125.
- Lonnerdal, B., Valencia, N., Graverholt, G., and Zavaleta, N. (2006) Effect of fortifying complementary food with a bioactive milk protein fraction with micronutrients on growth and micronutrient status of peruvian infants. *Journal of Pediatric Gastroenterology and Nutrition* **42**(5), E88.
- Lopez, C., Briard-Bion, V., Menard, O., Rousseau, F., Pradel, P., and Besle, J.-M. (2008) Phospholipid, sphingolipid, and fatty acid compositions of the milk fat globule membrane are modified by diet. *Journal of Agricultural and Food Chemistry* **56**(13), 5226–5236.
- Lopez, C., Madec, M.-N., and Jimenez-Flores, R. (2010) Lipid rafts in the bovine milk fat globule membrane revealed by the lateral segregation of phospholipids and heterogeneous distribution of glycoproteins. *Food Chemistry* **120**(1), 22–33.
- Mao, F.C., Chen, W., Chiang, Y., and Chiang, L.C. (2007) Composition for preventing and treating cardiovascular disorders. USPO.
- Martin, H.M., Hancock, J.T., Salisbury, V., and Harrison, R. (2004) Role of xanthine oxidoreductase as an antimicrobial agent. *Infection and Immunity* **72**(9), 4933–4939.
- Mather, I.H. (2000) A review and proposed nomenclature for major proteins of the milk-fat globule membrane. *Journal of Dairy Science* **83**(2), 203–247.
- Mather, I.H. and Jacks, L.J.W. (1993) A review of the molecular and cellular biology of butyrophilin, the major protein of bovine milk fat globule membrane. *Journal of Dairy Science* **76**(12), 3832–3850.
- Mather, I.H. and Keenan, T.W. (1975) Studies on the structure of milk fat globule membrane. *The Journal of Membrane Biology* **21**(1), 65–85.
- Mather, I.H. and Keenan, T.W. (1998) Origin and secretion of milk lipids. *Journal of Mammary Gland Biology and Neoplasia* **3**(3), 259–273.
- Mather, I.H. and Linington, C. (1999) Methods of modifying the BTN protein to eliminate an autoimmune response and products associated therewith. World Patent WO 99/05162A1.
- Michalski, M.-C. and Januel, C. (2006) Does homogenization affect the human health properties of cow's milk? *Trends in Food Science & Technology* **17**(8), 423–437.
- Michalski, M.C., Leconte, N., Briard-Bion, V., Fauquant, J., Maubois, J.L., and Goudedranche, H. (2006) Microfiltration of raw whole milk to select fractions with different fat globule size distributions: process optimization and analysis. *Journal of Dairy Science* **89**(10), 3778–3790.
- Mora-Gutiérrez, A. and Gurin, M.H. (2007) Bioactive complexes compositions and methods of use thereof. USPO. U.S. P. A. N. A1. USA.
- Morin, P., Pouliot, Y., and Jiménez-Flores, R. (2006) A comparative study of the fractionation of regular buttermilk and whey buttermilk by microfiltration. *Journal of Food Engineering* **77**(3), 521–528.
- Morin, P., Britten, M., Jiménez-Flores, R., and Pouliot, Y. (2007a) Microfiltration of buttermilk and washed cream buttermilk for concentration of milk fat globule membrane components. *Journal of Dairy Science* **90**(5), 2132–2140.
- Morin, P., Jiménez-Flores, R., and Pouliot, Y. (2007b) Effect of processing on the composition and microstructure of buttermilk and its milk fat globule membranes. *International Dairy Journal* **17**(10), 1179–1187.
- Murgiano, L., Timperio, A.M., Zolla, L., Bongiorno, S., Valentini, A., and Pariset, L. (2009) Comparison of milk fat globule membrane (MFGM) proteins of chianina and holstein cattle breed milk samples through proteomics methods. *Nutrients* **1**(2), 302–315.
- Nielsen, R.L., Andersen, M.H., Mabhout, P., Berglund, L., Petersen, T.E., and Rasmussen, J.T. (1999) Isolation of adipophilin and butyrophilin from bovine milk and characterization of a cDNA encoding adipophilin. *Journal of Dairy Science* **82**(12), 2543–2549.
- Pan, Y., Lee, A., Wan, J., Coventry, M.J., Michalski, W.P., Shiell, B., and Roginski, H. (2006) Antiviral properties of milk proteins and peptides. *International Dairy Journal* **16**(11), 1252–1261.
- Patton, S. (2004) *Milk: Its Remarkable Contribution to Human Health and Well-Being*. New Brunswick and London: Transaction.

- Patton, S. and Huston, G. (1986) A method for isolation of milk fat globules. *Lipids* **21**(2), 170–174.
- Reinhardt, T.A. and Lippolis, J.D. (2006) Bovine milk fat globule membrane proteome. *The Journal of Dairy Research* **73**(04), 406–416.
- Riccio, P. (2004) The proteins of the milk fat globule membrane in the balance. *Trends in Food Science & Technology* **15**(9), 458–461.
- Robenek, H., Hofnagel, O., Buers, I., Lorkowski, S., Schnoor, M., Robenek, M., Heid, H., Troyer, D., and Severs, N.J. (2006a) Butyrophilin controls milk fat globule secretion. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 10385–10390.
- Robenek, H., Hofnagel, O., Buers, I., Robenek, M.J., Troyer, D., and Severs, N.J. (2006b) Adipophilin-enriched domains in the ER membrane are sites of lipid droplet biogenesis. *Journal of Cell Science* **119**(20), 4215–4224.
- Rombaut, R., Camp, J.V., and Dewettinck, K. (2006a) Phospho- and sphingolipid distribution during processing of milk, butter and whey. *International Journal of Food Science & Technology* **41**(4), 435–443.
- Rombaut, R., Dejonckheere, V., and Dewettinck, K. (2006b) Microfiltration of butter serum upon casein micelle destabilization. *Journal of Dairy Science* **89**(6), 1915–1925.
- Roos, S. and Jonsson, H. (2002) A high-molecular mass cell-surface protein from *Lactobacillus reuteri* 1063 adheres to mucus components. *Microbiology* **148**, 433–442.
- Rutter, E.R.F. (2006) Multiple sclerosis and milk: to drink or not to drink? *International Journal of Dairy Technology* **59**(4), 223–228.
- Ryan, P.A., Pancholi, V., and Fischetti, V.A. (2001) Group A *Streptococci* bind to mucin and human pharyngeal cells through sialic acid-containing receptors. *Infection and Immunity* **69**(12), 7402–7412.
- Sanchez-Juanes, F., Alonso, J.M., Zancada, L., and Hueso, P. (2009) Distribution and fatty acid content of phospholipids from bovine milk and bovine milk fat globule membranes. *International Dairy Journal* **19**(5), 273–278.
- Scammell, A.W. (2001) Liquid colostrum for dairy products. USPO.
- Shefer, A. and Shefer, D. (2005) Multicomponent controlled release system for oral care, food products, nutraceutical and beverages. USPO. U.S. Patent 6, 493B2. U.S. Patent 6,887,493B2.
- Silanikove, N. and Shapiro, F. (2007) Distribution of xanthine oxidase and xanthine dehydrogenase activity in bovine milk: physiological and technological implications. *International Dairy Journal* **17**(10), 1188–1194.
- Silanikove, N., Merin, U., and Leitner, G. (2006) Physiological role of indigenous milk enzymes: an overview of an evolving picture. *International Dairy Journal* **16**, 533–545.
- Sillanpaa, J., Martinez, B., Antikainen, J., Toba, T., Kalkkinen, N., Tankka, S., Lounatmaa, K., Keranen, J., Hook, M., Westerlund-Wikstrom, B., Pouwels, P.H., and Korhonen, T.K. (2000) Characterization of the collagen-binding S-layer protein CbsA of *Lactobacillus crispatus*. *Journal of Bacteriology* **182**(22), 6440–6450.
- Singh, H. (2006) The milk fat globule membrane—a biophysical system for food applications. *Current Opinion in Colloid & Interface Science* **11**(2–3), 154–163.
- Snow, D.R., Jimenez-Flores, R., Ward, R.E., Cambell, J., Young, M.J., Nemere, I., and Hintze, K.J. (2010) Dietary milk fat globule membrane reduces the incidence of aberrant crypt foci in fischer-344 rats. *Journal of Agricultural and Food Chemistry* **58**(4), 2157–2163.
- Sodini, I., Morin, P., Olabi, A., and Jimenez-Flores, R. (2006) Compositional and functional properties of buttermilk: a comparison between sweet, sour, and whey buttermilk. *Journal of Dairy Science* **89**(2), 525–536.
- Spence, A.J., Jimenez-Flores, R., Qian, M., and Goddik, L. (2009) The influence of temperature and pressure factors in supercritical fluid extraction for optimizing nonpolar lipid extraction from buttermilk powder. *Journal of Dairy Science* **92**(2), 458–468.
- Spitsberg, V.L. (2005) Invited review: bovine milk fat globule membrane as a potential nutraceutical. *Journal of Dairy Science* **88**(7), 2289–2294.
- Spitsberg, V.L. and Gorewit, R.C. (1997) *In vitro* phosphorylated bovine milk fat globule membrane proteins. *The Journal of Nutritional Biochemistry* **8**(4), 181–189.
- Spitsberg, V.L. and Gorewit, R.C. (1998) Solubilization and purification of xanthine oxidase from bovine milk fat globule membrane. *Protein Expression and Purification* **13**(2), 229–234.
- Spitsberg, V.L. and Gorewit, R.C. (1999) Method of detecting expression of and isolating the protein encoded by the BRCA1 gene. USPO.
- Sprecher, H.W., Strong, F.M., and Swanson, A.M. (1965) Phospholipids of fresh milk and of sterile whole milk. *Journal of Agricultural and Food Chemistry* **13**, 17–21.

- Tao, N., DePeters, E.J., Freeman, S., German, J.B., Grimm, R., and Lebrilla, C. (2008) Bovine milk glycome. *Journal of Dairy Science* **91**(10), 3768–3778.
- Thompson, A.K. and Singh, H. (2006) Preparation of liposomes from milk fat globule membrane phospholipids using a microfluidizer. *Journal of Dairy Science* **89**(2), 410–419.
- Uzzan, N., Nechrebeki, J., and Labuza, T.P. (2007) Thermal and storage stability of nutraceuticals in a milk beverage dietary supplement. *Journal of Food Science* **72**, E109–E114.
- Vissac, C., Lemery, D., Le Corre, L., Fustier, P., Dechelotte, P., Maurizis, J.C., Bignon, Y.J., and Bernard-Gallon, D.J. (2002) Presence of BRCA1 and BRCA2 proteins in human milk fat globules after delivery. *Biochimica et Biophysica Acta* **1586**, 50–56.
- Wat, E., Tandy, S., Kapera, E., Kamili, A., Chung, R.W.S., Brown, A., Rowney, M., and Cohn, J.S. (2009) Dietary phospholipid-rich dairy milk extract reduces hepatomegaly, hepatic steatosis and hyperlipidemia in mice fed a high-fat diet. *Atherosclerosis* **205**(1), 144–150.
- Webb, B.H., Johnson, A.H., and Alford, J.A. (1974) *Fundamentals of Dairy Chemistry*, 2nd ed. Westport, CT: The Avi Publishing Company, Inc.
- Wilson, N.L., Robinson, L.J., Donnet, A., Bovetto, L., Packer, N.H., and Karlsson, N.G. (2008) Glycoproteomics of milk: differences in sugar epitopes on human and bovine milk fat globule membranes. *Journal of Proteome Research* **7**(9), 3687–3696.
- Wong, P.Y.Y. and Kitts, D.D. (2003) Chemistry of buttermilk solid antioxidant activity. *Journal of Dairy Science* **86**, 1541–1547.
- Wooding, F.B.P. and Kemp, P. (1975) Ultrastructure of the milk fat globule membrane with and without triglyceride. *Cell and Tissue Research* **165**(1), 113–127.
- Yano, Y., Masuda, S., and Hidaka, T. (1994) Stable pharmaceutical composition and method for its production. U.S. Patent 5, 246. US Patent 5,298,246.
- Ye, A., Singh, H., Taylor, M.W., and Anema, S. (2002) Characterization of protein components of natural and heat-treated milk fat globule membranes. *International Dairy Journal* **12**(4), 393–402.
- Ye, A., Anema, S.G., and Singh, H. (2004a) High-pressure-induced interactions between milk fat globule membrane proteins and skim milk proteins in whole milk. *Journal of Dairy Science* **87**(12), 4013–4022.
- Ye, A., Singh, H., James Oldfield, D., and Anema, S. (2004b) Kinetics of heat-induced association of [beta]-lactoglobulin and [alpha]-lactalbumin with milk fat globule membrane in whole milk. *International Dairy Journal* **14**(5), 389–398.
- Zavaleta, N., Valencia, N., Graverholt, G., Staudt-Kvistgaard, A., and Lonnerdal, B. (2006) Incidence and duration of diarrhea in peruvian infants consuming complementary food with bioactive milk fat globule membrane Proteins. *Journal of Pediatric Gastroenterology and Nutrition* **42**(5), E39.

4 Biofunctional Dairy Protein Fractions

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4.1 INTRODUCTION

Biologically active milk proteins and peptides have attracted considerable research attention in recent decades. The ultimate goal of this effort has been to develop biologically active dairy ingredients and complete dairy products that improve human health and nutrition.

A complex picture emerges from this research as some of the peptides studied elicit multiple but sometimes competing physiological responses. A number of products containing dairy-derived bioactive peptides are already on the market, such as fermented milk products with ACE-inhibitory peptides. Other products, such as GMP-based foods for people with special dietary needs, are likely to be commercialized in the near future.

Further studies should be undertaken on most dairy derived proteins and peptides to validate their physiological efficacy in humans. In addition, the gradually decreasing cost of alternative technologies to produce bioactive peptides (e.g., through chemical synthesis or recombination) will demand that the dairy industry develops economically competitive isolation, and where necessary, purification technologies. With a gradual progress in elucidating biological functions of dairy proteins and peptides and with development of new commercial products, further research will be required to achieve a better understanding of the interactions of bioactive proteins and peptides with other food components and their stability during processing, distribution, and storage.

4.2 PHYSIOLOGICALLY ACTIVE PEPTIDES FROM MILK

A host of physiological activities have been reported for peptides produced from hydrolysis of dairy proteins (Hayes et al., 2007). Bioactive peptides in milk may be present as a result of endogenous enzyme activity, for example, plasmin (Kostyra and Kostyra, 1992), and enzymes associated with microbiological quality of the milk. In addition, a wide opportunity for process-mediated manipulation of bioactivity arises because of the accepted use of exogenous cultures and enzymes associated with various traditional dairy products, such as yoghurt and cheese.

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4.2.1 Antihypertensive peptides

Antihypertensive peptides can be produced by direct treatment with enzymes (Hernández-Ledesma et al., 2004), by the proteolytic activity of lactic acid bacteria (Ashar and Chand, 2004), or fermenting with particular probiotic cultures (Donkor et al., 2007) and strains of *Lactobacillus* spp. (Nielsen et al., 2009), and are present in fresh cheeses (Hernández-Ledesma et al., 2004). Concentration of angiotensin-converting enzyme inhibitory (ACEi) peptides have been developed by combining fermentation or enzyme-mediated hydrolysis (Gobbetti et al., 2002) with membranes (Recio et al., 2000) and chromatography, for progressive purification of the bioactive fraction or individual peptides.

A rich literature has accumulated evidence for *in vitro* bioactivity of peptides present in milk and encrypted in milk proteins. However, in the case of peptides with antihypertensive activity, bioactive sequences are both released and potentially degraded (Hernández-Ledesma et al., 2004) by digestive enzymes, and this process would be expected to apply to peptides with other bioactive properties. In general, it is necessary for the bioactive sequence to survive digestion and reach the required biological target in adequate dose to exert physiological effect. In a few cases, for example, antihypertensive peptides from casein, *in vivo* efficacy has been demonstrated and consumer products developed, that is, Calpis and Evolus (Korhonen, 2009). For the most part, however, significant gaps exist in demonstrating the *in vivo* efficacy of dairy bioactive peptides. Research in this field represents a worthwhile pursuit so that dairy-derived bioactives can be marketed and exert benefits toward causes and symptoms of chronic disease, and delivered in convenient and safe consumer products. Indeed, if dairy industry-driven products are not available, recombinant forms of bioactive peptides from dairy can compete (Lv et al., 2003), should the commercial case be justified.

4.2.1.1 Biology of hypertension

Through its combined role in regulation of water, sodium balance, and blood pressure (BP), the renin-angiotensin-aldosterone system (RAAS) plays a key role in BP-related hypertension (Gong and Hubner, 2006). In the RAAS system, angiotensin-converting enzyme (ACE) catalyzes the cleavage of angiotensin and production of the physiologically active peptide angiotensin II (ATII). ATII antagonism of the AT1 receptor is associated with multiple G-protein coupled- and G-protein-independent signaling pathways that in addition to vasoconstriction can lead to blood vessel cell growth and thickening of the vessel wall by altering protein and fibrous tissue matrix (Siragy, 1999). ACE inhibitors lower circulating ATII and in turn BP by inducing relaxation and expansion of blood vessels. ACE inhibitors also achieve BP lowering by reducing blood volume. RAAS-system intervention medications (ACEi and AT1Ri) act at the adrenal cortex, which is the site of angiotensin production, at kidney tubules, and at angiotensin receptors in vessels. As such, absorption into the circulation is required for pharmacological activity of inhibitors of ACE, including peptides. ACE-inhibiting pharmaceuticals targeting the RAAS system (e.g., Captopril and Enalapril) are widely prescribed to control high BP and represent the most widely prescribed class of CVD medications. Dairy-derived peptides offer potential to regulate BP in this proven site of intervention. In addition, antihypertensive bioactivity may be also be due to the release of other vasodilatory mediators or action on opioid receptors (Erdmann et al., 2008).

4.2.1.2 *Antihypertensive peptides encrypted in dairy proteins*

A very wide number of dairy-derived (and nondairy-derived) peptides have been reported to exhibit ACE-inhibitory activity *in vitro* (Hong et al., 2008; Korhonen, 2009). The majority of sequences identified have been released from the major casein and whey proteins, and values of IC₅₀ ranged from 2 μ M to >2 mM. Summaries of known ACE inhibitory peptides can be found in Meisel (2005) and Hong et al. (2008).

4.2.1.3 *Substantiation of in vivo efficacy of antihypertensive peptides*

While proteins and peptides are equally effective inhibitors of ACE *in vitro*, absorption is favored for shorter peptides not degraded by normal digestion and brush-border peptidases, although mechanisms for transport of larger peptides and proteins also exist (Vermeirssen et al., 2004). Absorption of di- and tripeptides surviving digestion is favored through specific transport mechanisms (Pihlanto-Leppala, 2001). In studies testing either pure peptides or peptide present in prepared hydrolysates, using the spontaneous hypertensive rat model in either single oral dose or extended feeding trial, systolic BP lowering up to 24.6; 34.0 and 55.0 mm Hg were reported for treatment with fermented milks (i.e., whey plus caseins), casein, and whey hydrolysates, respectively (Meisel et al., 2006). Human studies with whole milk, casein, and whey-derived hydrolysates, in some cases enriched with established bioactive peptides (VPP, IPP, VAP, etc.), have subsequently shown moderate, sometimes significant, antihypertensive activity. Several products claiming antihypertensive bioactivity are now commercially available, based on this research.

4.2.2 **Biological role of antithrombotic peptides**

In all vertebrates, blood coagulation is an inducible process that is triggered by vascular injury in order to maintain hemostasis. The first reaction to injury is to initiate blood clotting (thrombus) followed by endothelial repair and finally dissolution of the thrombus (clot) or fibrinolysis (Gentry, 2004). In cardiovascular disease, anticoagulation processes can be deficient, leading to increased occurrence of blood clotting, and are treated using antithrombotic drugs that reduce platelet aggregation and increase fibrinolysis (Erdmann et al., 2008). The initiation of blood clotting by the action of thrombin on fibrinogen permits rapid assembly of a clot, comprising a covalent network of fibrin monomers (Gentry, 2004). Chymosin inhibitory peptides, released during the action of chymosin on kappa casein in cheesemaking, are also able to bind to the gamma chain of human fibrinogen and exert a clotting inhibitory effect.

4.2.2.1 *Antithrombotic peptides encrypted in dairy proteins*

A limited number of peptides encrypted in dairy proteins have been identified with antithrombotic activity. Peptides with antithrombotic activity corresponding to residues 106–116 (casoplatelin) of kappa casein and smaller subfragments (casoplatelins) contain selected sequence homologies to human fibrinogen, and thereby inhibit the interaction between both platelet receptors and the fibrinogen gamma chain (Maubois et al., 1991; Erdmann et al., 2008).

4.2.2.2 *Substantiation of in vivo efficacy of antithrombotic peptides*

Antithrombotic peptides derived from kappa casein, as present in infant formula, were detected at physiologically active concentrations in the plasma of 5-day-old infants, thereby supporting reasonable expectation of bioavailability (Chabance et al., 1995). Furthermore, processes for recovery of enriched preparations of antithrombotic peptides from dairy sources have been described (Bouhallab and Touze, 1995). In spite of this status of knowledge, further studies of *in vivo* efficacy of the antithrombotic properties of dairy peptides have not been forthcoming and may represent an opportunity to revisit in the future, perhaps in combination with peptides that can regulate hypertension.

4.2.3 **Biological role of immunomodulatory peptides**

Reported immunomodulatory properties of dairy-derived peptides are numerous and reflect the complexity of the innate and adaptive immune systems, and, in addition, the functional purpose of immune factors present in human milk (Hawkes et al., 1999), that is, to train the immune system of the neonate, as driven by transitional expression of particular cytokines as a function of stage of lactation from birth. In general, the approach to identify immunoactive peptides from dairy sources has been to identify if activity discovered in human milk (or colostrum) is present and active in bovine milk (or colostrum), or vice versa. In the context of this work, the discussion will focus on immunomodulatory properties of peptides produced as a result of protein hydrolysis, either exogenous or digestive, and not on the direct immunomodulatory properties of cytokines (Hawkes et al., 1999). Immunomodulatory properties of dairy proteins, including lactoferrin (LF), are discussed elsewhere. Likewise, adverse immunogenicity of dairy proteins causing allergic reactions in babies reflect the stimulation of B cell responses to specific epitopes, although T-cell aspects to the immune response are also active (Schade et al., 2000). While substantial ongoing research efforts seek to understand the basis for milk protein allergy, the principal practical approach to attenuate allergenicity has been to destroy epitopes by hydrolysis (partially or extensively). Depending on the degree of hydrolysis, hydrolyzed milk proteins can “train” the immune system and be used for prevention of allergy symptoms in at-risk babies (Bahna, 2008). The discovery of individual peptides that can induce immune tolerance may revolutionize the former approach to milk allergy and availability of products for allergic individuals. Immune responses surrounding allergy represent a particular category of immune system modulation and will not be discussed here.

4.2.3.1 *Immunomodulatory peptides encrypted in dairy proteins*

Immunomodulatory peptide sequences identified from bovine protein sources were reviewed in Meisel (2005). Such sequences were found to be present in β -casein (β -casomorphin-7; β -casokinin); α_{s1} -casein and α_{s2} -caseins (caseinophosphopeptides); LF (lactoferricin [LFcin]); α_{s1} -casein (immunocasokinin); and α -lactalbumin (α -La).

4.2.3.2 *Substantiation of in vivo efficacy of immunomodulatory peptides*

A body of evidence has shown the favorable immunoregulation of infants as a result of breast versus formula feeding (Lonnerdal, 2003). However, immunomodulation of dairy

peptide products has mostly been confined to the use of hydrolyzed milk proteins for both prevention and treatment of milk allergy in infants and babies (Bahna, 2008), and antimicrobial peptides enhancing the innate immune system (see Section 6.2.2). Otherwise, the substantiations of *in vivo* efficacy of immunomodulating peptide products from bovine sources that can potentiate the immune response, are mostly inferred from either cell-based studies, for example, Rusu et al. (2010), or comparative studies on human milk and bovine milk.

There are multiple opportunities for peptides to modulate the innate and adaptive aspects of the immune system, including via binding to receptors on B, T, natural killer cells, in either agonist, antagonist, or chemoattractant modalities; interceptive binding to or reactivity with, inflammatory mediators, including cytokines, prostaglandins, and NO. While antiinflammatory regulation may be useful for chronic inflammation, for other conditions, such as cancer, proinflammatory effects may be desirable. Clearly, in the case of a mixture of peptides produced by enzymatic hydrolysis of a mixture of proteins, carefully designed, hypothesis-driven studies are necessary to resolve multiple, potentially competing effects and ensure the desired bioactivity is achieved. To further complicate matters, it is possible that a peptide that can activate an immune receptor function may also activate an opioid receptor, as shown for β -casomorphin 7 (Meisel, 2005).

4.2.4 Biological role of opioid receptor-binding peptides

Opioid receptor activation produces a biological cascade of effects, including analgesia, pupil narrowing, sedation, lowering of BP and body temperature, and numbing of reflexes (Jordan and Devi, 1998). With multiple receptor types (μ , κ , and δ) and locations in nervous, endocrine, immune, and gastrointestinal tract, ligands of opioid receptors can exert multiple effects arising from a staged signaling pathway of which a key mechanism of heterogeneity of the response is due to regulation of G protein coupling. With widespread distribution over the mammalian organism, the opportunities for opioid receptor agonism are significant, with concomitant physiological effects. The fact that opioid-active peptides are produced in human milk implicates a functional purpose of these peptides for neonate development (Teschemacher et al., 1997) and highlights potential uses of opioid activities of dairy peptides, including: regulation of immunological, gastrointestinal functions, and stress responses of the nervous system. Indeed, effects on gut- and immune (gut-associated lymphoid tissue)-located receptors do not require peptide absorption, and the opportunity exists to selectively avoid effects mediated by nervous system activation (e.g., sedation) by selection of active peptides that are not absorbed or cannot cross the blood–brain barrier. Milk proteins encrypt peptides with both agonist and antagonist bioactivity with requirement to separate competing activities in order to observe targeted effects.

4.2.4.1 Opioid receptor-binding peptides encrypted in dairy proteins

A series of opioid-like peptides have been identified as beta-casomorphins, alpha-casomorphins, alpha-lactorphin, beta-lactorphin, and serorphin, all containing the structural element Tyr-X-Phe or Tyr-X1-X2-Phe (Zhou et al., 2006). These peptides are agonists or mimic ligands of opioid receptors. An additional series of peptides, the casoxins and lactoferoxins, have been identified from kappa casein and LF, respectively, and are antagonists of opioid receptors (Teschemacher et al., 1997)

The opioid active peptide beta casomorphin-7 was also released by proteolytic enzymes from cow's milk following intramammary infection with *Staphylococcus aureus*, as shown in isolated rat ileum (Kostyra and Kostyra, 1992). Both opioid agonist and antagonist peptides were detected in a selection of hard and soft cheeses (Sienkiewicz-Szlapka et al., 2009b) and in a digestive hydrolysate of an infant formula (Jarmolowska et al., 2007). The opioid agonist peptides discovered in milk have been used to develop peptide analogues with improved pharmacological activity and resistance to proteolytic digestion (Meisel, 2005).

4.2.4.2 Substantiation of *in vivo* efficacy of opioid receptor-binding peptides

The agonist capacity of dairy peptides has been demonstrated in several isolated organ preparations (Teschemacher et al., 1997), and recent data have shown capacity for intestinal uptake using a Caco2 monolayer model (Sienkiewicz-Szlapka et al., 2009a). Dairy peptides with opioid antagonism have the capacity for modulating gastrointestinal function (Teschemacher, 2003), fat metabolism, analgesic, and relaxation effects (Zhou et al., 2006), but not all studies proved specific regulation by opioid receptor via naloxone-mediated reversal. In addition, the opportunity for lowering BP by alpha-lactorphin without inducing effects on the nervous system was shown for hypertensive rats (Ijäs et al., 2004); however, BP lowering was also observed in normotensive rats, which is not usually the case for peptides acting on the ACE pathway, nor is desirable. The bioavailability of beta-casomorphins in human adults, babies, and animals appears to be variable (Teschemacher, 2003), while direct administration of beta-casomorphin to rats was not addictive (Reid and Hubbell, 1994). A product-based alpha-casomorphin for inducing relaxation in babies has been marketed as "Lactium," apparently based on successful *in vivo* research outcomes (Guesdon et al., 2006; Kim et al., 2007). Nevertheless, it appears that significant opportunities remain to be captured for relaxation and other non-nervous system opioid receptor-mediated activities of dairy peptides.

4.2.5 Biological role of metal-binding peptides

Metal-binding peptides represent a special category of bioactive agents that exert their biological effect through depletion of metals used by organisms, cells, cofactors, and other biochemical intermediates. Dairy caseins are well known for their capacity to bind and deliver bioavailable forms of calcium, iron, and zinc (Miquel et al., 2006), which is a primary function attributed to the caseins. The utilization of peptide-bound metals by immune and intestinal cells and regulation of cancer cell growth represent examples of demonstrated physiological functions (Meisel and FitzGerald, 2003). Commercial production of casein phosphopeptides (CPP) enriched with amorphous calcium phosphate (ACP), have been successfully used for oral antimicrobial and dental remineralization applications (Walker et al., 2009).

4.2.5.1 Metal-binding peptides encrypted in dairy proteins

The complexation of Ca by CPP involves the fully phosphorylated seryl-cluster motif, which is essential for the interaction between phosphate and Ca (Cross et al., 2005). Fe and Zn-binding sites involve different motifs from those binding Ca (Miquel et al., 2006).

Whey proteins and LF and encrypted peptides also have the capacity to bind Ca, Mg, Zn, Na, and K, particularly after enzymatic hydrolysis (Vegarud et al., 2000).

4.2.5.2 Substantiation of *in vivo* efficacy of metal-binding peptide

The CPP-ACP product (marketed as “Tooth Mousse”), when used in combination with a fluoridated toothpaste, remineralized dental caries lesions caused by demineralization treatment (Kumar et al., 2008). Capacity for stimulation of IL-5, IL-6, and IgA was observed for β casein (1-28) containing a phosphoserine-rich region in mouse spleen cell cultures (Otani and Watanabe, 2006). Furthermore, elevation of IgG and IgA levels in sows fed a CPP-rich diet lead to a relatively enhanced rate of bodyweight gain of piglets, compared with the control diet, which was attributed to the enhanced protective status of the immune system (Kitamura et al., 2002).

4.2.6 Conclusions

An extensive number of peptide fragments encrypted within milk proteins can be released through pre- or postingestion processes. Peptides so released have been shown to exert a very broad range of bioactive properties, by *in vitro* methods, applicable to a very broad range of biological pathways. Not surprisingly, *in vivo* substantiation of potential bioactive applications lags behind *in vitro* indications of bioactivity. To date, *in vivo* substantiation of dairy bioactive peptides in humans has been demonstrated for but a subset of potential applications, including peptide bioavailability nutrition and use in athletic recovery, BP, mineral absorption, growth hormone, and dental caries applications. Substantiation of new applications of dairy bioactive peptides is expected to be driven by innovative thinking and favorable business cases surrounding specific applications.

4.3 ANTIMICROBIAL AND ANTIVIRAL EFFECTS OF MILK PROTEINS AND PEPTIDES

Milk is not merely a source of nutrients for the newborn mammal, but also provides an effective protection against microbial and viral infections. Immunoglobulins present in colostrum and milk (reviewed later in this chapter) protect the neonate against specific pathogens. In addition, a number of components of colostrums and milk constitute a non-specific protective system, in which various proteins seem to play a dominant role.

Several milk proteins, such as lactoperoxidase (LP), LF, and lysozyme (LZ), have traditionally been recognized for their antimicrobial effects. It is not only intact proteins that have antimicrobial properties; enzymatic digestion of several milk proteins generates a variety of peptides, some of which produce antimicrobial effects. In addition, chemical modification of milk proteins and peptides can enhance their antimicrobial properties.

In recent decades, research in this field has largely focused on LF, which has diverse biological functions (Wakabayashi et al., 2006), including the recently suggested acid phosphatase activity (Miura et al., 2010). Some of the peptides released from LF by proteolysis are known to produce strong antimicrobial effects. However, a number of peptides generated from both caseins and the main whey proteins, α -La and β -lactoglobulin (β -Lg), also have antimicrobial properties.

4.3.1 Antimicrobial proteins

4.3.1.1 Lactoperoxidase

Lactoperoxidase (EC 1.11.1.7) is a peroxidase with a broad substrate specificity (it oxidizes, *inter alia*, halides such as I^-) and has been extensively studied, notably during the 1950s and 1960s. It is a part of the nonspecific protective system in milk, which relies on the presence of thiocyanate (SCN^-) and hydrogen peroxide.

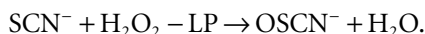
LP is a glycoprotein with a molecular weight (MW) of 72.5–88 kDa (Shakeel-ur-Rehman and Farkye, 2002), depending on the technique used to determine it. LP is also a heme protein, and its prosthetic group is either tetrapyrrol or protoporphyrin. LP is present in the milk of most mammals. It is always present in bovine milk, at approximately 30 mg/L on the average (Shakeel-ur-Rehman and Farkye, 2002), with the range from 1 to 60 mg/L (H. Korhonen, pers. comm.).

LP remains in whey after the separation of the casein curd from whey. Bovine LP is relatively heat resistant, retaining about 50% of its original activity after the HTST pasteurization.

LP is a cationic protein that can be removed from milk or whey (e.g., from cheese whey) by an ion-exchange process, together with LF.

In the batch process patented by the Swedish Dairies' Association (Tetra Pak Processing Systems, 2003), (1) particulate material is removed from cheese whey by cross-flow micro-filtration (pore size of 1.4 μm), and then (2) LP and LF are removed from cheese whey by an ion exchange (IE) process, followed by (3) the elution of LF and LP with a solution of sodium chloride. Typically, the IE resin absorbs 40–45 g LF and LP per L of resin. By adjusting elution conditions, primarily the NaCl concentration, separate and relatively pure fractions of LP and LF can be obtained. A bed volume of 100 L allows processing of 100,000 L whey per cycle/batch.

With hydrogen peroxide present in raw milk LP oxidizes thiocyanate:



The main product of this reaction is hypothiocyanite, $OSCN^-$. Higher oxyacids, namely, O_2SCN^- and even O_3SCN^- , which are very reactive and therefore short-lived species, are also produced if H_2O_2 is available at higher amounts than merely a concentration equimolar to that of SCN^- .

H_2O_2 is present in milk as a by-product of activity of various oxidases, at the concentration of up to 4 mg/L in raw milk (Reiter, pers. comm.).

SCN^- occurs in milk as a by-product of detoxification of cyanogenic glycosides present in *Cruciferae*; ≤ 8.1 mg/L SCN^- was measured in bulk milk in the state of Victoria, south-eastern Australia (Roginski et al., 1984).

$OSCN^-$, O_2SCN^- , and O_3SCN^- are highly reactive oxidants, which kill bacteria or cause the inhibition of bacterial growth. These effects are attributed to the oxidation of sulfhydryl (thiol) groups and nicotinamide nucleotides NADH and NADPH in bacterial cells.

It has been demonstrated that hypothiocyanate oxidizes thiol groups in cysteinyl residues of both membrane and cytoplasm proteins, impairing various physiological functions. For example, hexokinase, the enzyme catalyzing the first step of glycolysis, is completely inhibited in many bacteria, while aldolase is usually partly inhibited.

In general, LP is bactericidal against Gram-negative bacteria and bacteriostatic against Gram-positive bacteria.

It is known that SCN^- oxidation products (of which OSCN^- can be measured much easier than the species at the higher level of oxidation) inhibit succinate-dependent respiration system in *Escherichia coli*. Impairment of bacterial respiration chain affects the generation of trans-membrane proton gradient. However, terminal oxidases of the respiration chain remain largely unaffected (Shin et al., 2001).

In response to the stress caused by the LP system, some bacteria, for example, *E. coli*, are able to synthesize compounds containing $-\text{SH}$ groups, such as cysteine and glutathione, which help to alleviate oxidative stress. Sermon et al. (2005) identified 13 LP-induced genes in *E. coli*; the most strongly induced was the *cysJ* gene, which encodes the key enzyme in the synthesis of compounds with $-\text{SH}$ groups.

Activation of the LP system has been proposed for the preservation of raw milk in areas that lack refrigerated milk collection and delivery systems (Korhonen, 1980). The optimization of this antimicrobial system requires supplementation of milk with potassium or sodium thiocyanate and with a source of H_2O_2 . In most applications, the minimum concentration of thiocyanate required for the desired antibacterial effect is about $250\mu\text{M}$, which is lower than that observed in human body fluids, such as saliva or gastric juice. H_2O_2 can be provided in the form of either sodium percarbonate (which, when added to water, gives a burst of H_2O_2) or glucose oxidase (GluOx) with glucose (Glu). The combination of GluOx + Glu is preferred because it ensures a sustained generation of H_2O_2 for as long as glucose and the sufficient amount of oxygen are present in milk. In addition, GluOx is an approved food additive. Generally, any oxidase approved for food use, with an appropriate substrate, can be used to generate H_2O_2 for the LP system. LP, a very fast enzyme, is always present in milk to oxidize any amount of SCN^- present in milk.

Successful field trials conducted in the 1970s in Kenya, Sri Lanka, and Mexico, demonstrated that milk collected in locations remote from processing facilities could be transported at ambient temperatures (sometimes as high as 30°C) for up to 8 hours, without an increase in the total plate count. Following these trials, the LP system was recommended for use by the World Health Organization and the Food and Agriculture Organization of the United Nations.

The LP system, with either thiocyanate or iodide as a substrate, can also be successfully used in food and crop protection (reviewed by Boots and Floris, 2006). For such applications, LP system is usually supplemented with a vegetable oil, which ensures a uniform distribution and retention of LP system components on the plant and a good contact with lipophilic fungal colonies. For example, the infection of cucumbers by *Sphaerotheca fuliginea* (cucumber powdery mildew) is reduced by 90–95% (Boots and Floris, 2006).

4.3.1.2 Lactoferrin

In the past 50 years, since the isolation of “red protein” from milk by Groves (1960), great progress has been made in the studies of this protein, which seems to have multiple, sometimes quite unexpected, biological functions.

Bovine LF (bLF) is an ~80-kDa, single chain, iron-binding glycoprotein of the transferrin family. It is present in milk and other exocrine secretions, as well as in the neutrophil granulocytes (a class of leucocytes).

bLF and human LF (hLF) consist of 689 and 691 amino acid residues, respectively; the degree of sequence homology between these two proteins is 69% (Korhonen and Marnila, 2002). These are cationic proteins—isoelectric points (pI) of bLF and hLF are 9.4 and 9.5, respectively.

bLF contains two iron-binding sites, each of which can bind ferric iron with the concomitant incorporation of bicarbonate or carbonate ion. The affinity of bLF for iron is very high, about 300 times higher than that of transferrin, the function of which is to transport iron in the serum (Korhonen and Marnila, 2002). Iron saturation of “native” bLF is about 20–30%. The degree of glycosylation of bLF does not affect its affinity for iron. However, fully glycosylated bLF is more resistant to proteolysis. bLF also binds other metals: Cu, Co, Zn, and Mn (Korhonen and Marnila, 2002).

bLF concentration in bovine milk is normally about 100 mg/L (with the range of 70–120 mg/L), while colostrum is much richer in LF, at about 1.5 g/L (the range of 0.2–5.2 g/L) (Korhonen and Marnila, 2002).

After precipitation of casein coagulum, bLF remains in whey. bLF is commercially recovered either from milk or whey by an ion-exchange process described above.

Iron can be removed from bLF by chelating agents, giving apo-bLF. On the other hand, holo-bLF can be obtained by fully saturating bLF with iron.

LF from various species is active against Gram-positive and Gram-negative bacteria, viruses (both enveloped and naked), fungi, and even parasites; for details, see a recent review by Jenssen and Hancock (2009).

HTST pasteurization practically has no effect on bLF structure and its antibacterial properties, while preheating at 70°C/3 min, followed by 130°C/2 s, leads to only 3% loss in residual iron-binding capacity (Korhonen and Marnila, 2002).

UHT treatment destroys the ability of holo-bLF to bind to bacterial wall, as well as bacteriostatic effects of apo-bLF. During the heat treatment, apo-LF is denatured faster than holo-bLF. Spray-drying of milk causes only a negligible loss of antimicrobial properties of bLF (Korhonen and Marnila, 2002).

bLF's antimicrobial action relies on its ability to:

1. sequester available iron in the medium, affecting the growth of those microbial species that have relatively high requirements for iron and are unable to wrest iron out of bLF; and
2. bind to bacterial cell wall, thus disrupting its integrity and altering its permeability which affects essential physiological functions of the cell.

Initially, antimicrobial effects of bLF were thought to depend solely on iron sequestering. A number of studies investigated conditions that would maximize antimicrobial effects of bLF.

It was established, *inter alia*, that the ratio of bicarbonate to citrate concentration in the medium is critical, as it affects the antimicrobial action of bLF. With this ratio moving in favor of citrate, antibacterial effects against a number of bacterial species were significantly weaker or prevented entirely.

In experiments, *in vitro* iron deprivation by bLF led to inhibition of growth of: *E. coli*, *Klebsiella* spp., *Salmonella* spp., *Proteus* spp., *Pseudomonas* spp., *Listeria* spp., *Bacillus* spp., *Streptococcus* spp., and *Candida albicans* (Korhonen and Marnila, 2002).

LF from various species can chelate Ca²⁺ and binds strongly (at the neutral pH or slightly above it) to lipopolysaccharide (LPS) of Gram-negative bacteria, inducing its release from the wall.

Proteolytic activity against two autotransported proteins of *Haemophilus influenzae* has been identified in hLF. This proteolysis limits the virulence of the pathogen and prevents colonization (Qiu et al., 1998). Hendrixson et al. (2003) established that hLF is a serine

protease that hydrolyses *H. influenzae* surface proteins in arginine-rich regions; this activity is located in the N-terminal lobe.

Acid phosphatase activity of bLF fraction has recently been suggested by Miura et al. (2010).

The antimicrobial effects of bLF are enhanced in the presence of LZ or antibodies against the species in question. This observation has led to a suggestion that a combination of bLF with other antimicrobial agents should be utilized in the treatment of some microbial infections.

It is now known that high amounts of bLF, administered orally, reduce the viability of *Helicobacter pylori* colonizing the stomach (the pylorus area). bLF glycans may bind to bacterial adhesins, interfering with bacterial attachment to epithelial cells. An important effect has also been attributed to LFcin, a product of peptic digestion of bLF (Korhonen and Marnila, 2002).

In experiments with mice, rat, and guinea pig models (Wakabayashi et al., 2006), bLF and hLF, as well as LFcin B (see the section on antimicrobial peptides below), produced effects such as decrease in populations of intestinal pathogens, increase in counts of bifidobacteria, decrease of infection rate with *Helicobacter felis*, cure of oral candidiasis, decrease in *Staph. aureus* in kidney infection, and so on.

In addition to its application in infant formulae, which began in 1986 and is now well established, bLF is used in products such as yoghurt, skim milk, drinks, in skin and oral care products, as a supplement in tablets, and even in pet foods (Wakabayashi et al., 2006). Some of these applications are related to functions of bLF other than its antimicrobial or antiviral effects. See Wakabayashi et al. (2006) for a detailed review of effects of orally administered bLF-related compounds on bacterial flora and infections in animals and humans, as well as on cancer in animals.

The preventive and therapeutic applications of bLF continue to be a field of vigorous research.

4.3.1.3 Other antimicrobial proteins of milk

Lysozyme (LZ), also known as muramidase (peptidoglycan N-acetylmuramoyl hydrolase; EC 3.2.1.17) catalyses the hydrolysis of peptidoglycan, a component of bacterial cell wall (Farkye, 2002b). Peptidoglycan is particularly abundant in the cell wall of Gram-positive bacteria. LZ has been known for a long time as an effective antimicrobial agent. LZ from chicken eggs was traditionally used in the manufacture of Swiss cheese to prevent the late blowing of cheese caused by clostridia. However, its concentration in bovine milk is relatively low, at less than 3 mg/L, in contrast to human milk that contains between 100 and 120 mg of LZ per liter (Farkye, 2002b). Thus, while LZ undoubtedly adds to the nonspecific defense of the newborn, usually in a synergistic manner with some of the other antimicrobial agents in milk, practical applications of milk-derived LZ are unlikely.

Xanthine oxidase (XO), xanthine : oxygen oxidoreductase (EC 1.1.3.22), an oxidase with a broad substrate specificity, and present in bovine milk at a relatively high concentration of about 35 mg/L (Farkye, 2002a), plays a major role in the catabolism of purines. It is a dimeric metalloprotein, with a molecular mass of about 300 kDa. XO is particularly abundant in the milk fat globule membrane (MFGM), accounting for about 20% of all protein present in the MFGM. XO can also be considered an antimicrobial agent because it generates hydrogen peroxide as a by-product of reactions it catalyses. H_2O_2 is generated as a result of reduction of O_2 .

The H_2O_2 produced, in the absence of catalase, can either affect some bacteria directly or can be utilized by the LP (always present in milk, also after HTST pasteurization), if a suitable substrate is available. In experiments *in vitro*, enough H_2O_2 was generated to directly inhibit various bacterial species, if a sufficient amount of substrate was present, under aerobic conditions.

4.3.1.4 Enhancing antimicrobial effects of milk proteins through chemical modification

As positive charge on proteins can be increased by amidation of aspartyl and glutamyl residues to asparaginyll and glutaminyl residues, respectively, enhanced antimicrobial effects may be expected from amidated proteins. Amidation also increases amphipathicity of the molecule and leads to changes in charge distribution.

Sheehan and Hess (1955) were the first to have used NN'-dicyclohexylcarbodiimide for carbodiimide-mediated condensation of carboxyl groups in proteins in the presence of nucleophiles to amidate carboxyl groups in amino acid residues. This reaction, as modified by Mattarella et al. (1983), was used in the studies reported by Pan et al. (2007a,b).

Antimicrobial properties of amidated bLF (Pan et al., 2007a) and amidated bovine β -Lg (Pan et al., 2007b) were stronger than those of the respective native proteins. For example, when compared with the native bLF, amidated bLF (with a theoretical pI of 9.93) caused a 5 log decrease in the viability of resting cells of *Listeria monocytogenes*, and an almost 6 log decrease in the viability of resting cells of *Pseudomonas fluorescens* ATCC49838. The antimicrobial effects varied depending on the target organism. For example, neither native nor amidated bLF had any effect against *Penicillium candidum* and *Saccharomyces cerevisiae* (Pan et al., 2007a).

Amidation of β -Lg, a protein that does not have antimicrobial activity in its native state, converted it into a strong bactericidal agent against *P. fluorescens*, *Pseudomonas fragi*, and *Bacillus subtilis*. Amidated β -Lg, which had a theoretical pI of 9.83 (compared with the theoretical pI of 4.83 for the native β -Lg), caused an almost 6 log, 4 log, and 4.5 log reduction in the viable count of resting cells of *P. fragi*, *P. fluorescens*, and *B. subtilis*, respectively (Pan et al., 2007b).

These results confirm that the positive charge on the molecule is critical for antimicrobial features of proteins. Any cationic protein may be expected to possess antimicrobial properties or serve as a source of antimicrobial peptides.

4.3.2 Antimicrobial peptides

Peptides with antimicrobial properties continue to be the focus of a growing research attention. In many animal species, these peptides play an important role in the host defense. In addition, their mode of action seems to elude most, if not all, known drug resistance mechanisms (Mor, 2003). Medical practice is now faced with dangerous bacterial pathogens that no longer respond to the known antibiotics, for example, vancomycin-resistant enterococci (VRE) and methicillin-resistant *Staph. aureus* (MRSA), hence an added stimulus to search for novel antimicrobial agents.

The goal of research in this field is to identify and then commercially produce peptides that could be used to treat microbial infections and serve as food preservatives.

Antimicrobial peptides share the following features (Powers and Hancock, 2003): (1) generally less than 50 amino acid residues; (2) an overall positive charge; (3) substantial

($\geq 50\%$) proportion of hydrophobic residues, and (4) they may simultaneously affect multiple functions of bacterial cell.

Various modes of action for antibacterial peptides have been suggested, including their effect on functions of bacterial membranes by forming pores, causing the thinning of the membrane, and destabilizing the lipid bilayer. As these peptides are amphipathic molecules, they act in a detergent-like manner. They also inhibit synthesis of macromolecules and interact with various components in the cell interior (van der Kraan et al., 2005b).

When the action of antibiotics is compared with that of cationic peptides, an interesting observation emerges (Hancock, 2004). In general, it is easier to make antibiotics against Gram-positive than Gram-negative bacteria, because in the latter, there is: (1) a restricted uptake of antibiotics through the channels of porins, and (2) active efflux from the cell. In contrast, cationic peptides with broad-spectrum activity or specificity for Gram-negative bacteria are more common than those exclusively active against Gram-positive bacteria (Hancock, 2004).

Hancock and Chapple (1999) postulated a mechanism for the transfer of cationic peptides across the cell envelope of Gram-negative bacteria. In this mechanism, described as “self-promoted uptake,” a sequence of events ultimately leads to a cationic peptide transverse the cytoplasmic membrane of the bacterial cell.

A general model applicable to both Gram-positive and Gram-negative bacteria does not seem to exist.

The Hancock and Chapple’s model of self-promoted uptake of cationic peptides cannot be applied to cationic proteins, such as LF. Owing to their larger size, cationic proteins are unlikely to transverse across the outer membrane and insert into the cytoplasmic membrane. They may, however, bind to the divalent cation binding sites on the LPS and thus disrupt the membrane.

4.3.2.1 Antimicrobial peptides from milk

Lactoferricin

Lactoferricin contains distinct clusters of amino acid residues with basic side chains. In bLF, one of these clusters occurs between residues 17 and 41, making this fragment highly cationic. This fragment, called bovine LFc_{in} (LFc_{in} B), is liberated by hydrolysis of LF with gastric pepsin. A fragment of hLF, LFc_{in} H (f1-47), also released by pepsin, contains within its sequence a region homologous with LFc_{in} B (Korhonen and Marnila, 2002). Like LFc_{in} B, LFc_{in} H is cationic and has antimicrobial properties similar to those of LFc_{in} B.

LFc_{in} B contains a fragment each of an α -helix and a β -sheet structure from the original LF molecule. Within LFc_{in} B there is a loop of 18 amino acid residues, linked by a disulfide bond between Cys residues 19 and 36.

LFc_{in} B is a more potent antimicrobial agent than LF. LFc_{in} binds rapidly to the surface of *E. coli* and *B. subtilis* cells, upsetting their functions and killing them. The rate of killing of these bacteria corresponds to the rate of binding of LFc_{in} to cells; the killing effect is greatest near the optimal pH for binding to each bacterial strain. The killing effect against *E. coli* is more pronounced at 20°C than at 37°C.

The net positive charge of LFc_{in}, and of other peptides derived from LF, such as lactoferrampin, is essential for their interactions with the negatively charged membranes of bacteria.

LFcin binds to LPS in Gram-negative bacteria and teichoic acid in Gram-positive bacteria (Vorland et al., 1999), and translocates across the cytoplasmic membrane of both Gram-negative and Gram-positive bacteria (Haukland et al., 2001). The hydrophobic region of LFcin contains tryptophan, which is involved in membrane insertion (Schiffer et al., 1992). Ulvatne et al. (2004) reported that LFcin B, on reaching cytoplasm, inhibited protein synthesis in *E. coli* and *B. subtilis*; the authors did not propose a mechanism for this inhibition.

The asymmetry of the charge on the molecule of cationic peptide also plays a role. Increasing the charge asymmetry in synthetic analogs of LFcin B enhanced the effectiveness of these peptides against *E. coli*; increasing the lipophilicity of the noncationic part of the sequence had an even greater effect (Rekdal et al., 1999).

The minimum inhibitory concentrations (MIC) of an intact bLF and of LFcin against a strain of *E. coli* were 2 mM and 100 μ M, respectively (Bellamy et al., 1992).

In animal models, LFcin B protected mice against infection by methicillin-resistant *Staph aureus* (Nakasone et al., 1994) and parasite *Toxoplasma gondii* (Isamida et al., 1998).

Positive effects of orally administered LF in animal models and in humans may at least partly be attributed to LFcin, as LF is degraded by pepsin in the stomach.

Richardson et al. (2009) have recently demonstrated that the antimicrobial core (RRWQWR) of LFcin B, referred to as LfcinB6, when delivered to the cytosolic compartment by fusogenic liposomes, kills T-leukemia cells. This observation opens the way for further studies on potential applications of bLF's fragments as anticancer agents.

4.3.2.2 Lactoferrampin

When examining the amino acid sequence of cationic proteins, one can expect certain regions, containing both cationic and hydrophobic amino acid residues, to have promising antimicrobial features. However, it is not always possible to "excise" the candidate sequences precisely with the tools we have at our disposal (primarily proteases but also certain reagents that cleave specific peptide bonds). The only way in which such peptides can be studied is to synthesize them. Lactoferrampin (LFampin), LF (f268-284), is an example of such a synthetic peptide. This particular part (WKLLSKAQEKFGKNKSR) of the bLF sequence was selected on the basis of its putative antimicrobial properties (van der Kraan et al., 2004). Hydrolysis of bLF by pepsin, in addition to LFcin B, generates cationic peptides in the LFampin region, for example, LF (f277-288), LF (f265-285), and LF (f267-288), but LFampin cannot be obtained by proteolysis.

The LC₅₀ values for LFampin against *Ca. albicans*, *B. subtilis*, *E. coli* K12, and *Pseudomonas Paeruginosa* Pak were (in μ M): 2.1, 18, 5.8, and 7, respectively (van der Kraan et al., 2004).

Using confocal laser scanning microscopy, van der Kraan et al. (2005a) demonstrated that LFampin (f268-284), LF (f265-284) and part of LFcin b (f17-30), disrupted membrane integrity in *Ca. albicans*, and were internalized within a few minutes. The same group (van der Kraan et al., 2005b) studied the effect of amino acid substitutions on the candidacidal activity of LF (f265-284) using a glycine substitution scan. Each time glycine was substituted for a positively charged residue, a decrease in candidacidal activity was observed. These results confirm again that positive charge plays a key role in antimicrobial properties of peptides.

4.3.2.3 Antimicrobial peptides from caseins and major whey proteins

While bLF, a cationic protein that is commercially produced from milk or whey, was an obvious focus of the search for antimicrobial peptides, peptides liberated from caseins and the main whey proteins have also been investigated for their potential antimicrobial features.

4.3.2.4 Peptides from α_{s1} -casein

Isracidin (f1-23), obtained by digestion with chymosin, was effective *in vivo* (in mice) against *Staph. aureus*, *Streptococcus pyogenes*, *Listeria monocytogenes*, and *Ca. albicans*. Isracidin had a synergistic effect *in vitro* against antibiotic-resistant *Staph. aureus*, when combined with penicillin or streptomycin (Lahov and Regelson, 1996).

McCann et al. (2006) obtained a number of peptides through peptic hydrolysis of sodium caseinate. One of these peptides, liberated from α_{s1} -casein (f99-109), had a theoretical pI of 10.46 and inhibited pathogenic and spoilage bacteria, including (MIC, as $\mu\text{g/mL}$, in brackets): *B. subtilis* (125), *Listeria innocua* (125), *Salm. Typhimurium* (125), *E. coli* (250), *Salm. Enteritidis* (125), and *Citrobacter freundii* (500).

4.3.2.5 Peptides from α_{s2} -casein

Casocidin-I (f150-188), prepared by digestion with trypsin, pI 8.9, inhibited the growth of *E. coli* and *Staphylococcus carnosus* (Zucht et al., 1995), while peptide f166-203 inhibited *E. coli* (Zucht et al., 1996).

Fragments f164-179 and f183-207 inhibited growth of both Gram-positive and Gram-negative bacteria, with MIC values of 25–100 μM and 8–16 μM , respectively (Recio and Visser, 1999).

Three cationic peptides from the C-terminal region, isolated from a chymosin digest of Na caseinate, viz. f181-207, f175-207 and f164-207, were effective in the 0.1% peptone solution against Gram-positive and Gram-negative bacteria, with MIC values (in $\mu\text{g/mL}$) of 21–168, 10.7–171, and 4.8–76.2, respectively. However, the chymosin Na caseinate digest was not effective against *Salm. Typhimurium* in skim milk (McCann et al., 2005).

4.3.2.6 Peptides from κ -casein

Kappacin, a nonglycosylated, phosphorylated form of caseinomacropeptide (CMP), f138-158, was effective against *Streptococcus mutans*, *E. coli*, and *Porphyromonas gingivalis* (Malkoski et al., 2001). Kappacin affects bacterial membranes.

A product of tryptic digestion, κ -casecidin (f17-21), inhibits the growth of *Staph. aureus*, *E. coli* and *Salm. Typhimurium* (Matin and Otani, 2002).

Peptides liberated by pepsin, f18-24, f139-146, and f30-32, inhibit *List. innocua* and *E. coli* (Lopez-Exposito and Recio, 2006).

4.3.2.7 Peptides from α -Lactalbumin

α -Lactalbumin, a small (MW 14.2 kDa) whey protein (pI 4.8) that contains a tightly bound Ca^{2+} , is not antimicrobial in its native state, but some of the peptides liberated by trypsin and chymotrypsin have antibacterial properties (Pellegrini et al., 1999).

GYGGVSLPEWVCTTF ALCSEK, f[(17-31)S-S(109-114)] was most and EQLTK least effective against Gram-positive bacteria. Gram-negative bacteria were only weakly affected. Stabilization of the peptides' structure through disulfide bridges was essential for antibacterial effects. Replacing Leu23 with Ile in the f(17-31)S-S(109-114) peptide weakened the antibacterial properties of this peptide (Pellegrini et al., 1999).

4.3.2.8 Peptides from β -Lactoglobulin

β -Lactoglobulin (MW 18.3 kDa) is the main protein in whey, at ~3.0–3.5 g/L (~10% of milk proteins). It forms reversible dimers at the native pH of milk. β -Lg binds a range of small hydrophobic and amphipathic molecules (retinol, vitamin D2, fatty acids, etc.). It is not antimicrobial in its native state, but peptides liberated by trypsin have antibacterial properties (Pellegrini et al., 2001).

Fragments VAGTWY (f15-20), AASDISLLDAQSAPLR (f25-40), IPAVFK (f78-83), and VLVLDTDYK (f92-100) were effective against the Gram-positive bacteria tested.

Fragment VLVLDTDYK was modified by replacing the Asp98 residue by Arg and the addition of a Lys residue at the C-terminus; the resulting peptide VLVLDTRYKK was effective against Gram(–) organisms *E. coli* and *Bordetella bronchiseptica*, but had a weaker effect against *B. subtilis* (Pellegrini et al., 2001).

A synthetic peptide VLVATLRYKK was very effective against all the bacterial strains tested, and also against *Ca. albicans*, with log N_0/N_1 values about 3.0 (except 1.5 against *E. coli* and 1.8 against *Salm. Typhimurium*) (Pellegrini et al., 2001).

Antibacterial activity of peptides and folding variants from milk proteins has been comprehensively reviewed by Lopez-Exposito and Recio (2006).

With a fast progress in peptide synthesis techniques, it can be expected that the search for peptides with strong antimicrobial properties will focus on design and synthesis of sequences that will have the optimal combination and sequence of cationic and hydrophobic amino acid residues. However, liberating antimicrobial peptides from proteins still offers practical possibilities, as demonstrated by Bolscher et al. (2006), who used a single enzyme, endoproteinase AspN, to obtain a 32-mer fragment, which strongly inhibited both *E. coli* and *Ca. albicans*.

4.3.3 Antiviral proteins and peptides from milk

Several milk proteins have been investigated for their potential to interfere with viral infections. Research in this field has focused on LF, as well as on LFCin and other peptides derived from LF. A substantial body of evidence, gathered mainly in studies on cell lines *in vitro*, indicates that LF does interfere with viral infections by physically preventing viral particles from attaching to host cells, which is the first step in each viral infection.

In general, bLF is more effective against viral infections than hLF. LFCin and other peptides liberated from LF are not as effective as the intact LF molecule (McCann et al., 2003).

In experiments *in vitro* (Arnold et al., 2002) LF inhibited adenovirus replication in a dose-dependent manner. LF prevented viral replication both when added before and during the viral adsorption step.

LF interferes with infections caused by several enveloped viruses, such as herpes simplex type 1 and 2, human cytomegalovirus (HCMV), human immunodeficiency virus (HIV), hepatitis B, C, and G viruses, human papilloma virus (HPV), and alphavirus. It is

also effective against a number of nonenveloped viruses, such as rotavirus, enterovirus, poliovirus (PV), adenovirus, and feline calicivirus (FCV) (reviewed by Pan et al., 2006).

LF seems to interfere with viral infections by two mechanisms: (1) interacting with the receptors on the host cell's surface, such as glycosaminoglycans, and (2) binding directly to viral particles. Both mechanisms appear to rely on electrostatic attraction, opening the possibility for enhancing the protein's antiviral effects through its chemical modification in order to change both the charges on the protein and charge distribution across the molecule.

4.3.3.1 Effects of chemical modification

Chemical modification of whey proteins often leads to changes in their antiviral properties. Increasing the negative charge on the molecule, either through acylation (usually as a result of a reaction with the anhydride of either succinic acid or cis-aconitic acid) or through production of 3-hydroxyphthaloyl derivatives (in a reaction with the anhydride of 3-hydroxyphthalic acid) enhances antiviral effects of proteins and imparts antiviral properties to proteins that do not possess them in their native state.

Succinylated or aconitylated LF was two to four times more effective against human immunodeficiency virus-1 (HIV-1) than the native LF (Swart et al., 1996, 1998, 1999b).

β -Lg and α -La were more effective against HIV-1 and HIV-2 viruses when additional negative charges were introduced through modification of lysine residues (Swart and Meijer, 1994; Jiang et al., 1996; Swart et al., 1996, 1999b; Neurath et al., 1997).

Negatively charged proteins have a stronger affinity to the viral target cell receptors and/or to the viral envelope proteins (Marchetti et al., 1996; Neurath et al., 1996; Lüscher-Mattli, 2000). For example, β -Lg modified with 3-hydroxyphthalic acid had a high affinity toward the CD4 receptor for HIV particles (Zeder-Lutz et al., 1999).

Experiments *in vitro* demonstrated that the negatively charged compounds specifically interact with the positively charged V3 loop of gp120 envelope protein of HIV (Swart et al., 1996; Berkhout et al., 2002).

Succinylated and aconitylated human serum albumins (negatively charged), rapidly passed from the bloodstream into the lymphatic system, where most HIV replications occur (Swart et al., 1999a).

Modification of bovine serum albumin (BSA), α -La and β -Lg, and of the peptides derived from these proteins, by 3-hydroxyphthalic acid, produced compounds effective against herpes simplex virus-1 (HSV-1) (Oevermann et al., 2003). 3-Hydroxyphthaloyl β -Lg prevented HSV-2 infection in the mouse model of genital HSV-2 infection (Kokuba et al., 1998), while acylated milk proteins prevented adsorption of influenza virus to target cells (Schoen et al., 1997).

Acylation does not always improve the antiviral action of the protein. For example, acylation abolished anti-HCMV properties of LF (Swart et al., 1999b).

In general, reducing or totally removing the net negative charge on the protein molecule or increasing the net positive charge abolishes effects of this protein against most viruses.

The specific distribution of positively and negatively charged domains in the molecule appears to be important in both the anti-HIV and anti-HCMV effects (Swart et al., 1998). It is interesting that increasing the positive net charge on LF through amination abrogated its anti-HIV effect but enhanced its effect against HCMV (Swart et al., 1999b).

Antiviral properties of milk proteins have been comprehensively reviewed by Pan et al. (2006).

As chemical modification of proteins offers possibilities of considerable enhancement of their antiviral properties, new modification techniques may be employed in future studies. It can be expected that mechanisms of antiviral effects of modified proteins will be extensively studied *in vivo* in animal models before any practical applications can be considered.

4.3.4 Conclusions

LP and bLF, two main antimicrobial proteins of milk, are already recovered from whey or milk commercially. Of these two proteins, bLF, which has multiple biological functions, has been used more extensively. Its applications for various practical purposes can be expected to increase, for example, in foods for people with special dietary needs and for food preservation.

Antimicrobial peptides are a field of very intensive research, mainly owing to an expectation that novel peptides, either synthesized or liberated from native proteins, will offer an effective way of combating antibiotic-resistant bacteria, which claim an increasing share of nosocomial infections. Antimicrobial sequences encrypted in milk proteins will have a future in commercial applications if cost-effective technologies for their liberation, isolation, and, where necessary, purification are developed to the point where they can compete with peptide synthesis or production through the use of recombinant microorganisms.

4.4 IMMUNOGLOBULINS

Immunoglobulins (Ig), also called antibodies, are present in milk and colostrum of all lactating species. The biological function of milk Igs is to give the offspring an immunological protection against microbial pathogens and toxins and to protect the mammary gland against infections. Ruminant neonates are born virtually without Igs, and the colostral Igs are essential for survival. Thus, in ruminants, the colostrum contains remarkably higher amounts of Igs than the mature milk (Butler, 1998; Hurley, 2003). Igs are divided into classes with different physicochemical structures and biological activities. The major classes in bovine and human milk are IgA, IgG and IgM. The concentration of different Ig classes in milk and colostrum varies considerably according to species, breed, age, stage of lactation, and health status, and is often different from that in blood (Table 4.1).

Table 4.1. Concentration of immunoglobulins in bovine serum and mammary secretions.

Ig-class	Concentration (g/L)		
	Serum	Colostrum ^a	Milk
IgG-total	25.0	60 (20–200)	0.47 (0.15–0.8)
IgG ₁	14.0	15–180	0.35 (0.3–0.6)
IgG ₂	8–11	1–3	0.02–0.12
IgA ^b	0.4	3.5 (1–6)	0.05–0.14
IgM	3.1	5 (3–9)	0.04–0.10

Data compiled from Marnila and Korhonen (2002) and Mehra et al. (2006).

^aFirst milking.

^bPrimarily as SIgA in colostrum and milk.

4.4.1 Structure

Ig molecules of all classes are symmetrical multichain glycoproteins composed of two identical glycosylated heavy chains and two identical nonglycosylated light chains. This basic structure of all monomeric Igs is similar. The IgG class is a general model of a monomeric Ig. The MW of each light chain is around 23 kDa, and of the heavy chains 53 kDa. The MW of the complete Ig molecule varies around 160 kDa. Both the light and heavy chains contain domains referred to as constant (C_L , C_H) and variable (V_L , V_H) regions. Light chains are attached to the heavy chains by a disulfide bond, while the two heavy chains are held together by two disulfide bonds near a hinge region, giving the molecule structural flexibility needed in antibody–antigen interactions. The two identical antigen-binding sites needed in these interactions are formed by the N-terminal region of one heavy chain and the variable region of one light chain. V_L region determines the immunological specificity. Antigen binding occurs by the interactions between the antigen and these regions. The Ig classes and subclasses are determined by the genes encoding the constant regions of heavy chains (Butler, 1998).

Proteolysis of an Ig with papain cleaves it near the hinge region into two antigen-binding sites (Fab fragments), which are formed by the N-terminal part of one heavy and one light chain, and to the third C-terminal fragment termed the Fc part. The differences between Ig classes affect the cleavage patterns in enzymatic proteolysis. The bovine IgG molecule occurs predominantly in two subclasses: IgG₁ and IgG₂. Monomeric IgM and IgA have a similar basic structure to IgG except for the differences in heavy chain structures and the addition of a C-terminal octapeptide to the heavy chain of IgA. Monomeric IgA occurs in serum, but in milk, it is present as a dimer comprising two IgA molecules joined together by a polypeptide J-chain and an additional 75-kD secretory component. This secretory IgA (SIgA) has an MW of about 380 kDa, and, therefore, is more resistant to proteolysis in the gastrointestinal tract than the Ig molecules without the secretory component. IgM is a circular pentamer consisting of five subunits, similar to those of monomeric IgA or IgG, which are linked together in a circular mode by disulfide bonds and a J chain. The MW of IgM is approximately 900 kDa (Butler, 1998; Korhonen et al., 2000a; Marnila and Korhonen, 2002).

4.4.2 Recovery and purification

The use of bovine colostrum as a source of Ig (antibody) preparations for promoting the health of farm animals and humans has been an interesting research subject for many decades. One of the challenges in this research has been the enrichment or isolation of active Igs from colostrum due to its complex composition. Also, Igs are relatively heat-sensitive and will not survive ultrahigh temperature (UHT) treatment (138°C/4 s) (Li-Chan et al., 1995). In high temperature/short time (HTST) pasteurization (72°C/15 seconds), about 25–40% of the Igs, based on antibody activity, is lost (Mainer et al., 1997, 1999). A number of patented methods have been reported for pilot or industrial-scale separation of Igs from colostrum or cheese whey. Separation technologies, such as membrane filtration and chromatography, have been employed alone or in combination. With these methods, the recovery rate of Igs has varied from 40% to 70% of the amount present in the starting material (Elfstrand et al., 2002). Specific chromatographic techniques, such as immobilized metal chelate chromatography, immunoaffinity chromatography, and cation-exchange

chromatography have been applied to improve the yield and purity of immunoglobulin preparations further (Korhonen, 2004; Korhonen and Pihlanto, 2007).

4.4.3 Biological effects

Ig preparations obtained from bovine colostrum or cheese whey are nowadays commercially available in many countries. These products are designed for newborn farm animals as colostrum supplements and for humans as dietary supplements to boost or support various body functions, such as protection against microbial infections and general well-being, without any specific microbial target (Scammell, 2001; Tripathi and Vashishtha, 2006; Struff and Sprotte, 2007). The scientific clinical evidence related to colostrum supplements remains, however, disputed. At present, the proven benefits seem mainly to be associated with an improved recovery from long endurance physical training among athletes (Crittenden et al., 2009).

The biological function of Igs is to act as specific antibodies formed by the body as a response to an immunogenic stimulus. The concentration of specific antibodies against pathogenic microorganisms can be raised in colostrum and milk by immunizing cows with vaccines made of pathogens or their antigens (Korhonen et al., 2000b). These antibodies can be fractionated, enriched, and formulated as industrial preparations (Mehra et al., 2006; Korhonen and Marnila, 2009). Oral administration of such preparations has proven successful in combating many microbial infections in humans. The concept of “immune milk” originates from the studies of Petersen and Campbell, who in the 1950s first suggested that orally administered colostrum from hyperimmunized cows could provide passive immune protection for humans (Campbell and Petersen, 1963; Wheeler et al., 2007). Over the last 50 years, a great number of clinical studies have demonstrated that immune milk preparations can be effective in prevention of human and animal diseases caused by different pathogenic microbes, for example, rotavirus, *E. coli*, *Ca. albicans*, *Cl. difficile*, *Shigella flexneri*, *Streptococcus mutans*, *Cryptosporidium parvum*, and *H. pylori*. In respective therapeutic studies, the efficacy of immune milk preparations has not been proven equally good (Korhonen et al., 2000b; Korhonen and Marnila, 2006, 2009; Hammarström and Weiner, 2008). The unclear regulatory status of immune milk products has constrained their global commercialization. In the European Union and the United States, the Ig-containing preparations from immunized cows are regarded as pharmaceuticals, whereas in many other countries, their regulatory status is not defined (Hoerr and Bostwick, 2002; Mehra et al., 2006). A few immune milk products have, however, been launched on the market in some countries (Mehra et al., 2006; Korhonen and Marnila, 2009). The safety of immune milk products has been established by the U.S. Food and Drug Administration (FDA) (Gingerich and McPhillips, 2005; Krissansen, 2007). The globally increasing problem of antibiotic-resistant strains, which have caused serious endemic hospital infections, may add stimulus to the development of suitably designed immune milk products. It also should be noted that the documented synergistic effects of Igs with probiotics and other milk bioactives, such as LF, may open possibilities for developing novel means to prevent microbial diseases by nutritional intervention.

4.5 MILK GROWTH FACTORS

Bovine milk and colostrum contain a number of growth factors that have been exploited to develop new biofunctional ingredients. Growth factors are found at variable but very

low concentration (≤ 1000 ng/mL) in milk and colostrums, and they share some of common physicochemical features (low MW, cationic character, contain disulfide bridges, and occur as a larger complex with their binding protein). Designing cost-effective methods to extract growth factors from milk and substantiating the efficacy of extracts with unequivocal experimental evidence represent the two main challenges in the development of growth factor-rich protein ingredients.

4.5.1 Composition and characteristics

The composition growth factors in milk, their biological properties, and technological aspects have been reviewed by many authors (Grosvenor et al., 1993; Pakkanen and Aalto, 1997; Playford et al., 2000; Smithers, 2004; Gauthier et al., 2006; Pouliot and Gauthier, 2006). Milk growth factors are synthesized in the mammary gland and transferred to the milk in an active or modified (glycosylated, phosphorylated) form or complexed to other molecules. It is generally accepted that growth factors are responsible for regulation and tissue differentiation in the newborn (e.g., gastrointestinal tract, immune system). The concentrations of growth factors are highest in the first colostrum and then decrease drastically during the first 2 days of lactation.

The most abundant growth factors in bovine milk and colostrum are insulin-like growth factor-I (IGF-I), followed by transforming growth factor-beta 2 (TGF- β 2), epidermal growth factor (EGF), and basic fibroblast growth factor (bFGF or FGF2), respectively.

Table 4.2 summarizes comparative concentration values, MW, pI, and primary activities of the main milk growth factors in milk. The potential biological activities of milk growth factors cover a large spectrum of physiological functions (bone, skin, blood vessels, etc.).

4.5.2 Methods for extracting growth factors

The challenge of extracting growth factors from milk or colostrum results from their low concentration, their large range of MW, and pI relative to that of other constituents, but most importantly, because of the occurrence of interactions between growth factors and the other constituents in milk and colostrum.

As seen in Table 6.2, the average MW of the growth factors is between 6400 Da (EGF) and 30,000 Da (PDGF). These MW values do not take into account the fact that some of these growth factors are found as complexes with their specific binding protein. Therefore, considering that milk and colostrum also contain casein micelles and whey proteins, it is difficult to design a separation solely based on size exclusion, such as membrane separation processes.

However, considering that the pI values of the growth factors present in milk are in the range between 6.5 (IGF-II) and 9.6 (bFGF and PDGF), except for EGF that has its pI at 4.8, it is possible to use the cationic character of milk growth factors to separate them from the major whey proteins that have a pI between 4.7 and 5.1. This represents one of the most important physicochemical characteristics of milk growth factors with regards to their extraction from whey.

4.5.2.1 Sources: colostrum, milk, or whey?

Selecting the appropriate source for the extraction of milk growth factors represents a key aspect of growth factors-enriched ingredient development. It is known that the first

Table 4.2. Concentration of growth factors (ng/mL) reported for bovine colostrum and milk.

Growth factor	Concentration (ng/mL) ^a		Molecular weight (Da)	pI	Primary activity
	Colostrum	Milk			
Epidermal growth factor (EGF)	~300	~150	6,000	4.8	Stimulates the proliferation of epidermal, epithelial and embryonic cells; Inhibits secretion of gastric acid, wound healing, and bone resorption
Betacellulin (BTC)	<5	<5	22,000	7.7	Stimulates the proliferation of many cell types. IGF-I is a stronger mitogen than IGF-II. Influences differentiation of some cells. Causes hypoglycaemia, improvement of nitrogen balance, cholesterol and potassium lowering, and improvement of renal function
Insulin-like growth factor I (IGF-I)	100–2,000	5–100	7,650	7.8	
Insulin-like growth factor II (IGF-II)	150–600	5–100	7,530	6.5	
Transforming growth factor β 2 (TGF- β 2)	150–1,150	10–70	~25,000	7.7	Stimulates growth of cells, especially in connective tissue. Inhibits other cells (lymphocytes and epithelial). Important role in embryogenesis, wound healing, bone and cartilage formation, and control of the immune system
Platelet-derived growth factor (PDGF)	n/a	n/a	30,000	9.6	Plays a role in embryonic development, proliferation of mesenchymal cells, migration, wound healing, and angiogenesis
Fibroblast growth factor (FGF2)	n/a ^b	<1	~16,400	9.6	Role in proliferation, differentiation and survival of many cell types. Involved in angiogenesis, wound healing, and hematopoiesis

^aData collected from Gauthier et al. (2006).^bn/a: present but concentration data not available.

colostrum collected after parturition has the highest content in growth factors; however, it has been shown that the decrease in molecules, such as TGF- β 2 and IGF-I, was concomitant with a decrease in protein content, therefore, the changes in these growth factors reported on protein basis occurring within the first 48 hours were less pronounced (Montoni et al., 2009). An appropriate milking schedule can ensure high content of growth factors. However, colostrum yields low volumes of raw material, it is variable in composition, has a high viscosity, and contains a wide variety of molecules that are highly sensitive to heat. Alternatively, milk can be considered as a potential source, but the sensitivity of casein micelles to pH changes and their interactions with whey proteins upon heating interfere with the extraction of growth factors. Rogers et al. (1996) found comparable concentration of TGF- β in milk and whey. Akbache et al. (2009) compared the use of native whey (from microfiltration of raw milk) and cheese whey from thermized and pasteurized milk with concentrate TGF- β 2 and IGF-I using ultrafiltration (UF) membranes. The whey from pasteurized milk contained very low amounts of TGF- β 2, but the IGF-I content was comparable with that of milk, in agreement with the observations from Yun et al. (2007), suggesting that IGF-I

can resist pasteurization treatment. Ozawa et al. (2009) sampled seven commercial pasteurized milks, and found significant but variable amounts (0.5–3.0 µg/L) of active TGF-β2 in six of these samples. Peroni et al. (2009) showed that commercial pasteurized milk had significantly lower levels of TGF-β1, compared with unpasteurized milk.

4.5.2.2 Technological approaches

Chromatography

Cation-exchange chromatography has been used by several authors to extract growth factors from colostrum, milk, and whey. The first basic cation-exchange extraction process was developed by Francis et al. (1995) and was further refined by Rogers et al. (1996) and Ballard et al. (1999). Typically, whey is first clarified by microfiltration (MF) using a 0.1- or 0.8-µm pore size membrane, and the clarified whey is passed through a cation-exchange column. The adsorbed material, consisting of growth factors, basic proteins (LF and LP), and immunoglobulins, is eluted at alkaline pH. The eluate is thereafter concentrated by UF/DF, and a final sterile filtration is applied before freeze- or spray drying. The so-called mitogenic bovine whey extract (MBWE) contains 5% of the initial whey proteins, but 90% of its mitogenic activity.

Kivits et al. (2001) proposed the use of an alkaline eluate (pH 7.5–9.5) from LF and LP production process to proceed with the extraction of growth factors while minimizing cross-contamination with IGF-I. A milk basic protein (MBP) has also been developed from whey using cation-exchange chromatography (Takada et al., 1997), but little is known about the exact process conditions.

Membrane-based separations

Direct recovery of growth factors from milk or colostrum by membrane separation (UF or MF) is not possible because casein micelles are present. The main approach used to separate growth factors from colostrum has been to remove casein by acid or rennet treatments (Lefranc-Milot et al., 1996). This approach has never been tried with milk. Others (Piot et al., 2004) have made successful attempts at removing caseins using MF of diluted colostrum. Lachkar et al. (2008) used anionic polysaccharides, such as λ-carrageenan (λ-CG) and pectin, to generate a phase separation enabling the removal of casein micelles by centrifugal separation.

The recovery of milk growth factors from whey using membrane separations is possible. Hossner and Yemm (2000) achieved the separation of IGF-I and IGF-II on a 30,000 g/mol UF membrane by performing diafiltration (DF) at pH 8.0, which allowed the passage of IGF-II to the permeate due to its pI of 7.5. Akbache et al. (2009) have shown that a bioactive fraction containing TGF-β2 and IGF-I could be concentrated by UF from cheese whey. Using a 10 kDa membrane enabled the preferential concentration of TGF-β2, compared with IGF-I, and modified the TGF-β2/IGF-I ratio of whey protein extracts.

Other approaches

Maubois et al. (2003) proposed a combination of acidification and heat treatment of precipitate to obtain a TGF-β2-rich fraction from native (unheated) whey. This precipitated material was further concentrated using 0.1-µm MF membrane and was characterized by a predominant content of α-lactalbumin (α-La), had 15% of the initial protein and 70% of the initial TGF-β2. Juneau et al. (2008) patented a similar approach, but applied to

commercial whey protein sources, such as whey protein isolates (WPI) in solution. The final composition was, however, dominated by β -Lg.

Ben Ounis et al. (2008) separated major whey proteins from a WPI solution using a heparin sulfate affinity column that enabled the removal of cationic species, including TGF- β 2 and IGF-I. Earlier observations by Lachkar et al. (2008) suggested that TGF- β 2 could bind to (λ -CG), and a methodology to separate this growth factors from colostrum was developed (Lachkar et al., 2008). Using this approach, the complexes induced between λ -CG, caseins and growth factors were removed by centrifugal separation. It has later been shown (Ben Ounis et al., 2010) that adding λ -CG can increase the retention of TGF- β 2 during microfiltration of WPI solutions. The retention of TGF- β 2 by the MF-membrane was, however, greatly dependent on the physicochemical conditions set for this study (pH and added NaCl), and may also be related to its potential interactions with whey protein. In addition, this work suggested potential applications of MF in the presence of a ligand for the separation of IgGs from WPIs.

4.5.3 Health benefits of milk growth factors

4.5.3.1 Biological activities of extracts of milk growth factors

Mitogenic bovine whey extract, also referred to as Lacternin® (TGR Biosciences Pty Ltd, Thebarton, SA, Australia) represents the most documented milk growth factor extract in terms of biological activities related to wound repair (oral and gastrointestinal mucosa) following inflammatory bowel disease and radiotherapy or chemotherapy treatments. The most recent paper dealt with the potential effect of MBWE on the modulation of matrix metalloproteinase (MMP)-2 and -9 in the development of chronic ulcers (Varelias et al., 2006). A toxicological evaluation, including *in vitro* and *in vivo* mutagenicity of MBWE (Dyer et al., 2008), showed its safety over a 13-week oral treatment in rats.

Nestle Clinical Nutrition (Vevey, Switzerland) have developed an oral polymeric diet, namely CT3211, that is enriched in TGF- β 2, and that has been shown to favor mucosal healing in some children with active small bowel Crohn's disease (Fell et al., 2000). CT3211 utilizes a TGF- β 2-enriched casein ingredient as protein source.

Milk basic protein (MBP™, Snow Brand Milk Products, Tokyo, Japan) has been found to suppress bone resorption, promote bone formation (Kawakami, 2005), and increase radial bone mineral density in healthy adult women. Further investigations have led to the identification of cystatin C and kininogen fragment 1.2 as responsible for the reduction of bone resorption *in vitro* by inhibiting the action of cathepsin K. A third component that has not yet been identified could also be TGF- β 2.

Finally, XP-828L or Dermylex™ (Botaneco Corp., Calgary, AB, Canada), a whey protein extract from bovine milk, has been shown to reduce skin lesions caused by psoriasis vulgaris when taken orally (Poulin et al., 2005, 2006). XP-828L is characterized by a high TGF- β 2 content. *In vitro* experiments showed that XP-828L decreased the proliferation of concanavalin A (ConA)-stimulated murine splenocytes and their production of interleukin (IL)-2 and interferon (IFN)- γ (Drouin et al., 2007). It was thus hypothesized that XP-828L would improve inflammatory conditions in modulating some of the neutrophils' functions in healthy humans. Recent data on a similar extract (GFX) showed that it modulates, *in vitro*, some key proinflammatory functions of neutrophils (Rusu et al., 2009). It was demonstrated that β -Lg and α -lactalbumin present in XP-828L were responsible for these effects on human neutrophils (Rusu et al., 2010). The exact interpretation of these

unexpected results is complicated by the fact that the detailed composition of XP-828L has still not been completely elucidated.

4.5.4 Future developments

The potential of growth factor-rich protein ingredients as bioactive compounds has still not been fully exploited. Understanding the mechanisms by which complex ingredients, such as MBWE, MBP, or XP-828L, elicit a physiological response and ascribing specific activities to individual growth factors or compounds is yet to be achieved. Once this task is accomplished, development of even more potent and targeted ingredients for human health will be possible.

4.6 GLYCOMACROPEPTIDE

Caseins exist in milk as colloidal particles (micelles) that are stabilized by a hydrated, negatively charged surface layer. This surface layer is dominated by the hydrophilic C-terminal region of kappa-casein, while the N-terminal hydrophobic region is buried in the casein micelle's interior. During cheesemaking, kappa-casein is cleaved between phenylalanine₁₀₅ and methionine₁₀₆ by the enzyme chymosin (E.C. 3.4.23.4), releasing the hydrophilic 64 amino-acid C-terminal peptide into the whey and destabilizing the casein micelle. GMP is released into whey derived from bovine milk at 1.2–1.5 g per liter, representing approximately 15–25% of total whey proteins. There are a range of techniques that can be employed to isolate GMP from whey, including UF, gel chromatography, hydrophobic interaction chromatography, ion-exchange chromatography, membrane adsorption, and precipitation (DeSilva et al., 2003; Kelly et al., 2009; Taylor and Woonton, 2009).

4.6.1 Structure

GMP is a heterogeneous peptide, with amino acid substitutions possible at residues 135, 136, 148, 153 and 155, and phosphorylation sites and glycosylation sites as shown in Figure 4.1.

M₁₀₆ A₁₀₇ I₁₀₈ P₁₀₉ P₁₁₀ K₁₁₁ K₁₁₂ N₁₁₃ Q₁₁₄ D₁₁₅ K₁₁₆ T₁₁₇ E₁₁₈ I₁₁₉ P₁₂₀ T[^]₁₂₁ I₁₂₂ N₁₂₃

T₁₂₄ I₁₂₅ A₁₂₆ S^{*}₁₂₇ G₁₂₈ E₁₂₉ P₁₃₀ T[^]₁₃₁ S₁₃₂ T[^]₁₃₃ P₁₃₄ T^{^*}(I)₁₃₅ T[^](I)₁₃₆ E₁₃₇ A₁₃₈ V₁₃₉

E₁₄₀ S[^]₁₄₁ T[^]₁₄₂ V₁₄₃ A₁₄₄ T^{*}₁₄₅ L₁₄₆ E₁₄₇ D(A)₁₄₈ S^{*}₁₄₉ P₁₅₀ E₁₅₁ V₁₅₂ I(T)₁₅₃ E₁₅₄

S(G)₁₅₅ P₁₅₆ P₁₅₇ E₁₅₈ I₁₅₉ N₁₆₀ T₁₆₁ V₁₆₂ Q₁₆₃ V₁₆₄ T₁₆₅ S₁₆₆ T₁₆₇ A₁₆₈ V₁₆₉

Figure 4.1 Amino acid sequence of GMP. Superscript * represents possible phosphorylation sites and superscript ^ possible glycosylation sites and amino acids in parenthesis possible substitutions (Molle and Leonil 1995; Brody, 2000).

Of the genetic variants, A and B are by far the most common in commercial bovine milk. Variant A contains Thr₁₃₆ and Asp₁₄₈, whereas variant B contains Ile₁₃₆ and Ala₁₄₈ (Minkiewicz et al., 1996). Five saccharide structures are reported to be attached to bovine GMP through *O*-glycosidic linkages and contain *N*-acetylgalactosamine (GalNAc), *N*-acetylneuraminic acid (NeuNAc), and galactose (Gal) (Brody, 2000). The five saccharides are:

1. GalNAc – *O* – R (monosaccharide)
2. Gal β (1 \rightarrow 3)GalNAc – *O* – R (disaccharide)
3. NeuNAc α (2 \rightarrow 3)Gal β (1 \rightarrow 3)GalNAc – *O* – R (trisaccharide)
4. Gal β (1 \rightarrow 3)NeuNAc α (2 \rightarrow 6)GalNAc – *O* – R (trisaccharide)
5. NeuNAc α (2 \rightarrow 3)Gal β (1 \rightarrow 3)(NeuNAc α (2 \rightarrow 6))GalNAc – *O* – R (tetrasaccharide).

The variation in GMP amino acid sequence, glycosylation, and phosphorylation leads to significant heterogeneity. This can result in GMP preparations having different amino acid compositions, states of glycosylation, and carbohydrate quantity, as evident from the complex liquid chromatographic profiles shown in Figure 4.2.

GMP is often employed to describe the glycosylated peptide, while caseinomacropeptide (CMP) is sometimes employed to describe the nonglycosylated peptide. For clarity, GMP

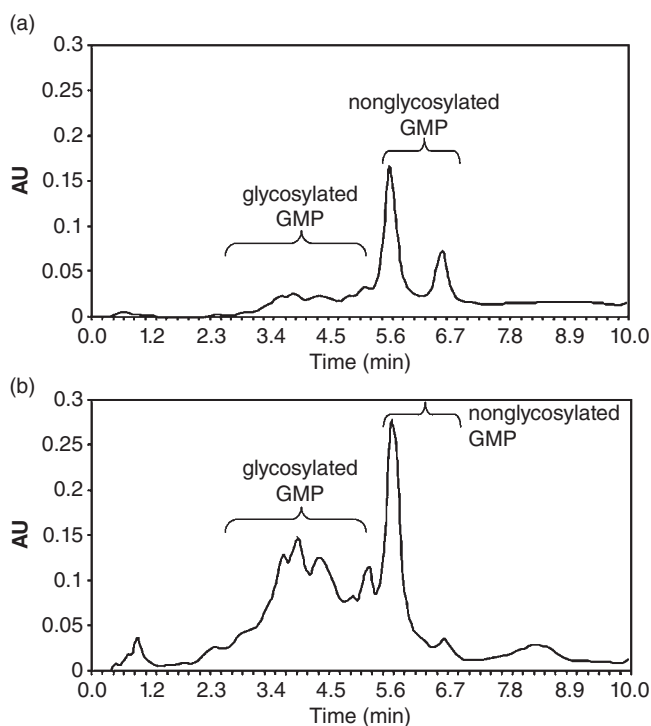


Figure 4.2 Reversed-phase styrene-divinylbenzene chromatographic profiles of precipitated (A) and soluble (B) glycomacropeptide (GMP) fractions. Marked peaks are those reported to be the glycosylated and nonglycosylated forms of GMP. Reprinted from Taylor and Woonton (2009). Copyright (2009), with permission from Elsevier.

has been used for both the glycosylated and nonglycosylated peptides throughout this chapter.

4.6.2 Physiological effects in humans and animals

GMP has been reported to have a wide range of biofunctional and health-promoting properties. In this chapter, the biofunctional properties of GMP that are well studied and if proven efficacious offer significant human health benefits are covered. These biofunctional properties of GMP are: as an ingredient for individuals with phenylketonuria (PKU), to promote growth of bifidobacteria, to reduce viral, bacterial and toxin pathogenesis, and to induce satiety and reduce food intake.

Phenylketonuria is a metabolic disease where affected individuals have a deficiency in the hepatic enzyme phenylalanine hydroxylase and are unable to metabolize phenylalanine (LaClair et al., 2009). Affected individuals must adhere to a diet low in phenylalanine upon diagnosis and throughout life, which can be extremely challenging due to the prevalence of this amino acid in almost all food proteins (Poustie and Wildgoose, 2010). GMP is the only natural source of dietary protein that does not contain the aromatic amino acid phenylalanine, and may therefore be suitable as a protein source for individuals with PKU (Ney et al., 2008). Although GMP contains 47% (w/w) indispensable amino acids, it does not contain histidine, tryptophan, tyrosine, arginine, or cysteine. PKU diets should therefore be supplemented with these amino acids. The potential of GMP-based foods to improve the quality of life for individuals with PKU is significant, as recent studies have indicated that foods made with GMP plus limiting amino acids are preferred to amino acid-only diets (van Calcar et al., 2009). GMP-based foods are likely to be available in the near future from specialized medical food companies, such as Cambrooke Foods (Ayer, MA).

Bifidobacteria predominate in the intestinal tract of breast-fed infants and inhibit the growth of intestinal pathogens, such as *E. coli*, thus protecting breast-fed infants against gastrointestinal disease (Azuma et al., 1984). *In vitro* research has indicated that GMP promotes the growth of bifidobacteria in milk (Idota et al., 1994; Janer et al., 2004), reduces the growth of pathogenic organisms (Bruck et al., 2003a), and inhibits the adhesion of verotoxigenic and enteropathogenic *E. coli* to stomach and epithelial cells (Rhoades et al., 2005; Bruck et al., 2006a). *In vivo* studies are limited; however, using infant rhesus macaques, Bruck and colleagues showed that a diet supplemented with GMP reduced the incidence of enteropathogenic *E. coli*-induced diarrhea (Bruck et al., 2003b). Studies with human infants have not been as conclusive (Bruck et al., 2006b), and further research is required to understand the role of GMP in enhancing growth of bifidobacteria and protecting infants against gastrointestinal disease.

GMP has various virus- and toxin-binding properties. For example, GMP inhibits hemagglutination induced by human influenza viruses (Kawasaki et al., 1993), the morphological transformation of peripheral blood lymphocytes that occurs with Epstein-Barr virus infection (Dosako et al., 1992) and the binding of cholera toxin to Chinese hamster ovary cells and to ganglioside GM1, the cell ganglioside to which cholera toxin binds (Kawasaki et al., 1992). GMP also inhibits splenocyte proliferation induced by *Salm. Typhimurium* LPS (Otani et al., 1992) and binds *E. coli* toxin (Isoda et al., 1999). The anti-viral and toxin-binding properties of GMP are most likely due to the attached carbohydrate structures which act as decoys, mimicking structures found on cells and reducing the extent of pathogen and enterotoxin adhesion. Oh et al. (2000) reported that glycosylated forms of GMP were the most effective at inhibiting cholera toxin cell binding (Oh et al., 2000). Further

clinical research is required to determine if GMP is suitable as an oral supplement to reduce the risk and extent of foodborne illness caused by viruses or bacterial toxins.

GMP has also been reported to incorporate into the salivary pellicle a layer of glycoproteins on the tooth enamel, and inhibit the adherence of oral bacterial pathogens, such as *Streptococcus spp.* (Neeser et al., 1994; Vacca-Smith et al., 1994). The nonglycosylated, phosphorylated variant B of GMP, designated “kappacin” is the active form (Malkoski et al., 2001), and divalent metal ions such as calcium and zinc increase its activity. A specific phosphorylated peptide within “kappacin” is reported to be responsible for the antibacterial activity (Dashper et al., 2007). With further research into the clinical relevance of this finding, there may be an opportunity to manufacture specific GMP variants or the active peptide and incorporate it into consumer products to reduce plaque formation and dental caries.

Various animal studies have suggested that consumption of GMP influences the release of gastrointestinal hormones, such as cholecystokinin (CCK), gastrin, and somatostatin, and influences gastric secretions, pancreatic secretions, gastrointestinal motility, and satiety (Stan et al., 1983; Beucher et al., 1994; Yvon et al., 1994; Pedersen et al., 2000; Dunshea et al., 2007; Royle et al., 2008; Guilloteau et al., 2010). However, studies with human subjects have not been as promising. For example, GMP preloads given to subjects prior to a test meal had no influence on subjective measures of satiety or subsequent food intake (Gustafson et al., 2001; Burton-Freeman, 2008). Studies have also reported that minimally glycosylated or glycosylated GMP intake prior to a test meal does not influence subjective measures of satiety or subsequent food intake (Clifton et al., 2009), and consuming CMP enriched whey protein does not increase weight loss over 6 or 12 months (Keogh and Clifton, 2008). In addition, consumption of alpha-lactalbumin may suppress hunger more than do other whey proteins (Hursel et al., 2010; Veldhorst et al., 2009). Additional studies are required to further elucidate how GMP preload quantity and GMP structure influence satiety, food intake, and long-term weight loss in various populations.

4.6.3 Future developments

GMP is emerging as an interesting dairy-derived molecule with unique biofunctional properties. However, studies to date are limited, and additional studies are required to validate its biological efficacy in humans. Further research is also necessary to develop cost-effective methods to manufacture specific biofunctional GMP fractions at commercial scale, and to understand the interaction of GMP with other food components and its stability in various food formulations throughout storage. Undertaking this research may lead to greater demand for GMP as a functional food ingredient and increased value of cheesemaking by-products.

REFERENCES

- Akbache, A., Lamiot, É., Moroni, O., Turgeon, S.L., Gauthier, S.F., and Pouliot, Y. (2009) Use of membrane processing to concentrate TGF- β 2 and IGF-I from bovine milk and whey. *Journal of Membrane Science* **326**, 435–440.
- Arnold, D., Di Biase, A.M., Marchetti, M., Pietrantoni, A., Valenti, P., Seganti, L., and Superti, F. (2002) Antiadenovirus activity of milk proteins: lactoferrin prevents viral infection. *Antiviral Research* **53**, 153–158.

- Ashar, M.N. and Chand, R. (2004) Fermented milk containing ACE-inhibitory peptides reduces blood pressure in middle aged hypertensive subjects. *Milchwissenschaft* **59**, 363–366.
- Azuma, N., Yamauchi, K., and Mitsuoka, T. (1984) Bifidus growth-promoting activity of a glycomacropeptide derived from human κ -casein. *Agricultural and Biological Chemistry* **48**(8), 2159–2162.
- Bahna, S.L. (2008) Hypoallergenic formulas: optimal choices for treatment versus prevention. *Annals of Allergy, Asthma & Immunology* **101**, 453–459.
- Ballard, F.J., Francis, G.L., and Regester, G.O. (1999) Milk protein mixture for promoting growth of animal cells or treating wounds and a method of making and methods employing the mixture. U.S. Patent 5, 866, 418.
- Bellamy, W., Takase, M., Wakabayashi, H., Kawase, K., and Tomita, M. (1992) Antibacterial spectrum of lactoferricin B, a potent bactericidal peptide derived from the N-terminal region of bovine lactoferrin. *Journal of Applied Bacteriology* **73**, 472–479.
- Ben Ounis, W., Gauthier, S.F., Turgeon, S.L., Roufik, S., and Pouliot, Y. (2008) Separation of minor protein from whey protein isolates by heparin affinity chromatography. *International Dairy Journal* **18**, 1043–1050.
- Ben Ounis, W., Gauthier, S.F., Turgeon, S.L., and Pouliot, Y. (2010) Separation of transforming growth factor-beta 2 (TGF- β 2) from whey protein isolates by crossflow microfiltration in the presence of a ligand. *Journal of Membrane Science* **351**, 189–195.
- Berkhout, B., van Wamel, J.L.B., Beljaars, L., Meijer, D.K.F., Visser, S., and Floris, R. (2002) Characterization of the anti-HIV effects of native lactoferrin and other milk proteins and protein-derived peptides. *Antiviral Research* **55**, 341–355.
- Beucher, S., Levenez, F., Yvon, M., and Corring, T. (1994) Effects of gastric digestive products from casein on Cck release by intestinal-cells in rat. *Journal of Nutritional Biochemistry* **5**(12), 578–584.
- Bolscher, J.G.M., van der Kraan, M.I.A., Nazmi, K., Kalay, H., Gruen, C.H., Van't Hof, W., Veerman, E.C.L., and Nieuw Amerongen, A.V. (2006) A one-enzyme strategy to release an antimicrobial peptide from the LFampin-domain of bovine lactoferrin. *Peptides* **27**, 1–9.
- Boots, J.-W. and Floris, R. (2006) Lactoperoxidase: from catalytic mechanism to practical applications. *International Dairy Journal* **16**, 1272–1276.
- Bouhallab, S. and Touze, C. (1995) Continuous hydrolysis of caseinoglycopeptide in a membrane reactor: kinetic study and gram-scale production of antithrombotic peptides. *Le Lait* **75**, 251–258.
- Brody, E.P. (2000) Biological activities of bovine glycomacropeptide. *British Journal of Nutrition* **84**, S39–S46.
- Bruck, W.M., Graverholt, G., and Gibson, G.R. (2003a) A two-stage continuous culture system to study the effect of supplemental alpha-lactalbumin and glycomacropeptide on mixed cultures of human gut bacteria challenged with enteropathogenic *Escherichia coli* and *Salmonella* serotype Typhimurium. *Journal of Applied Microbiology* **95**(1), 44–53.
- Bruck, W.M., Kelleher, S.L., Gibson, G.R., Nielsen, K.E., Chatterton, D.E., and Lonnerdal, B. (2003b) rRNA probes used to quantify the effects of glycomacropeptide and alpha-lactalbumin supplementation on the predominant groups of intestinal bacteria of infant rhesus monkeys challenged with enteropathogenic *Escherichia coli*. *Journal of Pediatric Gastroenterology and Nutrition* **37**(3), 273–280.
- Bruck, W.M., Kelleher, S.L., Gibson, G.R., Graverholt, G., and Lonnerdal, B.L. (2006a) The effects of alpha-lactalbumin and glycomacropeptide on the association of CaCo-2 cells by enteropathogenic *Escherichia coli*, *Salmonella typhimurium* and *Shigella flexneri*. *FEMS Microbiology Letters* **259**(1), 158–162.
- Bruck, W.M., Redgrave, M., Tuohy, K.M., Lonnerdal, B., Graverholt, G., Hernell, O., and Gibson, G.R. (2006b) Effects of bovine alpha-lactalbumin and casein glycomacropeptide-enriched infant formulae on faecal microbiota in healthy term infants. *Journal of Pediatric Gastroenterology and Nutrition* **43**(5), 673–679.
- Burton-Freeman, B.M. (2008) Glycomacropeptide (GMP) is not critical to whey-induced satiety, but may have a unique role in energy intake regulation through cholecystokinin (CCK). *Physiology & Behavior* **93**(1–2), 379–387.
- Butler, J.E. (1998) Immunoglobulin diversity, B-cell and antibody repertoire development in large farm animals. *Revue Scientifique et Technique* **17**, 43–70.
- Campbell, B. and Petersen, W.E. (1963) Immune milk—a historical survey. *Dairy Science Abstracts* **25**, 345–358.
- Chabance, B., Jollès, P., Izquierdo, C., Mazoyer, E., Francoual, C., Drouet, L., and Fiat, A.-M. (1995) Characterization of an antithrombotic peptide from α -casein in newborn plasma after milk ingestion. *British Journal of Nutrition* **73**, 583–590.

- Clifton, P.M., Keogh, J.B., Woonton, B.W., Taylor, C.M., Janakievski, F., and De Silva, K. (2009) Effect of glycomacropeptides (GMP) on satiety hormones and food intake. *Australian Journal of Dairy Technology* **64**(1), 29–31.
- Crittenden, R., Buckley, J., Cameron-Smith, D., Brown, A., Thomas, K., Davey, S., and Hobman, P. (2009) Functional dairy protein supplements for elite athletes. *Australian Journal of Dairy Technology* **64**, 133–137.
- Cross, K.J., Huq, N.L., Palamara, J.E., Perich, J.W., and Reynolds, E.C. (2005) Physicochemical characterization of casein phosphopeptide-amorphous calcium phosphate nanocomplexes. *Journal of Biological Chemistry* **280**, 15362–15369.
- Dashper, S.G., Liu, S.W., and Reynolds, E.C. (2007) Antimicrobial peptides and their potential as oral therapeutic agents. *International Journal of Peptide Research and Therapeutics* **13**(4), 505–516.
- DeSilva, K., Stockmann, R., and Smithers, G.W. (2003) Isolation procedures for functional dairy components—novel approaches to meeting the challenges. *Australian Journal of Dairy Technology* **58**(2), 148–152.
- Donkor, O.N., Henriksson, A., Singh, T.K., Vasiljevic, T., and Shah, N.P. (2007) ACE-inhibitory activity of probiotic yoghurt. *International Dairy Journal* **17**, 1321–1331.
- Dosako, S., Kusano, H., Deya, E., and Idota, T. (1992) Infection protectant. U.S. Patent 5147853.
- Drouin, R., Lamiot, É., Cantin, K., Gauthier, S.F., Pouliot, Y., Poubelle, P.E., and Juneau, C. (2007) XP-828L (Dermylex™), a new whey protein extract for the treatment of mild to moderate psoriasis. *Canadian Journal of Physiology and Pharmacology* **85**, 943–951.
- Dunshea, F.R., Ostrowska, E., Ferrari, J.M., and Gill, H.S. (2007) Dairy proteins and the regulation of satiety and obesity. *Australian Journal of Experimental Agriculture* **47**(9), 1051–1058.
- Dyer, A.R., Burdock, G.A., Carabin, I.G., Haas, M.C., Boyce, J., Alsaker, R., and Read, L.C. (2008) *In vitro* and *in vivo* safety studies of a proprietary whey extract. *Food and Chemical Toxicology* **46**, 1659–1665.
- Elfstrand, L., Lindmark-Månsson, H., Paulsson, M., Nyberg, L., and Åkesson, B. (2002) Immunoglobulins, growth factors and growth hormone in bovine colostrums and the effects of processing. *International Dairy Journal* **12**, 879–887.
- Erdmann, K., Cheung, B.W.Y., and Schröder, H. (2008) The possible roles of food-derived bioactive peptides in reducing the risk of cardiovascular disease. *The Journal of Nutritional Biochemistry* **19**, 643–654.
- Farkye, N.Y. (2002a) Enzymes indigenous to milk—xanthine oxidase. In: *Encyclopedia of Dairy Sciences*, edited by H. Roginski, J.F. Fuquay, and P.F. Fox, pp. 941–942. London: Academic Press/Elsevier Science.
- Farkye, N.Y. (2002b) Enzymes indigenous to milk—other enzymes. In: *Encyclopedia of Dairy Sciences*, edited by H. Roginski, J.F. Fuquay, and P.F. Fox, pp. 943–948. London: Academic Press/Elsevier Science.
- Fell, J.M., Paintin, M., Arnaud-Battandier, F., Beatties, R.M., Hollis, A., Kitching, P., Donnet-Hughes, A., Macdonald, T.T., and Walker-Smith, J.A. (2000) Mucosal healing and fall in mucosal pro-inflammatory cytokine mRNA induced by a specific oral polymeric diet in paediatric Crohn's disease. *Alimentary Pharmacology & Therapeutics* **14**, 281–289.
- Francis, G.L., Regester, G.O., Webb, H.A., and Ballard, F.J. (1995) Extraction from cheese whey by cation-exchange chromatography of factors that stimulate the growth of mammalian cells. *Journal of Dairy Science* **78**, 1209–1218.
- Gauthier, S.F., Pouliot, Y., and Maubois, J.L. (2006) Milk growth factors: a review. *Le Lait* **86**, 99–125.
- Gentry, P.A. (2004) Comparative aspects of blood coagulation. *The Veterinary Journal* **168**, 238–251.
- Gingerich, D.A. and McPhillips, C.A. (2005) Analytical approach to determination of safety of milk ingredients from hyperimmunized cows. *Regulatory Toxicology and Pharmacology* **41**, 102–112.
- Gobbetti, M., Stepaniak, L., De Angelis, M., Corsetti, A., and Di Cagno, R. (2002) Latent bioactive peptides in milk proteins: proteolytic activation and significance in dairy processing. *Critical Reviews in Food Science and Nutrition* **42**, 223–239.
- Gong, M. and Hubner, N. (2006) Molecular genetics of human hypertension. *Clinical Science* **110**, 315–326.
- Grosvenor, C.E., Picciano, M.F., and Baumrucker, C.R. (1993) Hormones and growth factors in milk. *Endocrine Reviews* **14**, 710–728.
- Groves, M.L. (1960) The isolation of a red protein from milk. *Journal of the American Chemical Society* **82**, 3345–3350.

- Guesdon, B., Messaoudi, M., Lefranc-Millot, C., Fromentin, G., Tomé, D., and Even, P.C. (2006) A tryptic hydrolysate from bovine milk [α]S1-casein improves sleep in rats subjected to chronic mild stress. *Peptides* **27**, 1476–1482.
- Guilloteau, P., Rome, V., Delaby, L., Mendy, F., Roger, L., and Chayvialle, J.A. (2010) Is caseinomacropptide from milk proteins, an inhibitor of gastric secretion? *Regulatory Peptides* **159**(1–3), 129–136.
- Gustafson, D.R., McMahon, D.J., Morrey, J., and Nan, R. (2001) Appetite is not influenced by a unique milk peptide: caseinomacropptide (CMP). *Appetite* **36**(2), 157–163.
- Hammarström, L. and Weiner, C.K. (2008) Targeted antibodies in dairy-based products. *Advances in Experimental Medicine and Biology* **606**, 321–343.
- Hancock, R.E.W. (2004) Bacterial structure and physiology: influence on susceptibility to cationic antimicrobial peptides. In: *Mammalian Host Defense Peptides*, edited by D.A. Devine and R.E.W. Hancock. Cambridge: Cambridge University Press.
- Hancock, R.E.W. and Chapple, D.S. (1999) Peptide antibiotics. *Antimicrobial Agents and Chemotherapy* **43**, 1317–1323.
- Haukland, H.H., Ulvatne, H., Sandvik, K., and Vorland, L.H. (2001) The antimicrobial peptides lactoferricin B and magainin 2 cross over the bacterial cytoplasmic membrane and reside in the cytoplasm. *FEBS Letters* **508**, 389–393.
- Hawkes, J.S., Bryan, D.-L., James, M.J., and Gibson, R.A. (1999) Cytokines (IL-1[β], IL-6, TNF-[α], TGF-[β]1, and TGF-[β]2) and prostaglandin E2 in human milk during the first three months postpartum. *Pediatric Research* **46**, 194–199.
- Hayes, M., Stanton, C., Fitzgerald, G.F., and Ross, R.P. (2007) Putting microbes to work: dairy fermentation, cell factories and bioactive peptides. Part II: bioactive peptide functions. *Biotechnology Journal* **2**, 435–449.
- Hendrixson, D.R., Qiu, J., Shewry, S.C., Fink, D.L., Petty, S., Baker, E.N., Plaut, A.G., and Geme, J.W. (2003) Human milk lactoferrin is a serine milk protease that cleaves *Haemophilus* surface proteins at arginine-rich sites. *Molecular Microbiology* **47**, 607–617.
- Hernández-Ledesma, B., Amigo, L., Ramos, M., and Recio, I. (2004) Release of angiotensin converting enzyme-inhibitory peptides by simulated gastrointestinal digestion of infant formulas. *International Dairy Journal* **14**, 889–898.
- Hoerr, R.A. and Bostwick, E.F. (2002) Commercializing colostrum based products: a case study of Galagen Inc. *International Dairy Federation Bulletin* **375**, 33–46.
- Hong, F., Ming, L., Yi, S., Zhanxia, L., Yongquan, W., and Chi, L. (2008) The antihypertensive effect of peptides: a novel alternative to drugs? *Peptides* **29**, 1062–1071.
- Hossner, K.L. and Yemm, R.S. (2000) Improved recovery of insulin growth factors (IGFs) from bovine colostrum using alkaline diafiltration. *Biotechnology and Applied Biochemistry* **32**, 161–166.
- Hurley, W.L. (2003) Immunoglobulins in mammary secretions. In: *Advances in Dairy Chemistry, Proteins*, edited by P.F. Fox and P.L.H. McSweeney, pp. 422–427. New York: Kluwer Academic/Plenum Publishers.
- Hursel, R., van der Zee, L., and Westerterp-Plantenga, M.S. (2010) Effects of a breakfast yoghurt, with additional total whey protein or caseinomacropptide-depleted α -lactalbumin-enriched whey protein, on diet-induced thermogenesis and appetite suppression. *British Journal of Nutrition* **103**(5), 775–780.
- Idota, T., Kawakami, H., and Nakajima, I. (1994) Growth-promoting effects of N-Acetylneuraminic acid-containing substances on bifidobacteria. *Bioscience, Biotechnology, and Biochemistry* **58**(9), 1720–1722.
- Ijäs, H., Collin, M., Finckenberg, P., Pihlanto-Leppälä, A., Korhonen, H., Korpela, R., Vapaatalo, H., and Nurminen, M.-L. (2004) Antihypertensive opioid-like milk peptide [α]-lactorphin: lack of effect on behavioural tests in mice. *International Dairy Journal* **14**, 201–205.
- Isamida, T., Tanaka, T., Omata, Y., Yamauchi, K., Shimazaki, K., and Saito, A. (1998) Protective effect of lactoferricin against *Toxoplasma gondii* infection in mice. *Journal of Veterinary Medical Science* **60**, 241–244.
- Isoda, H., Kawasaki, Y., Tanimoto, M., Dosako, S., and Idota, T. (1999) Use of compounds containing or binding sialic acid to neutralize bacterial toxins. European Patent 385112.
- Janer, C., Pelaez, C., and Requena, T. (2004) Caseinomacropptide and whey protein concentrate enhance *Bifidobacterium lactis* growth in milk. *Food Chemistry* **86**(2), 263–267.
- Jarmolowska, B., Szlapka-Sienkiewicz, E., Kostyra, E., Kostyra, H., Mierzejewska, D., and Marcinkiewicz-Darmochwał, K. (2007) Opioid activity of Humana formula for newborns. *Journal of the Science of Food and Agriculture* **87**, 2247–2250.
- Jenssen, H. and Hancock, R.E.W. (2009) Antimicrobial properties of lactoferrin. *Biochimie* **91**, 19–29.

- Jiang, S., Lin, K., Strick, N., Li, Y.-Y., and Neurath, A.R. (1996) Chemically modified bovine β -lactoglobulin blocks uptake of HIV-1 by colon- and cervix-derived epithelial cell lines. *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology* **13**, 461–462.
- Jordan, B. and Devi, L.A. (1998) Molecular mechanisms of opioid receptor signal transduction. *British Journal of Anaesthesia* **81**(1), 12–19.
- Juneau, C., Drouin, R., Pouliot, Y., Aattouri, N., Gauthier, S.F., and Lamiot, E. (2008) Composition for treating psoriasis. U.S. Patent 20080031969.
- Kawakami, H. (2005) Biological significance of milk basic protein (MBP) for bone health. *Food Science and Technology Research* **11**, 1–8.
- Kawasaki, Y., Isoda, H., Tanimoto, M., Dosako, S., Idota, T., and Ahiko, K. (1992) Inhibition by lactoferrin and kappa-casein glycomacropeptide of binding of cholera-toxin to its receptor. *Bioscience, Biotechnology, and Biochemistry* **56**(2), 195–198.
- Kawasaki, Y., Isoda, H., Shinmoto, H., Tanimoto, M., Dosako, S., Idota, T., and Nakajima, I. (1993) Inhibition by kappa-casein glycomacropeptide and lactoferrin of influenza-virus hemagglutination. *Bioscience, Biotechnology, and Biochemistry* **57**(7), 1214–1215.
- Kelly, P., Woonton, B.W., and Smithers, G.W. (2009) Improving the sensory quality, shelf-life and functionality of milk. In: *Functional and Speciality Beverage Technology*, edited by P. Paquin, pp. 170–231. Cambridge: Woodhead.
- Keogh, J.B. and Clifton, P. (2008) The effect of meal replacements high in glycomacropeptide on weight loss and markers of cardiovascular disease risk. *American Journal of Clinical Nutrition* **87**(6), 1602–1605.
- Kim, J.H., Desor, D., Kim, Y.T., Yoon, W.J., Kim, K.S., Jun, J.S., Pyun, K.H., and Shim, I. (2007) Efficacy of [alpha]s1-casein hydrolysate on stress-related symptoms in women. *European Journal of Clinical Nutrition* **61**, 536–541.
- Kitamura, H.O., Oshida, T., Otani, H., Wakaduki, S., and Kusuhara, S. (2002) Milk immunoglobulin levels in sows given a diet containing a commercially available casein phosphopeptide preparation (CPP-1) during pregnancy. *Milchwissenschaft. Milk Science International* **57**, 486–489.
- Kivits, M.G.C., Hendricks, A.W.J., and Malle, L.F. (2001) Process for obtaining growth factor preparations (TGF- β and IGF-I) from milk products having low mutual cross-contamination. PTC WO 01/25276 A1.
- Kokuba, H., Aurelian, L., and Neurath, A.R. (1998) 3-Hydroxyphthaloyl β -lactoglobulin. IV. Antiviral activity in the mouse model of genital herpesvirus infection. *Antiviral Chemistry and Chemotherapy* **9**, 353–357.
- Korhonen, H. (1980) A new method for preserving raw milk—the lactoperoxidase antibacterial system. *World Animal Review* **35**, 23–29.
- Korhonen, H. (2004) Isolation of immunoglobulins from colostrum. *International Dairy Federation Bulletin* **389**, 78–81.
- Korhonen, H. (2009) Bioactive components in bovine milk. In: *Bioactive Components in Milk and Dairy Products*, edited by Y.W. Park, pp. 15–42. Ames, IA: Wiley-Blackwell.
- Korhonen, H. and Marnila, P. (2002) Lactoferrin. In: *Encyclopedia of Dairy Sciences*, edited by H. Roginski, J.F. Fuquay, and P.F. Fox, pp. 1946–1950. London: Academic Press/Elsevier Science.
- Korhonen, H. and Marnila, P. (2006) Bovine milk antibodies for protection against microbial human diseases. In: *Nutraceutical Proteins and Peptides in Health and Disease*, edited by Y. Mine and F. Shahidi, Series: Nutraceutical Science and Technology 4. Series Editor Fereidoon Shahidi. pp. 137–159. Boca Raton, FL: CRC Press, Taylor & Francis Group.
- Korhonen, H. and Marnila, P. (2009) Bovine milk immunoglobulins against microbial human diseases. In: *Dairy-Derived Ingredients. Food and Nutraceutical Uses*, edited by M. Corredig, pp. 269–289. Cambridge: Woodhead Publishing Limited.
- Korhonen, H., Marnila, P., and Gill, H.S. (2000a) Milk immunoglobulins and complement factors. *British Journal of Nutrition* **84**(Suppl. 1), S75–S80.
- Korhonen, H., Marnila, P., and Gill, H. (2000b) Bovine milk antibodies for health: a review. *British Journal of Nutrition* **84**(Suppl. 1), S1–S7.
- Korhonen, H. and Pihlanto, A. (2007) Technological options for the production of health-promoting proteins and peptides derived from milk and colostrum. *Current Pharmaceutical Design* **13**, 829–843.
- Kostyra, E. and Kostyra, H. (1992) The opioid activity of peptide milk extracts from cows with *Staphylococcus mastitis*. *Acta Academiae Agriculturae. Ac Technicae Olstenesis, Veterinaria*.
- Krissansen, G.W. (2007) Emerging health properties of whey proteins and their clinical implications. *Journal of the American College of Nutrition* **26**, 713S–723S.

- Kumar, V.L.N., Itthagarun, A., and King, N.M. (2008) The effect of casein phosphopeptide-amorphous calcium phosphate on remineralization of artificial caries-like lesions: an *in vitro* study. *Australian Dental Journal* **53**, 34–40.
- Lachkar, D., Lamiot, E., Gauthier, S.F., Turgeon, S.L., Paquin, P., and Pouliot, Y. (2008) An experimental approach for the removal of caseins from bovine colostrum using anionic polysaccharides. Effects on TGF- β 2 concentration. *International Journal of Dairy Technology* **61**, 43–50.
- LaClair, C.E., Ney, D.M., MacLeod, E.L., and Etzel, M.R. (2009) Purification and Use of Glycomacropeptide for Nutritional Management of Phenylketonuria. *Journal of Food Science* **74**(4), E199–E206.
- Lahov, E. and Regelson, W. (1996) Antibacterial and immunostimulating casein-derived substances from milk: casecidin, isracidin peptides. *Food and Chemical Toxicology* **34**, 131–145.
- Lefranc-Milot, C., Canivez, O., and Quinque, B. (1996) Fraction sérocolostrale à forte activité anticorps, utilisation et procédé de fabrication d'une telle fraction. EP 0 722 952 A1.
- Li-Chan, E., Kummer, A., Losso, J.N., Kits, D.D., and Nakai, S. (1995) Stability of bovine immunoglobulins to thermal treatment and processing. *Food Research International* **28**, 9–16.
- Lonnerdal, B. (2003) Nutritional and physiologic significance of human milk proteins. *American Journal of Clinical Nutrition* **77**, 1537S–1543S.
- Lopez-Exposito, I. and Recio, I. (2006) Antibacterial activity of peptides and folding variants from milk proteins. *International Dairy Journal* **16**, 1294–1305.
- Lüscher-Mattli, M. (2000) Polyanions—a lost chance in the fight against HIV and other virus diseases? *Antiviral Chemistry and Chemotherapy* **11**, 249–259.
- Lv, G.S., Huo, G.C., and Fu, X.Y. (2003) Expression of milk-derived antihypertensive peptide in *Escherichia coli*. *Journal of Dairy Science* **86**, 1927–1931.
- Mainer, G., Sánchez, L., Ena, J.M., and Calvo, M. (1997) Kinetic and thermodynamic parameters for heat denaturation of bovine milk IgG, IgA and IgM. *Journal of Food Science* **62**, 1034–1038.
- Mainer, G., Dominguez, E., Randrup, M., Sánchez, L., and Calvo, M. (1999) Effect of heat treatment on anti-rotavirus activity of bovine colostrum. *Journal of Dairy Research* **66**, 131–137.
- Malkoski, M., Dashper, S.G., O'Brien-Simpson, N.M., Talbo, G.H., Macris, M., Cross, K.J., and Reynolds, E.C. (2001) Kappacin, a novel antibacterial peptide from bovine milk. *Antimicrobial Agents and Chemotherapy* **45**(8), 2309–2315.
- Marchetti, M., Longhi, C., Conte, M.P., Pisani, S., Valenti, P., and Seganti, L. (1996) Lactoferrin inhibits herpes simplex virus type 1 adsorption to Vero cells. *Antiviral Research* **29**, 221–231.
- Marnila, P. and Korhonen, H. (2002) Immunoglobulins. In: *Encyclopedia of Dairy Sciences*, edited by H. Roginski, J.F. Fuquay, and P.F. Fox, pp. 1950–1956. London: Academic Press/Elsevier Science.
- Matin, M.A. and Otani, H. (2002) Cytotoxic and antibacterial activities of chemically synthesized κ -casecidin and its partial peptide fragments. *Journal of Dairy Research* **69**, 329–334.
- Mattarella, N.L., Creamer, L.K., and Richardson, T. (1983) Amidation or esterification of bovine β -lactoglobulin to form positively charged proteins. *Journal of Agricultural and Food Chemistry* **31**, 968–972.
- Maubois, J.L., Trouve, R., and Bouhallab, S. (1991) Milk peptides with cardiovascular activity—antithrombotic and antihypertensive activities. *Le Lait* **71**, 249–255.
- Maubois, J.L., Fauquant, J., Jouan, P., and Bourtourault, M. (2003) Method for obtaining a TGF- β enriched protein fraction in activated form, protein fraction and therapeutic applications. PCT/WO 03/006500.
- McCann, K.B., Lee, A., Wan, J., Roginski, H., and Coventry, H. (2003) The effect of bovine lactoferrin and lactoferricin B on the ability of feline calicivirus (a norovirus surrogate) and poliovirus to infect cell cultures. *Journal of Applied Microbiology* **95**, 1026–1033.
- McCann, K.B., Shiell, B.J., Michalski, W.P., Lee, A., Wan, J., Roginski, H., and Coventry, M.J. (2005) Isolation and characterisation of antibacterial peptides derived from the f(164–207) region of bovine a_{2-} -casein. *International Dairy Journal* **15**, 133–143.
- McCann, K.B., Shiell, B.J., Michalski, W.P., Lee, A., Wan, J., Roginski, H., and Coventry, M.J. (2006) Isolation and characterisation of a novel antibacterial peptide from bovine a_{s1-} -casein. *International Dairy Journal* **16**, 316–323.
- Mehra, R., Marnila, P., and Korhonen, H. (2006) Milk immunoglobulins for health promotion: a review. *International Dairy Journal* **16**, 1262–1271.
- Meisel, H. (2005) Biochemical properties of peptides encrypted in bovine milk proteins. *Current Medicinal Chemistry* **12**, 1905.
- Meisel, H. and Fitzgerald, R.J. (2003) Biofunctional peptides from milk proteins: mineral binding and cytomodulatory effects. *Current Pharmaceutical Design* **9**(16), 1289–1295.

- Meisel, H., Walsh, D.J., Murray, B. and FitzGerald, R.J. (2006) ACE inhibitory peptides. In: *Nutraceutical Proteins and Peptides in Health and Disease*, edited by Y. Mine and F. Shahidi, pp. 269–315. Boca Raton, London, and New York: CRC Press, Taylor & Francis Group.
- Minkiewicz, P., Slangen, C.J., Lagerwerf, F.M., Haverkamp, J., Rollema, H.S., and Visser, S. (1996) Reversed-phase high-performance liquid chromatographic separation of bovine kappa-casein macropeptide and characterization of isolated fractions. *Journal of Chromatography. A* **743**(1), 123–135.
- Miquel, E., Alegría, A., Barberá, R., and Farré, R. (2006) Casein phosphopeptides released by simulated gastrointestinal digestion of infant formulas and their potential role in mineral binding. *International Dairy Journal* **16**, 992–1000.
- Miura, T., Ono, K., Izumi, T., and Akuzawa, R. (2010) Presence of lactoferrin in a bovine skim milk fraction with acid phosphatase activity. *International Dairy Journal* **20**, 67–71.
- Molle, D. and Leonil, J. (1995) Heterogeneity of the Bovine Kappa-Casein Caseinomacropptide, Resolved by Liquid-Chromatography Online with Electrospray-Ionization Mass-Spectrometry. *Journal of Chromatography. A* **708**(2), 223–230.
- Montoni, A., Gauthier, S.F., Pouliot, Y., Richard, C., Poubelle, P.E., and Drouin, R. (2009) Study of the transforming growth factor β (TGF- β) and insuline-like growth factors-I (IGF-I) content in colostrum samples from Holstein cows. *Dairy Science and Technology* **89**, 511–518.
- Mor, A. (2003) Introduction. *Peptides* **24**, 1645.
- Nakasone, Y., Adjei, A., Yoshise, M., Yamauchi, K., Takase, M., Yamauchi, K., Shimamura, S., and Yamamoto, S. (1994) Effect of dietary lactoferricin on the recovery of mice infected with methicillin-resistant *Staphylococcus aureus*. In *Abstracts of Annual Meeting of the Japanese Society of Nutritional Food Science*, p. 50 [in Japanese].
- Neeser, J.R., Golliard, M., Woltz, A., Rouvet, M., Dillmann, M.L., and Guggenheim, B. (1994) *In vitro* modulation of oral bacterial adhesion to saliva-coated hydroxyapatite beads by milk casein derivatives. *Oral Microbiology and Immunology* **9**(4), 193–201.
- Neurath, A.R., Jiang, S., Strick, N., Lin, K., Li, Y.-Y., and Debnath, A.K. (1996) Bovine β -lactoglobulin modified by 3-hydroxyphthalic anhydride blocks the CD4 cell receptor for HIV. *Nature Medicine* **2**, 230–234.
- Neurath, A.R., Debnath, A.K., Strick, N., Li, Y.-Y., Lin, K., and Jiang, S. (1997) 3-Hydroxyphthaloyl- β -lactoglobulin. I. Optimization of production and comparison with other compounds considered for chemoprophylaxis of mucosally transmitted human immunodeficiency virus type 1. *Antiviral Chemistry and Chemotherapy* **8**, 131–139.
- Ney, D.M., Hull, A.K., van Calcar, S.C., Liu, X.W., and Etzel, M.R. (2008) Dietary glycomacropptide supports growth and reduces the concentrations of phenylalanine in plasma and brain in a murine model of phenylketonuria(1,2). *Journal of Nutrition* **138**(2), 316–322.
- Nielsen, M.S., Martinussen, T., Flambard, B., Sørensen, K.I., and Otte, J. (2009) Peptide profiles and angiotensin-I-converting enzyme inhibitory activity of fermented milk products: effect of bacterial strain, fermentation pH, and storage time. *International Dairy Journal* **19**, 155–165.
- Oevermann, A., Engels, M., Thomas, U., and Pellegrini, A. (2003) The antiviral activity of naturally occurring proteins and their peptide fragments after chemical modification. *Antiviral Research* **59**, 23–33.
- Oh, S., Worobo, R.W., Kim, B.C., Rheem, S., and Kim, S. (2000) Detection of the cholera toxin-binding activity of kappa-casein macropeptide and optimization of its production by the response surface methodology. *Bioscience, Biotechnology, and Biochemistry* **64**(3), 516–522.
- Otani, H. and Watanabe, T. (2006) Enhancement of immunoglobulin A formation by a casein phosphopeptide, bovine beta-casein (1-28), via modulation of cytokines produced by Th2 type of lymphocytes in mouse spleen cell cultures. *Milchwissenschaft. Milk Science International* **61**(2), 123–126.
- Otani, H., Monnai, M., and Hosono, A. (1992) Bovine kappa-casein as inhibitor of the proliferation of mouse splenocytes induced by lipopolysaccharide stimulation. *Milchwissenschaft. Milk Science International* **47**(8), 512–515.
- Ozawa, T., Miyata, M., Nishimura, M., Ando, T., Ouyang, Y., Ohba, T., Shimokawa, N., Ohnuma, Y., Katoh, R., Ogawa, H., and Nakao, A. (2009) Transforming growth factor- β activity in commercially available pasteurized cow milk provides protection against inflammation in mice. *Journal of Nutrition* **139**, 69–75.
- Pakkanen, R. and Aalto, J. (1997) Growth factors and antimicrobial factors of bovine colostrum. *International Dairy Journal* **7**, 285–297.
- Pan, Y., Lee, A., Wan, J., Coventry, M.J., Michalski, W.P., Shiell, B., and Roginski, H. (2006) Antiviral properties of milk proteins and peptides. *International Dairy Journal* **16**, 1252–1261.

- Pan, Y., Shiell, B., Wan, J., Coventry, M.J., Roginski, H., Lee, A., and Michalski, W.P. (2007a) The molecular characterisation and antimicrobial activity of amidated bovine lactoferrin. *International Dairy Journal* **17**, 606–616.
- Pan, Y., Shiell, B., Wan, J., Coventry, M.J., Michalski, W.P., Lee, A., and Roginski, H. (2007b) The molecular characterisation and antimicrobial activity of amidated bovine β -lactoglobulin. *International Dairy Journal* **17**, 1450–1459.
- Pedersen, N.L.R., Nagain-Domaine, C., Mahe, S., Chariot, J., Roze, C., and Tome, D. (2000) Caseinomacropeptide specifically stimulates exocrine pancreatic secretion in the anesthetized rat. *Peptides* **21**(10), 1527–1535.
- Pellegrini, A., Thomas, U., Bramaz, N., Hunziker, P., and von Fellenberg, R. (1999) Isolation and identification of three bactericidal domains in the bovine α -lactalbumin molecule. *Biochimica et Biophysica Acta* **1426**, 439–448.
- Pellegrini, A., Dettling, C., Thomas, U., and Hunziker, P. (2001) Isolation and characterization of four bactericidal domains in the bovine β -lactoglobulin. *Biochimica et Biophysica Acta* **1526**, 131–140.
- Peroni, D.G., Piacentini, G.L., Bodini, A., Pigozzi, R., and Boner, A.L. (2009) Transforming growth factor- β 1 is elevated in unpasteurized cow's milk. *Pediatric Allergy and Immunology* **20**, 42–44.
- Pihlanto-Leppala, A. (2001) Bioactive peptides derived from whey proteins: opoid and ace-inhibitory peptides. *Trends in Food Science and Technology*, **11**, 347–356.
- Piot, M., Fauquant, J., Madec, M.-N., and Maubois, J.-L. (2004) Preparation of serocolostrum by membrane filtration. *Le Lait* **84**, 333–341.
- Playford, R.J., Macdonald, C.E., and Johnson, W.S. (2000) Colostrum and milk-derived peptide growth factors for the treatment of gastrointestinal disorders. *American Journal of Clinical Nutrition* **72**, 5–14.
- Poulin, Y., Pouliot, Y., Lamiot, E., Aattouri, N., and Gauthier, S.F. (2005) Safety and efficacy of a milk-derived extract in the treatment of plaque psoriasis: an open-label study. *Journal of Cutaneous Medicine and Surgery* **9**, 271–275.
- Poulin, Y., Bissonette, R., Juneau, C., Cantin, K., Drouin, R., and Poubelle, P.E. (2006) XP-828L in the treatment of mild-to-moderate psoriasis: an randomized, double blind, placebo-controlled study. *Journal of Cutaneous Medicine and Surgery* **10**, 241–248.
- Pouliot, Y. and Gauthier, S.F. (2006) Milk growth factors as health products: some technological aspects. *International Dairy Journal Special Issue: Milk as a Source of Functional Foods: Technological and Health Aspects* **16**, 1415–1420.
- Poustie, V.J. and Wildgoose, J. (2010) Dietary interventions for phenylketonuria. *Cochrane Database of Systematic Reviews* (1), CD001304.
- Powers, J.-P.S. and Hancock, R.E.W. (2003) The relationship between peptide structure and antibacterial activity. *Peptides* **24**, 1681–1691.
- Qiu, J., Hendrixson, D.R., Baker, E.N., Murphy, T.F., St Geme, III, J.W., and Plaut, A.G. (1998) Human milk lactoferrin inactivates two putative colonization factors expressed by *Haemophilus influenzae*. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 12641–12646.
- Recio, I. and Visser, S. (1999) Identification of two distinct antibacterial domains within the sequence of bovine α_2 -casein. *Biochimica et Biophysica Acta* **1428**, 314–326.
- Recio, I., Floris, R., and Visser, S. (2000) Bioactive peptides from food proteins: A new isolation method. *Agro Food Industry Hi-Tech* **11**, 9–11.
- Reid, L.D. and Hubbell, C.L. (1994) An assessment of the addiction potential of the opioid associated with milk. *Journal of Dairy Science* **77**(3), 672–675.
- Rekdal, Ø., Andersen, J., Vorland, L.H., and Svendsen, J.S. (1999) Construction and synthesis of lactoferricin derivatives with enhanced antibacterial activity. *Journal of Peptide Science* **5**, 32–45.
- Rhoades, J.R., Gibson, G.R., Formentin, K., Beer, M., Greenberg, N., and Rastall, R.A. (2005) Caseinoglycomacropeptide inhibits adhesion of pathogenic *Escherichia coli* strains to human cells in culture. *Journal of Dairy Science* **88**(10), 3455–3459.
- Richardson, A., de Antueno, R., Duncan, R., and Hoskin, D.W. (2009) Intracellular delivery of bovine lactoferricin's antimicrobial core (RRWQWR) kills T-leukemia cells. *Biochemical and Biophysical Research Communications* **388**, 736–741.
- Rogers, M.-L., Goddard, C., Regester, G.O., Ballard, F.J., and Belford, D.A. (1996) Transforming growth factor β in bovine milk: concentration stability and molecular mass forms. *Journal of Endocrinology* **151**, 77–86.
- Roginski, H., Broome, M.C., and Hickey, M.W. (1984) Non-phage inhibition of Group N streptococci in milk. I. The incidence of inhibition in bulk milk. *Australian Journal of Dairy Technology* **39**, 23–27.

- Royle, P.J., McIntosh, G.H., and Clifton, P.M. (2008) Whey protein isolate and glycomacropeptide decrease weight gain and alter body composition in male Wistar rats. *British Journal of Nutrition* **100**(1), 88–93.
- Rusu, D., Drouin, R., Pouliot, Y., Gauthier, S., and Poubelle, P.E. (2009) A bovine whey protein extract can enhance innate immunity by priming normal human blood neutrophils. *Journal of Nutrition* **139**, 386–393.
- Rusu, D., Drouin, R., Pouliot, Y., Gauthier, S., and Poubelle, P. (2010) A Bovine Whey Protein Extract Stimulates Human Neutrophils to Generate Bioactive IL-1Ra through a NF- κ B- and MAPK-Dependent Mechanism. *Journal of Nutrition* **140**(2), 382–391.
- Scammell, A.W. (2001) Production and uses of colostrum. *Australian Journal of Dairy Technology* **56**, 74–82.
- Schade, R.P., Van Ieperen-Van Dijk, A.G., Van Reijssen, F.C., Versluis, C., Kimpen, J.L.L., Knol, E.F., Bruijnzeel-Koomen, C.A.F.M., and Van Hoffen, E. (2000) Differences in antigen-specific T-cell responses between infants with atopic dermatitis with and without cow's milk allergy: relevance of TH2 cytokines. *Journal of Allergy and Clinical Immunology* **106**, 1155–1162.
- Schiffer, M., Chang, C.H., and Stevens, F.J. (1992) The functions of tryptophan residues in membrane proteins. *Protein Engineering* **5**, 213–214.
- Schoen, P., Corver, J., Meijer, D.K.F., Wilschut, J., and Swart, P.J. (1997) Inhibition of influenza virus fusion by polyanionic proteins. *Biochemical Pharmacology* **53**, 995–1003.
- Sermon, J., Vanoirbeek, K., De Spiegeleer, P., Van Houdt, R., Aertsen, A., and Michiels, C.W. (2005) Unique stress response to the lactoperoxidase-thiocyanate enzyme system in *E. coli*. *Research in Microbiology* **156**, 225–232.
- Shakeel-Ur-Rehman and Farkye, N.Y. (2002) Enzymes indigenous to milk—lactoperoxidase. In: *Encyclopedia of Dairy Sciences*, edited by H. Roginski, J.F. Fuquay, and P.F. Fox, pp. 938–941. London: Academic Press/Elsevier Science.
- Sheehan, J.C. and Hess, G.P. (1955) A new method of forming peptide bonds. *Journal of the American Chemical Society* **77**, 1067–1068.
- Shin, K., Hayasawa, H., and Lonnerdal, B. (2001) Inhibition of *E. coli* respiratory enzymes by the lactoperoxidase-hydrogen peroxide-thiocyanate antimicrobial system. *Journal of Applied Microbiology* **90**, 489–493.
- Sienkiewicz-Szlapka, E., Jarmolowska, B., Krawczuk, S., Kostyra, E., Kostyra, H., and Bielikowicz, K. (2009a) Transport of bovine milk-derived opioid peptides across a Caco-2 monolayer. *International Dairy Journal* **19**, 252–257.
- Sienkiewicz-Szlapka, E., Jarmolowska, B., Krawczuk, S., Kostyra, E., Kostyra, H., and Iwan, M. (2009b) Contents of agonistic and antagonistic opioid peptides in different cheese varieties. *International Dairy Journal* **19**, 258–263.
- Siragy, H.M. (1999) The role of the AT(2) receptor in hypertension. *American Journal of Hypertension* **12**, 221a–a.
- Smithers, G.W. (2004) Isolation of growth factors from whey and their application in food and biotechnology industries—a brief review. In: *Advances in Fractionation and Separation Processes for Novel Dairy Applications*, IDF Bulletin 389, pp. 16–19. Brussels: International Dairy Federation.
- Stan, E., Groisman, S.D., Krasil'shchikov, K.B., and Chernikov, M.P. (1983) Effect of kappa-casein glycomacropeptide on gastrointestinal motility in dogs. *Biulleten' Eksperimental'noi Biologii i Meditsiny* **96**(7), 10–12.
- Struff, W.G. and Sprotte, G. (2007) Bovine colostrum as a biologic in clinical medicine: a review. *International Journal of Clinical Pharmacology and Therapeutics* **45**, 193–202.
- Swart, P.J. and Meijer, D.K.F. (1994) Negatively-charged albumins: a novel class of polyanionic proteins with a potent anti-HIV activity. *Antiviral News* **2**, 69–71.
- Swart, P.J., Kuipers, M.E., Smit, C., Pauwels, R., De Béthune, M.-P., De Clercq, E., Meijer, D.K.F., and Huisman, J.G. (1996) Antiviral effects of milk proteins: acylation results in polyanionic compounds with potent activity against human immunodeficiency virus types 1 and 2 *in vitro*. *AIDS Research and Human Retroviruses* **12**, 769–775.
- Swart, P.J., Kuipers, M.E., Smit, C., Van Der Strate, B.W.A., Harmsen, M.C., and Meijer, D.K.F. (1998) Lactoferrin: antiviral activity of lactoferrin. In: *Advances in Lactoferrin Research*, edited by G. Spik, D. Legrand, J. Mazurier, A. Pierce, and J.-P. Perraudin, pp. 205–213. New York: Plenum Press.
- Swart, P.J., Beljaars, L., Kuipers, M., Smit, C., Nieuwenhuis, P., and Meijer, D.K.F. (1999a) Homing of negatively charged albumins to the lymphatic system. *Biochemical Pharmacology* **58**, 1425–1435.

- Swart, P.J., Harmsen, M.C., Kuipers, M.E., Van Dijk, A.A., Van Der Strate, B.W.A., Van Berkel, P.H.C., Huijens, J.H., Smit, C., Witvrouw, M., De Clercq, E., De Béthune, M.-P., Pauwels, R., and Meijer, D.K.F. (1999b) Charge modification of plasma and milk proteins results in antiviral active compounds. *Journal of Peptide Science* **5**, 563–576.
- Takada, Y., Kobayashi, N., Kato, K., Yamamura, J., Yahiro, M., Kumegawa, M., and Aoe, S. (1997) Whey protein suppresses the osteoclast-mediated bone resorption and osteoclast cell formation. *International Dairy Journal* **7**, 821–825.
- Taylor, C.M. and Woonton, B.W. (2009) Quantity and carbohydrate content of glycomacropeptide fractions isolated from raw and heat-treated milk. *International Dairy Journal* **19**(12), 709–714.
- Teschemacher, H. (2003) Opioid receptor ligands derived from food proteins. *Current Pharmaceutical Design* **9**, 1331–1334.
- Teschemacher, H., Koch, G., and Brantl, V. (1997) Milk protein-derived opioid receptor ligands. *Peptide Science* **43**, 99–117.
- Tetra Pak Processing Systems (2003) Chromatographic isolation of lactoperoxidase and lactoferrin. In: *Dairy Processing Handbook*, pp. 352–353. Malmoe: Tetra Pak Processing Systems AB.
- Tripathi, V. and Vashishtha, B. (2006) Bioactive compounds of colostrum and its application. *Food Review International* **22**, 225–244.
- Ulvatne, H., Samuelsen, Ø., Haukland, H.H., Kramer, M., and Vorland, L.H. (2004) Lactoferricin B inhibits bacterial macromolecular synthesis in *Escherichia coli* and *Bacillus subtilis*. *FEMS Microbiology Letters* **237**, 377–384.
- Vacca-Smith, A.M., Van Wuyckhuysse, B.C., Tabak, L.A., and Bowen, W.H. (1994) The effect of milk and casein proteins on the adherence of *Streptococcus mutans* to saliva-coated hydroxyapatite. *Archives of Oral Biology* **39**(12), 1063–1069.
- van Calcar, S.C., MacLeod, E.L., Gleason, S.T., Etzel, M.R., Clayton, M.K., Wolff, J.A., and Ney, D.M. (2009) Improved nutritional management of phenylketonuria by using a diet containing glycomacropeptide compared with amino acids. *American Journal of Clinical Nutrition* **89**(4), 1068–1077.
- van der Kraan, M.I.A., Groenink, J., Nazmi, K., Veerman, E.C.I., Bolscher, J.G.M., and Nieuw Amerongen, A.V. (2004) Lactoferrampin: a novel antimicrobial peptide in the N1-domain of bovine lactoferrin. *Peptides* **25**, 177–183.
- van der Kraan, M.I.A., van Marle, J., Nazmi, K., Groenink, J., Van't Hof, W., Veerman, E.C.I., Bolscher, J.G.M., and Nieuw Amerongen, A.V. (2005a) Ultrastructural effects of antimicrobial peptides from bovine lactoferrin on the membrane of *Candida albicans* and *Escherichia coli*. *Peptides* **26**, 1537–1542.
- van der Kraan, M.I.A., van der Made, C., Nazmi, K., Van't Hof, W., Groenink, J., Veerman, E.C.I., Bolscher, J.G.M., and Nieuw Amerongen, A.V. (2005b) Effect of amino acid substitutions on the candidacidal activity of LFampin 265–284. *Peptides* **26**, 2093–2097.
- Varelias, A., Cowin, A.J., Adams, D., Harries, R.H.C., Cooter, R.D., Belford, D.A., Fitridge, R.A., and Rayner, T.E. (2006) Mitogenic bovine whey extract modulates matrix metalloproteinase-2, -9, and tissue inhibitor of matrix metalloproteinase-2 levels in chronic leg ulcer. *Wound Repair and Regeneration* **14**, 28–37.
- Vegarud, G.E., Langsrud, T., and Svenning, C. (2000) Mineral-binding milk proteins and peptides; occurrence, biochemical and technological characteristics. *British Journal of Nutrition* **84**, 91–98.
- Veldhorst, M.A., Nieuwenhuizen, A.G., Hochstenbach-Waelen, A., Westerterp, K.R., Engelen, M.P., Brummer, R.J., Deutz, N.E., and Westerterp-Plantenga, M.S. (2009) A breakfast with alpha-lactalbumin, gelatin, or gelatine + TRP lowers energy intake at lunch compared with a breakfast with casein, soy, whey, or whey-GMP. *Clinical Nutrition* **28**(2), 147–155.
- Vermeirssen, V., Camp, J.V., and Verstraete, W. (2004) Bioavailability of angiotensin I converting enzyme inhibitory peptides. *British Journal of Nutrition* **92**, 357–366.
- Vorland, L.H., Ulvatne, H., Rekdal, Ø., and Svendsen, J.S. (1999) Initial binding sites of antimicrobial peptides in *Staphylococcus aureus* and *E. coli*. *Scandinavian Journal of Infectious Diseases* **31**, 467–473.
- Wakabayashi, H., Yamauchi, K., and Takase, M. (2006) Lactoferrin research, technology and applications. *International Dairy Journal* **16**, 1241–1251.
- Walker, G.D., Cai, F., Shen, P., Bailey, D.L., Yuan, Y., Cochrane, N.J., Reynolds, C., and Reynolds, E.C. (2009) Consumption of milk with added casein phosphopeptide-amorphous calcium phosphate remineralizes enamel subsurface lesions in situ. *Australian Dental Journal* **54**, 245–249.
- Wheeler, T.T., Hodgkinson, A.J., Prosser, C.G., and Davis, S.R. (2007) Immune components of colostrum and milk—a historical perspective. *Journal of Mammary Gland Biology and Neoplasia* **12**, 237–247.

- Yun, Z.Y., Zhang, H.P., Cai, X.Z., Wang, A.P., and Zhang, L.B. (2007) Kinetic and thermodynamic studies on the thermal denaturation of bovine milk insulin-like growth factor-I in model systems. *Le Lait* **87**, 139–148.
- Yvon, M., Beucher, S., Guilloteau, P., Lehuereouluron, I., and Corring, T. (1994) Effects of Caseinomacropptide (Cmp) on Digestion Regulation. *Reproduction, Nutrition, Development* **34**(6), 527–537.
- Zeder-Lutz, G., Neurath, A.R., and Van Regenmortel, M.H.V. (1999) Kinetics of interaction between 3-hydroxyphthaloyl- β -lactoglobulin and CD4 molecules. *Biologicals* **27**, 29–34.
- Zhou, J.Q., Lu, J.P., and Ren, R.X. (2006) Review on the opioid peptide derived from milk. *China's Dairy Industry* **34**(10), 45–48.
- Zucht, H.-D., Raida, M., Adermann, K., Magert, H.J., and Forssmann, W.G. (1995) Casocidin-I: a casein- α_{s2} derived peptide exhibits antibacterial activity. *FEBS Letters* **372**, 185–188.
- Zucht, H.-D., Forssmann, W.G., Raida, M., and Adermann, K. (1996) Verfahren zur Gewinnung eines antibiotisch wirksamen Praeparates aus der bovinen Milch und zu deren synthetischen Darstellung. German Patent DE 4444 753 A1.

5 Modern Chromatographic Separation Technologies for Isolation of Dairy Ingredients

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5.1 INTRODUCTION

Chromatography (from the Greek words for “color” and “to write”) is a technology that can separate, isolate, analyze, and purify various components in a heterogeneous mixture. The essence of modern chromatography was discovered, and the practical basis for its operation established more than 100 years ago through the pioneering work of M.S. Tswett (see Ettre and Sakodynskii [1993] for a review of Tswett’s work between 1903 and 1910). Chromatographic separation of mixtures of various compounds is based upon their distribution between a stationary and a mobile phase. The technology relies on different molecules with different physical and chemical characteristics (e.g., boiling point, polarity, electric charge [for ionic compounds], molecular size, stereochemistry, and various combinations of these) behaving in different ways when they are dissolved in a mobile phase (usually a solvent or a gas) and moved across a stationary phase (adsorbent medium) usually housed in a column. Even subtle differences in a molecule’s partition coefficient between the stationary and mobile phases will result in differential retention on the stationary phase and thus lead to separation. Such differences can be exploited to separate the molecules for both analytical and preparative purposes. An excellent review, recently revised and updated, of the principles and applications of chromatography is that by Lederer and Lederer (1957).

There are a number of different types of chromatography in use today for both analytical and preparative purposes, including gas, liquid, thin-layer, and gel permeation chromatography, and several of these are in widespread use within the food, pharmaceutical, and other manufacturing industries. An excellent recent review of the applications of chromatography in the industrial sector has been authored by Schoenmakers (2009).

Within the dairy processing industry, chromatography has several advantages for a processor looking to isolate commercial quantities of a dairy component (e.g., protein) within a heterogeneous and complex mixture, such as milk or whey. These advantages include specificity, reproducibility, robustness, and mildness. However, until recently, a number of real and/or perceived shortcomings of the technique have restricted the more widespread application of large-scale chromatography in dairy processing. Such

limitations, whether real or perceived, include high cost, restricted throughput, inflexibility, low productivity, and excessive complexity.

Recent advances in hardware, stationary phases, and the methodology used during the chromatographic process have all helped to address the shortcomings and to continue to capture the advantages of chromatography. These advances have perhaps given chromatography a new “lease of life” as a viable industrial processing technology for the manufacture of dairy ingredients (De Silva et al., 2003). This chapter reviews modern chromatographic separation technologies, including both the hardware and the stationary phases, and their potential and practical application in the isolation of dairy ingredients.

5.2 ISOLATION OF DAIRY COMPONENTS USING RESIN-BASED CHROMATOGRAPHY

The most vital elements in achieving efficient separation and isolation of targeted compounds, including dairy components, using chromatography are the adsorbent and the method of contacting the fluid containing the components with the adsorbent.

5.2.1 Chromatographic hardware

There are many techniques used to contain the adsorbent and achieve contact between the adsorbent and the fluid. The stirred tank technique involves mixing the adsorbent with the mobile phase in a large tank with agitation, holding to allow adsorption to take place and then separating the liquid and solid phases. This method is associated with poor adsorbent utilization efficiency, the potential for loss of adsorbent during processing, and adsorbent damage during mixing. The time taken to complete a batch is long, and it is very labor intensive. Overall, this method of chromatography is inefficient, costly, and therefore not often employed at commercial scale.

Batch or conventional column chromatography is an established method of containing the resin and contacting it with the fluid. In this system, the adsorbent is contained within the column with the use of frits or retaining screens fitted to flanges at both ends of the column, and the fluid (mobile phase) is passed through. Since the bed is tightly packed and the fluid generally flows in one direction, the adsorbent bed tends to act as a filter. Using fluids that contain particulate matter may foul the adsorbent bed, hence the fluid should be clarified prior to loading when using such columns. The efficiency of adsorbent utilization in conventional batch chromatography is low, and the volume of adsorbent needed to achieve the required duty is large. For the isolation of small quantities of valuable molecules, adsorbent utilization efficiency may not be a concern. An example of a conventional column design is the “Resolute” chromatography columns manufactured by the Pall Corporation, where a three-position nozzle valve enables packing, operation, and unpacking of the column (Figure 5.1). Such valves are not a necessity to enable batch column chromatography, but may improve loading, operating, and unpacking cycle times.

Radial flow columns (RFCs) are a variation of the conventional batch columns, in which the fluid, instead of flowing in a vertical direction, flows radially. The effective bed height of RFCs is low, as the fluid travels only the distance between the inner and outer surfaces. The area available for flow in a RFC is also greater than that of a conventional batch column. These characteristics result in low pressure drops across the bed and the ability to operate at higher flow rates. This technology has been commercialized in the dairy industry



Figure 5.1 An example of a batch chromatography column is the “Resolute” column manufactured by Pall Corporation. This has a three-position nozzle valve to enable packing, unpacking, and running of the column. Provided with compliments of Pall Corporation. Copyright Pall Corporation, 2010.

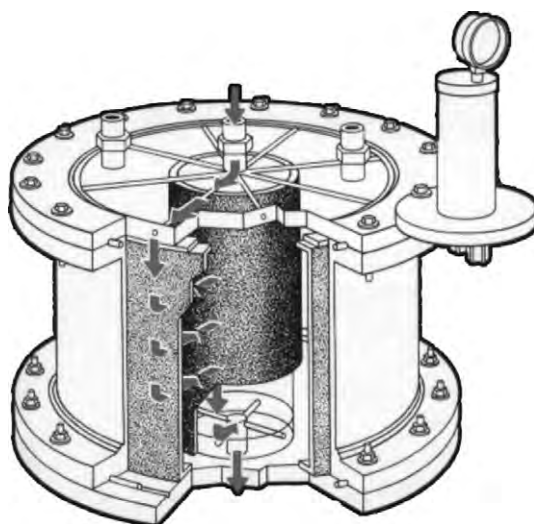


Figure 5.2 An example of a radial flow column (RFC). This RFC was developed and commercialized by Sepragen Corporation (Hayward, CA). The arrows indicate feed and permeate flow (<http://www.sepragen.com/files/SP/superflo.pdf>). Reproduced with permission from Sepragen Corporation.

in New Zealand and Ireland. Sepragen Corporation (Hayward, CA) have commercialized RFC technology for large-scale applications. Their columns have three annular channels. Fluid is introduced from the outer annular channel and flows across to the middle annular channel containing the resin and into the inner annular channel, which is connected to an outlet (Figure 5.2). Another example is the RFC commercialized by Handtmann Armaturenfabrik GmbH & Co. KG (Biberach, Germany). Handtmann columns are designed

so that fluid flows from the center radially toward the outer edge of the column. Handtmann columns are widely used in the brewing industry, where very large volumes of liquid must be processed.

A method to improve resin utilization and separation efficiency is to move the resin bed in a counter-current direction to the mobile phase. As such movement is impractical, simulated moving bed (SMB) chromatography was developed by Broughton and Gerhold and patented in 1961 (Broughton and Gerhold, 1961). This technique involves switching the liquid mobile phase through various columns to simulate moving of the resin bed. SMB chromatography captures the benefits of column chromatography and overcomes the inefficiencies associated with batch column chromatography. SMB technology is capable of achieving high efficiencies with respect to resin utilization assisted by its counter-current operation and its productive use of all the columns simultaneously. Due to its high efficiency of resin utilization, SMB technology requires significantly less volumes of resin compared with conventional column chromatography. This translates to lower operating costs by savings made in the lower volumes of buffers, eluents, water, and CIP chemicals required. An example of an SMB system is CSEP, developed by advanced separation technologies and now owned by Calgon Carbon Corporation. This system has been commercially established in the water treatment, chemical, and dairy industries (Figure 5.3).

An alternative SMB approach available at commercial scale is the VariCol technology developed by NovaSep. This technology uses four zones (feed, rinse, extract, and regenerate) and achieves SMB operation with nonsynchronous switching of inlet and outlet valves and recirculation pumps. The number of columns varies periodically in each zone.

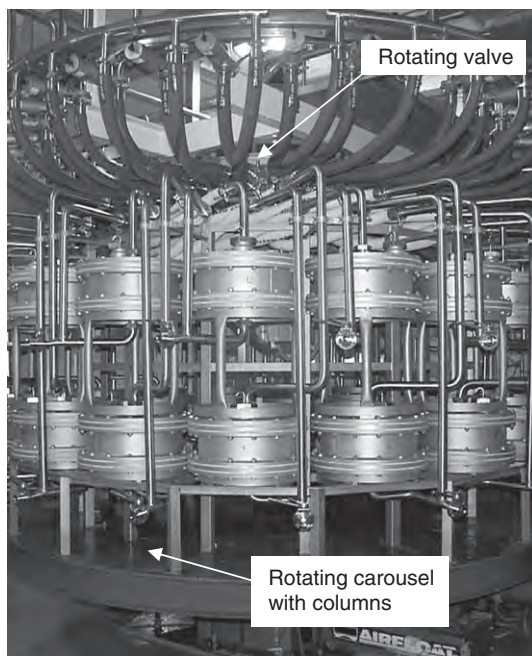


Figure 5.3 An example of a simulated moving bed (SMB) chromatography system is the CSEP, which was developed by Advanced Separation Technologies (now owned by Calgon Carbon Corporation, Pittsburgh, PA). The columns rotate on a carousel, which causes them to “switch” between the inlet and outlet ports and achieve SMB chromatography. Reproduced with permission from CSIRO.

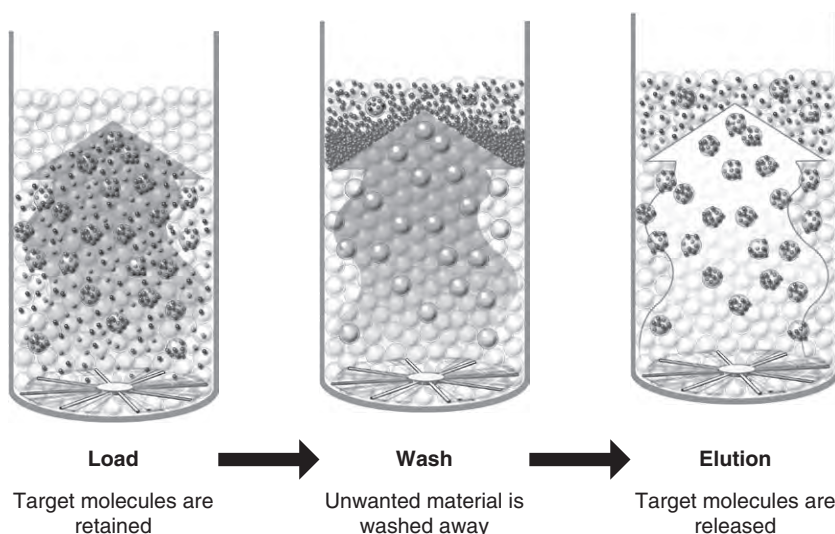


Figure 5.4 Schematic showing the loading, washing, and elution of an expanded bed chromatography column. The resins are designed to enable expansion and suspension in the loading, washing, and elution buffer. Reproduced with permission from UpFront Chromatography A/S.

Achieving cost-effective separations using chromatography in the dairy industry is dependent on the scale of operation, the nature of the fluid, and chosen contacting system. SMB chromatography is a cost-effective technology, and its efficiency could be further improved if it is combined with RFC technology where the mass transfer advantages of SMB is combined with the hydraulic advantages of RFC.

Expanded bed chromatography is a technology of contacting the adsorbent with the fluid where the fluid flows in an upward direction, causing the resin bed to expand but not fluidize (Figure 5.4). This technology enables the use of crude feed stocks without the need for preclarification as the fouling of the bed is minimized due to bed expansion. The mass transfer achieved with this technology can be very efficient. The disadvantage of this technology is the need to use adsorbents tailored for expanded bed operation, as the density of the adsorbent particle is a critical factor in achieving expanded bed status. A number of companies manufacture adsorbents with densities suitable for expanded bed chromatography, including GE Healthcare and UpFront Chromatography.

Apart from the columns required to achieve contact between the adsorbent and the fluid, chromatographic systems need instrumentation control systems to measure and control variables such as the volume and the rate of flow of fluid into the columns. Chromatographic separation design is based on the application of bed volumes; hence, its measurement is important for successful operation. In addition, maintenance of temperature, ionic strength, and pH are important factors that need to be measured and controlled to achieve consistent and repeatable production.

5.2.2 Chromatographic adsorbents

Central to the chromatographic process and the efficiency of a dairy separation is the adsorbent (solid or stationary phase). The adsorbent provides the surface at which partitioning of dairy molecules between the mobile phase and the adsorbent takes place,

Table 5.1. Adsorbent characteristics to consider when selecting an adsorbent for use in the dairy industry.

Adsorbent characteristic	Target	Reason
Surface area	Maximum	Increases adsorption capacity and decreases the quantity of resin required.
Adsorption capacity	Maximum	Decreases quantity required, operating costs, and capital equipment required.
Specificity	Maximum	Improves purification power and decreases further processing requirements.
Shape	Spherical	Improves bed packing uniformity, reduces tendency to compact under pressure and restrict mobile phase flow and improves mobile phase flow uniformity.
Hydrodynamic properties	Minimal pressure drop	Allows higher mobile phase linear velocities, reduces energy requirements, increases adsorbent operating life, and reduces adsorbent cost during manufacturing.
Pore size	Maximum	Increases diffusion and mass transfer rates, especially for large molecules, such as dairy proteins. Reduces risk of pore blockage and adsorbent fouling.
Stability (chemical, thermal, mechanical and enzyme)	Maximum	Increased adsorbent operating life, reducing adsorbent replacement frequency and operating costs. Reduces risk of adsorbent breakdown and leaching, improving adsorbent safety.
Regulatory status	Approved	Adsorbent should be approved for use as a food contact substance or food processing aid by the local regulatory agency.
Cost	Low	Reduces operating costs when resin is replaced.

thus affecting the separation. There are many different support matrices, synthesis methodologies, and surface functionalities available, leading to a large number of possible adsorbents that can be chosen for a chromatographic process. However, only a small number of these are suitable for use in the commercial scale separation of dairy components. A summary of the characteristics that should be considered when selecting an adsorbent for the large-scale chromatographic separation of dairy components is shown in Table 5.1.

The material the adsorbent is made from is an important consideration for any dairy application as it affects a number of the adsorbent characteristics and separation efficiencies, as summarized in Table 5.1. A large range of materials are possible, including inorganic silica, natural organic materials, such as agarose and cellulose, and synthetic organic materials, such as polyacrylamide or hybrids of inorganics and organics.

5.2.2.1 Available adsorbent materials

Due to their well-defined structure, rigidity, and ease of chemical modification (Berthod, 1991), inorganic silica beads are widely employed as adsorbents in analytical chromatography applications. However, the covalent bonds within silica are inherently unstable at high pH, which is the pH of cleaning agents often employed in the dairy industry. Silica-based matrices are therefore avoided, and adsorbents made from organic materials that have

good chemical, temperature, and mechanical stabilities are often chosen for large-scale dairy applications. For example, adsorbents made from natural cross-linked carbohydrates, such as agarose (a carbohydrate polymer isolated from red algae) and cellulose, have good stability, shape, pore size, and hydrodynamic characteristics, making them ideally suited for use in the dairy industry. Importantly, adsorbents made from such natural organic materials with specific surface chemistries are approved as food contact substances (FCS) by the U.S. Food and Drug Administration (e.g., FCS Nos. 45, 52, 528, 531, 156, 157, 443; Food and Drug Administration, 2011).

Synthetic organic polymeric matrices made from styrene, divinylbenzene, acrylates, and their copolymers can also be used in the large-scale commercial separation of food and dairy components (Manlan et al., 1990; Diaz-Reinoso et al., 2010). These matrices have the advantage of being easy to manufacture at large scale and are stable over a broad pH and temperature range. In addition, a number of these synthetic organic adsorbents with specific surface chemistries are approved as food contact substances by the U.S. Food and Drug Administration (FCS Nos. 55, 74, 254, 325, 564; Food and Drug Administration, 2011). Columns made from continuous solid organic polymers with interconnected flow-through paths (termed monoliths; Figure 5.5) are the subject of ongoing research and development (Nordborg and Hilder, 2009). Diffusion mass transfer is minimized, and convective mass transfer is maximized in such monolithic adsorbents. As convective mass transfer is faster than diffusive transport, higher mobile phase flow rates can be employed, and faster separation of molecules can be achieved. BIA Separations (Villach, Austria) are an example of one of few companies manufacturing large-scale organic monolithic columns (up to 8L) for large-scale separation applications. Although not currently employed by the dairy industry, as their cost of production decreases, synthetic organic monolithic columns are likely to find utility in the large-scale isolation of dairy molecules at high flow rates.

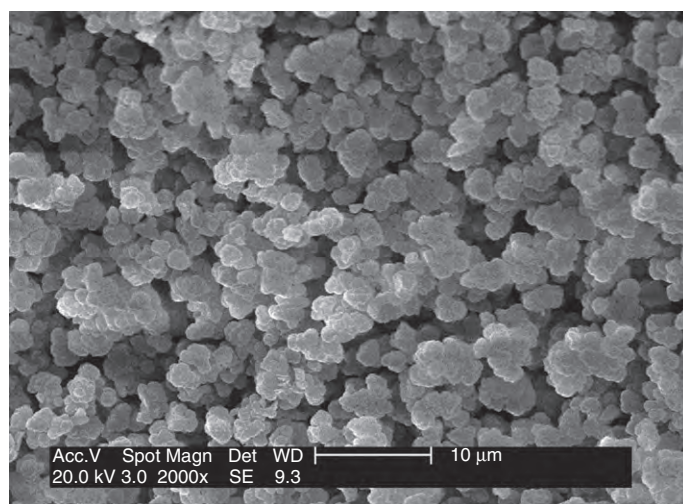


Figure 5.5 SEM image of a glycidyl methacrylate-co-ethylene glycol dimethacrylate monolith prepared in the presence of the organic porogens dodecanol and cyclohexanol (Du et al., 2007). Reproduced with permission. Copyright Elsevier (2007).

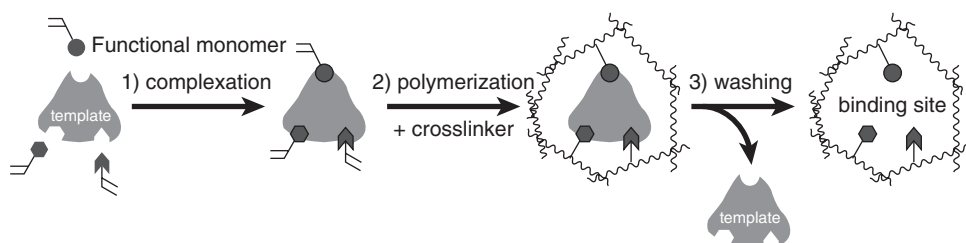


Figure 5.6 Schematic illustration of the methodology employed to synthesize molecular imprinted polymers (MIPs). (Takeuchi and Haginaka, 1999). Reproduced with permission. Copyright Elsevier (1999).

5.2.2.2 Future adsorbent materials

Molecular imprinted polymers (MIPs) are a novel class of organic polymeric adsorbent materials that are manufactured by polymerizing selected monomers around a template molecule. The template molecule is subsequently removed, leaving cavities in the polymer that are specific for the template (Figure 5.6). MIPs are reported to have good selectivity, capacity, and stability in various chemical and physical environments (Kriz and Mosbach, 1995; Ciardelli et al., 2006) and can be designed for the isolation of small organic molecules, amino acids, peptides, sugars, and high molecular weight proteins (Sellergren, 1994; Andersson et al., 1995; Nicholls et al., 1995; Steinke et al., 1995; Shi et al., 1999; Rachkov and Minoura, 2001; Bolisay et al., 2006; Bossi et al., 2007). MIPs are available from Sigma-Aldrich (SepelMIP™, St. Louis, MO) for the solid phase extraction (SPE) of selected molecules prior to analytical quantification (Rejtharová and Rejthar, 2009). However, MIPs are not yet available commercially for the large-scale isolation or removal of molecules from food or dairy streams. If MIPs can be manufactured cost-effectively at industrial scale, are selective in aqueous environments and robust for large commercial-scale applications, they may offer the dairy industry the ability to selectively isolate minor components in a cost-effective manner.

5.2.2.3 Adsorbent surface chemistries

The partitioning between the mobile phase and the adsorbent and thus the separation is not only influenced by the adsorbent material, but also its surface chemistry. There are a large number of surface modifications that can be imparted to affect the interactions taking place between the adsorbent and the mobile phase, including the addition of hydrophobic groups (e.g., octadecyl), carbohydrates (e.g., heparin), glycoproteins (e.g., concanavalin A), proteins (e.g., protein G) and charged groups (e.g., carboxylic acid). The addition of charged groups, such as those detailed in Table 5.2 produces ion-exchange adsorbents. Using such adsorbents relies on the charge differential between the adsorbent and the molecule of interest to affect adsorption and separation. Ion-exchange adsorbents have found widespread use in the dairy industry for the isolation of proteins and peptides (De Silva et al., 2003), as outlined in the application section below.

Biological molecules, such as peptides, proteins, and glycosaminoglycans, can also be attached to adsorbent materials to produce affinity adsorbents. These adsorbent rely on the specific interaction between the ligand attached to the matrix and the molecule of interest, similar to the specific interaction between an antigen and antibody or a substrate and an

Table 5.2. Common matrix modifications that impart ion-exchange functionality to chromatographic adsorbents.

Cation exchangers (CEX)	Chemistry	Dairy applications ^a
Carboxymethyl (CM)	-O-CH ₂ -COO ⁻	Adsorption of high pI proteins, such as immunoglobulins, lactoferrin, and lactoperoxidase
Sulfopropyl (SP)	-O-CH ₂ -CHOH-CH ₂ -O-CH ₂ -CH ₂ -CH ₂ SO ₃ ⁻	
Methyl sulfonate (S)	-O-CH ₂ -CHOH-CH ₂ -O-CH ₂ -CHOH-CH ₂ SO ₃ ⁻	
Anion exchangers (AEX)	Chemistry	Dairy applications ^a
Diethylaminoethyl (DEAE)	-O-CH ₂ -CH ₂ -N ⁺ H(CH ₂ CH ₃) ₂	Adsorption of low pI proteins, such as GMP, α-lactalbumin, and β-lactoglobulin.
Quaternary ammonium (Q)	-O-CH ₂ -CHOH-CH ₂ -O-CH ₂ -CHOH-CH ₂ -N ⁺ (CH ₃) ₃	
Quaternary aminoethyl (QAE)	-O-CH ₂ -CH ₂ -N ⁺ (C ₂ H ₅) ₂ -CHOH-CH ₃	

^aAt the natural pH of milk or whey.

enzyme. Such specific interactions greatly improve the specificity of the adsorbent for a particular molecule and significantly improve the separation efficiency. An example of a commercially available affinity adsorbent is “Protein G Sepharose™ 4 Fast Flow” (GE Healthcare, Uppsala, Sweden). The immobilized *Streptococcal* surface protein (Protein G) has a high affinity for the Fc region of immunoglobulins and can be used to isolate immunoglobulins from dairy streams with a minimal number of processing steps (Chen and Wang, 1991). Although such affinity adsorbents offer great potential, their use within the dairy industry for large-scale separations is limited due to their high costs and concerns about their ability to withstand industrial operating and cleaning environments. With further research and development to cost-effectively identify and synthesize affinity peptides, there may be an opportunity for affinity adsorbents to reduce the number of processing steps and thus the cost of producing minor dairy components.

Novel adsorbent chemistries are continuously being developed to enable the isolation of molecules with fewer processing steps and fewer environmental issues. The application of stimuli responsive polymers to reduce the environmental impact of chromatographic separations in the dairy and other industries is one such area of development (Maharjan et al., 2008). An ion-exchange adsorbent with the ability to adsorb and desorb dairy proteins (e.g., lactoferrin) by changing the temperature has recently been reported (Maharjan et al., 2010). They reported that lactoferrin could be isolated from a model whey solution simply by changing the temperature of the resin and mobile phase within the column (Figure 5.7; Maharjan et al., 2010). Such resins may allow the dairy industry to isolate molecules without the use of salt, thus reducing downstream processing requirements, reducing energy inputs, and reducing salt disposal. Such technologies should not only reduce the cost of separation processes in the dairy industry, but also reduce the environmental impact of dairy ingredient manufacture.

5.2.3 Commercial applications of resin chromatography in the dairy industry

Preparative batch chromatography is used widely in the healthcare, pharmaceutical, and biotechnology industries (Schoenmakers, 2009). In these industries, the focus has been on

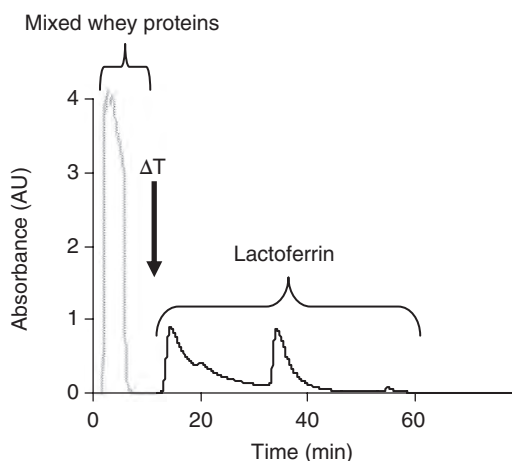


Figure 5.7 Chromatogram produced when a mixture of whey proteins (lactoferrin, α -lactalbumin, and β -lactoglobulin) were separated on a column containing a novel temperature responsive adsorbent. The arrow represents when the temperature of the column and mobile phase were changed from 50 to 20°C and pure lactoferrin eluted. Reproduced with permission. Copyright Elsevier (2009).

the application of chromatography in a robust, reliable, rapid, and, preferably, automatic manner. Chromatography is inherently a “batch” process whether conducted in a column or other device holding the stationary phase, and this “batch” or noncontinuous nature results in a technique that is inherently costly. In the pharmaceutical and related arenas, such costs are not usually an impediment to the use of a purification technology, rather the focus is on maximizing the purity of an isolated component, and high margins on the isolate (drug or drug precursor) usually mitigate the costs. By contrast, the dairy industry operates on much lower margins, and the extreme purity of an ingredient is not usually the primary aim. Rather, in dairy processing, a technology must be capable of handling very large volumes of milk or whey in a timely, continuous, and cost-effective manner, and to generate a saleable ingredient that is fit for purpose. These requirements have often eliminated preparative chromatography as a viable commercial processing technology option in the manufacture of dairy ingredients. One early exception to this rule was the use of ion exchange chromatography in the demineralization of whey in order to generate demineralized whey powder for application in formulated food products, notably infant formulas (Houldsworth, 1980). In this application, simple mixed anion/cation exchange resins were used to remove mono- and divalent cations and anions (e.g., Na^+ , Cl^- , Ca^{2+} , K^+ , and Mg^{2+}) from the whey in a process analogous to softening water. The need for copious amounts of concentrated acid (e.g., HCl) and alkali (e.g., NaOH) solutions to regenerate the used resins, and associated handling and safety concerns, has nowadays often given way to the use of modern membrane processing technologies, such as nanofiltration, for demineralization of whey and other dairy fluids (van der Horst et al., 1995).

Over the past 30 years, the marketplace for dairy ingredients has become more sophisticated, and nowadays, end users of these ingredients are increasingly demanding more “natural,” functional, and bioactive products (Smithers, 2008). The latter is being driven by a functional foods market worth about \$80 billion annually. While many of these dairy biofunctional ingredients (e.g., lactoferrin) fall into a niche market category, they are commanding a higher price as are more functional varieties of traditional commodities, such as whey protein concentrate (WPC), whey protein isolate (WPI), and even lactose. This

increasing sophistication of the market and the demand for ever more functional dairy ingredients has “opened the door” for more widespread application of chromatography in dairy processing. These changes in the marketplace have also been paralleled by enhancements in chromatographic processing that have addressed issues like cost, throughput, productivity, flexibility, and complexity (De Silva et al., 2003; Etzel, 2004; Smithers, 2008).

It has been in the processing of cheese and acid whey for the manufacture of whey protein and peptide ingredients that industrial applications of ion exchange chromatography have been most widespread and successful in the recent past (De Silva et al., 2003). Market demands for whey protein ingredients containing an increasing content of protein together with lower levels of lactose and fat have seen ion exchange chromatography, both in fixed columns and stirred tanks, used commercially in the production of WPI. For example, Davisco Foods International, Inc. (Eden Prairie, MN) in the United States has been something of a pioneer in the application of ion exchange chromatography for the commercial manufacture of WPI. Their BiPRO™ WPI, produced through a proprietary ion exchange technology, claims to provide superior nutrition, enhanced functionality, complete solubility, and neutral flavor (Davisco Foods, 2011). Similarly, an Australian dairy company has manufactured various WPI products with tailored compositions for different end uses since the early 2000s. This company also relied upon ion exchange chromatography but implemented it using an incarnation of SMB chromatography (continuous separation or CSEP; Calgon Carbon Corporation, Pittsburgh, PA) (Calgon Carbon, 2011). In this way the company captured the processing benefits of chromatography but at the same time made the technology truly continuous, enhanced throughput, productivity and flexibility, and lowered the cost (De Silva et al., 2003). This chromatography plant was capable of processing in excess of 1 million L of cheese whey per day (Figure 5.8).

The flexibility of the CSEP plant allowed the Australian company to manufacture tailored WPI products, enriched in β -lactoglobulin or in caseinomacropeptide for example, with different targeted applications, through a very simple change in operational parameters. The CSEP technology has also been used by the same Australian dairy company to



Figure 5.8 Continuous ion exchange chromatography plant for processing cheese whey at an Australian dairy company (see also De Silva et al., 2003). Reproduced with permission of CSIRO.

manufacture minor protein/peptide fractions (e.g., lactoferrin and growth factors) from milk and whey (De Silva et al., 2003; Rowney et al., 2005).

While ion exchange chromatography has recently made great advances in the processing of dairy streams, particularly whey, for the manufacture of high-value and functional protein ingredients, the technology has not been without competition. Parallel advances in membrane processing technology have also allowed for greater application of microfiltration (MF) and ultrafiltration (UF) in the production of high-protein whey ingredients, both in isolation and also as a complement to chromatographic techniques (Yang, 2004). Glanbia Nutritionals, for example, uses a proprietary MF/UF process to manufacture their WPI that the company claims has virtually no denatured proteins, a superior amino acid profile, and more calcium and less sodium (Glanbia Nutritionals, 2011).

A major challenge in the industrial chromatographic processing of dairy streams through resin bead adsorbents is fouling. The high solids, fat, and particulate materials in dairy streams make such fluids good foulants. Industrial approaches to addressing this challenge include the use of counter-current flow, as can be used in the CSEP process (see above) and the use of expanded bed chromatography (Anspach et al., 1999). In the latter, feed material is introduced to the resin adsorbent housed in a column using upward flow. Through careful selection of specialized resin beads, the adsorbent becomes unpacked and the beads are fluidized or expanded. Such expansion of the resin beads reduces the tendency to foul using feedstocks like whey and milk. The Upfront Chromatography company in Copenhagen, Denmark has commercialized their version of expanded bed chromatography (Rhobust® EBA) for the processing of dairy fluids and the industrial manufacture of high-value ingredients (Upfront, 2011a). The Rhobust EBA technology has or has the potential to be applied to manufacture of WPI, β -lactoglobulin and α -lactalbumin fractions, lactoferrin, lactoperoxidase, bovine serum albumin and immunoglobulins from unclarified, crude milk and/or whey feedstocks. The Rhobust EBA technology has been commercialized by the Dairy Farmers company in Australia for the industrial-scale production of lactoferrin, lactoperoxidase, and immunoglobulins from cheese whey for use in food and healthcare applications (Upfront, 2011b). The Dairy Farmers plant represents the world's largest expanded bed adsorption system and is capable of processing more than 200,000 L of cheese whey per day (Upfront, 2011b).

Both edible and pharmaceutical grade lactose represent important commercial commodities manufactured from whey. While the typical methodology for production of lactose from whey has involved crystallization (Gaenzle et al., 2008), a more recent development in the use of ion exclusion chromatography has provided the industry with an alternative to traditional crystallization and a simple and cost-effective process for manufacture of high-purity lactose suitable for the most demanding pharmaceutical applications (Durham et al., 2004). The ion exclusion process is "green," as it generates no waste and a surplus of potable water. The technology has been licensed to Groupe Novasep, and this French company is commercializing the process around the globe (Theobald, 2007).

5.3 MEMBRANE ADSORPTION CHROMATOGRAPHY (MAC)

5.3.1 Principles of MAC technology and technical description

Membrane separation is widely applied in the dairy industry. The separation principle is based on size exclusion of substances relative to the size of the membrane pores. Therefore,

separation effects using membrane technology are rather unspecific as far as other properties beyond size are concerned. Separation tasks are often achieved in a more targeted way by applying chromatographic techniques, often in conjunction with other separation unit operations prior to chromatography. Chromatographic processes, however, are often limited by the low velocity of diffusional processes, which are responsible for the transport of substances into and out of the chromatographic beads.

Novel hybrid systems have been developed combining the features of both processes, namely the specificity of functional ligands in chromatography and the high throughput of membranes. Such a system is called membrane adsorption chromatography (MAC). Similar to adsorbent beads, the surfaces of such membranes can carry charged or otherwise adsorptive ligands, which are convectively brought in contact with the substrates in the product stream passing along them. Using MAC systems, diffusional processes are much less limiting as compared with conventional chromatographic systems.

The MAC technology was developed in the 1990s for pharmaceutical industry applications. The membranes can be seen as an alternative stationary phase for conventional resin bead-based chromatographic separations, mainly affinity and ion exchange chromatography (IEC), but also reversed phase and hydrophobic interaction chromatography. A large number of bioseparation processes have been described using MAC technology, for example, virus clearance (Czermak et al., 2008) or DNA removal (Zhang et al., 2003), antibody purification (Knudsen et al., 2001; Zhou and Tressel, 2006), or protein separation (Ghosh, 2002; Suck et al., 2006). The ligands are the same as in resin-based stationary phases. They are covalently bound to porous membranes made of reinforced cellulose or hydrophilic polyethersulfone. The active membranes are produced in all common variants of anionic (Q; DEAE) and cationic (S; CM) type of ligands (see Table 5.2 in previous section of this chapter).

A variety of modules from lab to production scale are available in flat sheet, hollow fiber, radial flow, and cross-flow types (Figure 5.9; Kreuss and Kulozik, 2009). Most dominant are the flat sheet modules, consisting of stacks of disc-shaped membranes, typically with a large radial dimension when compared with the axial length. The low diameter-to-length-ratio leads to problems of flow distribution and hold-up volume, while higher flow rates result in a jetting effect, that is, a higher mass stream through the module center. These problems have been addressed by special module designs and special flow distribution devices at the inlet and outlet of the modules.

MAC technology possesses several significant advantages in comparison with conventional bead-based stationary phases. The main difference is that the limitation of slow diffusional mass transfer toward and from the ligands in resin-based systems are overcome in MAC. This is due to the macroporous structure of the membrane and the open flow channels, resulting in predominately convective over diffusion mass transport. The molecules are transported to the ligands by the liquid flowing along or through the membranes, depending on the module type used (see Figure 5.9). As a result of the comparably open flow path, the back pressure decreases and the separation speed increases. The binding efficiency is generally independent of the feed flow rate across a wide range of operating conditions. Therefore, high feed flow rates may be used (Ghosh, 2002). Figure 5.10 illustrates the typical features and the flow through the various chromatographic systems.

In comparison with resin bead adsorption techniques, MAC devices have a lower binding capacity (BC), as the surface area and the ligand density of the macro-porous membranes are lower when compared with the small beads of packed bed chromatography columns. However, this drawback is significantly reduced at large scale as the speed of the

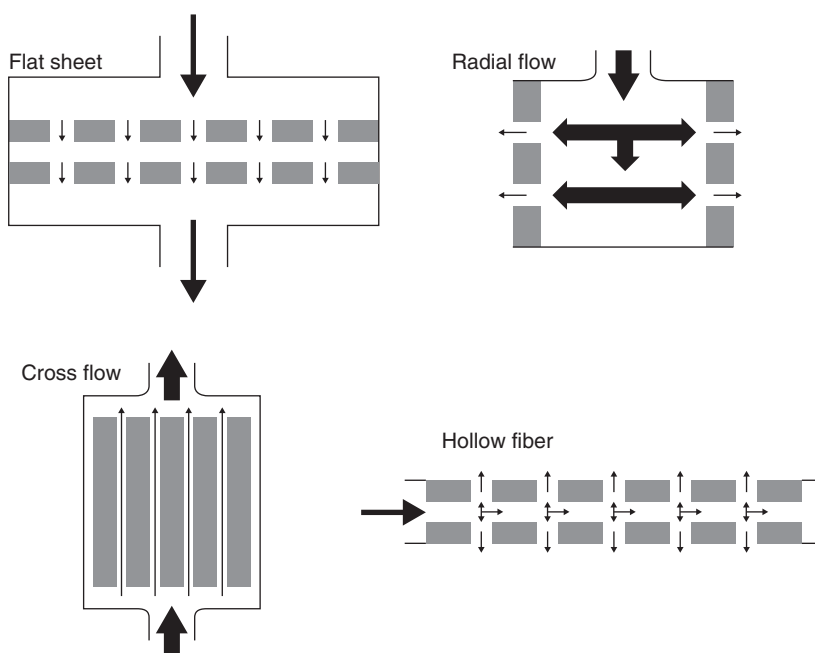


Figure 5.9 Membrane adsorber modules.

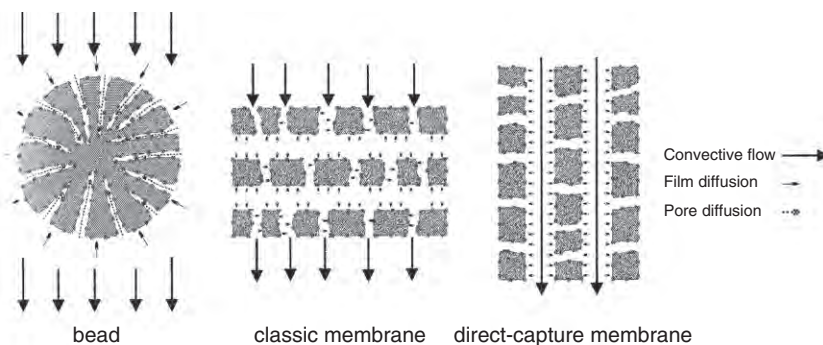


Figure 5.10 Comparison of bead-based, classical membrane MAC and direct capture MAC. While the molecules have to be transported to the ligands by diffusion inside the pores of the beads, they are mainly transported by convective flow and film diffusion inside or along a membrane.

process results in a higher throughput, suggesting MAC is a good alternative for large-scale processes (Reif and Freitag, 1993; Bhattacharjee et al., 2006; Faber et al., 2006; Ghosh and Wong, 2006; Van Reis and Zydney, 2007). This is especially the case when the target substance is present at comparably low concentrations (e.g., caseinomacropetide) in high volume streams like rennet whey.

Care has to be taken in the module design to ensure an equal flow distribution across the entire module, since in large scale systems, the feed flow would have a greater tendency to flow through the module centre, thus not making full use of all available BC. Feed flow distribution is optimized by means of a solid core inside the module and by special flow distributors at the module inlet and outlet as illustrated in Figure 5.11.

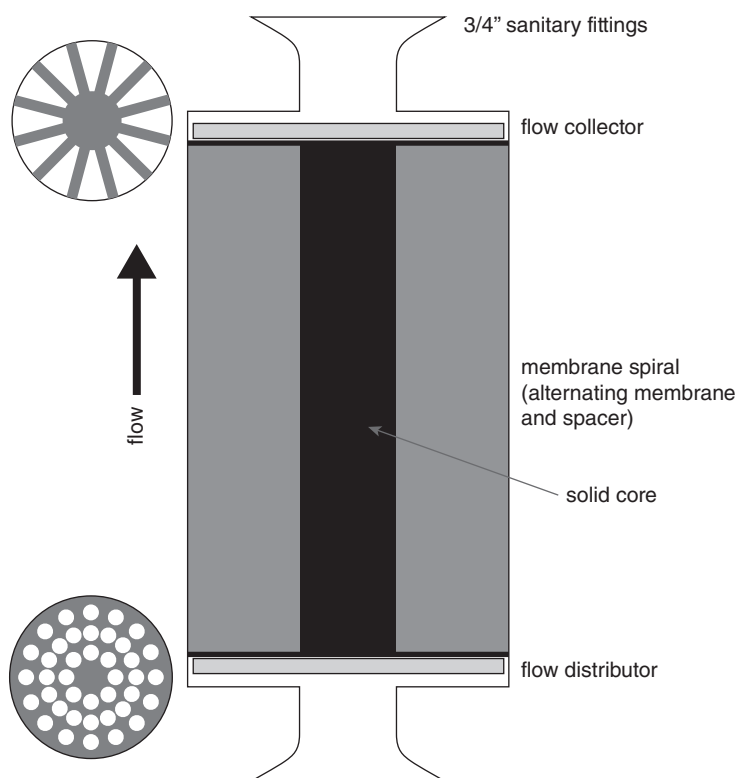


Figure 5.11 Design of a direct capture membrane adsorber with a solid core and flow distributors at the module inlet and outlet (courtesy of Sartorius Stedim Biotech, Göttingen, Germany).

While in pharmaceutical applications of MAC, the modules are disposed after use; for economic use of MAC in the food industry, the modules are required to be repeatedly used. The systems are therefore designed for multiple cleaning cycles and reuse and for application in a direct capture mode to bind substances from undiluted, unfiltered, and turbid feed solutions, such as fermentation broths or whey.

By means of this new type of MAC module, purification of proteins from turbid feed solutions like milk or whey could be envisaged. Promising studies have been performed on the purification of lactoferrin (Plate et al., 2006; Schwarz, 2006). The following section demonstrates the separation of milk-derived molecules by means of MAC, using the fractionation of caseinomacropепptide (CMP) into its main fractions, namely glycosylated CMP, (gCMP), and nonglycosylated or aglyco CMP (aCMP). This could be of interest because of reported biofunctional and technofunctional properties of CMP (Thomä et al., 2006). CMP is the peptide that is cleaved from κ -casein at the outer shell of the casein micelle by chymosin during the renneting process. CMP normally consists of these two fractions approximately in a ratio of 1 : 1 (Thomä et al., 2006).

5.3.2 Separation of aCMP and gCMP by means of MAC

Besides its biological activity, CMP has several interesting technofunctional properties (Moreno et al., 2002; Martin-Diana et al., 2004, 2005; Thomä-Worringer et al., 2006;

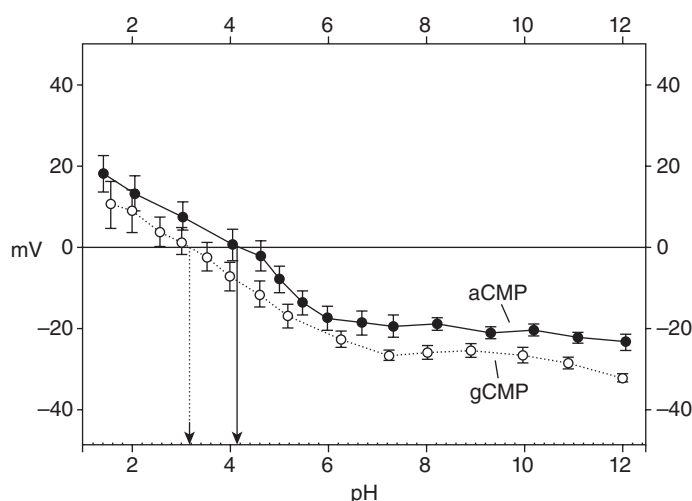


Figure 5.12 Charge properties of aCMP and gCMP as a function of pH (Kreuss, 2010). Reproduced with permission.

Kreuss et al., 2009b). These properties are mainly related to its amphiphilic character, which results from a hydrophobic amino acid backbone and highly charged polar and diverse glycan side chains with a prebiotic character, as well as phosphate groups. Ion exchange chromatography can be used for the separation of CMP out of sweet whey (Etzel, 2004; Tek et al., 2005), because aCMP and gCMP differ in their isoelectrical points (IEP) (Figure 5.12) (Kreuss et al., 2009a). Charge-based or hydrophobicity/hydrophilicity-based separation methods are therefore best suited for the fractionation of the diverse CMP fractions. With the perspective of scaling up the chromatographic fractionation process, the use of MAC devices was investigated and compared against conventional bead-based columns (Kreuss et al., 2008). For details and results beyond those presented in this chapter, see Kreuss (2010).

The pilot plant setup used in this study consisted of a prototype of a strong anionic 1000-mL Sartobind Direct-Capture Q MAC-device from Sartorius Stedim Biotech (Göttingen, Germany). The spiral packed membrane (stabilized reinforced cellulose with a pore size of 3.0 μm) module of 500-mm length and 85-mm diameter is coiled around a solid core (diameter of 58.5 mm), with an active surface area of about 36 cm^2 . An overall scheme of the setup is shown in Figure 5.13. It consisted additionally of two 150-L tanks for the buffers and two 50-L tanks for the stock solution and the eluate. The stock solution (solution of CMP or, in a second step, sweet whey) was pumped through the MAC device in a recirculation mode in order to achieve the maximum BC of gCMP.

After each loading step, the MAC module was flushed with buffer 1 for 10 column volumes (CV) to wash out unbound molecules. Finally, the gCMP was eluted within 6 CV of buffer 2 (0.02M NaAc, 0.7M NaCl, pH 4.1) in a single pass mode. All loading and elution profiles were monitored via pH, conductivity, and flow volume. Detailed profiles of CMP and whey protein concentrations were obtained by RP-HPLC and sialic acid analysis. The experiment was performed at a maximum pump speed (8.0 L/min) and at a constant temperature of $20 \pm 1^\circ\text{C}$. After each loading step, unbound material trapped inside the membrane pores was completely washed out of the module using buffer 1. The elution was done in a single step (step gradient) without recirculation and resulted in a gCMP

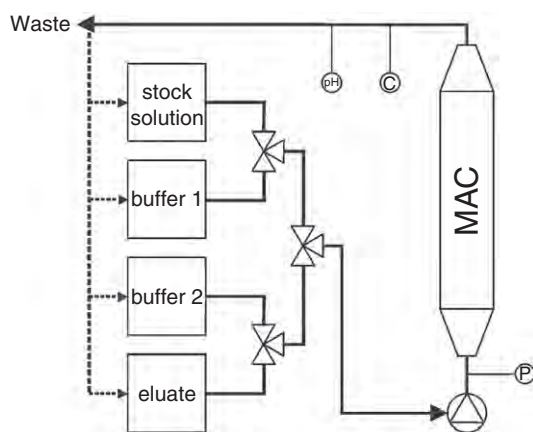


Figure 5.13 Schematic diagram of the preparative MAC plant. Solid lines represent permanent solid pipe tubing and dotted lines represent flexible pipe tubing. P, pressure gauge; C, conductivity meter (Kreuss and Kulozik 2009). Reproduced with permission from Elsevier.

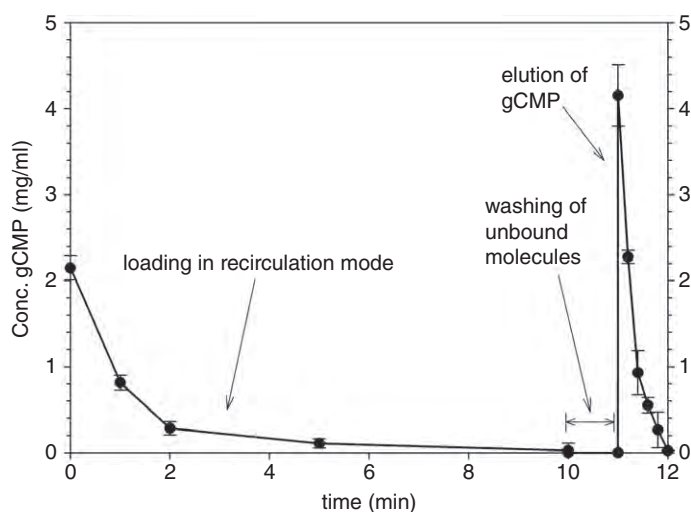


Figure 5.14 Loading and elution cycle tracked by inline sampling at the outlet of the MAC module. The curve represents the gCMP concentration measured by RP-HPLC analysis of samples taken from the feed solution, the washing phase, and the eluate as a function of time (Kreuss and Kulozik 2009). Reproduced with permission from Elsevier.

fraction (fraction 1) of 97% purity. The purity was assessed by RP-HPLC using protein analysis, as well as sialic acid analysis. Figure 5.14 shows the complete cycle of loading and elution.

Figure 5.15 shows the separation achieved using a MAC unit in comparison with conventional ion exchange chromatography column system. The diagram depicts the elution curves detected by a UV detector as a function of elution time.

As can be seen, the separation effect is very similar between the systems. They both achieve a baseline separation of the substances. The IEX bead-based chromatogram shows that the glyco-CMP, as is known from other studies, consists of various subfractions due to the diversity of glycans bound to gCMP molecules. However, the target here was to separate

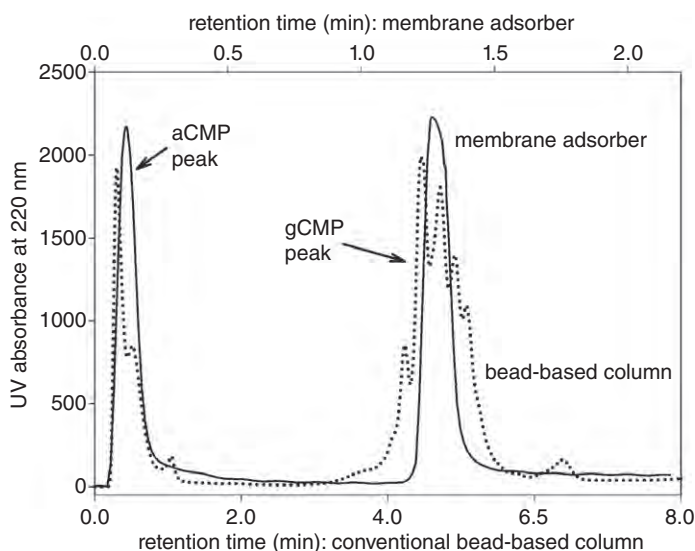


Figure 5.15 Elution curves from a separation process of aglyco- and glyco-CMP from whey using ion exchange chromatography and membrane adsorption chromatography, both carrying quaternary ammonium ligand groups.

the two main fractions, aCMP and gCMP. More importantly, it was shown that in fact, MAC achieved the separation effect in a quarter of the time required by conventional IEX chromatography, which is a significant operational advantage.

In accordance to the experiments using CMP isolate, the total amount of gCMP and aCMP in the eluate plus the residual solution was exactly the same as in the feed solution, resulting in a sample recovery of 100%.

5.3.3 Separation of aCMP and gCMP in a direct capture mode

Efficient downstream processing reduces the number of processing steps and enables high volume throughput. To assess the performance of MAC, the method was adopted for direct capture of gCMP, starting with sweet whey instead of a purified CMP isolate. The intention was to capture the gCMP in one single step. Using the optimized binding conditions, the original pH of the sweet whey was adjusted to 4.1, the conductivity to 2.0 mS/cm. Thereafter, the adsorption process was performed the same way as when using the CMP isolate solution. Using the MAC method, gCMP with a purity of 91% was obtained in the eluate.

Using the unfiltered sweet whey resulted in a significantly lower MAC BC when compared to the pure CMP solution (approximately 7.5 mg/mL with 0.21 mg/cm² of membrane surface area during the initial loading/elution cycle). The purity of the gCMP was also somewhat lower than when using the pure CMP solution as a starting material. This implies a possible inhibition of MAC ligands, possibly by other minor protein or peptide molecules, which were not detectable by RP-HPLC analysis. Another explanation might be the high amount of lactose, oligosaccharides, and salts which are found in sweet whey. They could possibly act as counter-ions or competitive ions and thus have influenced the retention behavior of gCMP.

Repeated loading/elution experiments with sweet whey showed a decrease of the initial binding, especially during the first four to five cycles, and an increase in system back pressure (Figure 5.16). The final capacity was around 4.1 mg/mL ($0.11 \text{ mg gCMP/cm}^2$), approximately 46% lower than the initial BC. Ongoing loading/elution steps showed no further decrease in the BC (up to at least 10 cycles).

This result indicates that in direct capturing mode, there is a loss of capacity from cycle to cycle. In order to prevent this capacity loss, the whey was pretreated by microfiltration prior to the MAC operation. The loss of BC was largely avoided, as demonstrated in Figure 5.17. Alternatively, the MAC system can be CIP cleaned in order to recover the original BC.

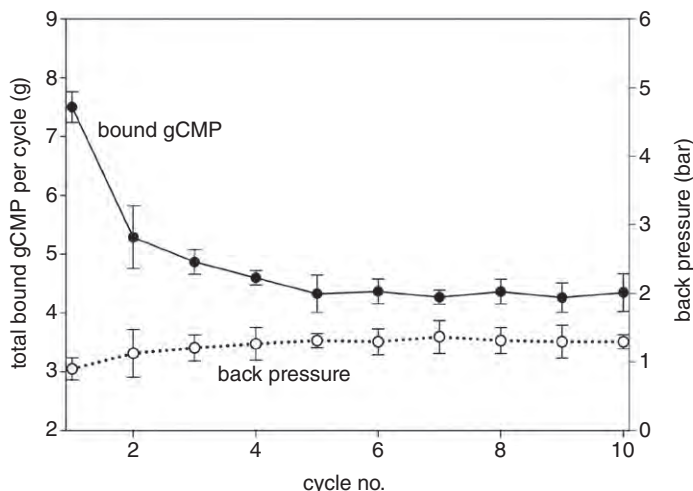


Figure 5.16 Binding capacity of the MAC module for gCMP (solid line) and back pressure of the module (dotted line) as a function of loading/elution cycles (Kreuss and Kulozik 2009). Reproduced with permission from Elsevier.

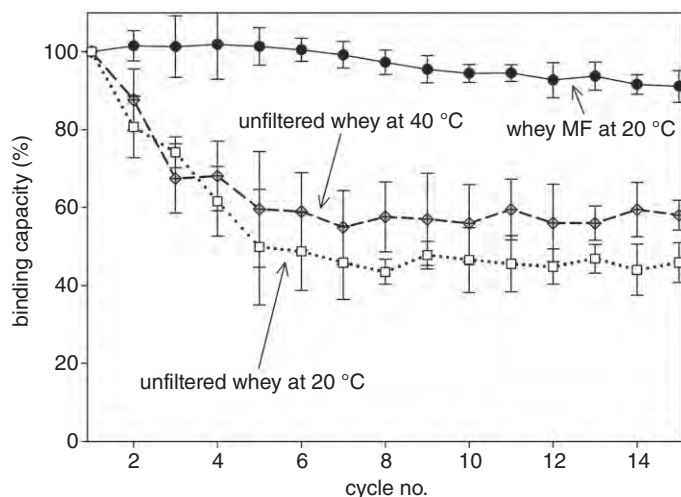


Figure 5.17 Effect of sweet whey pretreatment on the gCMP binding capacity as a function of loading/elution cycle number (Kreuss and Kulozik 2009). Reproduced with permission from Elsevier.

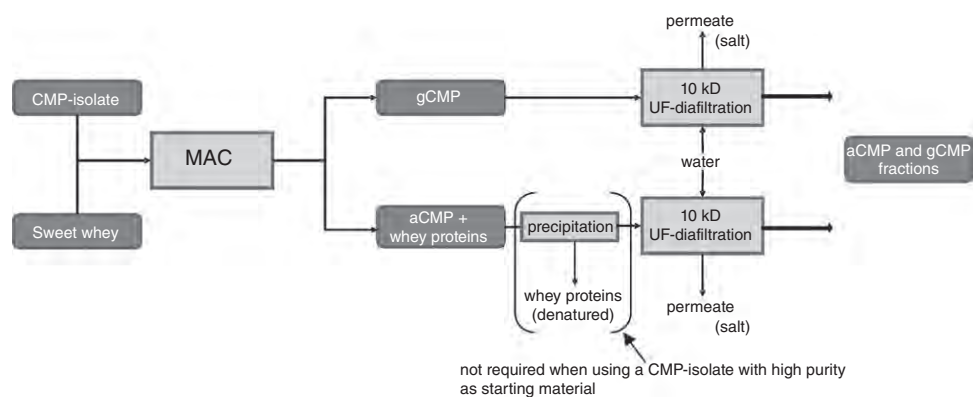


Figure 5.18 Overall processing scheme for the fractionation of CMP.

5.3.4 Processing scheme for the separation of CMP

A processing scheme, including the diafiltration step, for the separation of gCMP from a buffered system with a very high ionic strength is depicted in Figure 5.18. Depending on the starting material, a MF pretreatment can be performed if the capacity loss during the first four to five cycles is not acceptable. However, the costs associated with an additional pretreatment step as compared with the installation of additional MAC capacity would need further evaluation.

Based on the results presented and other works on MAC fractionation, it can be predicted that MAC technology will have further applications in food or pharmaceutical industries. As different ligands can be linked with the carrier surfaces, a wide spectrum of separation tasks could be successfully be undertaken using MAC.

5.4 CONCLUSIONS

The development and application of techniques that enable the isolation of dairy molecules is important to improve the overall value gained from dairy streams. Chromatography is one such technique that until recently had a number of perceived shortcomings and thus found limited application in the dairy industry. The recent advances in chromatography hardware, adsorbent technologies, and chromatographic methodologies detailed in this chapter provide the dairy industry with the opportunity to isolate a broader range of functional molecules and add significantly more value to dairy streams. Of particular interest should be the large-scale SMB (CSEP) and membrane adsorber (MAC) technologies described. This information should help the modern dairy process engineer to identify and then develop cost-effective chromatographic processes to manufacture functional and differentiated ingredients from dairy streams.

REFERENCES

- Andersson, L.I., Muller, R., Vlatakis, G., and Mosbach, K. (1995) Mimics of the binding-sites of opioid receptors obtained by molecular imprinting of enkephalin and morphine. *Proceedings of the National Academy of Sciences of the United States of America* **92**, 4788–4792.

- Anspach, F.B., Curbelo, D., Hartmann, R., Garke, G., and Deckwer, W.D. (1999) Expanded-bed chromatography in primary protein purification. *Journal of Chromatography A* **865**, 129–144.
- Berthod, A. (1991) Silica: backbone material of liquid chromatographic column packings. *Journal of Chromatography A* **549**, 1–28.
- Bhattacharjee, S., Bhattacharjee, C., and Datta, S. (2006) Studies on the fractionation of β -lactoglobulin from casein whey using ultrafiltration and ion-exchange membrane chromatography. *Journal of Membrane Science* **275**, 141–150.
- Bolisay, L.D., Culver, J.N., and Kofinas, P. (2006) Molecularly imprinted polymers for tobacco mosaic virus recognition. *Biomaterials* **27**, 4165–4168.
- Bossi, A., Bonini, F., Turner, A.P.F., and Piletsky, S.A. (2007) Molecularly imprinted polymers for the recognition of proteins: the state of the art. *Biosensors and Bioelectronics* **22**, 1131–1137.
- Broughton, D.B. and Gerhold, C.G. (1961) Continuous Sorption Process Employing Fixed bed of sorbent and moving inlets and outlets. U.S. Patent 2,985,589.
- Calgon Carbon (2011) Engineered solutions—making water and air safer and cleaner. http://www.calgoncarbon.com/solutions/pdf/ISEP_CSEP_EngSolutions-09-02-09.pdf. Accessed October 28, 2011.
- Chen, J.Y.H.-P. and Wang, C.-H. (1991) Microfiltration affinity purification of lactoferrin and immunoglobulin G from cheese whey. *Journal of Food Science* **56**, 701–706.
- Ciardelli, G., Borrelli, C., Silvestri, D., Cristallini, C., Barbani, N., and Giusti, P. (2006) Supported imprinted nanospheres for the selective recognition of cholesterol. *Biosensors and Bioelectronics* **21**, 2329–2338.
- Czermak, P., Grzenia, D.L., Wolf, A., Carlson, J.O., Specht, R., Han, B., and Wickramasinghe, S.R. (2008) Purification of the densovirus by tangential flow ultrafiltration and by ion exchange membranes. *Desalination* **224**, 23–27.
- Davisco Foods (2011) BiPRO™ whey protein isolate. <http://www.daviscofoods.com/specialty/bipro.html>. Accessed on June 18, 2012.
- De Silva, K.J., Stockmann, R., and Smithers, G.W. (2003) Isolation procedures for functional dairy components—novel approaches to meeting the challenges. *Australian Journal of Dairy Technology* **58**, 148–152.
- Diaz-Reinoso, B., Gonzalez-Lopez, N., Moure, A., Dominguez, H., and Parajo, J.C. (2010) Recovery of antioxidants from industrial waste liquors using membranes and polymeric resins. *Journal of Food Engineering* **96**, 127–133.
- Du, K.F., Yang, D., and Sun, Y. (2007) Fabrication of high-permeability and high-capacity monolith for protein chromatography. *Journal of Chromatography A* **1163**, 212–218.
- Durham, R.J., Sleight, R.W., and Hourigan, J.A. (2004) Pharmaceutical lactose: a new whey with no waste. *Australian Journal of Dairy Technology* **59**, 138–141.
- Ettre, L.S. and Sakodyskii, K.I. (1993) M. S. Tswett and the discovery of chromatography II: completion of the development of chromatography (1903–1910). *Chromatographia* **35**, 329–338.
- Etzel, M.R. (2004) Manufacture and use of dairy protein fractions. *Journal of Nutrition* **134**, 996S–1002S.
- Faber, R., Demmer, W., Hörl, H.-H., and Nußbaumer, D. (2006) Microporous membrane adsorbents: basic concepts and recent developments. *Desalination* **199**, 553–554.
- Food and Drug Administration (2011) Food contact materials database. <http://www.fda.gov/Food/FoodIngredientsPackaging/FoodContactSubstancesFCS/ucm116567.htm>. Accessed on June 18, 2012.
- Gaenzle, M.G., Haase, G., and Jelen, P. (2008) Lactose: crystallization, hydrolysis and value-added derivatives. *International Dairy Journal* **18**, 685–694.
- Ghosh, R. (2002) Protein separation using membrane chromatography: opportunities and challenges. *Journal of Chromatography A* **952**, 13–27.
- Ghosh, R. and Wong, T. (2006) Effect of module design on the efficiency of membrane chromatographic separation processes. *Journal of Membrane Science* **281**, 532–540.
- Glanbia Nutritionals (2011) Ion exchange vs. cross-flow microfiltration. http://www.sportswhey.com/science_ion_exchange.htm. Accessed October 25, 2011.
- Houldsworth, D.W. (1980) Demineralization of whey by means of ion exchange and electrodialysis. *International Journal of Dairy Technology* **33**, 45–51.
- Knudsen, H.L., Fahrner, R.L., Xu, Y., Norling, L.A., and Blank, G.S. (2001) Membrane ion-exchange chromatography for process-scale antibody purification. *Journal of Chromatography A* **907**, 145–154.
- Kreuss, M. (2010) Ion exchange fractionation and structure-function relationship of caseinomacropptide as a function of glycosylation. Dissertation Technische Universität München Verlag Dr. Hut, München, Germany.

- Kreuss, M. and Kulozik, U. (2009) Separation of glycosylated caseinomacropeptide at pilot scale using membrane adsorption in direct-capture mode. *Journal of Chromatography A* **1216**, 8771–8777.
- Kreuss, M., Krause, I., and Kulozik, U. (2008) Separation of a glycosylated and non-glycosylated fraction of caseinomacropeptide using different anion-exchange stationary phases. *Journal of Chromatography A* **1208**, 126–132.
- Kreuss, M., Krause, I., and Kulozik, U. (2009a) Influence of glycosylation on foaming properties of bovine caseinomacropeptide. *International Dairy Journal* **19**, 715–720.
- Kreuss, M., Strixner, T., and Kulozik, U. (2009b) The effect of glycosylation on the interfacial properties of bovine caseinomacropeptide. *Food Hydrocolloids* **23**, 1818–1826.
- Kriz, D. and Mosbach, K. (1995) Competitive amperometric morphine sensor based on an agarose-immobilized molecularly imprinted polymer. *Analytica Chimica Acta* **300**, 71–75.
- Lederer, E. and Lederer, M. (1957) *Chromatography: A Review of Principles and Applications*. New York: Elsevier Publishing.
- Maharjan, P., Woonton, B.W., Bennett, L.E., Smithers, G.W., DeSilva, K., and Hearn, M.T.W. (2008) Novel chromatographic separation—the potential of smart polymers. *Innovative Food Science and Emerging Technologies* **9**, 232–242.
- Maharjan, P., Hearn, M.T.W., Jackson, W.R., De Silva, K., and Woonton, B. (2010) Development of a temperature-responsive agarose-based ion-exchange chromatographic resin. *Journal of Chromatography A* **1216**, 8722–8729.
- Manlan, M., Matthews, R.F., Rouseff, R.L., Littell, R.C., Marshall, M.R., Moye, H.A., and Teixeira, A.A. (1990) Evaluation of the properties of polystyrene divinylbenzene adsorbents for debittering grapefruit juice. *Journal of Food Science* **55**(2), 440–445.
- Martin-Diana, A.B., Pelaez, C., and Requena, T. (2004) Rheological and structural properties of fermented goat's milk supplemented with caseinomacropeptide and whey protein concentrate. *Milchwissenschaft* **59**, 383–386.
- Martin-Diana, A.B., Frias, J., and Fontecha, J. (2005) Emulsifying properties of whey protein concentrate and caseinomacropeptide of cow, ewe and goat. *Milchwissenschaft* **60**, 363–366.
- Moreno, F.J., Lopez-Fandino, R., and Olano, A. (2002) Characterization and functional properties of lactosyl caseinomacropeptide conjugates. *Journal of Agricultural and Food Chemistry* **50**, 5179–5184.
- Nicholls, I.A., Ramstrom, O., and Mosbach, K. (1995) Insights into the role of the hydrogen-bond and hydrophobic effect on recognition in molecularly imprinted polymer synthetic peptide receptor mimics. *Journal of Chromatography A* **691**, 349–353.
- Nordborg, A. and Hilder, E.F. (2009) Recent advances in polymer monoliths for ion-exchange chromatography. *Analytical and Bioanalytical Chemistry* **394**, 71–84.
- Plate, K., Beutel, S., Buchholz, H., Demmer, W., Fischer-Fruhholz, S., Reif, O., Ulber, R., and Scheper, T. (2006) Isolation of bovine lactoferrin, lactoperoxidase and enzymatically prepared lactoferricin from proteolytic digestion of bovine lactoferrin using adsorptive membrane chromatography. *Journal of Chromatography A* **1117**, 81–86.
- Rachkov, A. and Minoura, N. (2001) Towards molecularly imprinted polymers selective to peptides and proteins. The epitope approach. *Biochimica et Biophysica Acta*, **1544**, 255–266.
- Reif, O.-W. and Freitag, R. (1993) Characterization and application of strong ion-exchange membrane adsorbents as stationary phases in high-performance liquid chromatography of proteins. *Journal of Chromatography A* **654**, 29–41.
- Rejtharova, M. and Rejthar, L. (2009) Determination of chloramphenicol in urine, feed water, milk and honey samples using molecular imprinted polymer clean-up. *Journal of Chromatography A* **1216**, 8246–8253.
- Rowney, M., Hobman, P., Read, L., and Denichilo, M. (2005) Commercialisation of whey growth factor extract: a case study. *Australian Journal of Dairy Technology* **60**, 183–184.
- Schoenmakers, P. (2009) Chromatography in industry. *Annual Review of Analytical Chemistry* **2**, 333–357.
- Schwarz, J. (2006) Downstream Processing und Biotransformation von nachwachsenden Rohstoffen. Dissertation Universität Hannover, Germany.
- Sellergren, B. (1994) Direct drug determination by selective sample enrichment on an imprinted polymer. *Analytical Chemistry* **66**, 1578–1582.
- Shi, H.Q., Tsai, W.B., Garrison, M.D., Ferrari, S., and Ratner, B.D. (1999) Template-imprinted nanostructured surfaces for protein recognition. *Nature* **398**, 593–597.
- Smithers, G.W. (2008) Whey and whey proteins—from gutter to gold. *International Dairy Journal* **18**, 695–704.

- Steinke, J., Sherrington, D.C., and Dunkin, I.R. (1995) Imprinting of synthetic polymers using molecular templates. *Advances in Polymer Science* **123**, 81–125.
- Suck, K., Walter, J., Menzel, F., Tappe, A., Kasper, C., Naumann, C., Zeidler, R., and Scheper, T. (2006) Fast and efficient protein purification using membrane adsorber systems. *Journal of Biotechnology* **121**, 361–367.
- Takeuchi, T. and Haginaka, J. (1999) Separation and sensing based on molecular recognition using molecularly imprinted polymers. *Journal of Chromatography B* **728**, 1–20.
- Tek, H.N., Turhan, K.N., and Etzel, M.R. (2005) Effect of conductivity, pH, and elution buffer salinity on glycomacropeptide recovery from whey using anion exchange chromatography. *Journal of Food Science* **70**, E295–E300.
- Theobald, J. (2007) High purity lactose production with chromatography: Industrial scale and green process. http://lactose.ru/present/3Jerome_Theobald.pdf. Accessed October 25, 2011.
- Thomä, C., Krause, I., and Kulozik, U. (2006) Precipitation behaviour of caseinomacropeptides and their simultaneous determination with whey proteins by RP-HPLC. *International Dairy Journal* **16**, 285–293.
- Thomä-Worringner, C., Sorensen, J., and Lopez-Fandino, R. (2006) Health effects and technological features of caseinomacropeptide. *International Dairy Journal* **16**, 1324–1333.
- Upfront (2011a) Rhobust® EBA chromatographic separation technology. <http://biomine.upfront-dk.com/dairy.aspx>. Accessed October 25, 2011.
- Upfront (2011b) EBA installation at Dairy Farmers, Australia. <http://biomine.upfront-dk.com/CasestoriesDairyfarmers.aspx>. Accessed October 25, 2011.
- van der Horst, H.C., Timmer, J.M.K., Robbertsen, T., and Leenders, J. (1995) Use of nanofiltration for concentration and demineralization in the dairy industry: model for mass transport. *Journal of Membrane Science* **104**, 205–218.
- van Reis, R. and Zydney, A. (2007) Bioprocess membrane technology. *Journal of Membrane Science* **297**, 16–50.
- Yang, J. (2004) Whey protein processing methods. *Bodybuilding for You*. <http://www.bodybuildingforyou.com/protein/whey-protein-processing.htm>. Accessed October 25, 2011.
- Zhang, S., Krivosheyeva, A., and Nochumson, S. (2003) Large-scale capture and partial purification of plasmid DNA using anion-exchange membrane capsules. *Biotechnology and Applied Biochemistry* **37**, 245–249.
- Zhou, J.X. and Tressel, T. (2006) Basic concepts in Q-membrane chromatography for large-scale antibody production. *Biotechnology Progress* **22**, 341–349.

6 Nonthermal Technologies in Dairy Processing

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6.1 INTRODUCTION

In recent years, there have been major developments in nonthermal technologies as alternatives or adjuncts to thermal processing. This is well illustrated by the number of applications of one of the technologies, high pressure (HP) (Carole Tonello, NC Hyperbaric, pers. comm., 2009). There are several reasons for this dramatic development. First, the developments have been driven by a consumer demand for foods that are perceived to be less processed and more natural than those on the market. In general, the degree of processing equates with the extent of heat treatment that causes obvious changes to sensory characteristics, especially color and flavor, as well as less obvious changes to the nutritional value of the foods. Secondly, physiologically functional foods which contain ingredients with health-giving or disease-prevention properties have become a significant market segment of the food industry. Many of these bioactive ingredients are sensitive to heat and hence the use of nonthermal processing is attractive (Wan et al., 2005). Thirdly, as the potential of the different technologies has been explored, new applications have been identified which have allowed new product and process concepts to be developed. Fourthly, the equipment relating to these technologies has become accessible to researchers and more recently scaled up versions are available for industrial adoption.

Many of the technologies have been investigated as possible replacements for pasteurization and other heat processes that are universally used in food processing. However, there have now been many discoveries of other beneficial effects that had not been anticipated and may be able to be exploited commercially, for example, the release of meat from oysters by HP and modifications to whey proteins and their functional properties by ultrasound and HP homogenization. While some of the technologies can be used satisfactorily alone and at low or room temperature, it is becoming increasingly clear that some become much more versatile or effective when applied with some heat or in conjunction with other technologies or additives, such as antimicrobial substances (Ross et al., 2003).

Nonthermal technologies vary in their extent of commercial adoption as indicated in Table 6.1. Even the four technologies discussed in this chapter (i.e., High pressure processing [HPP], high pressure homogenization (HPH), ultrasonication, and pulsed electric field) vary in their commercial readiness. HPP has been widely adopted in food processing, as

Table 6.1. Nonthermal technologies.

Technology (Key references)	Possible dairy applications	Readiness status for commercial adoption
High-pressure processing (Huppertz et al., 2006)	Enhanced shelf-life of yogurts, especially containing probiotics, and products containing colostrum and bioactives Ready-to-eat and food service products containing dairy ingredients	Several ready, patents exist Colostrum drinks and cheese spreads have been commercialized Science largely known; processing capacity an issue
High-pressure homogenization including microfluidization (Hayes et al., 2005)	Treatment of liquid milk—pasteurization, extended shelf life (ESL) Pretreatment for manufacture of cheese, yogurt, and ice cream Extraction of metabolites from microbial cultures Production of liposomes Modifying proteins to alter functional properties	Microfluidization and HPH used in nonfood industries, nutraceuticals and functional foods Needs development of larger commercial-scale equipment at higher pressures Promising but needs more research and development
Ultrasound (Ashokkumar et al., 2010)	Mostly nonpreservation applications Modifying functional properties of proteins Enhanced mass and energy transfer	Adoption commencing for some applications, for example, defoaming, emulsification, viscosity alteration, and waste water treatment Promising wider applications but needs more research
Pulsed electric field treatment (Bendicho et al., 2002a; Wan et al., 2009)	Liquid milk processing; pretreatment of milk for cheese manufacture Treatment of bioactive (functional) products	Promising but needs more research on use with heat; regulatory issues; Commercial trials required Waste water sludge treatment in food industry commencing
Microfiltration (Hoffmann et al., 2006; Gésan-Guiziou, 2010)	"Pasteurization" by removal of bacteria Separation of casein micelles	In use in dairy industry for spore reduction; Used commercially
Bactofugation (Wolfschoon-Pombo, 2001; Gésan-Guiziou, 2010).	"Pasteurization" by removal of bacteria	In use in dairy industry
Carbon dioxide (Hotchkiss et al., 2006)	"Thermization"-like treatment of milk	Some early adoption in dairy industry
UV light (Guerrero-Beltran and Barbosa-Cánovas, 2004)	Microbial reduction of non-opaque liquids and surfaces	Commercialized in juice industry, potential use with whey, waste water
High intensity pulsed light (Gómez-López et al., 2007)	Microbial reduction of non-opaque liquids and surfaces	Potential use for sterilizing packaging material, water
Cold plasma (Schneider et al., 2005; Locke et al., 2006; Wan et al., 2009)	Microbial reduction of surfaces	Potential use for sterilizing packaging material, equipment and powders; more research required
Oscillating magnetic field (Barbosa-Cánovas et al., 2000b)	Microbial reduction	Research only to date, no obvious applications yet

Table 6.1. (Continued)

Technology (Key references)	Possible dairy applications	Readiness status for commercial adoption
High voltage arc discharge (Barbosa-Cánovas et al., 2000b)	Microbial reduction	Research only to date, no obvious applications yet
Chemical technologies—nisin, ozone, hydrogen peroxide (Khadre and Yousef, 2001; Odoi et al., 2003; Søløft-Jensen and Hansen, 2005; Sobrino-Lopez and Martín-Belloso, 2008b)	As standalone antimicrobials or used with other technologies	Used as regulations permit
Ionizing radiation (Mollins, 2001)	Microbial reduction	Has possible applications but faces consumer opposition and regulatory restrictions

indicated above, but has been adopted very little by the dairy industry; HPH, in the form of microfluidization, has been applied in the pharmacy and cosmetics industries but very little in the food industry; ultrasonication is being increasingly used in the food industry, including the dairy industry, for applications such as defoaming and waste treatment; while pulsed electric field technology has not yet been adopted, despite some promising research results and industrial trials.

In general, new technologies, both thermal and nonthermal, will only be commercially attractive if they offer one or more of the following: greater economic efficiency in terms of both capital and operating costs; and/or ability to perform a task that is very difficult or impossible by other technologies. Most of the adopted applications fall into the latter category and include defoaming and waste treatment by ultrasonication, with estimated payback times of 6 weeks and 3 months, respectively (Patist and Bates, 2008), and treatment of yogurt to destroy spoilage bacteria but retain probiotic bacteria (Carroll et al., 2004).

A significant reason for the tardiness of industry to adopt these technologies, especially for “pasteurization” of liquid foods, is the uncertain regulatory situation. In the dairy industry, thermal pasteurization can be monitored by the alkaline phosphatase test, as this enzyme is inactivated at approximately the same conditions as required to inactivate the two major pathogens of concern, *Mycobacterium tuberculosis* and *Coxiella burnetti* (Juffs and Deeth, 2007). However, no such simple test exists for the nonthermal technologies and alternative ways of monitoring the efficacy of the technologies, in particular their equivalence with thermal technologies have been considered by many government and industry bodies, including the International Dairy Federation (Bishop, 2002).

Another reason for the slow adoption of some technologies is the insufficient capacity of the available equipment. While small-scale equipment is suitable for low volume, high value, or niche products, it is not suitable for high volume processing, such as commercial pasteurization of milk.

There are several nonthermal technologies that are possible candidates for use with dairy ingredients (Table 6.1). In this chapter, the first four technologies, HPP, HPH, ultrasonication, and pulsed electric field technology, are discussed in detail, as this is where there has been the most development.

6.2 HIGH PRESSURE PROCESSING

Food and dairy processing with HP rather than by conventional heating technologies can offer significant advantages to both consumers and the food industries. Processing with this method not only destroys microorganisms and viruses without causing significant changes to the sensory and nutritional attributes of the processed foods, it also provides opportunities for developing new ingredients by modifying the structure of food components, particularly the tertiary structure of proteins (Altuner et al., 2006). HPP technology has been commercialized in Japan since the early 1990s; however, in recent years, it has gained popularity and acceptance worldwide. Pressure-treated (PT) foods receive high preference scores from consumers when assessed against foods processed with heat or other technologies (Cardello et al., 2007). In 2009, there were 61 companies using 130 HPP units for food production—72 units are located in the United States and 31 in Europe (Carole Tonello, NC Hyperbaric, pers. comm., 2009).

Although HPP is an effective and globally recognized preservation technique that has become popular for processing certain fruits, vegetables, seafood, meat products, and ready-to-eat meals, to date, very few commercial applications in milk and dairy products have been demonstrated. Batch and semi-continuous processing modes, with limited throughput capacity, inability to eliminate bacterial spores completely, and the high cost of equipment, are major hurdles to its application in dairy processing.

HPP of milk and dairy products has been reviewed by Trujillo et al. (1997, 2002), Datta and Deeth (1999), Messens et al. (2003), Huppertz et al. (2002, 2006), López-Fandiño (2006), Stewart et al. (2006), and Considine et al. (2007). In addition, HPP of foods has been reviewed by several authors (Barbosa-Cánovas and Rodríguez, 2005; Heinz and Knorr, 2005; Hogan et al., 2005; Welte-Chanes et al., 2005; Tewari, 2007).

6.2.1 Principle

HPP is usually carried out by subjecting food, sealed in flexible packaging, to a pressure of 300–1000 MPa for a holding time of 0–30 minutes, depending upon the product application. It can be used to process liquid, semi-solid, and solid foods. The chamber temperature for HPP is usually maintained at lower than 40°C, but some applications require higher temperatures.

HP disrupts noncovalent bonds such as ionic and hydrophobic bonds but has little effect on covalent bonds. As a consequence, large biomolecules, such as proteins and polysaccharides, are affected through alterations of their secondary, tertiary, and quaternary structures, but small molecules are generally not affected. Since color and flavor compounds and vitamins are, in general, small molecules, HPP has little effect on these components in foods.

In HPP, the applied pressure spreads instantaneously and uniformly throughout the food (based on Pascal's Principle), and is independent of the size and geometry of the food mass. The compressed product then returns to its original shape after the pressure is released. Thus, food is treated evenly throughout and no particle escapes the treatment; pressure gradients that would lower the processing efficiency are not formed. This contrasts with heating technologies, which often result in temperature gradients and uneven heating risking local underprocessing or causing some parts of the food to be overheated.

Pressurization is accompanied by a uniform temperature increase known as adiabatic heat of compression. The adiabatic heating rate is specific for each chemical compound

(e.g., at 20°C: water ~3–5°C/100 MPa, fats and oils ~6–8°C/100 MPa [Ting et al., 2002]) and is temperature and pressure dependent. The level of adiabatic heating can vary considerably and for water it increases to over 5°C/100 MPa at 90°C at pressures around 200 MPa (Knoerzer et al., 2010). The adiabatic heating is completely reversed upon pressure release. Though this temperature increase is relatively small, it can contribute substantially to the overall lethality of the process on microorganisms and has significant implications when pressure is applied at elevated temperature. However, in small pressure vessels in laboratories where the food is surrounded by a relatively large amount of metal, heat is absorbed by the metal, and consequently the adiabatic heating in the product is less than that experienced in industrial-size vessels (Hjelmqvist, 2005).

Recently, high pressure, high temperature (HPHT) processing or pressure-assisted thermal processing (PATP) has been investigated for improved inactivation of bacteria, especially spores, with a view to manufacturing shelf-stable, low acid products (in this case, the process is called pressure-assisted thermal sterilization, or PATS). HPHT uses pressures of around 600 MPa and initial chamber temperatures of between 60 and 90°C, which, with adiabatic heating, increases to processing temperatures of 90–130°C (Barbosa-Cánovas and Juliano, 2008). The combination of adiabatic heating and pressure enhances destruction of spores. An advantage of this approach is the rapid cooling in the pressure release phase, which minimizes heat-induced damage to the foods (Fryer and Versteeg, 2008). In 2009, mashed potato was the first FDA-approved, shelf-stable, low acid food produced by PATS.

A fundamental principle of pressure-induced changes in foods is the Le Chatelier-Braun principle, which states that chemical reactions that result in a decrease in total volume are enhanced by an increase in pressure (Masson, 1992). Therefore, the rates of reactions, such as proteolysis, which result in products of smaller volume, are enhanced by HP (Stapelfeldt et al., 1996), while reactions resulting in an increased total volume are retarded by HP (Ludikhuyze et al., 2001).

6.2.2 Equipment and operation

A typical HP system comprises at least three main parts: (1) a HP vessel and its closure; (2) a pressure-generation system; and (3) a materials-handling system. Frequently, there is also a temperature-control device. The pressure transmitting liquid in most isostatic pressure applications is simply water mixed with a small percentage of oil for lubrication and anticorrosion purposes, but it can also be an oil or an alcohol, such as propylene glycol. Liquid food can be compressed directly in the pressure vessel, eliminating the use of a pressure-transmitting medium. However, the success of such systems relies heavily on the integrity of the seals between the liquid being treated and the pressure transmitting liquid and the effectiveness of the packaging systems.

The packaging used for food subjected to HPP needs to be sufficiently flexible to compensate for the complete compression of the head space, as well as some reduction in the product volume. Only one part of the package has to deform due to limited volume reduction of the food (~12% volume reduction of water at 500 MPa and 22°C); therefore, rigid containers with flexible laminate or coated aluminum foil lids can be used. No migration of chemicals to the food from the plastic containers has been detected (Mertens, 1993; Cole, 1997). An innovative packaging for pressure treating juice uses a triangular-shaped plastic bottle. Six such bottles form a hexagon that fits neatly into the cylindrical pressure chamber (Steeman, 2009).

Two kinds of pressure treatment are used in food processing: batch and semi-continuous. Batch processing is the most common, with the capacity of the largest pressure vessel being 420L for pressures up to 600MPa and 687L for pressures up to 310MPa. Most vessels are specified for temperatures up to about 50°C. The largest HP, high-temperature vessel available is 150L for pressures up to 700MPa, and initial vessel temperatures of up to 95°C, enabling processing temperatures of over 121°C to be attained internally through adiabatic heating. In a typical batch process, the prepackaged product is loaded into a pressure chamber and subjected to HP for a specific time. The whole process has three distinct phases: pressurizing, holding and depressurizing. In semi-continuous processing, several vessels are connected in series; while some are under constant pressure, others are being pressurized, unloaded, or loaded. This minimizes the operation time per batch and allows a portion of the energy in the vessel under pressure to be used to pressurize another vessel, thus reducing operating costs (Vardag et al., 1995).

6.2.3 Effect on milk components

6.2.3.1 Microorganisms

Of the nonthermal technologies considered in this chapter, HPP is the only proven technology for production of high-quality “pasteurized” foods on an industrial scale, although its full potential for milk and dairy products is yet to be realized. The technology is effective against vegetative bacteria, both spoilage and pathogenic, as well as yeasts, molds, and several viruses. Bacterial spores and viruses are much more resistant than vegetative cells. Though most yeasts and molds are easily inactivated by pressure treatment, spores of heat-resistant molds, such as *Byssoschlamys nivea*, are extremely pressure resistant (Butz et al., 1996). The risk of the presence of barotolerant mold spores in a product needs to be considered when this technology is adopted commercially. The effects of HPP on microorganisms in milk have been reviewed by Guamis et al. (2005), Patterson (2005) and Black et al. (2007).

Like other nonthermal technologies, HPP is more effective when combined with a thermal treatment. Treatments equivalent to different levels of thermal treatment of milk: thermization, pasteurization, higher pasteurization, or extended shelf life processing, can be devised by manipulating the process parameters of the pressure treatment. Thus, treatment of raw milk at 300MPa, which has been reported to achieve around a 4 log reduction in bacteria and an extension of shelf life of up to 18 and 12 days at 5 and 10°C respectively (Mussa and Ramaswamy, 1997), is equivalent to thermization; treatment at 400MPa for 15 minutes or 500MPa for 5 minutes at room temperature achieves a similar bacterial reduction as thermal pasteurization (but produces a fresher tasting milk) (Rademacher and Kessler, 1997); and treatment at 586MPa at 55°C for 3 or 5 minutes produces the equivalent of higher pasteurized milk with a shelf-life extended to more than 45 days under refrigeration (Tovar-Hernandez et al., 2005). Production of shelf-stable milk equivalent to ultrahigh temperature (UHT) milk is not possible with pressure alone due to the resistance of bacterial spores. However, when combined with heat in PATS, it can produce shelf-stable milk with higher quality than conventional UHT milk (Lamela and Torres, 2008; Ramirez et al., 2009).

The effect of heat treatment when used in conjunction with HP is variable. For example, in an investigation of 40 isolates representing eight *Bacillus* species subjected to HPP at 600MPa for one minute at 72 to 95°C, Scurrah et al. (2006) found inactivation levels of 0 to 6 logs; one isolate was activated. In general, more inactivation occurred at higher

temperatures with many isolates achieving a high level of inactivation, for example, 5–6 log reductions of *Bacillus licheniformis* and *Bacillus cereus* spores. The relative sensitivity of the isolates to HP varied with treatment temperature.

Another study of combined pressure–heat treatments used two different strategies for reduction of four strains of *B. cereus* spores, spiked into UHT skim milk at 10^6 and 10^8 cfu/mL (van Opstal et al., 2004). Treatments involved pressurizing in a single step at 600 MPa for 30 minutes at 60°C or in two steps, first at 200 MPa for 30 minutes at 45°C, and subsequent heat treatment at 60°C for 10 minutes. More than 6 log reduction of spores was achieved in both methods. The two-step method using low pressure and temperature initially induced germination of spores that were then inactivated by the mild heat treatment. This study found a synergistic effect of pressure and free amino acids present in UHT milk in the germination stage.

Bacteriocins exert a synergistic bactericidal effect with HPP and can cause significant reduction of pathogens and bacterial spores in milk (García-Graells et al., 1999; Alpas and Bozoglu, 2000; Morgan et al., 2000). The synergistic effect of HPP and nisin was observed by Black et al. (2005) on *E. coli*, *Pseudomonas fluorescens*, *Listeria innocua*, and *Lactobacillus viridescens* grown in milk. Pressure up to 500 MPa was applied at room temperature for 5 minutes, and nisin was used at a concentration of up to 500 IU/mL. Substantial reductions (≥ 8 logs) were obtained for all bacteria at 500 MPa with 500 IU/mL nisin. However, even lower pressures of 400 and 250 MPa for 5 minutes with 500 IU/mL nisin caused a >8 log reduction of *Escherichia coli* and *P. fluorescens*, respectively. The Gram-negative bacteria were more sensitive to pressure than the Gram-positive bacteria.

Masschalck et al. (2001) investigated the combined effects of HP and bovine lactoferrin, lactoferricin and a lactoferrin hydrolysate on eight bacteria (two *E. coli* strains, *Salmonella enteritidis*, *Salmonella* Typhimurium, *Shigella sonnei*, *Shigella flexneri*, *P. fluorescens*, and *Staphylococcus aureus*) at room temperature. At the dosages used, the lactoferrin and derivatives had no bactericidal function without pressure treatment, but with HP (155–400 MPa, chosen to cause a 1- to 3-log reduction of each bacterium without antimicrobials), they had bactericidal activity against some of the test bacteria. The Gram-positive *Staphylococcus* was not affected. Lactoferricin was much more effective than lactoferrin, causing significant sensitization of all Gram-negative bacteria, except *E. coli* 0157:H7. The increased susceptibility of some of the bacteria to the antimicrobials appeared to be transient, and it only occurred during pressure treatment. This differs from the report by Black et al. (2005), where the sensitization by HP of some of the bacteria was persistent in that nisin was still effective when added to cell suspensions after HPP treatment.

HPP is also capable of inactivating (by 5 logs) heat-resistant lactococcal phage using a pressure treatment at 600 MPa for 2 hours at 25°C. The phage, in calcium-enriched phosphate buffer, was reduced by only 2 logs by treatment at 550 MPa for 2 hours, while treatment at 450 MPa caused no reduction (Müller-Merbach et al., 2005).

While HPP shows promise for extending the shelf life of milk, there are some issues of concern. First, some strains of the pathogenic bacteria *Listeria monocytogenes* and *Staph. aureus* are quite pressure-resistant and may not be sufficiently inactivated (Alpas et al., 1999). In addition, some mutant strains of *E. coli* are particularly barotolerant being reduced by only 0.4 log at 700 MPa for 15 minutes (García-Graells et al., 1999). Second, HPP causes sublethal injury to a proportion of cells that may then grow slowly at refrigeration temperatures (Bozoglu et al., 2004). This suggests that rigorous storage trials of PT products should be performed before this technology is commercially adopted.

6.2.3.2 Fat

The crystallization of fat in cream can be accelerated, induced, or initiated by pressure treatment via a shift in the phase transition temperature. HP treatment increases the solid fat content of emulsified fats, and thus can be used to reduce the aging time of ice cream mixes and augment the physical ripening of cream for making butter (Bucheim and El-Nour, 1992; Bucheim et al., 1996).

With UHT-sterilized cream, HPP at 450 MPa and 25°C for 15 or 30 minutes, or at 10 or 40°C for 30 minutes, induced aggregation of fat globules, and markedly increased viscosity. This aggregation was partly reversed during chilled storage (Dumay et al., 1996).

HPP on bovine milk at 100–600 MPa for up to 60 minutes at 20°C does not significantly alter the size of the fat globules. However, the creaming properties are affected; pressures ≤ 250 MPa increase creaming, whereas treatment at ≥ 400 MPa decreases creaming (Huppertz et al., 2003). Similarly, HPP of ovine milk at 500 MPa at 25°C and 50°C decreased creaming, while pressure treatment at 4°C increased creaming (Gervilla et al., 2001). The reduction in creaming is probably related to inactivation of agglutinins and interaction of fat globules with milk proteins, although Gervilla et al. (2001) considered it may be related to an increase in the number of small fat particles in the range 1–2 μm that they observed after treatment at 500 MPa at 25 and 50°C. The improved creaming of milk pressure treated at 4°C could be useful in butter manufacturing (Trujillo et al., 2002).

6.2.3.3 Proteins

HPP of milk has significant effects on the proteins. Casein micelles may be unaffected, disintegrated, or aggregated, whey proteins may be either unaffected or denatured, and certain bioactive proteins, such as immunoglobulin, may be affected or denatured depending on the applied pressure and pH of the medium (Huppertz et al., 2006). These phenomena, in turn, can alter the functional properties, enhance processing characteristics, and improve the physicochemical properties of PT milk and milk products, such as cheese, yogurt, beverages containing bioactive protein, low fat whipping cream, and ice cream mixes.

Casein

Three types of change occur in the casein micelle when milk is subjected to HP. There is very little or no change in the micelle after treatment at 100–200 MPa at 20°C for 30 minutes, an increase in size after treatment at ~ 250 MPa for >15 minutes, due to aggregation of micelles, and a reduction in average size by about 50% at ≥ 400 MPa, even for short treatment times and irrespective of temperature (Gaucheron et al., 1997; López-Fandiño et al., 1998; Huppertz et al., 2006). Formation of the smaller casein particles is due to breaking of hydrophobic bonds and solubilization of micellar calcium phosphate and some caseins from the casein micelles (Huppertz et al., 2006). After HP treatment at ≥ 400 MPa, skim milk is almost transparent in appearance (Gaucheron et al., 1997). The semi-transparency is retained for several days under refrigeration conditions (Considine et al., 2007).

At HP (≥ 400 MPa), the caseins are solubilized from the casein micelles in the order: β -casein $>$ κ -casein $>$ α_{s1} -casein $>$ α_{s2} -casein (López-Fandiño et al., 1998). The order has been attributed to the serine phosphate content of the proteins, but may also be related to their hydrophobicity; β -casein, the most hydrophobic casein, is solubilized first,

while α_{s2} -casein, which has a high serine phosphate content, is released last. It has been suggested that this differential solubilization of caseins by pressure could be exploited in the manufacture of casein isolates or ingredients enriched in certain caseins (DeSilva et al., 2003).

Gaucheron et al. (1997) demonstrated some interesting effects of pressure on the physicochemical characteristics of skim milk. Treatment at 250 MPa and 40°C resulted in two distinct populations of casein particles with average diameters of 50 and 250 nm, while after treatments at 4 and 20°C, the average particle size was 50 nm (cf. 100 nm for unpresurized). Similar phenomena of coexistence of small and large casein micelles in skim milk pressure treated at 150–300 MPa were demonstrated by Knudsen and Skibsted (2009). The pressure-modified micelles were shown by cryo-transmission electron microscopy to have a similar substructure to micelles of untreated skim milk.

Whey proteins

HP has a disruptive effect on the quaternary (at <150 MPa) and tertiary (at >200 MPa) structures of most globular proteins, but has little or no effect on their secondary structure due to the relative stability of hydrogen bonds to the applied pressure (Velez-Ruiz et al., 1998).

Whey proteins can undergo partial, but fully reversible, unfolding of their native molecular structures under appropriate pressures. Denaturation of β -lactoglobulin commences at ~150 MPa and increases with increasing pressure and temperature. Significant aggregation of whey proteins occurs during the holding phase in HPP. Almost complete denaturation occurs after treatment at 750 MPa at 30°C for 30 minutes, or 450 MPa at 60°C for 15 minutes (Rademacher et al., 1997). α -Lactalbumin (α -La) and bovine serum albumin (BSA) are more resistant to denaturation by pressure than β -lactoglobulin. Higher numbers of intramolecular disulfide bonds (Hinrichs et al., 1996) and the absence of a free sulfhydryl group (López-Fandiño et al., 1996) cause α -La to have the most pressure stability. The sensitivity of the whey proteins to denaturation by pressure is in the order: lactoferrin > β -Lg > immunoglobulin > BSA > α -La (Patel et al., 2006). The difference in sensitivity of β -Lg and α -La to pressure can be used to separate α -La from (denatured) β -Lg (Rademacher and Hinrichs, 2002).

There is considerable interest in pressure treatment of the bioactive immunoglobulin (IgG), which is less sensitive to pressure than to heat, particularly at low pH. It is not denatured at 200 MPa, partially denatured at 500 MPa, and completely inactivated at 700 MPa at pH 7.0 (Felipe et al., 1997; Wan et al., 2005). However, IgG is more stable to pressure at acidic pH than at neutral (Carroll, 2008). Furthermore, certain food constituents, such as sucrose, protect it from inactivation during pressure treatment, retaining full activity at 500 and 700 MPa in the presence of 50% sucrose (Zhang et al., 1998; Wan et al., 2005). An application from this finding is the preservation of IgG in colostrum using HPP rather than heat treatment (Carroll, 2008). A New Zealand dairy manufacturer has patented an HPP process for producing a ready-to-drink colostrum beverage (Carroll et al., 2008).

Whey protein solutions increase in viscosity and turbidity when subjected to pressure. These changes are due to denaturation of the β -lactoglobulin, which undergoes polymerization reactions through disulfide linkages to form protein aggregates, and the concomitant increase in water-binding capacity; this phenomenon can be used to advantage in yogurt manufacture (Rademacher et al., 1997).

β -lactoglobulin is a major allergen in milk. An estimated 3% of the world population, mainly children, suffer from bovine milk allergy (Kelly and Zeece, 2009). β -lactoglobulin can be selectively eliminated from whey protein concentrates (WPCs) by hydrolysis with thermolysin at elevated hydrostatic pressure. This method can be utilized in making modified milk for infants (Kunugi, 1993; Dufour et al., 1995; Stapelfeldt et al., 1996). α -La is resistant to hydrolysis due to the presence of four disulfide bonds. When the thermolysin digestion is performed under HP, β -Lg is hydrolyzed faster and more completely than at atmospheric pressure, without affecting α -La. Thus, human milk, which contains α -La but no β -Lg, could be simulated by using thermolysin in conjunction with pressure treatment of bovine milk (Hayashi et al., 1987). Nakamura et al. (1993) demonstrated that if HP treatment (200 MPa for 10 minutes at 28°C) was applied to WPCs prior to enzymatic hydrolysis, hypoallergenic hydrolysates could be produced and excess hydrolysis avoided. Similar approaches have been used for reduction of allergenicity of bovine milk by hydrolysis of whey proteins with other proteolytic enzymes, such as alcalase, neutrase, papain, pepsin, and trypsin, at pressures of 100–300 MPa (Penas et al., 2006a,b).

Lim et al. (2008a,b) examined the functional properties of PT WPC (35% protein, WPC-35). Treatments at 300 or 400 MPa for 15 minutes and 600 MPa with no holding time showed higher overrun and significantly greater foam stability than untreated WPC. The protein solubility was enhanced at pH 7.0, indicating that PT WPC would have useful applications in food products of neutral pH. The addition of WPC, pressure treated at 300 MPa for 15 minutes, to low fat whipping cream improves its foaming properties (Padiernos et al., 2009), suggesting that PT WPC is a potentially useful ingredient for low fat foods requiring a stable foam.

Pressure treatment at ≥ 400 MPa induced gel formation of whey protein isolate (WPI) at 10%, but at higher concentrations ($>18\%$), with WPC ($\sim 75\%$ protein). However, the viscosity of WPI and WPC increased at 6% and $>12\%$, respectively, at 400 MPa (Kanno et al., 1998). Similar results were obtained by Hinrichs and Kessler (1997), who noted that gelation of WPI occurred at $>10\%$ at 600 MPa, while commencement of gelation and change of viscosity of WPC occurred at concentrations of $>18\%$ and $>12\%$, respectively, at 400 MPa.

Pressure treatment (600 MPa for 0–30 minutes and 50°C) of WPC ($\sim 85\%$ protein) has been shown to increase the number of binding sites for flavor molecules. This suggests that PT WPC may be a useful ingredient for enhancing the flavor of low fat products (Liu et al., 2005; Kühn et al., 2006).

A high value functional dairy ingredient of emerging interest is lactoferrin, which has antimicrobial and other beneficial physiological properties (Baldi et al., 2005). Pressure treatment of lactoferrin (5% in water) at 600 MPa for 5 minutes did not affect its bactericidal activity (Wan et al., 2005).

6.2.3.4 Enzymes

In general, milk enzymes are much less affected by HP treatment than by thermal treatment; however, the effect on individual enzymes is variable. Reviews have been published on this topic by López-Fandiño (2006), Rademacher and Hinrichs (2006), Huppertz et al. (2004c), and Stepaniak (2004).

Alkaline phosphatase (ALP) is quite resistant to HP inactivation and hence is not a suitable indicator of pasteurization effectiveness of milk, as it is for thermal pasteurization. Little if any inactivation occurs at 20°C up to 400 MPa (López-Fandiño et al., 1996) and

a treatment at 800 MPa for 8 minutes is required for complete inactivation (Rademacher et al., 1998). Ludikhuyze et al. (2000) observed increased *D*-values of ALP at 64°C with increasing pressure up to 450 MPa, and then a fall in *D*-values with further pressure increase. A similar trend was observed by Seyderhelm et al. (1996). Treatment of goat milk at 500 MPa for 10 minutes did not cause any reduction in ALP activity (Felipe et al., 1997). Acid phosphatase activity in bovine milk is much more sensitive to pressure than ALP, being substantially inactivated at 200 MPa (Balci et al., 2002).

Plasmin can be inactivated by HPP with synergetic effects being observed between pressure, temperature, and times of treatment (García-Risco et al., 1998; Huppertz et al., 2004c; López-Fandiño, 2006). It is resistant up to 400 MPa for 30 minutes at 25°C, reduced by 30 and 75% at 400 and 600 MPa, respectively, for 30 minutes at 20°C (Huppertz et al., 2004c), and by 86.5% at 400 MPa for 15 minutes at 60°C (García-Risco et al., 1998). In addition, HP transfers plasmin and plasminogen activator (PA) from the micelles to the soluble phase of milk. This occurs at 200, 400, or 650 MPa at room temperature with loss of enzyme activity, although the loss of activity at 200 MPa was small (Moatsou et al., 2008a). The inactivation of the enzymes closely follows the denaturation of the whey proteins, especially β -Lg, and it appears that as β -Lg is denatured, it interacts with plasmin via sulfhydryl—disulfide interchange reactions and inhibits the enzyme activity. The authors concluded that reductions in total plasmin and PA activity and the transfer of plasmin and PA to the serum phase will produce some beneficial effects on milk quality and on cheese yield (Moatsou et al., 2008a). Interestingly, ovine milk plasmin is more resistant to inactivation by pressure than bovine milk plasmin and PA; around one-third of the activities of these enzymes remain after treatment at 650 MPa at 55°C. Moreover, pressure treatment does not cause a transfer of plasmin to the serum phase in ovine milk (Moatsou et al., 2008b).

The barostability of lactoperoxidase (LPO) has been observed by several researchers. Rademacher et al. (1998) found >50% LPO activity in PT milk after treatment at 800 MPa for 4 hours at 25–60°C, Wan et al. (2005) found ~90% LPO activity was retained in 0.2% solution after pressure treatment at 600 MPa for 5 minutes at room temperature, and Ludikhuyze et al. (2001) observed little or no inactivation by treatments up to 700 MPa at 20–65°C. In fact, the last authors found that pressure up to 700 MPa at 73°C, a temperature at which rapid inactivation occurs at atmospheric pressure, had a protective effect on the enzyme. These phenomena suggest that HPP could be useful in the manufacture of dairy ingredients or foods containing LPO to ensure retention of its antimicrobial activity. Despite the barostability of LPO at pressures up to 300 MPa and temperatures up to 60°C, its activity in the presence of substrate is lower under pressure than at atmospheric pressure. This reflects the increase in volume of LPO during the reaction with the substrate, in accordance with the Le Chatelier Braun principle (Ludikhuyze et al., 2001).

Pandey and Ramaswamy (2004) demonstrated that lipoprotein lipase activity in milk was resistant to pressures up to 400 MPa at 3°C. In fact, they observed an increase in activity with a pressure pulse treatment (zero holding time) at 350 and 400 MPa. This suggests the use of HP treatment of milk used for making cheeses where lipolysis by the native lipase may be beneficial. An earlier study by Seyderhelm et al. (1996) showed that activity of a lipase was reduced by 40% in Tris-buffer at pH 7.0 at 600 MPa for 10 minutes at ambient temperature. However, the nature of the lipase was not specified.

Like lipoprotein lipase, gamma-glutamyl transferase activity was enhanced by short-term pressure treatment, but was inactivated by longer treatments. Inactivation was 90% at 600 MPa after 8-minute hold time (Pandey and Ramaswamy, 2004).

Lysozyme in both bovine and human milk is stable to HP (Trujillo et al., 1997; Viazis et al., 2007). This suggests that milk treated with HP may retain some antimicrobial activity that may, in the case of banked human milk, be useful for neonatal nutrition.

6.2.3.5 Milk minerals

The major effect of HPP of milk on milk minerals is solubilization of micellar calcium phosphate, which increases the levels of soluble calcium and phosphate. According to Kielczewska et al. (2009), both soluble calcium and soluble phosphorus increase significantly at pressures of 150–350 MPa; the percentage increases at 350 MPa were 42 and 63%, respectively. Despite the increase in soluble calcium, ionic calcium shows no change (H.C. Deeth and M.J. Lewis, unpublished data).

6.2.4 Applications

Several recent reviews (Trujillo et al., 2002; López-Fandiño, 2006; Stewart et al., 2006; Kelly and Zeece, 2009) and patents (Carroll et al., 2004, 2008; Palmano et al., 2006) have addressed possible applications of HPP in the production and use of dairy products and ingredients. Some PT novel foods with functional dairy ingredients are already commercialized. For example, a PT cheese spread was commercialized in Spain in 2007, and has achieved market success due to its significantly extended shelf life (Voigt et al., 2007; Patel et al., 2008).

6.2.4.1 Effects on coagulation of milk proteins

Rennet coagulation of HP-treated milk

The effect of HP on milk coagulation by rennet has been studied in relation to three parameters, rennet coagulation time (RCT), curd formation time, and curd firmness (Desobry-Banon et al., 1994; López-Fandiño et al., 1996). These parameters are affected by the pressure, time, and temperature of treatment.

Rennet coagulation time (RCT)

RCT is not affected by treatment of milk up to 150 MPa, but is reduced by treatment at higher pressures up to ~300 MPa. This reduction has been attributed to disintegration of the casein micelle. Further increases in pressures to >400 MPa cause an increase in RCT, largely due to the denaturation of β -Lg and its subsequent complexation with the casein micelle, which hinders access of the rennet to κ -casein. This effect appears to outweigh the casein disintegration effect at the higher pressures (Huppertz et al., 2006).

Curd formation and firming

The effects of pressure treatment on rate of rennet curd formation and firmness of the resulting curd are similar to the effects on RCT. At lower pressures of 100–300 MPa (Ohmiya et al., 1989; López-Fandiño et al., 1996) or 200–400 MPa (Zobrist et al., 2005), the rate is high, but at higher pressures, the rate decreases. Similarly, HPP at 200 and 400 MPa for 90 minutes increased the curd firmness over that obtained at atmospheric treatment, but further increases in pressure decreased curd firmness (Ohmiya et al., 1989). Treatment at 600 MPa for 30 minutes also increased the rate of curd formation by lactic

acid bacteria due to higher growth rate of the bacteria. This growth stimulation may be due to the production of nonmicellar caseins in PT milk, providing more available nitrogen to the bacteria (Huppertz et al., 2004b).

Acid coagulation of high pressure-treated milk

HPP of milk improves the mechanical (gel rigidity and gel breaking strength) and textural (syneresis) properties of acid-set gels; this has important implications for dairy processing, particularly in the manufacture of yogurt (Johnston et al., 1993; Needs et al., 2000). Interestingly, milk HPP-treated at 200 or 400 MPa coagulates at a higher pH (5.3) than unpresurized milk (5.0) (Desobry-Banon et al., 1994; Ferragut et al., 2000). Furthermore, the microstructure of the curd shows a more densely packed protein network of finer strands of casein particles compared with dispersed casein particles with surface filamentous projections in curd from heat-treated milk (Johnston et al., 1992; Needs et al., 2000). The dense protein network with small pores is believed to be responsible for the resistance to syneresis of acid-set gel from PT milks (Johnston et al., 1992).

Coagulation of concentrated milk proteins

One of the novel applications of HP treatment is the formation of protein gels with concentrated milk protein preparations without addition of rennet or acidulant. Milk concentrated by microfiltration (MF) or ultrafiltration (UF) to 6.6–11.4% protein gelled at pH 5.9 under HP (200 and 400 MPa). The gelling appears to result from partial loss of colloidal calcium phosphate from the micelle and the consequent reduction in electrostatic repulsion between the micellar particles. This is supported by the fact that caseinate does not gel. WPCs (9.7–12.73% protein) also gelled at 200 and 400 MPa, but only at pH 9 (Famelart et al., 1998; López-Fandiño, 2006). The firmness of pressure-induced gels depends on the pressure, holding time, and the pressure release rate in the HPP treatment cycle (Famelart et al., 1998; Fertsch et al., 2003).

6.2.4.2 Cheese

Pressure treatment of cheese

Extensive research with HPP in cheese making has focused on three main areas: inactivation of pathogenic and spoilage microorganisms in cheese for shelf life enhancement; acceleration of cheese ripening for lowering storage costs; and improvement in the quality of cheese by using HPP-treated milk. In addition, some properties of particular cheeses are modified by HP treatment, for example, acceleration of shreddability in Cheddar cheese (Torres and Velazquez, 2005). An excellent review of the effect of HP on cheese was published by Stewart et al. (2006).

Inactivation of microorganisms in cheese

HPP may be useful for reducing postpasteurization contaminant in cheese and extending its shelf life. For example, treatment of a fresh goat's cheese at 500 MPa for 2–15 minutes caused a 5–6 log reduction of mesophiles and complete removal of *E. coli* inoculated into the cheese (Capellas et al., 1996). O'Reilly et al. (2000a) reported that HPP of Cheddar cheese at 400 MPa for 20 minutes at 20°C reduced *E. coli* and *Penicillium roqueforti* by 7 and 6 logs, respectively, and *Staph. aureus* by 3-logs. The refrigerated shelf life of

pressurized fresh lactic curd cheese was extended up to 8 weeks using HPP at ≥ 300 MPa for 5 minutes at ambient temperature to inactivate spoilage yeasts (Daryaei et al., 2008). Inhibition of outgrowth of yeasts for the whole 8 weeks was observed after pressure treatment at ≥ 400 MPa (Daryaei et al., 2008).

A combination of pressure (400 MPa at 30°C for 15 minutes) and nisin inactivated *B. cereus* spores in cheeses after germination was initiated by treatment at 60 MPa at 30°C for 210 minutes. HPP, together with nisin, also caused ~ 2.4 log reduction of *B. cereus* spores in raw milk soft curd cheeses (López-Pedemonte et al., 2003). The combination of HPP (300–500 MPa, for 5–10 minutes at 5°C) with bacteriocin, generated by the lactic acid bacteria, has also been shown to reduce *L. monocytogenes* during the ripening of cheeses inoculated with the bacterium (Arques et al., 2005).

Acceleration of cheese ripening

The application of pressure to cheese, usually soon after manufacture, has been reported by some authors to accelerate the ripening of cheese through enhanced proteolysis (Yokoyama et al., 1992); however, others have found no stimulation of proteolysis (O'Reilly et al., 2000b). Several factors are involved that may affect the outcome of the pressure treatment. For example, the pH of PT cheese is often greater than that of control unpressurized cheese (Saldo et al., 2002), the temperature of the pressure treatment may cause the enhancement (Sendra et al., 1999; Stewart et al., 2006), the pressure may alter the microstructure without accelerated proteolysis (Johnston and Darcy, 2000), pressure may enhance proteolysis by plasmin (Messens et al., 1999) but may inactivate endocellular enzymes of starter bacteria after the bacterial cells are disrupted (Messens et al., 1999).

Moderate pressure treatment of fresh Cheddar cheese curd facilitates the shreddability of the cheese by eliminating the ripening period. Treatment at 345 MPa for 3 minutes creates a microstructure similar to that of ripened cheese. Fresh curd treated in this way has textural and sensory qualities that are equivalent to those of unpressurized 27-day-old Cheddar cheese (Serrano et al., 2004a,b). Thus, this treatment could improve the efficiency and profitability of the shredded cheese manufacturing industry (Serrano et al., 2005). PT (200 MPa for 2 hours) half-fat Cheddar cheese shows improved texture and functional properties approaching those of full-fat Cheddar (Johnston et al., 2002). A similar acceleration of texture development occurs when fresh Mozzarella cheese is HP treated. Treatment at 200 MPa for 60 minutes at 20°C altered the texture, as measured by modulus of deformability, to be similar to that of 25-day-old untreated cheese. In addition, it greatly reduced the level of expressible serum due to the enhanced water-binding capacity of the milk proteins (Johnston and Darcy, 2000).

However, pressure treatment can also be used to arrest or delay the ripening of quick-ripening cheeses (such as mold-ripened cheeses). This occurs through inactivation of microorganisms and enzymes responsible for the ripening (Clark, 2007).

Cheese from HPP milk

Several authors have reported an increase in curd yield during cheesemaking from HPP-treated milk. Some of this increase can be accounted for by higher moisture content caused by the increased water-holding capacity of the treated proteins, but some increase is due to incorporation of denatured β -Lg into the curd, as evidenced by the reduced level of the whey protein in the whey (Gaucheron et al., 1997). Huppertz et al. (2004a) found an

increase in yield of up to 25% after pressure treatment of milk at 600 and 800 MPa; however, no increase in yield was observed after pressure treatments of ≤ 250 MPa.

Generally, pressure treatment of low fat milk prior to cheesemaking improves cheese texture and overall acceptability. Cheese made from milk subjected to a combined treatment of heat (65°C for 30 minutes) and pressure (400 MPa, for 15 minutes at 22°C) was more acceptable than that made from either pressurized or pasteurized milk. A softer texture and enhanced flavor in cheese made from pressurized milk was attributed to increased protein breakdown (Molina et al., 2000). Queso Fresco cheese made from PT milk (400 MPa for 20 minutes at 20°C) was softer, less crumbly, and stickier than the corresponding raw milk cheese. Thus, HPP technology may find application for production of fresh, soft cheese like Queso Fresco with acceptable textural qualities (Sandra et al., 2004).

6.2.4.3 Yogurt

HPP has attracted attention in yogurt making for two major reasons: shelf-life enhancement by selective inactivation of spoilage microorganisms while maintaining starter bacteria alive; and improvement of texture with reduction of syneresis (Penna et al., 2007; Shah et al., 2008).

A HP process for enhancing the shelf life of probiotic yogurt by inactivating spoilage agents, for example, yeasts and molds, while retaining the viability of specially selected pressure-resistant probiotics has been patented (Carroll et al., 2004). The optimum pressure parameters, 350–650 MPa for 1 second to 10 minutes at 10°C to 15°C, cause >5 log reduction of spoilage microflora and produce a probiotic yogurt with a shelf life of 4 weeks at 4°C.

Penna et al. (2007) compared the microstructures of three types of skim milk probiotic yogurts: heat treated (85°C for 30 minutes); pressure treated (676 MPa for 5 minutes); and treated with a combination of pressure and heat. The combined pressure–heat treatment produced compact yogurt gels with higher water-holding capacity and exhibited less syneresis compared with treatments with heat or pressure only.

A yogurt with added lactoferrin has been produced by a patented technology in which lactoferrin was added to the yogurt after fermentation and pressure treated at 500 MPa with no holding time. No coliforms, yeasts, or molds were detected, and very few colonies of mesophilic spores and aerobic bacteria were found in the yogurt after 130 days' storage at 4°C (Carroll et al., 2008).

6.2.4.4 Milk

Pressure treatments of 400 MPa for 15 minutes or 500 MPa for 3 minutes at ambient temperature resulted in pasteurized milk, which was equivalent to thermally pasteurized milk with respect to microbiological safety. Treatment at 586 MPa for 5 minutes at 55°C extended the shelf life of milk beyond 45 days, which is more than double that of thermally pasteurized milk (Vazquez-Landaverde et al., 2006).

Milk processed by PATP at pressures of 482, 586, 620, and 655 MPa, and temperatures of 45, 55, 60, and 75°C for 1, 3, 5, and 10 minutes produced “pasteurized” or “sterilized” milks, depending on the process conditions, with very different flavor profiles from those of corresponding thermally processed milk. PATP milk contained saturated aldehydes, such as hexanal, heptanal, octanal, nonanal, and decanal, similar to those produced in thermally

pasteurized milks, but much lower concentrations of methyl ketones, 2-methyl propanal, 2,3 butanedione, and volatile sulfur compounds, such as carbon disulfide, dimethyl disulfide, and dimethyl trisulfide. Sterile milk produced by PATS at ~650 MPa and >100°C also had different flavor profiles from those of thermally processed UHT milk (Pérez Lamela and Torres, 2008). This suggests a possible solution to the ongoing problem of the unfavorable flavor of UHT-sterilized milk; whether it offers a practical solution will largely depend on the economic viability of the PATS system.

6.2.4.5 Commercial developments

The New Zealand dairy company Fonterra has patented several applications of HPP including: improving the clarity and/or stability of protein gels without and with other ingredients such as sugars or fruit juice (Fletcher et al., 2004); retardation of casein degradation in cheese to maintain physical functionality (Johnston et al., 2004); extending the shelf-life of cultures, for example, in probiotic yogurts, where the desirable organism(s) has/have been selected to be relatively pressure-resistant and undesirable spoilage organisms are inactivated (Carroll et al., 2004); and processing compositions containing bioactive ingredients, including lactoferrin (Palmano et al., 2006) and immunoglobulins (in colostrum) (Carroll et al., 2008), while maintaining bioactivity

There has been a range of related press releases and articles (e.g., Halliday, 2007; Anon, 2008; Hembry, 2008) about the production of a safe ready-to-drink colostrum beverage with an ambient shelf life of up to 6 months based on the patented HPP process (Carroll et al., 2004). The products was first commercialized successfully in 2009 by New Image Natural Health Ltd., also from New Zealand (Anon, 2008), and in 2009, in Singapore, a range of 100 mL ready-to-drink acidified fruit-flavored colostrum products, each containing 1500 mg colostrums, was available in a range of stores under the name Col⁺™ (Colplus, 2010).

Since 2006, Grupo Rodilla in Spain has been successfully using HPP to improve the safety and shelf life of cheese-based vegetable-, meat-, and fish-containing sandwich fillings (Barros, 2008). By in-pack HPP treatment of the final products, the shelf life was extended from 7 to 12 days to 22 days. As a result, the risk of *Listeria* and other pathogens was greatly reduced, and the costs associated with defective products eliminated. The shelf-life extension also enabled larger production runs and distribution over the whole of Spain. During the development of these products other methods of pasteurization, such as heating had been considered. The benefits of the HPP process over heat treatments were found to include: the simplicity of the process, as no heating and cooling steps are required; no organoleptic changes occurred; no reformulation or additives were needed to give heat stability; and the pack size did not have to be reduced to facilitate the process (Barros, 2008).

With the decreasing costs and increasing throughput of HPP equipment, and increasing product-specific understanding, the authors believe that several more commercial HPP dairy and dairy-containing products can be expected in the coming years. Examples may include safe HPP-treated raw milk and products made without heat treatment (these will be attractive to some consumers because of the retention of much of the bioactives, including the natural enzyme systems); products such as yogurt and cheese treated by HPP to increase their microbial shelf life or physical functionality (e.g., stretchability of pizza cheese); products in which HPP dairy protein acts synergistically with other food polymer ingredients, such as starch to give firmer textures (Oh et al., 2007); and ready-to-eat snack

foods based on dairy and nondairy ingredients where HPP extends the shelf life, while maintaining taste, texture, and other functional properties.

In 2009, the FDA approved a mashed potato product produced by pressure-assisted thermal sterilization (PATS) (Bricher, 2009a,b). The low acid mashed potato product is ambient shelf stable and has superior sensory attributes to a comparable shelf-stable retorted product. The process was developed by the Dual Use Science and Technology (DUST) consortium, which aims to drive the use of the new PATS platform for food product innovation. Dairy products are expected make an important contribution to this exciting new development. For example, in shelf-stable egg patties processed through a PATS-like process, cheese was found to improve sensory and water-holding attributes (Juliano et al., 2006, 2007). We anticipate that in the near future, formulated PATS products will be developed in which dairy ingredients play an important functional role.

6.3 HIGH PRESSURE HOMOGENIZATION

Homogenization is an important process in the dairy industry for producing stable fat emulsions. Conventional homogenization at ~ 18 MPa reduces the average size of the fat globules in milk from ~ 3 to ~ 1 μm in diameter by shear, turbulence, and cavitation forces caused by the pressure difference across the homogenizing valve and high-speed collisions with the impact ring. It has little effect on bacteria or milk components.

HPH was introduced in the 1980s to improve the effectiveness of homogenization and emulsification. Also called ultra-high pressure homogenization (UHPH) or dynamic HPP, it differs considerably from conventional homogenization as it operates at pressures up to 20 times higher. Furthermore, in addition to achieving efficient homogenization and emulsification, it also causes destruction of bacteria and viruses, inactivation of enzymes, and changes to casein micelles and whey proteins. Thus, HPH has the potential to become a versatile processing technology for dairy products and ingredients (Hayes et al., 2005). The potential was encapsulated by Leadley (2003) who stated that “The use of HP in the homogenization of milk could have potential as a means of enhancing product stability and inducing novel textures or functionality for product development purposes.” However, although being increasingly used in the chemical, pharmaceutical, and biochemical industries (Floury et al., 2000), HPH has found little application in dairy processing. One reason for this is the relatively small capacity of the equipment available to date. Aspects of the technology have been reviewed by Paquin (1999), Hayes et al. (2005), and Diels and Michiels (2006).

While its alternative name of dynamic HPP suggests HPH is closely related to (static) HPP, it is distinctly different. The pressures used in HPH are lower and the liquid products, which are passed through the homogenizing valve in a continuous flow, are subjected to the HP for a much shorter time, a fraction of a second. A comparison of the two pressure-related technologies given in Table 6.2 demonstrates the considerable differences.

6.3.1 Principle

There are two major types of HP homogenizer: a HP version of the conventional valve homogenizer and the microfluidizer that operates on completely different principles. HP valve homogenizers are based on the same design principle as conventional homogenizers, but utilize far higher pressures (up to 400 MPa but typically 100–300 MPa). They have a

Table 6.2. Comparison of high pressure homogenization and high pressure processing.

Parameter	High pressure homogenization (HPH)	High pressure processing (HPP)
Mode of operation	Continuous flow	Batch and semicontinuous
Operating pressures	~100–350 MPa	100–1000 MPa
Residence time under pressure	Very short, $\sim 10^{-4}$ second	1–30 minutes
Capacity	Up to 8000 L per hour at 100 MPa and 5000 L per hour at 150 MPa ^a	Pressure vessels up to 420 L at 600 MPa, 5–10 batches per hour
Heating effect	17–24°C per 100 MPa	~4°C per 100 MPa, same decrease on pressure release
Energy recovery	Possible if heat regeneration system used	Not possible in single batch mode of operation, some possible when multiple vessels are in use
Filling and packaging	Product packaged after treatment	Products in flexible packaging can be treated directly
Microorganism inactivation	No sublethal injury	Partial inactivation or sublethal injury possible
Physical forces	Shear, turbulence, cavitation	Compression and decompression
Bactericidal mechanism	Rupture of the cell membrane and release of cytoplasmic material	Protein denaturation and membrane damage
Emulsification effect	Yes	No
Color of treated milk	Little effect on color	Milk becomes clear due to casein micelle dissociation

^aInformation supplied by GEA Process Engineering Pty. Ltd.

primary valve with a needle and seat arrangement, and, usually, a secondary valve of similar arrangement operating at a considerably lower pressure, typically ~10% of the pressure in the primary valve. By contrast, microfluidization involves division of the liquids feed stream into two streams that are forced to collide with each other at high velocity (up to 50 m/s) at 180° in an interaction chamber. Maximum pressures achieved in microfluidizers depend on the model, but are commonly 100–200 MPa, although some may operate at pressures up to 500 MPa (Paquin, 1999).

In HP valve homogenizers, the pressure is maintained for a very short time (approximately 10^{-4} second), but the force-induced phenomena of cavitation, shear, and turbulence are responsible for the physical effects observed. Microfluidization exerts its effects mainly through cavitation generated within an interaction chamber (Paquin, 1999). The high velocity of the product streams is responsible for the high force of impact (Hardham et al., 2000). An important feature of both technologies that is exploited in many of the applications is the almost instantaneous temperature rise in the treated product, which is linear with pressure; the rate of increase is ~17–23°C per 100 MPa (Asano et al., 2000; Desrumaux and Marcand, 2002; Bouaouina et al., 2006; Roach and Harte, 2008). However, the exact temperature rise depends on the matrix; for example, Hayes and Kelly (2003a) observed that the rate of temperature rise increased with fat content of the treated product.

6.3.2 Effect on milk components

6.3.2.1 Microorganisms

While destruction of microorganisms was not the major driver for this technology, it has now been shown to be effective to some extent against a wide range of organisms (Diels

and Michiels, 2006). In general, the higher the homogenization pressure and the higher the inlet temperature, the greater the inactivation. In addition, multiple passes, commonly used with microfluidizers, add to the inactivation effect (Picart et al., 2006). The product matrix can also influence the bactericidal efficiency; for example, the effect on strains of *E. coli* has been reported to be greater in whole milk than in skim milk (Brinez et al., 2006; Diels and Michiels, 2006).

The temperature increase during HPH treatment has a major synergistic effect with the shear, turbulence and cavitational forces. In fact, it has been suggested that the heat generated instantaneously in the homogenization process together with the physical effects of homogenization could be used to produce extended shelf life (Pereda et al., 2007) and even long shelf life (Asano et al., 2000) products with better sensory properties than those produce by heat processes alone because of the lower heat input in the process.

Two examples illustrate the bactericidal effect of HPH in milk. Pereda et al. (2007) treated whole milk with a valve-type HPH at up to 300 MPa with inlet temperatures of 30 and 40°C, and found that lactococci, and psychrotrophic and total bacteria were reduced by ~3.5 logs, while coliforms, lactobacilli, and enterococci were reduced to undetectable levels. The milk was reported to have a microbial shelf life at 4°C of 14–18 days. Picart et al. (2006) reported a 2–3 log reduction in total count after homogenizing raw milk at 250 or 300 MPa at an inlet temperature of 24°C with a valve-type homogenizer. They also observed maximum log reductions of 4.0, 2.6, and 1.8 for *P. fluorescens*, *Micrococcus luteus*, or *Listeria innocua*, respectively, inoculated into milk, after processing at 290 MPa. These results support other reports that Gram-negative bacteria are more susceptible to HPH than Gram positives (Wuytack et al., 2002; Thiebaud et al., 2003). For example, Wuytack et al. (2002) investigated the resistance of five Gram-positive (*Enterococcus faecalis*, *Staph. aureus*, *Lactobacillus plantarum*, *L. innocua*, and *Leuconostoc dextranicum*) and six Gram-negative bacteria (*Salmonella* Typhimurium, *Shigella flexneri*, *Yersinia enterocolitica*, *P. fluorescens*, and two strains of *E. coli*) to HPH at 100–300 MPa in a valve-type homogenizer and also to static HP at 200–400 MPa. The Gram-negative group was more susceptible to HPH than the Gram-positive group, and there was little difference in susceptibility within the two groups. By contrast, large differences in susceptibility to static HP were observed among the bacteria tested, and the Gram-positive and Gram-negative groups overlapped in susceptibility. Interestingly, a pressure-resistant strain of *E. coli* was not resistant to HPH.

A further difference between the effects of HPH and HPP is that sublethal injury is not observed with HPH, but is with HPP (Wuytack et al., 2002). The lack of sublethal injury by HPH has been noted by other authors also (Brinez et al., 2006; Jose-Brinez et al., 2007). This has important implications for growth of bacteria during storage of the treated products.

The lower susceptibility of Gram-positive bacteria, such as *Listeria* and *Staphylococcus* to HPH (Wuytack et al., 2002; Diels et al., 2003; Jose-Brinez et al., 2007), is worthy of note because of the pathogenicity of some species. Jose-Brinez et al. (2007) suggested that inlet temperatures >20°C should be used to achieve reductions of at least 4 logs, but indicated that some resistant strains of *Staphylococcus* may require even more severe treatment.

Bacterial spores are quite resistant to HPH with levels of inactivation of ≤1 log usually reported (Feijoo et al., 1997; Pereda et al., 2007). However, it is possible that the shear stresses suffered by the spores during HPH can reduce their heat resistance and enable sterilized products to be produced with less heat input than by conventional thermal

sterilization. Another approach to achieving the same goal is to use the instantaneous rise in temperature that occurs during HPH to momentarily expose the spores to a (lethal) high temperature without substantially increasing the total heat input (Asano et al., 2000); this minimizes the chemical damage to milk components responsible for changes, such as development of cooked flavor (Datta et al., 2002).

HPH with valve-type homogenizers has been shown to enhance the antimicrobial properties of lactoperoxidase, lysozyme (Vannini et al., 2004; Iucci et al., 2007), and lactoferrin (Iucci et al., 2007) toward bacteria. When HPH is applied to cultures of bacteria in the presence of these enzymes, it damages the cell membranes, facilitates the penetration of the enzymes through the damaged membranes, and increases the intrinsic activity of the enzymes, possibly through conformational changes and increased exposure of hydrophobic regions of the proteins. A similar synergistic effect has been observed with nisin and HPH (Taylor et al., 2007).

HPH and other methods of disrupting microbial cells have potential applications in the dairy industry (Geciova et al., 2002). The efficacy of HPH, sonication, and bead milling in disrupting cells of *Lactobacillus delbrueckii* subsp. *bulgaricus* to release intracellular β -galactosidase was assessed by Bury et al. (2001). HPH at 135 MPa with a valve-type homogenizer with three passes (or a single pass at 200 MPa) and bead milling for 2–3 minutes were more effective than sonication. Consequently, HPH, being a continuous flow process, may find application in large-scale operations for extracting intracellular material from bacterial and other cells.

HPH is also able to inactivate bacteriophages, which can cause failure of dairy fermentations. Treatment at 200 MPa (inlet temperature of 25°C, 5 passes) with a valve-type HP homogenizer inactivated three lactococcal bacteriophages in phosphate buffered saline by 5 logs. Lower inactivation rates occurred at 100 MPa or with fewer passes. Furthermore, the treatments were less effective when the phages were treated in milk or whey; the maximum reduction in milk was then 3 logs (Moroni et al., 2002)

6.3.2.2 Fat

HPH decreases the size of the fat globules in milk more than normal homogenization by a two-stage homogenizer operating at 18 MPa. Hayes and Kelly (2003a) reported a reduction in average fat globule size ($d_{4,3}$) from $\sim 3.2 \mu\text{m}$ to $0.70 \mu\text{m}$ by homogenization at 18 MPa and to $0.47 \mu\text{m}$ by two-stage HPH at 200 MPa. However, the inlet (and outlet temperature) for the conventional homogenizer was 50°C, while the inlet temperature for the HPH was 9.5°C; the outlet temperature was 53.9°C. The reduction in fat globule size increased with both the pressure of the HPH treatment and the inlet temperature (Hayes and Kelly, 2003a). However, Kielczewska et al. (2003) reported that as the homogenization pressure was increased, the size of fat globules decreased to a minimum and then increased with higher pressures. They concluded that HPH of whole milk at $>80 \text{ MPa}$ led to coalescence of the fat globules and hence homogenization efficiency of HPH at $>100 \text{ MPa}$ was doubtful. Coalescence was also indicated in the studies of Serra et al. (2007) and Thiebaud et al. (2003). Serra et al. (2007), using a valve-type homogenizer, observed a reduction of fat globule size ($d_{3,2}$) to $0.12\text{--}0.16 \mu\text{m}$ after HPH at $100\text{--}200 \text{ MPa}$, but no further reduction at 300 MPa. In fact, from 230–330 MPa, the size distribution became polydisperse, and some large particles were present. These may be fat aggregates that form due to a lack of sufficient casein to cover the new small globules. However, they may also be related to whey proteins, which denature under these conditions (Serra et al., 2007). Thiebaud et al. (2003)

considered the larger particles observed after treatment at 300 MPa rather than at 200 MPa to be fat clusters.

According to Paquin (1999), the emulsions produced by HPH, in particular microfluidization, are finer, and the size distribution is narrower. Fewer large fat globules were found in milk after HPH treatment than after conventional homogenization. This is important as the stability of the fat emulsion is largely determined by these large globules (Hillbrick et al., 1998). This was clearly demonstrated by Hardham et al. (2000) where UHT milk homogenized by microfluidization showed greater stability during storage than conventionally homogenized milk.

Thompson and Singh (2006) used HPH (microfluidization) to prepare liposomes from milk phospholipids and sphingolipids obtained from milk fat globule membrane material. The size of the liposomes decreased with increasing pressure and number of passes through the microfluidizer; the smallest average size (hydrodynamic diameter), 95 nm, was obtained after 10 passes at 103 MPa. Such liposomes may be a convenient means of supplying these unique membrane lipids as a food ingredient and also as a delivery vehicle for other bioactive ingredients.

HPH of raw milk induces considerable lipolysis of triglycerides due to the destruction of the milk fat globule membrane, allowing access to the fat by the native lipoprotein lipase (Lanciotti et al., 2006; Serra et al., 2008). This also occurs with conventional homogenization (Deeth, 2005). However, induction of lipolysis by HPH of raw milk can be avoided if the temperature reached during the treatment is sufficient to completely inactivate the native lipase. This was achieved in a study by Serra et al. (2008), where HPH was used at combinations of pressure and inlet temperature of 200 MPa/40°C and 300 MPa/30°C (outlet temperatures: 79.6 and 95.3°C, respectively). At 200 MPa and a lower inlet temperature of 30°C, considerable lipolysis was induced. These last conditions raised the temperature to 73.6°C, but with a residence time of only <0.7 second was insufficient to completely inactivate the lipase.

6.3.2.3 Proteins

There have been several recent publications, including patents (Paquin et al., 2003), on the effect of HPH on milk proteins. The structures of both the casein micelles and whey protein particles are affected by HPH, which suggests this technology may be useful for modifying milk proteins to suit specific applications.

Casein

Hayes and Kelly (2003a) reported that the average size of casein micelles in milk was decreased slightly by HPH at 200 MPa, while Roach and Harte (2008) found that the size varied according to the pressure of the treatment. It decreased by about 30% to 171 nm when the pressure was increased to 200 MPa, but at higher pressures, it increased, reaching 200 nm at 350 MPa. Roach and Harte (2008) also showed that when a microfiltered casein micelle suspension was HPH treated at different pressures, the micelle size remained stable until 250 MPa, and then increased at 300 and 350 MPa, reaching 363 nm. Similar, but much more marked, effects were observed when calcium chloride was added; for example, at 10 mM added CaCl₂, HPH at 350 MPa increased the micelle size to 16,200 nm compared with 244 nm at 250 MPa.

In a detailed study of the effect of HPH (microfluidization) of reconstituted skim milk powder (RSMP) on casein micelle size, with and without heating, Sandra and Dalgleish

(2005) used pressures of 41–186 MPa with up to six passes. They found that the size of the micelles decreased and the amount of nonsedimentable caseins increased with increasing pressure and number of passes. The micelle size decrease was greater for unheated RSMP than for RSMP, which had been heated to 85°C for 10 minutes either before or after HPH treatment.

Whey proteins

The effect of HPH on whey proteins has been studied by several groups (Paquin et al., 2003; Datta et al., 2005; Bouaouina et al., 2006; Dissanayake and Vasilejvic, 2009). In the absence of a heat treatment sufficient to cause thermal denaturation, some have reported no denaturation of whey proteins by HPH (Bouaouina et al., 2006; Dissanayake and Vasilejvic, 2009), while others have reported some denaturation (Datta et al., 2005; Hayes et al., 2005). The apparent discrepancy between the findings is attributable to the homogenization pressures and inlet temperatures used.

A more interesting and potentially useful effect of HPH on whey proteins is on their physical state and functional properties. Even in the studies that did not show denaturation, significant physical changes were observed. When Bouaouina et al. (2006) HPH treated WPI at pressures up to 300 MPa, they observed a marked reduction in particle size. Before treatment, only 9% (by volume) of particles had diameters less than 1 μm , while after treatment at pressures >200 MPa, 90% of the particles were in this size range. A similar reduction was reported by Dissanayake and Vasilejvic (2009) using microfluidization at 140 MPa with 5 passes. These particle size data were obtained using a laser-scattering Malvern Mastersizer. However, in size-exclusion HPLC, which does not detect the large particles because the samples are microfiltered before analysis, the generation of high molecular weight aggregates were observed. The aggregate peak increased with the homogenization and number of passes. The amount of these aggregates was relatively small; Bouaouina et al. (2006) reported that they comprised 2.8% of the total proteins analyzed after HPH at 300 MPa. The microfluidization treatment of Dissanayake and Vasilejvic (2009) increased the solubility of the whey proteins, but such an increase was not found by Bouaouina et al. (2006). One functional property that showed the same trend in both of these studies was foaming, which showed significant increases in both overrun and foam stability after HPH treatment. This effect has been attributed to the exposure on the surface of previously buried hydrophobic groups. Another interesting effect noted by Dissanayake and Vasilejvic (2009) was an increase in heat stability as measured by heat coagulation index (HCT).

Whiteley and Muir (1996) reported that HPH (microfluidization) at 68 MPa markedly increased the heat stability of concentrated milk if applied after concentration. When applied before concentration, the heat stability recovered, but only when the size of the fat globules was reduced to <~0.4 μm . This effect was considered to be related to the protein adsorbed to the fat globules. However, HPH of infant formula mix before in-can sterilization induced age thickening and gelation of an infant formula (Pouliot et al., 1990). The increase in viscosity during storage was proportional to the homogenization pressure.

HPH treatment of heat-treated whey protein solutions has a marked effect on the solubility of the whey proteins. HPH (microfluidizer) treatment at 150 MPa of 5% WPC (WPC80) solutions heat treated at 90°C for 10 minutes decreased the size of the heat-induced aggregates and substantially increased their solubility. Furthermore, subsequent spray or freeze-drying of the HPH/heat-treated WPC solutions produced powders with substantially improved solubility. Thus, HPH of heat-treated whey may be a useful strategy for

increasing the solubility of products containing heat-denatured whey proteins (Iordache and Jelen, 2003).

Enzymes

The effect of HPH on enzymes is variable. Picart et al. (2006), using HPH up to 300 MPa with an inlet temperature of 24°C, observed a slight increase in the activity of alkaline phosphatase at 100–150 MPa, a slight decrease at 175–200 MPa, and marked decreases at 250 MPa (80%) and 300 MPa (94%). The loss of activity was attributed almost entirely to mechanical forces (e.g., shear and cavitation) and not heat. By contrast, Datta et al. (2005), using HPH at 200 MPa and milk inlet temperatures of 10–50°C, found similar inactivation profiles of alkaline phosphatase for HPH and isothermal heating, and concluded that the loss of enzyme activity was due to heating alone. A similar result was obtained by Hayes et al. (2005) using inlet temperatures of 40–50°C. However, HPH at low temperatures causes little inactivation of alkaline phosphatase (4°C, Picart et al., 2006; 6–9°C, Hayes and Kelly, 2003b).

Plasmin has been reported to be inactivated by HPH, the extent of which depends on the initial temperature and the homogenization pressure (Datta et al., 2005; Hayes et al., 2005). Hayes et al. (2005), using an inlet temperature of 40–50°C, obtained 85 and 95% inactivation at 200 and 250 MPa, respectively; however, Hayes and Kelly (2003b) had found a maximum of 65% inactivation with a lower inlet temperature of 6–9°C. Iucci et al. (2008) refuted these findings and showed that the plasmin becomes associated with the milk fat globule membrane during homogenization, and hence the apparent decrease in activity previously reported was due to centrifugal removal of the enzyme with the fat during the analytical procedure. They showed there was no loss of plasmin activity by HP homogenization.

Datta et al. (2005) reported that native milk lipoprotein lipase was activated by a maximum of 240% in HPH treated samples. This occurred when the outlet temperature was 58°C. Less activation followed by inactivation occurred as the outlet temperature increased. Complete inactivation occurred after HPH at 200 MPa when the outlet temperatures reached >71°C.

Lactoperoxidase is partially inactivated (up to ~20%) by HPH at outlet temperatures lower than those that cause thermal inactivation, that is, ~70°C. Above this temperature, inactivation is largely a thermal effect; both HPH-treated and corresponding thermally treated samples are completely inactivated at 80°C (Datta et al., 2005). However, as indicated above, the antibacterial activity of lactoperoxidase toward bacteria is enhanced by HPH under conditions that do not cause thermal inactivation (Vannini et al., 2004).

6.3.3 Effect on products

6.3.3.1 Cheese

HPH has been reported to enhance (Kiełczewska et al., 2003; Sandra and Dalgleish, 2007; Zamora et al., 2007) or have no effect (Hayes and Kelly, 2003b) on rennet coagulation time of milk during cheesemaking. Interestingly, Zamora et al. (2007) found a positive effect on coagulation time after a single-stage HPH treatment at 200 and 300 MPa, but a negative effect when a two-stage treatment was applied. Sandra and Dalgleish (2007) attributed their observed (slight) decrease in rennet coagulation time to the loss of some κ -casein from the surface of the micelle, which facilitated coalescence of the micelles.

Zamora et al. (2007) obtained a higher wet curd yield after HPH of the raw milk partly because of a higher curd moisture (up to 18% higher after HPH at 300 MPa) and partly because of an increased yield of total curd solids, which was 11% (at 300 MPa) higher than for raw untreated milk; the corresponding increase in yield for pasteurization with conventional homogenization was reported to be 7%. A significant increase in yield of cheese (Caciotta) from cows' milk subjected to HPH at 100 MPa was also observed by Lanciotti et al. (2006). Zamora et al. (2007) attributed the yield improvement to changes to the protein—fat interactions due to both homogenization and the accompanying heat caused by the HPH treatment.

6.3.3.2 Yogurt

Milk subjected to HPH treatment at 200 and 300 MPa has been found to produce yogurt with better quality (higher gel strength and less syneresis) than milk treated conventionally and fortified with 3% skim milk powder (Serra et al., 2007). The aggregation rate was also higher in milk HPH treated at these pressures. These pressures also produced the smallest fat particles that the authors suggested act like large casein micelles with a large surface area available for interaction in gel formation, thereby enhancing the aggregation rate. Serra et al. (2007) concluded that HPH at 200 and 300 MPa causes modifications to the protein, such as denaturation of the whey proteins, and facilitates protein—protein and fat—protein interactions that lead to the formation of a strong gel network with enhanced water-binding capacity. Similar beneficial effects of HPH on the texture of fermented milks were reported by Patrignani et al. (2007).

6.3.3.3 Ice cream

The improved quality attributes of products treated with HPH were also evident in ice cream. Hayes et al. (2003) homogenized ice cream mixes containing 3–8% fat at 100 or 200 MPa, and found that the viscosity of HPH-treated 5% fat ice cream mixes was similar to that of 8% fat standard ice cream mix (homogenized at 18 MPa). The ice cream produced from the HPH-treated 5% fat ice cream mixes had similar textural characteristics to standard ice cream samples, suggesting that HPH may be useful for improving the texture of low fat ice cream.

6.3.4 Commercial developments

To the knowledge of the authors, HP homogenization and microfluidization are not applied on a large scale in the dairy industry or for traditional dairy products. However, these technologies are increasingly being used for high value functional foods and nutraceuticals, and for the preparation of liposomes and extraction of microbial cell contents from cultures; several of these contain or use dairy-derived ingredients. High equipment cost and low capacities at pressures over 100 MPa will limit wide adoption and applications of this technology for most dairy products.

6.4 ULTRASONICATION

The use of ultrasonication in the food industries, including dairy, has received considerable interest in recent years. This is largely attributable to the development of commercial-scale

equipment enabling laboratory applications to be scaled up for industrial use (Patist and Bates, 2008), but also to the increased awareness of its usefulness in a range of applications. In contrast to other nonthermal technologies, the emphasis has not been on destruction of microorganisms and extension of shelf life, but on a range of physical effects that improve processing efficiency and/or product quality. Several recent reviews have been published on the topic (Villamiel et al., 1999; Leadley and Williams, 2002; Pardeshi et al., 2005; Zheng and Sun, 2006; Ulusoy et al., 2007; Vilkhov et al., 2008; Ashokkumar et al., 2010).

6.4.1 Principle

Ultrasonication involves the use of sound waves of high frequency (18 kHz–20 MHz) that is beyond the range of human hearing. Two forms of ultrasonication are recognized: high frequency, low power and low frequency, high power (McClements, 1995). The former operates at $\sim 1 \text{ W/cm}^2$ and 0.1–20 MHz, while the latter operates at $10\text{--}1000 \text{ W/cm}^2$ and 18–100 kHz. The low power form is suitable for imaging and diagnostic applications and leaves most food systems unchanged, while the high-power form is physically disruptive and finds application in a wide variety of operations in the food industry. The high-power form is the focus of this section; however, several interesting studies and opportunities using frequencies higher than 100 MHz will also be discussed.

When ultrasound is applied to a food, the sound waves cause a series of rapid longitudinal compressions and rarefactions that cause mechanical vibrations, and, in liquids, strong eddies in the area surrounding the sonotrode that spread into the liquid. This effect, known as microstreaming or acoustic streaming, causes large localized forces that can effectively “rub” surfaces and cause physical damage. In addition, the sound waves create tiny bubbles that grow in size over several cycles and eventually implode violently. This phenomenon, which is known as cavitation, causes severe physical effects due to high shear and turbulence in the food in the immediate vicinity of the imploding bubbles. It also generates high localized temperatures (up to 5000 K) and pressures (up to 100 MPa). Cavitation is considered to be the major mechanism responsible for the effects, including bactericidal effects, observed in high power, low frequency ultrasonication. The three physical phenomena, mechanical vibration, microstreaming, and cavitation, are the major effects of ultrasonication in the lower frequency range, for example, <100 kHz, but chemical phenomena due to the generation of free radicals are more significant in the higher frequency range, 100 kHz–1 MHz. These free radicals can cause chemical changes in food, such as oxidation and hydroxylation, when oxygen is available. The changes can be detrimental or beneficial (Ashokkumar et al., 2008).

6.4.2 Setup

The physical setup for ultrasonication consists of three major parts: the frequency generator and power supply, the transducer, and the horn or sonotrode. The transducer transfers the vibrational energy from the frequency generator to the sonotrode, which transmits the vibrations to the material (food or air) with which it is in contact and generates the sound waves in that material. Most commonly, ultrasound treatment is applied to liquids in flow-through cells, but it also has applications when applied in batch mode and also in air and on solid material.

Much of the ultrasound energy is converted to heat, which causes an increase in temperature of the treated material. The amount of heat generated is dependent on the power output of the sonicator and the duration of the treatment, and can be used to obtain a measure of the actual power input into the sample. The power (W) and wave amplitude are governed by the power supply, while the power intensity (W/cm^2) is determined by both the power output of the equipment and the area of the sonotrode.

The outcome of ultrasonic treatment largely depends on the total energy input per volume of product (kW h/L) and the power or energy intensity (W/cm^2). The volumetric ultrasound energy density (W/cm^3) is now utilized where the volume of treated liquid around the sonotrode is considered (Ugarte-Romero et al., 2007). Both parameters are independent of scale, thus these two parameters are used for scale-up purposes (Patist and Bates, 2008). Unfortunately, different parameters of the ultrasonic treatment are reported by different researchers, making it difficult to make comparisons between reports. This is apparent in the discussion below.

6.4.3 Effect on milk components

6.4.3.1 Microorganisms

Ultrasound is capable of destroying microorganisms, but this ability depends on the time and temperature of treatment, the intensity of the ultrasound, the nature of the microorganism, and the nature of the medium (Scherba et al., 1991). In general, large cells are more sensitive to ultrasound than small cells, Gram-negative bacteria are more sensitive than Gram-positive, and rod-shaped organisms are more sensitive than coccus shaped (Jacobs and Thornley, 1954; Ahmed and Russell, 1975; Villamiel and de Jong, 2000). Ultrasonication has little effect on bacterial spores, even at elevated temperatures and pressures, but it can reduce their heat stability and hence enable sterile products to be produced at lower temperatures than by heat alone (Sanz et al., 1985).

For vegetative cells, at temperatures less than $\sim 51^\circ\text{C}$, the effects of ultrasound treatment are almost completely nonthermal, at ~ 52 to $\sim 60^\circ\text{C}$, the effects are both thermal and non-thermal while at $>60^\circ\text{C}$ the effects are almost entirely thermal (Raso et al., 1998; Villamiel and de Jong, 2000; D'Amico et al., 2006). Villamiel and de Jong (2000) found that continuous-flow, high-intensity ultrasonic treatment of milk at $\sim 62^\circ\text{C}$ had a similar effect to thermization (thermal treatment at 60 – 65°C for 5–15 seconds), but had the additional benefit of reducing the size of the fat globules in the treated milk. However, the extra effect came at a cost in energy input; for ultrasonic thermization at 62°C in continuous mode, the energy required was 270–830 kJ/L, while conventional heating required only 105–220 kJ/L.

Pasteurization of milk has been shown to be possible at 57°C with ultrasound treatment at 20 kHz, power intensity of $118 \text{ W}/\text{cm}^2$ and treatment time of 18 minutes (D'Amico et al., 2006). Longer treatment times are required for fat-containing milk than non-fat milk because of the protective effect of the fat. The long treatment times required for ultrasonic pasteurization are in contrast to the very short treatment times used by some other non-thermal technologies such as pulsed electric field technology and HP for inactivating microorganisms. The long treatment times increase the risk of off-flavor production and degradation of dairy components. Mason et al. (2005) reported an extreme cooked flavor, similar to that of freshly processed UHT milk, Riener et al. (2009a) observed a “rubbery” aroma of sonicated reduced-fat milk while Chouliara et al. (2010) found the flavor of

ultrasonicated full-fat milk to lie between “burnt” and “foreign.” Riener et al. (2009a) identified a range of chemical compounds that appeared after ultrasonication at 100–400 W at 24 kHz for 2.5–20 minutes. These included 1,3-butadiene and 1-buten-3-yne, which may have originated from pyrolysis of milk components, pentanal, hexanal, and heptanal, which were probably produced from hydroperoxides resulting from photooxidation, C6–C9 alk-1-enes, which could be formed from pyrolytic cleavage of fatty acid chains, and aromatic hydrocarbons such as benzene, toluene and xylene, whose origin is unclear. The formation of such compounds could limit the application of ultrasound in dairy processing, particularly for “pasteurization” where the energy input to achieve an acceptable reduction (>5 logs) of pathogenic bacteria is quite high.

At temperatures >~60°C, sonication has to be performed under pressure to negate the cushioning effect of the higher vapor pressure on the collapse of the cavitation bubbles. The higher pressures provide the dual benefits of enabling sonication to be carried out at high temperatures, even >100°C, and also increasing the power provided to the food product. Thus, sonication treatment at 112°C at a pressure of 0.3 MPa was 6–30 times more lethal on the spores of *B. cereus*, *Bacillus stearothermophilus*, and *Bacillus coagulans* than the corresponding heat treatment at the same temperature (Sala et al., 1995). During sonication at high temperatures under pressure, calcium, dipicolinic acid, and low molecular weight (7 kDa) glycopeptides are released from the cortex of the spores into the medium (Palacios et al., 1991). The loss of these substances from the cortex is believed to induce hydration of the protoplast, thereby reducing the spores’ heat resistance (Sala et al., 1995).

One interesting effect of ultrasound on some bacteria is the disruption of clusters of cells. Thus, short treatment times of some bacteria result in an increase in counts and apparent numbers before a decrease with longer incubation times (Joyce et al., 2003). However, this phenomenon is not observed with all bacteria.

6.4.3.2 Fat

Major effects of ultrasound on fat are homogenization with formation of emulsions and enhancement of crystallization. Several authors have shown that ultrasonication reduces the size of the fat globules in milk to <1 µm and forms a stable emulsion (Villamiel and de Jong, 2000; Wu et al., 2001; Ertugay et al., 2004). Both the size and the size range of the fat globules are smaller after ultrasonication than after conventional homogenization. Villamiel and de Jong (2000) reported that the temperature of ultrasound treatment affected the size distribution with treatments at room temperature, 55 and 61°C showing a bimodal distribution with maximum at 2–3 µm and ~0.6 µm, while treatments at 70 and 75.5°C showed a unimodal distribution with a maximum at 0.6–0.7 µm.

Ultrasonication has been shown to accelerate crystal formation through nucleation around the cavitation microbubbles. This leads to both improved efficiency of crystallization and development of smaller and more uniform-sized crystals, which is beneficial for some products (Martini et al., 2008). It has been suggested that ultrasound may be beneficial in ice cream production (Acton and Morris, 1992; Zheng and Sun, 2006); however, there is the risk of generation of off-flavors in such a high-fat product.

6.4.3.3 Lactose

Ultrasound has also been used to facilitate lactose crystallization (Bund and Pandit, 2007; Dhumal et al., 2008). Bund and Pandit (2007) demonstrated faster crystal growth and higher

crystal yield in the presence of ultrasound, while Dhumal et al. (2008) were able to engineer the size and shape of lactose crystals by manipulating various factors, including the temperature and the frequency, intensity, and duration of the ultrasonic treatment.

Another reported effect of ultrasonication is the enhanced hydrolysis and fermentation of lactose. Toba et al. (1990) found that when a sonication treatment (20 kHz, 60 W, 20 minutes, 0°C, 5 mL samples) was introduced in the fermentation of milk by *Lactobacillus delbrueckii* var. *bulgaricus* or *L. helveticus* after incubation for 4 hours and before further incubation for 12 hours. The percentage of lactose hydrolyzed was 71–74% compared with 39–51% in nonsonicated milk. This was attributed to release of the β -galactosidase from starter bacteria by the ultrasonic treatment. Wu et al. (2001) reported accelerated acid production and reduced yogurt fermentation time (by 0.5 hour), when inoculated yogurt milk was ultrasonicated (20 kHz, up to 450 W, 8 minutes, 15°C, 150 mL samples). Sener et al. (2006) investigated the effect of pulsed ultrasonication on lactose hydrolysis by a commercial β -galactosidase. The treatment resulted in a higher degree of lactose hydrolysis (90 cf. 84%); however, the ultrasound caused a 25% decrease in enzyme activity.

6.4.3.4 Proteins

Ultrasonication has been shown to have significant effects on milk proteins. Whey proteins are denatured to some extent by ultrasound treatment and this effect is synergistic with heat (Villamiel et al., 2000). The quaternary and/or tertiary structures of caseins are altered, and casein micelles are partially disrupted, releasing free caseins (Taylor and Richardson, 1980). However, Villamiel et al. (2000) found no changes in the electrophoretic pattern of the caseins after ultrasonication of milk, indicating little or no change in the primary structure of these proteins.

Considerable research has been carried out on the effect of ultrasonication on the physical properties of whey protein products. Krešić et al. (2008), using 20 kHz ultrasound at a power intensity of 43–48 W/cm² (determined by calorimetry; nominal power output of unit was 600 W) for 15 minutes, found an increase in solubility of WPI and WPC60 and an increase in viscosity of 10% solutions of these products. The enhanced solubility was attributed to the unfolding of the proteins, exposing hydrophilic groups and enabling increased interaction with water. This sonication treatment also improved the foaming and emulsification properties of the whey solutions (Jambrak et al., 2008). Ashokkumar et al. (2009a) have reported that when WPC80 solutions were subjected to ultrasound treatment at 20 kHz following a preheating step, there was a decrease in viscosity. This differs from the results of Krešić et al. (2008) above, where sonication of unheated WPC60 and WPI solutions increased their viscosity. Ashokkumar et al. (2009a) have also reported that sonication of reconstituted (preheated) WPC80 significantly increased its water solubility, largely through altering the particle size distribution through disruption of protein aggregates, and increased the heat stability of the whey proteins. Heat-set gels made from these sonicated WPC solutions were stronger and showed less syneresis than gels made from unsonicated WPC. The above effects were not observed when the sonication was carried out at frequencies >200 kHz (Ashokkumar et al., 2009b), suggesting that the changes are due to physical rather than chemical effects.

The effects of ultrasound on the functional properties of films made from sodium caseinate and WPC were examined by Banerjee et al. (1996). Sonication was performed at 168, 522, and 860 kHz for 0.5–1 hour. Sonication of solutions of caseinate had a marked effect on the tensile strength of the caseinate film, increasing it by up to 441% (average of 224%).

In general, longer process times and lower ultrasound frequencies increased the tensile strength of the films. By contrast, sonication of WPC solutions did not increase the strength of the whey protein films produced from them.

6.4.3.5 Enzymes

Enzymes are known to show a range of effects when ultrasonicated, often at elevated temperature and pressure. The sensitivity of the enzymes appears to relate to their heat resistance, molecular structure and size, and also to the nature of the treatment medium (Vercet et al., 2001). Villamiel and de Jong (2000) reported that ultrasound alone had little or no effect on alkaline phosphatase (AP), gamma-glutamyltranspeptidase (GGTP), and lactoperoxidase (LP), but there was a synergistic effect between ultrasound and heat that caused inactivation of AP at 61°C, GGTP at 70°C, and LP at 75.5°C. Ertugay et al. (2003) observed inactivation of AP and LP, which increased with increasing ultrasound amplitude, temperature, and time of treatment.

Of particular interest for dairy ingredients is the effect of ultrasonication on heat-resistant proteases and lipases, which can degrade milk proteins and lipids, respectively, and cause stability and flavor defects. Mauer and Hayes (2003) reported that milk plasmin, a heat-stable endogenous enzyme, was 55% inactivated by ultrasonication treatment at 20kHz for 15 seconds at an amplitude of 305 μ m in Tris buffer (pH 7.6, 0.1 M NaCl). Less inactivation was found after treatment at lower amplitudes. In a later report by the same research group, the endogenous milk plasminogen activator, which converts native plasminogen to plasmin, was shown to be inactivated by up to 52% by ultrasonication (Burbrink et al., 2004). The heat-resistant protease produced by the psychrotroph *P. fluorescens*, which can also cause defects in dairy products, was very resistant to sonication at 30°C but very sensitive to a manothermosonication (MTS) treatment at 76–109°C (Vercet et al., 2002). MTS at 20kHz, 117 μ m and 350kHz reduced the *D* (decimal reduction time)-values at ~110°C of the protease in whey at pH 6.6 and the lipase in buffer at pH 6.6 by about threefold; however, the reduction in *D*-value varied with pH and temperature for both enzymes.

Another approach to reducing the activities of extracellular lipase and protease of *P. fluorescens* in products is to prevent their production during growth of the bacterium in raw milk. Jaspe and San Jose (1999) found that this was possible if the raw milk was homogenized by ultrasonication before growth of the *Pseudomonas*. Lipase production was completely inhibited and protease production was reduced by 88%. The mechanism of this inhibition by ultrasonic treatment is unknown.

6.4.4 Applications

6.4.4.1 Processes

Ultrasonication has numerous applications relating to processing of dairy products and ingredients (For an extensive list, see Patist and Bates [2008]). These include: homogenization and emulsification; fermentation; crystallization; defoaming; reduction of fouling; and drying. The first three have been discussed in previous sections dealing with milk components; the others are discussed below. Some other relevant process applications of ultrasound not discussed in this chapter include cleaning, cutting, and waste treatment.

Defoaming

Defoaming applications of ultrasound include defoaming packages on filling machines and fermentation vessels. For these, the ultrasonic waves are transmitted through the air rather than through a liquid or solid. Defoaming by ultrasound results in improved efficiency and reduced waste, and hence the payback time for introducing the technology is quite short. Patist and Bates (2008) estimated the payback time could be as short as 6 weeks, considerably shorter than for all other applications considered.

Reduction of fouling

Ultrasound has been shown to be effective in reducing fouling of membranes during membrane filtration (Muthukumaran et al., 2006), and metal surfaces of heat exchangers (Lin and Chen, 2007), and extruders (Akbari Mousavi et al., 2007).

Muthukumaran et al. (2005, 2007) reported enhanced permeate flux rate in a cross-flow ultrafiltration system equipped with ultrasound (50 kHz) for processing whey. They also showed it to be beneficial during membrane cleaning cycles (Muthukumaran et al., 2004). The effects have been attributed to: mechanical vibration in the system, which maintains particles in suspension, resulting in more free channels for liquid flow through the membrane; cavitating bubbles rubbing the membrane surface and dislodging deposits; and acoustic streaming causing turbulence in the vicinity of the membrane and the cake layer. The ultrasonic power required for the process in the lab scale unit was low at ~2 W/L which suggests the treatment would cause minimal damage to the membranes. It also indicates that the process may be commercially economic (Muthukumaran et al., 2005). The efficacy of the ultrasound treatment depends on the type of ultrasound used. For example, continuous high power, low frequency (50 kHz) ultrasound was more effective than intermittent, low power, high frequency (1 MHz) ultrasound in reducing fouling and cleaning of ultrafiltration membranes used for whey processing (Muthukumaran et al., 2007).

The amount of fouling deposit during heat processing of milk is reduced under the influence of ultrasound (Lin and Chen, 2007). The ultrasound can either be delivered through the liquid itself or via the heated solid surface. The cavitation and acoustic streaming in the liquid as well as vibration of the heat exchanger prevent the deposit from attaching to the heat exchanger surface, and dislodge any attached deposit. As well as reducing the fouling layer and hence preventing a decline in the heat transfer coefficient of the heat exchanger during processing, the ultrasound also enhances heat transfer between the heated solid surface and the liquid being heated; both improve the efficiency of the process.

Ultrasound also enhances extrusion processes that can be used for producing products containing dairy ingredients. In this application, the ultrasonic energy is provided to the metal extrusion barrel or die by perpendicular attachment of the sonotrode. The resulting mechanical vibration reduces friction between the product and the metal and thus improves flow behavior and process efficiency (Akbari Mousavi et al., 2007).

Drying

Another application in which ultrasonic energy can improve efficiency is enhancing drying. A system developed by Fuente-Blanco et al. (2006) uses ultrasonic energy together with hot air to enhance drying of semi-solid foods at low temperature. Also, a low cost spray dryer with an ultrasonic atomizer has been developed (Avvaru et al., 2006; Luz et al., 2007),

which produces smaller droplets, and hence smaller dried particles, than conventional mechanical atomizers. For example, dextrin particles spray dried with this dryer spray had diameters in the range 0.2–2.6 μm , with an average of 1.7 μm (Luz et al., 2007). For comparison, the particles of milk powders produced by conventional spray dryers have diameters of 10–250 μm (Carić and Milanović, 2003).

6.4.4.2 Products

The production of some dairy products for which ultrasonication may be used have been mentioned above. These include homogenized milk, ice cream, fermented milks, and lactose-hydrolyzed products. Other applications are in yogurt and cheese manufacture.

Yogurt

Application of ultrasound to milk in yogurt production before inoculation increases consistency, gel strength, and viscosity, and decreases syneresis in the yogurt (Wu et al., 2001; Vercet et al., 2002; Riener et al., 2009b, 2010). Wu et al. (2001) attributed the improvement in physical characteristics to increased water-holding capacity, while Vercet et al. (2002), who used an MTS treatment of milk at 20 kHz, 0.2 MPa and 40 for 12 seconds, attributed it to increased whey protein denaturation caused by the ultrasound. The latter authors considered the possibility that the improved texture could be due to the homogenization effect, but dismissed this since milk used in the control yogurt had also been homogenized. Riener et al. (2009b) found differences in the microstructure of yogurt made from thermosonicated (24 kHz, 400 W nominal power output, 45°C, 10 minutes) milk compared with yogurt made from conventionally heated (90°C/10 minutes) milk. It had a more porous, honeycomb-like network and smaller-sized particles (<1 μm).

The improvement in the properties of yogurt from thermosonicated milk led Riener et al. (2010) to suggest that the use of thermosonication may reduce the need for addition of texture modifying to enhance the rheological characteristics of low fat yogurt. It is interesting to note that the effects of ultrasonication on milk for yogurt manufacture are similar to those reported for HP homogenization (Serra et al., 2007).

Cheese

A limited number of publications have appeared on the use of ultrasound in cheesemaking despite some early work suggesting it may be beneficial in accelerating ripening (Nelson and Winder, 1954), emulsification of processed cheese (Von der Heide, 1961), and production of fresh cheese with a smooth consistency and minimal whey separation (Schlager and Gervais, 1970).

Ultrasound treatment (30 kHz, 300 W at 12°C) during brining of Mahon cheese increased proteolysis and lipolysis during cheese ripening. The acoustically brined cheeses exhibited greater firmness and more intense aroma, odor, and flavor than conventionally brined cheeses (Sánchez et al., 2001a,b).

Gaffney (1987, 1996, 1997) patented novel processes for making various cheese-like products using ultrasound. In one process, a concentrated fat and protein emulsion is sprayed into a chamber containing an ultrasonic probe (called a *sonolator*), and an almost instantaneous cheese-like emulsion is formed (Gaffney, 1987, 1996). This process is claimed to be suitable for producing a range of coagulated products, such as soft cheeses,

yogurt, and cheese analogues. The mechanism of the sonication-induced coagulation was not reported.

6.4.5 Commercial developments

Ultrasonics have many potential and several realized applications in the dairy industry. Patist and Bates (2008) list nine that have been realized and three potential applications of ultrasonics for the food industry. Most of these are applicable to the dairy industry, including emulsification/homogenization, crystallization, filtration/screening, separation, viscosity alteration, defoaming, enzyme and microbial inactivation, fermentation, and heat transfer. As reported by Versteeg and Sanguansri (2009), many different ultrasonic systems and components are available commercially, but are not specifically designed or optimized for food (including dairy) applications. Consequently, commercial applications are developed under proprietary arrangements, which typically are confidential. Nevertheless, to our understanding of these listed applications, defoaming and viscosity reduction of proteins have been commercialized in the dairy industry (Bates, pers. comm., 2010). Another application, “ultrasonically assisted deaeration,” has also been commercialized, and “enhanced hydration processes” for milk powders are in an advanced stage of implementation (Bates, pers. comm., 2010). Based on recent research and patent activities, commercialization of ultrasonically enhanced processes is expected to continue and that ultrasonics will become a relatively common technology in the food and dairy industries. Applications may include formation of nanoemulsions (Kentish et al., 2008), improvement of functionality, including heat stability, of fresh and reconstituted dairy ingredients (Ashokkumar et al., 2009a,b), viscosity reduction of several dairy products (Bates and Bagnall, 2008), and enhancing the flux and cleaning of membranes (Muthukumaran et al., 2007).

6.5 PULSED ELECTRIC FIELD TECHNOLOGY

Pulsed electric field (PEF) technology involves treatment of a pumpable food with very short pulses (microseconds) at very high electric field strengths. In general the treatments are performed at temperatures around ambient when retention of the natural attributes of the food is the key consideration; however, higher temperatures up to ~60° can be used when specific effects are desired. For this technology, the research on milk has largely focused on its effects on microorganisms and shelf-life extension, although its effect on other milk constituents such as proteins, including enzymes, has recently received attention.

Several factors affect the outcome of PEF treatments. These include both PEF conditions such as electric field strength, pulse width, pulse frequency, number of pulses, shape of the pulse wave, flow rate and flow conditions (laminar or turbulent), and food factors, including electrical conductivity, temperature, viscosity, pH, and composition. When destruction of microorganisms is the objective, the type of microorganism and stage of growth are also important parameters. Because of the large number of factors which can influence the effectiveness of PEF treatments, it is difficult to make valid comparisons between the results from different researchers because all parameters are usually not specified. As suggested by Alvarez et al. (2003), total specific energy input (in kJ/kg or kJ/L), electric field strength, and treatment times are essential parameters that should be reported in order to be able to compare treatments from different laboratories. Unfortunately, this has been

seldom done in the past. In addition, as the results of a PEF treatment may be very temperature-dependent, and temperatures increase during treatment, the temperatures at various stages of the process should also be reported.

The electrical conductivity of the liquid being treated is an important factor. In general, PEF is more effective in liquids with low conductivity (Jayaram et al., 1993). Milk and fruit juices have conductivities in the range 4–6 mS/cm and are suitable for PEF, although some researchers have diluted milk before PEF treatments to enhance the effectiveness (de Luis et al., 2009).

Some authors have reported that PEF treatment is a low energy process compared with thermal treatments (Ho et al., 1995; Qin et al., 1995). According to Toepfl et al. (2006), the use of PEF for some applications, such as permeabilization of plant cells, requires very little energy, ~1.5 kJ/kg, but other applications, such as microbial inactivation for pasteurization purposes, requires 40–1000 kJ/kg. The energy requirement for pasteurization can be substantially reduced if: the PEF treatment is performed at an elevated temperature, such as 50°C; use is made of the synergistic heat effects of the PEF treatment (Sepulveda et al., 2009); and heat recovered through a regeneration system is of the order of 95%. When these modifications are enacted, the energy input can approach 20 kJ/kg, which is the energy required for thermal pasteurization.

A large number of publications on PEF treatment of milk or liquid dairy products have appeared in the last 20 years. These include several review articles, book chapters, and books (de Jong and van Heesch, 1998; Barbosa-Cánovas et al., 1999; Barbosa-Cánovas and Zhang, 2001; Wouters et al., 2001; Bendicho et al., 2002a; Rastogi, 2003; Martín-Belloso and Elez-Martínez, 2005; Mittal and Griffiths, 2005; Sampedro et al., 2005; Toepfl et al., 2005, 2006; Deeth et al., 2007; Mosqueda-Melgar et al., 2008; Huang and Wang, 2009; Soliva-Fortuny et al., 2009; Wan et al., 2009; Shamsi, 2010).

6.5.1 Principle

PEF treatments are typically carried out at high electric field strength (10–50 kV/cm) in multiple short pulses (1–5 μ s) at frequencies of 200–400 Hz (Wan et al., 2009). The total treatment time, which is the product of the pulse width and the number of pulses, is usually much less than 1 second, and typically between 10 and 50 μ s. The field strength can be determined by the voltage produced by the power supply and the distance between the electrodes in the treatment chamber. Thus, a voltage of 10 kV across a distance of 0.25 cm yields a field strength of 40 kV/cm.

Several authors cite, in addition to the electric field strength and treatment time, the overall energy input from a treatment in kJ/kg or kJ/L; this facilitates comparison of treatments (Alvarez et al., 2003). However, the shape of the pulse wave also affects the outcome of the treatment. Common shapes are the exponential decay and square wave, both of which can be administered as mono- or bipolar forms. Higher field intensities are reached, momentarily, in exponential decay pulses than in square wave pulses where a moderate field intensity is maintained for a longer time than in exponential decay pulses.

As for the other technologies discussed in this chapter, the rise in temperature during the so-called nonthermal treatment must be taken into account. As an indication of this effect, the following results from Craven et al. (2008) are a useful guide. Milk processed at a field intensity of 28 kV/cm and specific energy input of 111.6 kJ/L reached final temperatures of 15, 40, 50, or 55°C from inlet temperatures of 10.5, 30.5, 40.5, and 45.5°C.

6.5.1.1 Setup

The equipment used in PEF processing consists of:

- a. a high-voltage power supply (up to 50kV);
- b. an energy storage capacitor;
- c. a pulse generator and switching system;
- d. a treatment chamber, containing two electrodes between which the liquid food is pumped;
- e. a temperature measurement and control system;
- f. a unit for controlling and monitoring voltage, current, and electric field strength; and
- g. a product handling and packaging system

The energy from the high-voltage power supply is stored in the capacitor, discharged at high levels of power at an extremely fast rate (1–5 μ s). The time between discharges is from 1 millisecond to seconds. When the electrical energy is applied as pulses, the disadvantages of continuous high voltage current treatments, electrolysis and ohmic heating, are minimized.

The design of the treatment chamber containing the electrodes, which is the heart of the operation, is important for the efficiency of the PEF unit. It is important that it does not contain “dead spots,” in which the product receives little treatment. To alleviate this problem, two or more treatment chambers in series can be used. Various types of laboratory and pilot-scale treatment chambers have been designed. These can be either static or continuous. Common configurations for static chambers are parallel plate and coil, while common continuous chambers are parallel plate, coaxial, and cofield chambers. The chambers may be maintained at a slight positive pressure to suppress bubble formation that would cause electrical arcing. The designs of treatment chambers have been reviewed by Huang and Wang (2009).

6.5.2 Effect on milk components

6.5.2.1 Microorganisms

PEF technology is a promising alternative technique to thermal pasteurization of liquid milk products as it is effective against a range of vegetative bacteria, as well as yeasts and molds. Bacterial spores are much more resistant than vegetative cells. The effects of PEF on microorganisms in milk have been extensively reviewed by Sampedro et al. (2005) and Deeth et al. (2007).

PEF has been shown to be more effective when combined with some thermal treatment. Some authors have considered the effects to be synergistic, others additive. Under these conditions, PEF can inactivate vegetative cells of most pathogenic and spoilage microorganisms by up to 5–6 logs after short treatment times (Sampedro et al., 2005). It is now apparent that PEF treatments can be designed to produce effects equivalent to the different levels of thermal treatment: thermization, pasteurization, higher pasteurization or extended shelf life processing, and UHT sterilization (Lewis and Deeth, 2009). In brief, PEF treatments at less than or equal to room temperature or a little higher effect 2–4 log reductions in bacteria, similar to thermization; PEF at 50–60°C treatment results in the equivalent of pasteurization (Craven et al., 2008; Shamsi et al., 2008); PEF at 65°C (Sepulveda et al., 2009) or combined with a separate HTST pasteurization (Sepulveda et al., 2005) produces

extended-shelf-life products; and PEF treatment followed by heat treatment at 105–112°C can yield a shelf-stable product (Evrendilek et al., 2001).

The temperature of PEF treatment has emerged as a crucial factor affecting the bactericidal effect of a PEF treatment. This is well illustrated in the study of Craven et al. (2008) in which sterile milk inoculated with spoilage *Pseudomonas* isolates was PEF treated (electric field strength, 31 kV/cm and specific energy input, 139.4 kJ/L) at 15, 40, 50, and 55°C (final temperatures). The effects at 15 and 40°C were much less than those at 50 and 55°C. The highest inactivation of >5 logs was achieved at 55°C. Treatment at this temperature extended the microbial shelf life (time for the count to reach 10⁷ cfu/mL) of the milk by at least eight days at 4°C over that of untreated inoculated milks. In this treatment, the milk had a residence time of about 5 seconds at the final temperature of 55°C; such thermal treatment alone caused a minimal reduction (0.2–0.3 logs for a 10⁵ cfu/mL inoculum) in *Pseudomonas* counts. Shamsi et al. (2008), using similar PEF conditions to those of Craven et al. (2008), but conducting the treatments at a final temperature of 60°C, achieved a reduction in *Pseudomonas* of 5.9 logs. However, this included a significant thermal effect, as a control heat treatment at 60°C caused a 2.4 log reduction in *Pseudomonas*. From these data, it appears that thermal effects become significant for this type of organism between 55 and 60°C.

Sepulveda et al. (2009) used PEF (35 kV/cm, total time 11.5 µs) at a treatment temperature of 65°C with a residence time of 10 seconds and achieved a shelf-life extension of milk of at least 24 days. They concluded that with the use of a thermal regeneration system, the energy efficiency of such a PEF treatment process would be highly competitive with thermal pasteurization. Using the same PEF treatment conditions, Sepulveda et al. (2005) adopted two other approaches to extending the shelf-life of milk. They applied the PEF treatment at 65°C immediately after thermal (HTST) pasteurization or 8 days after pasteurization. The two approaches achieved shelf-life extensions of 60 and 78 days, respectively. The major effect of the PEF was the extension of the lag phase of bacterial growth in the milk. The added benefit of PEF treatment 8 days after HTST treatment could be attributed to inactivation of bacteria, including *Bacillus* species, which had begun to multiply during storage. The shelf-lives attained in this work were similar to those achieved by thermal treatments of 120–130°C for 1–4 seconds used for producing extended shelf life milk products (Lewis and Deeth, 2009).

Evrendilek et al. (2001) adopted a similar approach to that of Sepulveda et al. (2005) in exploring the use of PEF for producing chocolate-flavored milk with shelf stability equivalent to its UHT counterpart. They used a PEF treatment (35 kV/cm, total treatment time of 45 µs at 11.9 to 24°C in a pilot plant operating at 105 L/h) followed by heat treatment at 112°C for 31.5 seconds. Chocolate milk treated in this way had a shelf-life of 119 days when stored at 4, 22, or 37°C. No degradation in color of the milk was observed during storage.

It is therefore apparent that the shelf life of a particular liquid product can be tailored by PEF treatment combined with a judicious choice of heat treatment before, during, or after the PEF treatment. The synergistic hurdle effect of PEF and heat can therefore be exploited to advantage for specific applications.

Another hurdle approach to enhancing the bactericidal effectiveness of PEF is to combine it with other technologies or antibacterial agents (Ross et al., 2003). Pagan et al. (1998) found that HP can initiate germination of spores, which, on outgrowth into vegetative cells, are sensitive to PEF, while PEF and ultrasonication have a synergistic effect in inactivating *B. subtilis* spores, achieving up to a 4 log reduction (Su et al., 1996; Jin et al., 1998). A

combination of PEF and ultrasonication is also more effective than either technology alone in inactivating *Listeria innocua* in milk. PEF and ultrasonication treatments alone caused reductions of 1.1–3.3 and 1.2 logs, respectively, but when combined, resulted in a 6.8 log reduction (Noci et al., 2009).

Both nisin and lysozyme exert a synergistic bactericidal effect with PEF, achieving up to 7-log reduction of the native flora of milk (Smith et al., 2002; Sobrino-Lopez and Martin-Belloso, 2008b). However, the order of addition and treatment is important (Sobrino-Lopez and Martin-Belloso, 2008a,b), as PEF has a destructive effect on nisin in the absence of microorganisms (Terebiznik et al., 2000).

6.5.2.2 Fat

PEF treatment of milk does not alter the average size of the fat globules in milk (Barsotti et al., 2001; García-Amezquita et al., 2009), but a tendency of small globules to clump together to form larger fat particles has been observed (García-Amezquita et al., 2009). In contrast, it is also able to dissociate aggregates of milk fat globules of 60–70 μm in 35% fat cream (Barsotti et al., 2001). However, the shear induced during circulation of the samples during PEF treatment may also have an effect (Hemar et al., 2011).

Unlike HPH, PEF does not appear to disrupt the milk fat globule membrane, as treatment (35.5 kV/cm for 1000 or 300 μs , temperature $<40^\circ\text{C}$) of raw milk did not induce lipolysis and increase the level of free fatty acids during refrigerated storage (Odrizola-Serrano et al., 2006). This lack of lipolysis may have been due to inactivation of the lipase as under similar PEF conditions, but for a much shorter treatment time (20 μs), Shamsi (2010) observed 40% inactivation of the enzyme.

Zulueta et al. (2007) investigated the effect of PEF on lipids in a milk-orange juice beverage fortified with ω -3 fatty acids and oleic acid. After the PEF treatments (35 and 40 kV/cm for times 40–180 μs) no significant changes in the fatty acids or peroxides, indicative of oxidation, were observed. Zulueta et al. (2007) concluded that PEF has potential as an alternative preservation technology for such functional foods.

6.5.2.3 Proteins

In contrast to HPP, HP homogenization and ultrasonication, PEF has little effect on the proteins in milk. The size of casein micelles has been reported to be decreased slightly (45–55 kV/cm at $<50^\circ\text{C}$; Floury et al., 2006) or to be not affected (38 kV/cm at 30°C , 35 kV/cm at 60°C ; Shamsi, 2010). de Luis et al. (2009) reported no change in β -lactoglobulin, α -lactalbumin, lactoperoxidase, IgG, and lactoferrin after treatments of skim or whey (diluted 1/3 to adjust conductivity to 2 mS/cm at 20°C) with up to 200 pulses at 37.6 kV/cm, corresponding to a specific energy input of 920 kJ/kg. The temperatures reached in the treatments were $<35^\circ\text{C}$. Similarly, Barsotti et al. (2002) found no significant unfolding or aggregation of β -lactoglobulin at 30 kV/cm for 260 μs , and Li et al. (2003, 2005) found no significant change in secondary structure or immunoactivity of IgG after PEF treatment at 41.1 kV/cm for 54 μs , suggesting potential use of PEF for treatment of foods containing bioactive proteins. By contrast, Odrizola-Serrano et al. (2006) reported denaturation levels of serum albumin, β -lactoglobulin and α -lactalbumin of 24.5, 20.1, and 40%, respectively, using 35 kV/cm for 1000 μs . Floury et al. (2006) suggested PEF treatment of milk at 45–55 kV/cm with energy inputs up to 100 kJ/kg had an effect on the proteins of milk, since viscosity decreased and coagulation properties were enhanced. The different results from

different researchers may relate to the different PEF conditions used, including different wave forms (Odriozola-Serrano et al. [2006] used bipolar pulses, while Barsotti et al. [2001] used exponential decay pulses) and/or different analytical methods, for example, electrophoresis (Odriozola-Serrano et al., 2006), and UV spectroscopy (Barsotti et al., 2002) and radioimmunoassay (de Luis et al., 2009), for determining change in proteins. Mechanical shear conditions in continuous PEF systems can also play a role in changing physical and other properties of milk constituents (Hemar et al., 2011).

PEF (25 kV/cm, 19.2 μ s) had little if any effect on lactoferrin (Wan et al., 2008). However, interesting effects on the iron-binding capacity of lactoferrin were reported by Lu et al. (2008). In general, the iron-binding capacity decreased with increasing electric field strength, treatment time, and pulse width, but curiously increased by up to 3.8- and 1.2-fold when the number of pulses was increased to 256 or the treatment temperature was increased to 55°C, respectively. Sui et al. (2010) found that iron binding of lactoferrin decreased with increasing concentrations of the treatment medium (simulated milk ultrafiltrate, SMUF) after PEF treatment at 35 kV/cm for 19 μ s. At one-fifth strength SMUF, no iron was released by PEF; however, at double strength SMUF, only half the iron remained bound. Iron-depleted lactoferrin (apo-LF) can be more effective against microorganisms than natural or iron-saturated lactoferrin (holo-LF). Sui et al. (2010) propose that PEF could be a suitable physical method for preparing apo-LF. These results suggest that PEF can be beneficial or detrimental to the functional properties of bioactive proteins, depending on the treatment parameters.

Sui et al. (2011) treated WPIs in SMUF at 30–35 kV/cm for 19–211 μ s at temperatures between 30 and 75°C. These were compared with controls treated at the same time temperature profiles and pumping shear as the PEF treatments. Protein aggregation, surface hydrophobicity, sulfhydryl groups, and thermal stability were not affected by the PEF component of the treatments; however, heat-induced gel strengths decreased and gelation times increased as a result of PEF.

6.5.2.4 Enzymes

There have been reports of inactivation, no effect and even activation of enzymes by PEF. As a general rule, inactivation of enzymes requires more severe PEF conditions than those required for inactivation of (vegetative) bacteria (Ho et al., 1997).

The effect on milk alkaline phosphatase is of interest, because of its use as an indicator of the effectiveness of thermal pasteurization. However, it is not suitable as an indicator of effective “pasteurization” by PEF even though the more severe the PEF treatment conditions, the more alkaline phosphatase is inactivated. There have been reports of only trace inactivation (Grahl and Markl, 1996) through to 42% inactivation (Shamsi et al., 2008) of this enzyme. A higher inactivation rate, 74%, was reported by van Loey et al. (2001), but this included some thermal inactivation. Of particular significance is the report by Shamsi et al. (2008) that the inactivation of alkaline phosphatase corresponding to effective “pasteurization,” 5.9 log reduction of *Pseudomonas*, was 67%; the corresponding figure for HTST pasteurization measured in the same study was 98%. No doubt, complete inactivation of alkaline phosphatase during PEF treatment would indicate effective pasteurization but the product would be unnecessarily overprocessed.

Proteases appear to be more sensitive to PEF than to heat; however, the extent of inactivation is dependent on the severity of the treatment, that is, higher electric field strengths, longer treatment times, and higher treatment temperatures enhance inactivation. *B. subtilis*

protease activity was reduced by more than 80% after PEF treatment at 35.5 kV/cm for 866 μ s (Bendicho et al., 2003) and *P. fluorescens* protease in tryptic soy broth was reduced by 80% at 18 kV/cm with 20 pulses (Vega-Mercado et al., 2001). However, the components of the treatment medium can affect the extent of inactivation, as no inactivation of *P. fluorescens* protease was observed when treated in a casein-Tris buffer. Milk plasmin was reduced by 90% at both 30 and 45 kV/cm with 50 pulses at a treatment temperature of 15°C (Vega-Mercado et al., 1995); however, Shamsi (2010) found a 42% inactivation of plasmin after PEF treatment at 29 kV/cm and at 55°C with a specific energy input of 163 kJ/L. The inactivation effects of PEF on *Pseudomonas* proteases and plasmin are of considerable practical significance because of their high heat stability and their role in causing serious defects, such as bitterness and age gelation in milk products, especially long shelf life products, such as UHT milk (Datta and Deeth, 2001).

Lactoperoxidase activity was not affected by PEF at 37.6 kV/cm of 920 kJ/kg (de Luis et al., 2009), at 19 kV/cm of 500 kJ/kg (van Loey et al., 2001), or at 21 kV/cm of 400 kJ/kg (Grahl and Markl, 1996). This is a significant finding, as lactoperoxidase isolated from milk is used as an ingredient, and hence liquid products containing it could be PEF treated without causing its inactivation.

Both milk lipase and xanthine oxidase are inactivated to some degree by PEF. Using 35 kV/cm at 35°C with an energy input of 163 kJ/L, Shamsi (2010) found inactivation rates for these enzymes of 33 and 23%, respectively. Higher levels of inactivation occurred when the treatment temperature was increased, although the contribution of thermal inactivation became increasingly significant. For example, at 55°C, the corresponding PEF inactivation rates were 56 and 42%, but the thermal inactivation levels were 22 and 18%, respectively.

When a *P. fluorescens* lipase in SMUF was treated with PEF at 27.4 kV/cm with 80 pulses in batch mode, a reduction of 62.1% was observed; however, in continuous mode at 37.4 kV/cm with 80 pulses, only 13% loss of activity occurred (Bendicho et al., 2002c).

Vitamins

No changes in the content of water-soluble vitamins (thiamine and riboflavin) and fat-soluble vitamins (cholecalciferol and tocopherol) were observed after PEF treatments (18.3–27.1 kV/cm for up to 400 seconds, at room or moderate temperature), but some loss in ascorbic acid occurred. However, the loss of ascorbic acid in milk after a 400- μ s treatment at 22.6 kV/cm was only 6.6%; the loss from a thermal pasteurization treatment (63°C for 30 minutes) was ~50%. The PEF-induced loss of ascorbic acid followed first-order rate kinetics. Skim milk components, mainly proteins, protected the ascorbic acid from the electric pulses, since more ascorbic acid was destroyed in SMUF than in skim milk (Bendicho et al., 2002b).

6.5.3 Effects on products and processes

6.5.3.1 Cheese

After PEF treatments of 38 kV/cm at 30°C and 35 kV/cm at 60°C, the rennet coagulation time (RCT) increased slightly compared with the RCT for a raw milk control, but was similar to the RCT for HTST-pasteurized milk (Shamsi, 2010). However, Yu et al. (2009) found that in most cases, PEF-treated (30 kV/cm, 120 pulses, temperature up to 50°C) milk

showed better rennetability compared with thermally pasteurized milk. This led these authors to suggest that PEF treatment combined with mild heat may be a suitable “pasteurization” method for milk used in cheese production. One attraction of such nonthermal “pasteurization” is the potential to produce milk that is technically suitable for the manufacture of microbiologically safe raw-like product.

Milk treated with PEF at 30, 40, and 50 kV/cm at 60°C formed firmer rennet gels than thermally pasteurized milks with the gel firmness decreasing slightly as the electric field intensity increased from 30 to 50 kV/cm (Shamsi, 2010). Sepulveda-Ahumada et al. (2000) also reported increased hardness (and springiness) in cheese made from PEF-treated milk and concluded that the use of milk “pasteurized” by PEF may be a feasible option to improve the quality of cheese.

6.5.3.2 Yogurt

Little research has been performed on the effect of PEF on yogurt or yogurt production. However, Yeom et al. (2004) reported a marked improvement in the microbial stability of a yogurt-based product (low fat yogurt mixed with fruit jelly and corn syrup) by PEF treatment (30 kV/cm, 32 μ s, 30°C) following a mild heat treatment (60°C for 30 seconds). During storage at 4°C, the total aerobic bacterial, and yeast and mold counts of the treated yogurt product did not exceed 10 cfu/mL over 60 days’ storage. By contrast, these microbial counts in a control product subjected to only the heat treatment (60°C for 30 seconds) reached 10⁶/mL and 10⁴/mL, respectively, after 28 days. The combined PEF—heat treatment had no significant effect on color, pH, Brix, or sensory attributes.

6.5.4 Commercial developments

There has been general interest in PEF technology by the dairy industry over many years, as may be deduced from the support researchers have received in several countries over the last decade or more. Braakman (2003) reported that commercial PEF installations would be available from 2004, with several dairy products (yogurt, milk products, and puddings) given as likely applications. However to our knowledge pulsed electric field had not been commercialized by the dairy industry by the end of 2009. One reason may be that early expectations of researchers for spore inactivation by PEF alone have not been realized. Other reasons may be the very large scale of most dairy operations, which makes the required scale up difficult, and the well-established and -regulated thermal pasteurization protocols in most countries. Nevertheless, there appear to be clear opportunities for PEF in pasteurizing heat-sensitive proteins to provide enhanced safety and extended shelf-life while maintaining functionality (Wan et al., 2008; Versteeg and Sanguansri, 2009). Also, the work by Sepulveda et al. (2005), which showed that sequential combinations of PEF and normal pasteurization could extend the shelf life of pasteurized milk by 2–4 weeks has commercial potential, for example, for long distance exports of fresh milk, with few if any regulatory hurdles, as the milk will be heat pasteurized at least once. In a more recent publication, Sepulveda et al. (2009) reported a synergistic interaction between PEF and a mild heat treatment (65°C for 10 seconds), which produced a “remarkable” increase in shelf life.

A food industry application that has been commercialized, and also could be valuable for the dairy industry, is wastewater sludge treatment. PEF is reported to transform biosolids to an effective carbon source for denitrification and thereby improve the efficiency and

effectiveness of biomass to energy conversion. Benefits claimed include the reduction of biosolids disposal by up to 50%, increase in biogas production by up to 75%, as well as increased digester capacity and improved sludge safety (OpenCEL, 2009).

6.6 FURTHER READING

Nonthermal technologies have been the subject of several reviews, book chapters and books in recent years. These include Barbosa-Cánovas et al. (1998, 2000a, 2005), Alvarez and Ji (2003), Senorans et al. (2003), Clark (2005), Manas and Pagan (2005), Sun (2005), Wan et al. (2005), Leadley and Williams 2006, Tewari and Juneja (2007), Patel et al. (2008), Wan et al. (2009), Barbosa-Cánovas and Bermúdez-Aguirre (2010) and Gésan-Guiziu (2010).

REFERENCES

- Acton, E. and Morris, G.J. (1992) Method and apparatus for the control of solidification in liquids. Patent WO9220420-A1.
- Ahmed, F.I.K. and Russell, C. (1975) Synergism between ultrasonic waves and hydrogen peroxide in the killing of microorganisms. *Journal of Applied Bacteriology* **39**, 31–40.
- Akbari Mousavi, S.A.A., Feizi, H., and Madoliat, R. (2007) Investigations on the effects of ultrasonic vibrations in the extrusion process. *Journal of Materials Processing Technology* **187–188**, 657–661.
- Alpas, H. and Bozoglu, F. (2000) The combined effect of high hydrostatic pressure, heat and bacteriocins on inactivation of foodborne pathogens in milk and orange juice. *World Journal of Microbiology and Biotechnology* **16**, 387–392.
- Alpas, H., Kalchayanand, N., Bozoglu, F., Sikes, A., Dunne, C.P., and Ray, B. (1999) Variation in resistance to hydrostatic pressure among strains of food-borne pathogens. *Applied and Environmental Microbiology* **65**(9), 4248–4251.
- Altuner, E.M., Alpas, H., Erdem, Y.K., and Bozoglu, F. (2006) Effect of high hydrostatic pressure on physicochemical and biochemical properties of milk. *European Food Research and Technology* **222**, 392–396.
- Alvarez, I., Pagan, R., Condon, S., and Raso, J. (2003) The influence of process parameters for the inactivation of *Listeria monocytogenes* by pulsed electric fields. *International Journal of Food Microbiology* **87**, 87–95.
- Alvarez, V.B. and Ji, T.Y. (2003) Emerging processing and preservation technologies for milk and dairy products. In: *Food Science and Food Biotechnology*, edited by G.F. Gutierrez-Lopez and G.V. Barbosa-Canovas, pp. 313–327. Boca Raton, FL: CRC Press.
- Anon (2008) Fonterra, New Image to tackle Asian colostrum markets. *The National Business Review* 24/7, June 14.
- Arques, J.L., Rodriguez, E., Gaya, P., Medina, M., and Nunez, M. (2005) Effect of combinations of high-pressure treatment and bacteriocin-producing lactic acid bacteria on the survival of *Listeria monocytogenes* in raw milk cheese. *International Dairy Journal* **15**, 893–900.
- Asano, Y., Ihara, K., Mori, T., Mizota, T., Iwatsuki, K., and Sotoyama, K. (2000) Flash sterilization system using a high-pressure homogenizer. *Journal of the Japanese Society for Food Science and Technology (Nippon Shokuhin Kagaku Kogaku Kaishi)* **47**, 130–135.
- Ashokkumar, M., Sunartio, D., Kentish, S., Mawson, R., Simons, L., Vilku, K., and Versteeg, C. (2008) Modification of food ingredients by ultrasound to improve functionality: a preliminary study on a model system. *Innovative Food Science and Emerging Technologies* **9**, 155–160.
- Ashokkumar, M., Kentish, S., Lee, J.Y.-T., Zisu, B., Palmer, M., and Augustin, M.A. (2009a) Processing of dairy ingredients by ultrasonication. Patent Application No. WO 2009/079691-A1.
- Ashokkumar, M., Lee, J., Zisu, B., Bhaskaracharya, R., Palmer, M., and Kentish, S. (2009b) Sonication increases the heat stability of whey proteins. *Journal of Dairy Science* **92**, 5353–5356.

- Ashokkumar, M., Bhaskaracharya, R., Kentish, S., Lee, J., Palmer, M., and Zisu, B. (2010) The ultrasonic processing of dairy products - an overview. *Dairy Science and Technology* **90**, 147–168.
- Avvaru, B., Patil, M.N., Gogate, P.R., and Pandit, A.B. (2006) Ultrasonic atomization: effect of liquid phase properties. *Ultrasonics* **44**, 146–158.
- Balci, A.T., Ledward, D.A., and Wilbey, R.A. (2002) Effect of high pressure on acid phosphatase in milk. *High Pressure Research* **22**, 639–642.
- Baldi, A., Ioannis, P., Chiara, P., Eleonora, F., Roubini, C., and Vittorio, D. (2005) Biological effects of milk proteins and their peptides with emphasis on those related to the gastrointestinal ecosystem. *Journal of Dairy Research* **72**, 66–72.
- Banerjee, R., Chen, H., and Wu, J. (1996) Milk protein-based edible film mechanical strength changes due to ultrasound process. *Journal of Food Science* **61**, 824–828.
- Barbosa-Cánovas, G. and Bermúdez-Aguirre, D. (2010) Other novel milk preservation technologies: ultrasound, irradiation, microwave, radio frequency, ohmic heating, ultraviolet light and bacteriocins. In: *Improving the Safety and Quality of Milk*, edited by M. Griffiths, pp. 420–450. Cambridge: Woodhead Publishing Ltd.
- Barbosa-Cánovas, G.V. and Juliano, P. (2008) Food sterilization by combining high pressure and thermal energy. In: *Food Engineering Integrated Approaches*, edited by G.F. Gutierrez-Lopez, G.V. Barbosa-Cánovas, J. Welti-Chanes, and E. Parada-Arias, pp. 9–46. New York: Springer.
- Barbosa-Cánovas, G.V. and Rodriguez, J.J. (2005) Thermodynamic aspects of high hydrostatic pressure food processing. In: *Novel Food Processing Techniques*, edited by G.V. Barbosa-Cánovas, M.S. Tapia, and M.P. Cano, pp. 183–205. Boca Raton, FL: CRC Press.
- Barbosa-Cánovas, G.V. and Zhang, Q.H., eds. (2001) *Pulsed Electric Fields in Food Processing. Fundamental Aspects and Applications*. Lancaster, CA: Technomic Publishing.
- Barbosa-Cánovas, G.V., Pothakamury, U.R., Palau, E., and Swanson, B.G. (1998) *Non-Thermal Preservation of Foods*. New York: Marcel Dekker Inc.
- Barbosa-Cánovas, G.V., Góngora-Nieto, M.M., Pothakamury, U.R., and Swanson, B.S. (1999) *Preservation of Foods with Pulsed Electric Fields*. San Diego, CA: Academic Press.
- Barbosa-Cánovas, G.V., Schaffner, D.W., Pierson, M.D., and Zhang, Q.H. (2000a) Oscillating magnetic fields. *Journal of Food Science (Special Supplement)* **65**(4), 86–89.
- Barbosa-Cánovas, G.V., Zhang, Q.H., Pierson, M.D., and Schaffner, D.W. (2000b) High voltage arc discharge. *Journal of Food Science (Special Supplement)* **65**(4), 80–81.
- Barbosa-Cánovas, G.V., Tapia, M.S., Cano, M.P., Martín-Belloso, O., and Martínez, A. (2005) *Novel Food Processing Technologies*. Boca Raton, FL: CRC Press.
- Barros, M. (2008) Industrial implementation of nonthermal technologies: a high pressure case study. In: *Proceedings of IFT Nonthermal Processing Workshop in Madrid, Spain*, November 19, 2008.
- Barsotti, L., Dumay, E., Mu, T.H., Diaz, M.D.F., and Cheftel, J.C. (2001) Effects of high voltage electric pulses on protein-based food constituents and structures. *Trends in Food Science and Technology* **12**, 136–144.
- Barsotti, L., Dumay, E., Mu, T.H., Diaz, M.D.F., and Cheftel, J.C. (2002) Effects of high voltage electric pulses on protein-based food constituents and structures. *Trends in Food Science and Technology* **12**, 136–144.
- Bates, D.M. and Bagnall, W. (2008) Viscosity reduction. Patent WO 2008903844.
- Bendicho, S., Barbosa-Cánovas, G.V., and Martin, O. (2002a) Milk processing by high intensity pulsed electric fields. *Trends in Food Science and Technology* **13**, 195–204.
- Bendicho, S., Espachs, A., Arantegui, J., and Martin, O. (2002b) Effect of high intensity pulsed electric fields and heat treatments on vitamins of milk. *Journal of Dairy Research* **69**, 113–123.
- Bendicho, S., Estela, C., Giner, J., Barbosa-Cánovas, G.V., and Martin, O. (2002c) Effects of high-intensity pulsed electric field and thermal treatments on a lipase from *Pseudomonas fluorescens*. *Journal of Dairy Science* **85**, 19–27.
- Bendicho, S., Barbosa-Cánovas, G.V., and Martin, O. (2003) Reduction of protease activity in milk by continuous flow high-intensity pulsed electric field treatments. *Journal of Dairy Science* **86**, 697–703.
- Bishop, R. (2002) Alternative methods to heat treatment. *Bulletin of the International Dairy Federation* **374**, 8–11.
- Black, E.P., Kelly, A.L., and Fitzgerald, G.F. (2005) The combined effect of high pressure and nisin on inactivation of microorganisms in milk. *Innovative Food Science and Emerging Technologies* **6**, 286–292.

- Black, E.P., Setlow, P., Hocking, A.D., Stewart, C.M., Kelly, A.L., and Hoover, D.G. (2007) Response of spores to high-pressure processing. *Comprehensive Reviews in Food Science and Food Safety* **6**, 103–119.
- Bouaouina, H., Desrumaux, A., Loisel, C., and Legrand, J. (2006) Functional properties of whey proteins as affected by dynamic high-pressure treatment. *International Dairy Journal* **16**, 275–284.
- Bozoglu, F., Alpas, H., and Kaletunc, G. (2004) Injury recovery of foodborne pathogens in high hydrostatic pressure treated milk during storage. *FEMS Immunology and Medical Microbiology* **40**, 243–247.
- Braakman, L. (2003) Breakthrough in pasteurisation: pulsed electric fields. *Food Engineering and Ingredients* **June**, 34–38.
- Bricher, J.L. (2009a) NCFST receives regulatory acceptance of novel food sterilization process. Press release by National Centre for Food Safety and Technology, February 27, 2009.
- Bricher, J.L. (2009b) NCFST PATS process wins 2009 IFT Food Expo Innovation Award. Press release by National Centre for Food Safety and Technology, June 7, 2009).
- Brinez, W.J., Roig-Sagues, A.X., Hernandez-Herrero, M.M., and Guamis-Lopez, B. (2006) Inactivation of two strains of *Escherichia coli* inoculated into whole and skim milk by ultrahigh-pressure homogenisation. *Le Lait* **86**, 241–249.
- Buchheim, W. and El-Nour, A.M.A. (1992) Induction of milkfat crystallisation in the emulsified state by high hydrostatic pressure. *Fat Science Technology* **94**, 369–373.
- Buchheim, W., Schütt, M., and Frede, E. (1996) High pressure effects on emulsified fats. In: *High Pressure Bioscience and Biotechnology*, edited by R. Hayashi and C. Balny, pp. 331–336. Amsterdam: Elsevier Science.
- Bund, R.K. and Pandit, A.B. (2007) Sonocrystallization: effect on lactose recovery and crystal habit. *Ultrasonics Sonochemistry* **14**, 143–152.
- Burbrink, C.N., Mauer, L.J., and Hayes, K.D. (2004) Inactivation of plasminogen activators using sonication. Paper 17A-16 presented at 2004 IFT Annual Meeting, Las Vegas. http://www.ift.confex.com/ift/2004/techprogram/paper_23018.htm.
- Bury, D., Jelen, P., and Kalab, M. (2001) Disruption of *Lactobacillus delbrueckii* ssp. *bulgaricus* 11842 cells for lactose hydrolysis in dairy products: a comparison of sonication, high-pressure homogenization and bead milling. *Innovative Food Science and Emerging Technologies* **2**, 23–29.
- Butz, P., Funtenberger, S., Haberditzl, T., and Tauscher, B. (1996) High pressure inactivation of *Byssoschlamys* ascospores and other heat-resistant molds. *Lebensmittel Wissenschaft und Technologie* **29**, 404–410.
- Capellas, M., Mor-Mur, M., Sendra, E., Pla, R., and Guamis, B. (1996) Populations of aerobic mesophiles and inoculated *E. coli* during storage of fresh goat's milk cheese treated with high pressure. *Journal of Food Protection* **59**, 582–587.
- Cardello, A.V., Schutz, H.G., and Leshner, L.L. (2007) Consumer perceptions of foods processed by innovative and emerging technologies: a conjoint analytic study. *Innovative Food Science and Emerging Technologies* **8**, 73–83.
- Čarić, M. and Milanović, S. (2003) Physical and functional properties of milk powders. In: *Encyclopedia of Dairy Sciences*, edited by H. Roginski, J.W. Fuquay, and P.F. Fox, pp. 1874–1880. London: Academic Press.
- Carroll, T.J., Chen, P., Harnett, M., and Harnett, J. (2004) Pressure treating foods to reduce spoilage. International Patent WO 2004/032655 A1.
- Carroll, T.J., Patel, H., Gonzalez-Martin, M.A., Dekker, J.W., Collett, M.A., and Lubbers, M.W. (2008) High pressure processing of bioactive compositions. International Patent. U.S. 2008/0317823A1.
- Carroll, T. (2008) High pressure processing of colostrum. *Journal of Dairy Science* **91**, 554–555.
- Chouliara, E., Georgogianni, K.G., Kanellopoulou, N., and Kontominas, M.G. (2010) Effect of ultrasonication on microbiological, chemical and sensory properties of raw, thermized and pasteurized milk. *International Dairy Journal* **20**, 307–313.
- Clark, J.P. (2005) Evaluating non-thermal processes. *Food Technology* **59**(12), 79–81.
- Clark, J.P. (2007) High pressure effects on foods. *Food Technology* **61**(5), 69–71.
- Cole, R. (1997) High pressure processing: a technology for the future. *Food Manufacture* **72**, 21–22.
- Colplus (2010) Colostrum based functional beverage processed through high pressure pasteurisation launched by New Image. <http://www.colplus.com>. Accessed January 5, 2010.
- Considine, T., Patel, H.A., Anema, S.G., Singh, H., and Creamer, L.K. (2007) Interactions of milk proteins during heat and high hydrostatic pressure treatments—a review. *Innovative Food Science and Emerging Technologies* **8**, 1–23.

- Craven, H.M., Swiergon, P., Ng, S., Midgely, J., Versteeg, C., Coventry, M.J., and Wan, J. (2008) Evaluation of pulsed electric field and minimal heat treatments for inactivation of pseudomonads and enhancement of milk shelf-life. *Innovative Food Science and Emerging Technologies* **9**, 211–216.
- D'Amico, D.J., Silk, T.M., Wu, J.R., and Guo, M.R. (2006) Inactivation of microorganisms in milk and apple cider treated with ultrasound. *Journal of Food Protection* **69**, 556–563.
- Daryaei, H., Coventry, M.J., Versteeg, C., and Sherkat, F. (2008) Effect of high pressure treatment on starter bacteria and spoilage yeasts in fresh lactic curd cheese of bovine milk. *Innovative Food Science and Emerging Technologies* **9**, 201–205.
- Datta, N. and Deeth, H.C. (1999) High pressure processing of milk and milk products. *Australian Journal of Dairy Technology* **54**, 41–48.
- Datta, N. and Deeth, H.C. (2001) Age gelation of UHT milk—a review. *Transactions of the Institute of Chemical Engineers C. Food and Bioproducts Processing* **79**, 197–210.
- Datta, N., Elliott, A.J., Perkins, M.L., and Deeth, H.C. (2002) Ultra-high temperature (UHT) treatment of milk: comparison of direct and indirect modes of heating. *Australian Journal of Dairy Technology* **57**, 211–227.
- Datta, N., Hayes, M.G., Deeth, H.C., and Kelly, A.L. (2005) Significance of frictional heating for effects of high pressure homogenisation on milk. *Journal of Dairy Research* **72**, 393–399.
- de Jong, P. and van Heesch, E.J.M. (1998) Review: effect of pulsed electric fields on the quality of food products. *Milchwissenschaft* **53**, 1–8.
- de Luis, R., Arias, O., Puertolas, E., Benede, S., Sanchez, L., Calvo, M., and Perez, M.D. (2009) Effect of high-intensity pulse electric fields on denaturation of bovine whey proteins. *Milchwissenschaft* **64**, 422–426.
- Deeth, H.C. (2005) Lipoprotein lipase and lipolysis in milk. *International Dairy Journal* **16**, 555–562.
- Deeth, H.C., Datta, N., Ross, A.I.V., and Dam, X.T. (2007) Pulsed electric field technology: effect on milk and fruit juices. In: *Advances in Thermal and Non-Thermal Food Preservation*, edited by G. Tewari and V.K. Juneja, pp. 241–267. Ames, IA: Blackwell Publishing.
- DeSilva, K., Stockmann, R., and Smithers, G.W. (2003) Isolation procedures for functional dairy components—novel approaches to meeting the challenges. *Australian Journal of Dairy Technology* **58**, 148–152.
- Desobry-Banon, S., Richard, F., and Hardy, J. (1994) Study of acid and rennet coagulation of high pressurised milk. *Journal of Dairy Science* **77**, 3267–3274.
- Desrumaux, A. and Marcand, J. (2002) Formation of sunflower oil emulsions stabilized by whey proteins with high-pressure homogenization (up to 350 MPa): effect of pressure on emulsion characteristics. *International Journal of Food Science and Technology* **37**, 263–269.
- Dhumal, R.S., Biradar, S.V., Paradkar, A.R., and York, P. (2008) Ultrasound assisted engineering of lactose crystals. *Pharmaceutical Research* **25**, 2835–2844.
- Diels, A.M.J. and Michiels, C.W. (2006) High-pressure homogenization as a non-thermal technique for the inactivation of microorganisms. *Critical Reviews in Microbiology* **32**, 201–216.
- Diels, A.M.J., Wuytack, E.Y., and Michiels, C.W. (2003) Modelling inactivation of *Staphylococcus aureus* and *Yersinia enterocolitica* by high-pressure homogenisation at different temperatures. *International Journal of Food Microbiology* **87**, 55–62.
- Dissanayake, M. and Vasilejvic, T. (2009) Functional properties of whey proteins affected by heat treatment and hydrodynamic high-pressure shearing. *Journal of Dairy Science* **92**, 1387–1397.
- Dufour, E., Hervé, G., and Haertlé, T. (1995) Hydrolysis of β -lactoglobulin by thermolysin and pepsin under high hydrostatic pressure. *Journal of Dairy Research* **63**, 119–130.
- Dumay, E., Lambert, C., Funtenberger, S., and Cheftel, J.C. (1996) Effects of high pressure on the physico-chemical characteristics of dairy creams and model oil/water emulsions. *Lebensmittel Wissenschaft und Technologie* **29**, 606–625.
- Ertugay, M.F., Yuksel, Y., and Sengul, M. (2003) The effect of ultrasound on lactoperoxidase and alkaline phosphatase enzymes from milk. *Milchwissenschaft* **58**, 593–595.
- Ertugay, M.F., Sengul, M., and Sengul, M. (2004) Effect of ultrasound treatment on milk homogenization and particle size distribution of fat. *Turkish Journal of Veterinary and Animal Science* **28**, 303–308.
- Evrendilek, G.A., Dantzer, W.R., Streaker, C.B., Ratanatriwong, P., and Zhang, Q.H. (2001) Shelf-life evaluations of liquid foods treated by pilot plant pulsed electric field system. *Journal of Food Processing and Preservation* **25**, 283–297.
- Famelart, M.H., Chapron, L., Piot, M., Brulée, G., and Durier, C. (1998) High pressure-induced gel formation of milk and whey concentrates. *Journal of Food Engineering* **36**, 149–164.

- Feijoo, S.C., Hayes, W.W., Watson, C.E., and Martin, J.H. (1997) Effects of Microfluidizer(R) technology on *Bacillus licheniformis* spores in ice cream mix. *Journal of Dairy Science* **80**, 2184–2187.
- Felipe, X., Capellas, M., and Law, A.J.R. (1997) Comparison of the effect of high-pressure treatments and heat pasteurization on the whey proteins in goat's milk. *Journal of Agricultural and Food Chemistry* **45**, 627–631.
- Ferragut, V., Martinez, V., Trujillo, A., and Guamis, B. (2000) Properties of yogurt made from whole ewe's milk treated by high hydrostatic pressure. *Milchwissenschaft* **55**, 267–269.
- Fertsch, B., Müller, M., and Hinrichs, J. (2003) Firmness of pressure induced casein and whey protein gels modulated by holding time and rate of pressure release. *Innovative Food Science and Emerging Technologies* **4**, 143–150.
- Fletcher, K., Chen, P., and Carroll, T. (2004) Enhancing clarity and/or stability properties of protein-containing liquids and gels. PCT WO 2004/091309.
- Floury, J., Desrumaux, A., and Lardières, J. (2000) Effect of high pressure homogenization on droplet size distribution and rheological properties of model oil-in-water emulsions. *Innovative Food Science and Emerging Technologies* **1**, 127–134.
- Floury, J., Grosset, N., Leconte, N., Pasco, M., Madec, M.N., and Jeantet, R. (2006) Continuous raw skim milk processing by pulsed electric field at non-lethal temperature: effect on microbial inactivation and functional properties. *Le Lait* **86**, 43–57.
- Fryer, P.J. and Versteeg, C. (2008) Processing technology innovation in the food industry. *Innovation: Management, Policy and Practice* **10**, 74–90.
- Fuente-Blanco, S., Riera-Franco De Sarabia, E., Acosta-Aparicio, V.M., Blanco-Blanco, A., and Gallego-Juárez, J.A. (2006) Food drying process by power ultrasound. *Ultrasonics* **44**, e523–e527.
- Gaffney, B. (1996) Sonic converting sounds good to cheesemakers. *Food Engineering May*, 21–24.
- Gaffney, B.J. (1987) Sonic process for converting proteinaceous raw materials in situ into semi-solid food products. Patent U.S. 4675194.
- Gaffney, B.J. (1997) Flow process for converting proteinaceous mix to enzymatic curd for cheese product in which mixing takes place in acoustic resonating chamber for very rapid curd formation. Patent U.S. 5629037-A.
- García-Amezquita, L.E., Primo-Mora, A.R., Barbosa-Cánovas, G.V., and Sepulveda, D.R. (2009) Effect of non-thermal technologies on the native size distribution of fat globules in bovine cheese-making milk. *Innovative Food Science and Emerging Technologies* **10**, 491–494.
- García-Graells, C., Masschalck, B., and Michiels, C.W. (1999) Inactivation of *Escherichia coli* in milk by high-hydrostatic-pressure treatment in combination with antimicrobial peptides. *Journal of Food Protection* **62**, 1248–1254.
- García-Risco, M.R., Cortés, E., Carrascosa, A.V., and López-Fandiño, R. (1998) Microbiological and chemical changes in high-pressure-treated milk during refrigerated storage. *Journal of Food Protection* **61**, 735–737.
- Gaucher, F., Famelart, M.H., Mariette, F., Raulot, K., Michel, F., and Le Graet, Y. (1997) Combined effects of temperature and high-pressure treatments on the physicochemical characteristics of skim milk. *Food Chemistry* **59**, 439–447.
- Geciova, J., Bury, D., and Jelen, P. (2002) Methods for disruption of microbial cells for potential use in the dairy industry—a review. *International Dairy Journal* **12**, 541–553.
- Gervilla, R., Ferragut, V., and Guamis, B. (2001) High hydrostatic pressure effects on colour and milk-fat globule of ewe's milk. *Journal of Food Science* **66**, 880–885.
- Gésan-Guizieu, G. (2010) Removal of bacteria, spores and somatic cells from milk by centrifugation and microfiltration techniques. In: *Improving the Safety and Quality of Milk*, edited by M. Griffiths. Cambridge: Woodhead Publishing Ltd.
- Gómez-López, V.M., Ragaert, P., Debevere, J., and Devlieghere, F. (2007) Pulsed light for food decontamination: a review. *Trends in Food Science and Technology* **18**, 464–473.
- Grahl, T. and Markl, H. (1996) Killing of microorganisms by pulsed electric fields. *Applied Microbiology and Biotechnology* **45**, 148–157.
- Guamis, B., Pla, R., Trujillo, A.J., Capellas, M., Gervilla, R., Daldó, J., and Yuste, J. (2005) High-pressure processing of milk and dairy and egg products. In: *Novel Food Processing Technologies*, edited by G.V. Barbosa-Cánovas, M.S. Taipia, and M.P. Cano, pp. 343–359. Boca Raton, FL: CRC Press Inc.
- Guerrero-Beltran, J.A. and Barbosa-Cánovas, G.V. (2004) Review: advantages and limitations on processing foods by UV light. *Food Science and Technology International* **10**, 137–147.
- Halliday, J. (2007) Fonterra innovation takes colostrum into functional beverages. *Food Navigator*, March 29.

- Hardham, J.F., Imison, B.W., and French, H.M. (2000) Effect of homogenisation and microfluidisation on the extent of fat separation during storage of UHT milk. *Australian Journal of Dairy Technology* **55**, 16–21.
- Hayashi, R., Kawamura, Y., and Kunugi, S. (1987) Introduction to high pressure to food processing: preferential proteolysis of β -lactoglobulin in milk whey. *Journal of Food Science* **52**, 1107–1108.
- Hayes, M.G., and Kelly, A.L. (2003a) High pressure homogenisation of raw whole bovine milk (a) effects on fat globule size and other properties. *Journal of Dairy Research* **70**, 297–305.
- Hayes, M.G., and Kelly, A.L. (2003b) High pressure homogenisation of milk (b) effects on indigenous enzymatic activity. *Journal of Dairy Research* **70**, 307–313.
- Hayes, M.G., Lefrancois, A.C., Waldron, D.S., Goff, H.D., and Kelly, A.L. (2003) Influence of high pressure homogenisation on some characteristics of ice cream. *Milchwissenschaft* **58**, 519–523.
- Hayes, M.G., Fox, P.F., and Kelly, A.L. (2005) Potential applications of high pressure homogenisation in processing of liquid milk. *Journal of Dairy Research* **72**, 25–33.
- Heinz, V. and Knorr, D. (2005) High-pressure-assisted heating as a method for sterilizing foods. In: *Novel Food Processing Techniques*, edited by G.V. Barbosa-Cánovas, M.S. Tapia, and M.P. Cano, pp. 207–232. Boca Raton, FL: CRC Press.
- Hemar, Y., Augustin, M.A., Cheng, L.J., Sanguansri, P., Swiergon, P., and Wan, J. (2011) The effect of pulsed electric field processing on the particle size and viscosity of milk and milk concentrates. *Milchwissenschaft* **66**, 126–128.
- Hembry, O. (2008) Fonterra health drink thrives under pressure. *NZ Herald*, July 14.
- Hillbrick, G.C., McMahon, D.J., and Deeth, H.C. (1998) Electrical impedance particle size method (Coulter counter) detects the large fat globules in poorly homogenised UHT processed milk. *Australian Journal of Dairy Technology* **53**, 17–21.
- Hinrichs, J. and Kessler, H.G. (1997) Kinetics of pressure-induced denaturation of whey proteins at different temperatures and functional properties. In: *High Pressure Research in the Biosciences and Biotechnology*, edited by K. Heremans, pp. 407–410. Leuven, Belgium: Leuven University Press.
- Hinrichs, J., Rademacher, B., and Kessler, H.G. (1996) Food processing of milk products with ultra high pressure. In *Heat Treatments and Alternative Methods*, IDF Doc 9602, 185–201.
- Hjelmqvist, J. (2005) Commercial high pressure equipment. In: *Novel Food Processing Techniques*, edited by G.V. Barbosa-Cánovas, M.S. Tapia, and M.P. Cano, pp. 361–373. Boca Raton, FL: CRC Press.
- Ho, S.Y., Mittal, G.S., Cross, J.D., and Griffiths, M.W. (1995) Inactivation of *Pseudomonas fluorescens* by high voltage electric fields. *Journal of Food Science* **60**, 1337–1340, 1343.
- Ho, S.Y., Mittal, G.S., and Cross, J.D. (1997) Effects of high field electric pulses on the activity of selected enzymes. *Journal of Food Engineering* **31**, 69–84.
- Hoffmann, W., Kiesner, C., Clawin-Radecker, I., Martin, D., Einhoff, K., Lorenzen, P.C., Meisel, H., Hammer, P., Suhren, G., and Teufel, P. (2006) Processing of extended shelf life milk using microfiltration. *International Journal of Dairy Technology* **59**, 229–235.
- Hogan, E., Kelly, A.L., and Sun, D.-W. (2005) High pressure processing of foods. An overview. In: *Emerging Technologies for Food Processing*, edited by D.-W. Sun, pp. 1–32. Amsterdam: Elsevier Academic Press.
- Hotchkiss, J.H., Werner, B.G., and Lee, E.Y.C. (2006) Addition of carbon dioxide to dairy products to improve quality: a comprehensive review. *Comprehensive Reviews in Food Science and Food Safety* **5**, 158–168.
- Huang, K. and Wang, J.P. (2009) Designs of pulsed electric fields treatment chambers for liquid foods pasteurization process: a review. *Journal of Food Engineering* **95**, 227–239.
- Huppertz, T., Kelly, A.L., and Fox, P.F. (2002) Effects of high pressure on constituents and properties of milk. *International Dairy Journal* **12**, 561–572.
- Huppertz, T., Fox, P.F., and Kelly, A.L. (2003) High pressure-induced changes in the creaming properties of bovine milk. *Innovative Food Science and Emerging Technologies* **4**, 349–359.
- Huppertz, T., Fox, P.F., and Kelly, A.L. (2004a) Effects of high pressure treatment on the yield of cheese curd from bovine milk. *Innovative Food Science and Emerging Technologies* **5**, 1–8.
- Huppertz, T., Fox, P.F., and Kelly, A.L. (2004b) Influence of high pressure treatment on the acidification of bovine milk by lactic acid bacteria. *Milchwissenschaft* **59**, 246–249.
- Huppertz, T., Fox, P.F., and Kelly, A.L. (2004c) Plasmin activity and proteolysis in high pressure-treated bovine milk. *Le Lait* **84**, 297–304.
- Huppertz, T., Smiddy, M.A., Upadhyay, V.K., and Kelly, A.L. (2006) High-pressure-induced changes in bovine milk: a review. *International Journal of Dairy Technology* **59**, 58–66.

- Iordache, M. and Jelen, P. (2003) High pressure microfluidization treatment of heat denatured whey proteins for improved functionality. *Innovative Food Science and Emerging Technologies* **4**, 367–376.
- Iucci, L., Patrignani, F., Vallicelli, M., Guerzoni, M.E., and Lanciotti, R. (2007) Effects of high pressure homogenization on the activity of lysozyme and lactoferrin against *Listeria monocytogenes*. *Food Control* **18**, 558–565.
- Iucci, L., Lanciotti, R., Kelly, A., and Huppertz, T. (2008) Plasmin activity in high-pressure-homogenised bovine milk. *Milchwissenschaft* **63**, 68–70.
- Jacobs, S.E. and Thornley, M.J. (1954) The lethal action of ultrasonic waves on bacteria suspended in milk and other liquids. *Journal of Applied Bacteriology* **17**, 38–55.
- Jambrak, A.R., Mason, T.J., Lelas, V., Herceg, Z., and Herceg, I.L. (2008) Effect of ultrasound treatment on solubility and foaming properties of whey protein suspensions. *Journal of Food Engineering* **86**, 281–287.
- Jaspe, A. and San Jose, C. (1999) Extracellular enzyme production by *Pseudomonas fluorescens* in homogenized cold milk. *Milchwissenschaft* **54**, 493–495.
- Jayaram, S., Castle, G.S.P., and Margaritis, A. (1993) The effects of high field DC pulse and liquid medium conductivity on survivability of *Lactobacillus brevis*. *Applied Microbiology and Biotechnology* **40**, 117–122.
- Jin, Z.T., Su, Y., Tuhela, L., Singh, B., and Zhang, Q.H. (1998) Inactivation of *Bacillus subtilis* using high voltage pulsed electric fields and ultrasonication. Paper 59C-15 presented at 1998 IFT Annual Meeting, Atlanta.
- Johnston, D.E. and Darcy, P.C. (2000) The effects of high pressure treatment on immature Mozzarella cheese. *Milchwissenschaft* **55**, 617–620.
- Johnston, D.E., Austin, B.A., and Murphy, R.J. (1992) Effects of high hydrostatic pressure on milk. *Milchwissenschaft* **47**, 760–763.
- Johnston, D.E., Austin, B.A., and Murphy, R.J. (1993) Properties of acid-set gels prepared from high pressure treated skim milk. *Milchwissenschaft* **48**, 206–209.
- Johnston, D.E., O'Hagan, M., and Balmer, R.W. (2002) Effects of high pressure treatment on the texture and cooking performance of half-fat Cheddar cheese. *Milchwissenschaft* **57**, 198–201.
- Johnston, K., Carroll, T., Honore, C., Crow, V., White, N., and Chen, P. (2004) Dairy product and process. International Patent WO 2004/045295.
- Jose-Brinez, W., Roig-Sagues, A.X., Hernandez-Herrero, M.M., and Guamis-Lopez, B. (2007) Inactivation of *Staphylococcus* spp. strains in whole milk and orange juice using ultra high pressure homogenisation at inlet temperatures of 6 and 20°C. *Food Control* **18**, 1282–1288.
- Joyce, E., Phull, S.S., Lorimer, J.P., and Mason, T.J. (2003) The development and evaluation of ultrasound for the treatment of bacterial suspensions. A study of frequency, power and sonication time on cultured *Bacillus* species. *Ultrasonics Sonochemistry* **10**, 315–318.
- Juffs, H. and Deeth, H.C. (2007) Scientific evaluation of pasteurisation for pathogen reduction in milk and milk products. Canberra: FSANZ.
- Juliano, P., Toldra, M., Koutchma, T., Balasubramaniam, V.M., Clark, S., Mathews, J.W., Dunne, C.P., Sadler, G., and Barbosa-Cánovas, G.V. (2006) Texture and water retention improvement in high pressure thermally sterilized scrambled egg patties. *Journal of Food Science* **71**(2), 52–61.
- Juliano, P., Clark, S., Koutchma, T., Ouattara, M., Mathews, J.W., Dunne, C.P., and Barbosa-Cánovas, G.V. (2007) Consumer and trained panel evaluation of high-pressure thermally treated scrambled egg patties. *Journal of Food Quality* **30**, 57–80.
- Kanno, C., Mu, T.-H., Hagiwara, T., Ametani, M., and Azuma, N. (1998) Gel formation from industrial milk whey proteins under hydrostatic pressure: effects of hydrostatic pressure and protein concentration. *Journal of Agriculture and Food Chemistry* **46**, 417–424.
- Kelly, A.L. and Zeece, M. (2009) Applications of novel technologies in processing of functional foods. *Australian Journal of Dairy Technology* **64**, 12–15.
- Kentish, S., Wooster, T.J., Ashokkumar, M., Blachandran, S., Mawson, R., and Simons, L. (2008) The use of ultrasonics for nanoemulsion preparation. *Innovative Food Science and Emerging Technologies* **9**, 170–175.
- Khadre, M.A. and Yousef, A.E. (2001) Sporicidal action of ozone and hydrogen peroxide: a comparative study. *International Journal of Food Microbiology* **71**, 131–138.
- Kielczewska, K., Kruk, A., Czerniewicz, M., Warminska, M., and Haponiuk, E. (2003) The effect of high-pressure homogenization on changes in milk colloidal and emulsifying systems. *Polish Journal of Food and Nutrition Sciences* **12**, 43–46.

- Kielczewska, K., Kruk, A., Czerniewicz, M., and Kopeć_UNDEFINED, M. (2009) Effect of pressure on constituents of the colloidal phase of milk. *Milchwissenschaft* **64**, 358–360.
- Knoerzer, K., Buckow, R., Sanguansri, P., and Versteeg, C. (2010) Adiabatic compression heating coefficients for high pressure processing of water, propylene-glycol and mixtures—a combined experimental and numerical approach. *Journal of Food Engineering* **96**, 229–238.
- Knudsen, J.C. and Skibsted, L.H. (2009) High pressure effects on the structure of casein micelles in milk as studied by cryo-transmission electron microscopy. *Food Chemistry* **119**, 202–208.
- Krešić, G., Lelas, V., Jambrak, A.R., Herceg, Z., and Brnč_UNDEFINEDiĆ_UNDEFINED, S.R. (2008) Influence of novel food processing technologies on the rheological and thermophysical properties of whey proteins. *Journal of Food Engineering* **87**, 64–73.
- Kühn, J., Considine, T., and Singh, H. (2006) Interactions of milk proteins and volatile flavor compounds: implications in the development of protein foods. *Journal of Food Science* **71**, R72–R82.
- Kunugi, S. (1993) Modification of biopolymer functions by high pressure. *Progress in Polymer Science* **18**, 805–838.
- Lamela, C.P. and Torres, J.A. (2008) Pressure processing of foods: an alternative for high flavour quality foods retaining health enhancing factors—part 1. *Agro Food Industry Hi-Tech* **19**, 60–62.
- Lanciotti, R., Vannini, L., Patrignani, F., Lucci, L., Vallicelli, M., Ndagijimana, M., and Guerzoni, M.E. (2006) Effect of high pressure homogenisation of milk on cheese yield and microbiology, lipolysis and proteolysis during ripening of Caciotta cheese. *Journal of Dairy Research* **73**, 216–226.
- Leadley, C. (2003) High pressure homogenisation of milk. *Campden and Chorleywood Food Research Association (CCFRA) Newsletter* **3**(2), 4.
- Leadley, C. and Williams, A. (2002) *Power Ultrasound: Current and Potential Applications for Food Processing*. Chipping Campden: Campden and Chorleywood Food Research Association Group.
- Leadley, C.E. and Williams, A. (2006) Pulsed electric field processing, power ultrasound and other emerging technologies. In: *Food Processing Handbook*, edited by J.G. Brennan. Weinheim: Wiley-VCH.
- Lewis, M.J. and Deeth, H.C. (2009) Heat treatment of milk. In: *Market Milks—Processing and Quality Management*, edited by A.Y. Tamime, pp. 168–204. Oxford: Blackwell Publishing.
- Li, S.Q., Zhang, Q.H., Lee, Y.Z., and Pham, T.V. (2003) Effects of pulsed electric fields and thermal processing on the stability of bovine immunoglobulin G (IgG) in enriched soymilk. *Journal of Food Science* **68**, 1201–1207.
- Li, S.Q., Bomser, J.A., and Zhang, Q.H. (2005) Effects of pulsed electric fields and heat treatment on stability and secondary structure of bovine immunoglobulin G. *Journal of Agricultural and Food Chemistry* **53**, 663–670.
- Lim, S.Y., Swanson, B.G., and Clark, S. (2008a) High hydrostatic pressure modification of whey protein concentrate for improved functional properties. *Journal of Dairy Science* **91**, 1299–1307.
- Lim, S.Y., Swanson, B.G., Ross, C.F., and Clark, S. (2008b) High hydrostatic pressure modification of whey protein concentrate for improved body and texture of low-fat ice cream. *Journal of Dairy Science* **91**, 1308–1316.
- Lin, S.X.Q. and Chen, X.D. (2007) A laboratory investigation of milk fouling under the influence of ultrasound. *Transactions of the Institute of Chemical Engineers C. Food and Bioproducts Processing* **85**(C1), 57–62.
- Liu, X., Powers, J.R., Swanson, B.G., Hill, H.H., and Clark, S. (2005) Modification of whey protein concentrate hydrophobicity by high hydrostatic pressure. *Innovative Food Science and Emerging Technologies* **6**, 310–317.
- Locke, B.R., Sato, M., Sunka, P., Hoffmann, M.R., and Chang, J.S. (2006) Electrohydraulic discharge and nonthermal plasma for water treatment. *Industrial and Engineering Chemistry Research* **45**, 882–905.
- López-Pedemonte, T., Roig-Sagués, A.X., Trujillo, A.J., Capellas, M., and Guamis, B. (2003) Inactivation of spores of *Bacillus cereus* in cheese by high hydrostatic pressure with the addition of nisin or lysozyme. *Journal of Dairy Science* **86**, 3075–3081.
- López-Fandiño, R. (2006) High pressure-induced changes in milk proteins and possible applications in dairy technology. *International Dairy Journal* **16**, 1119–1131.
- López-Fandiño, R., Carrascova, A.V., and Olano, A. (1996) The effects of high pressure on whey protein denaturation and cheese-making properties of raw milk. *Journal of Dairy Science* **79**, 929–936.
- López-Fandiño, R., De La Fuente, M.A., Ramos, M., and Olano, A. (1998) Distribution of minerals and proteins between soluble and colloidal phases of pressurized milks from different species. *Journal of Dairy Research* **65**, 69–78.

- Lu, R.R., Chen, M.X., Yang, R.J., and Zhang, W.B. (2008) Effects of high-intensity pulsed electric fields on the iron-binding capacity of lactoferrin. *Transactions of the Chinese Society of Agricultural Engineering* **24**, 279–283.
- Ludikhuyze, L., Claeys, W., and Hendrickx, M. (2000) Combined pressure–temperature inactivation of alkaline phosphatase in bovine milk: a kinetic study. *Journal of Food Science* **65**, 155–160.
- Ludikhuyze, L., Claeys, W., and Hendrickx, M. (2001) Effect of temperature and/or pressure on lactoperoxidase activity in bovine milk and acid whey. *Journal of Dairy Research* **68**, 625–637.
- Luz, P.P., Pires, A.M., and Serra, O.A. (2007) A low cost ultrasonic spray dryer to produce spherical microparticles from polymeric matrices. *Quimica Nova* **30**, 1744–1746.
- Manas, P. and Pagan, R. (2005) Microbial inactivation by new technologies of food preservation: dairy and food microbiology: challenges and opportunities. *Journal of Applied Microbiology* **98**, 1387–1399.
- Martín-Belloso, O. and Elez-Martínez, P. (2005) Enzymatic inactivation by pulsed electric fields. In: *Emerging Technologies for Food Processing*, edited by D.-W. Sun, pp. 155–181. Amsterdam: Elsevier Academic Press.
- Martini, S., Suzuki, A.H., and Hartel, R.W. (2008) Effect of high intensity ultrasound on crystallization behavior of anhydrous milk fat. *Journal of the American Oil Chemists' Society* **85**, 621–628.
- Mason, T.J., Riera, E., Vercet, A., and Lopez-Buesa, P. (2005) Application of ultrasound. In: *Emerging Technologies for Food Processing*, edited by D.-W. Sun, pp. 323–351. Amsterdam: Elsevier Academic Press.
- Masschalck, B., van Houdt, R.V., and Michiels, C.W. (2001) High pressure increases bactericidal activity and spectrum of lactoferrin, lactoferricin and nisin. *International Journal of Food Microbiology* **64**, 325–332.
- Masson, P. (1992) Pressure denaturation of proteins. In: *High Pressure and Biotechnology*, edited by C. Balmy, R. Hayashi, K. Heremans, and P.J. Masson, pp. 38–44. London: Libbey Eurotext.
- Mauer, L.J. and Hayes, K.D. (2003) Inactivation of plasmin by sonication. Paper 14A-2 presented at 2003 *IFT Annual Meeting*, Chicago. http://www.ift.confex.com/ift/2003/techprogram/paper_16724.htm.
- McClements, D.J. (1995) Advances in the application of ultrasound in food analysis and processing. *Trends in Food Science and Technology* **6**, 293–299.
- Mertens, B. (1993) Developments in high pressure food processing. *Internationale Zeitschrift fuer Lebensmittel Technik, Marketing, Verpackung und Analytik* **44**, 100–104.
- Messens, W., Estepar-Garcia, J., Dewettinck, K., and Huyghebaert, A. (1999) Proteolysis of high pressure treated Gouda cheese. *International Dairy Journal* **9**, 775–782.
- Messens, W., Van Camp, J., and Dewettinck, K. (2003) High pressure processing to improve dairy quality. In: *Dairy Processing: Improving Quality*, edited by G. Smit, pp. 310–332. New York: CRC Press.
- Mittal, G.S. and Griffiths, M.W. (2005) Pulsed electric field processing of liquid foods and beverages. In: *Emerging Technologies for Food Processing*, edited by D.-W. Sun, pp. 99–139. Amsterdam: Elsevier Academic Press.
- Moatsou, G., Bakopanos, C., Katharios, D., Katsaros, G., Kandarakis, I., Taoukis, P., and Politis, I. (2008a) Effect of high-pressure treatment at various temperatures on indigenous proteolytic enzymes and whey protein denaturation in bovine milk. *Journal of Dairy Research* **75**(3), 262–269.
- Moatsou, G., Katsaros, G., Bakopanos, C., Kandarakis, I., Taoukis, P., and Politis, I. (2008b) Effect of high-pressure treatment at various temperatures on activity of indigenous proteolytic enzymes and denaturation of whey proteins in ovine milk. *International Dairy Journal* **18**, 1119–1125.
- Molina, E., Alvarez, M.D., Ramos, M., Olano, A., and López-Fandiño, R. (2000) Use of high pressure treated milk for the production of reduced-fat cheese. *International Dairy Journal* **10**, 467–475.
- Mollins, R.A., ed. (2001) *Food Irradiation, Principles and Applications*. New York: Wiley-Interscience, John Wiley and Sons, Inc.
- Morgan, S.M., Ross, R.P., Beresford, T.P., and Hill, C. (2000) Combination of hydrostatic pressure and lactacin 3147 causes increased killing of *Staphylococcus* and *Listeria*. *Journal of Applied Microbiology* **88**, 414–420.
- Moroni, O., Jean, J., Autret, J., and Fliss, I. (2002) Inactivation of lactococcal bacteriophages in liquid media using dynamic high pressure. *International Dairy Journal* **12**, 907–913.
- Mosqueda-Melgar, J., Elez-Martínez, P., Raybaudi-Massilia, R.M., and Martín-Belloso, O. (2008) Effects of pulsed electric fields on pathogenic microorganisms of major concern in fluid foods: a review. *Critical Reviews in Food Science and Nutrition* **48**, 747–759.
- Müller-Merbach, M., Rauscher, T., and Hinrichs, J. (2005) Inactivation of bacteriophages by thermal and high-pressure treatment. *International Dairy Journal* **15**, 777–784.

- Mussa, D.M. and Ramaswamy, H.S. (1997) Ultra high pressure pasteurization of milk: kinetics of microbial destruction and changes in physico-chemical characteristics. *Lebensmittel Wissenschaft und Technologie* **30**, 551–557.
- Muthukumar, S., Kentish, S., Ashokkumar, M., Vivekanand, V., and Mawson, R. (2004) Power ultrasound offers an environmentally friendly approach to cleaning dairy UF membranes. *Australian Journal of Dairy Technology* **59**, 193.
- Muthukumar, S., Kentish, S.E., Ashokkumar, M., and Stevens, G.W. (2005) Mechanisms for the ultrasonic enhancement of dairy whey ultrafiltration. *Journal of Membrane Science* **258**, 106–114.
- Muthukumar, S., Kentish, S.E., Ashokkumar, M., and Stevens, G.W. (2006) Application of ultrasound in membrane separation processes: a review. *Reviews in Chemical Engineering* **22**, 155–194.
- Muthukumar, S., Kentish, S.E., Stevens, G.W., Ashokkumar, M., and Mawson, R. (2007) The application of ultrasound to dairy ultrafiltration: the influence of operating conditions. *Journal of Food Engineering* **81**, 364–373.
- Nakamura, T., Sado, H., and Syuknobe, Y. (1993) Production of low antigenic whey protein hydrolysates by enzymatic hydrolysis and denaturation with high pressure. *Milchwissenschaft* **48**, 141–144.
- Needs, E.C., Capellas, M., Bland, A.P., Manoj, P., MacDougall, D., and Paul, G. (2000) Comparison of heat and pressure treatments of skim milk, fortified with whey protein concentrate, for set yoghurt preparation: effects on milk proteins and gel structure. *Journal of Dairy Research* **67**, 329–348.
- Nelson, J.H. and Winder, W.C. (1954) Further studies on the use of ultrasonic waves to accelerate the ripening rate of cheese. *Journal of Dairy Science* **37**, 638.
- Noci, F., Walkling-Ribeiro, M., Cronin, D.A., Morgan, D.J., and Lyng, J.G. (2009) Effect of thermosonication, pulsed electric field and their combination on inactivation of *Listeria innocua* in milk. *International Dairy Journal* **19**, 30–35.
- Odoi, A., Siefert, L., and Opuda-Asibo, J. (2003) Effectiveness and affordability of hydrogen peroxide in milk preservation under tropical conditions. *Milchwissenschaft* **58**, 65–67.
- Odrozola-Serrano, I., Bendicho-Porta, S., and Martin-Belloso, O. (2006) Comparative study on shelf life of whole milk processed by high-intensity pulsed electric field or heat treatment. *Journal of Dairy Science* **89**, 905–911.
- Oh, H.E., Anema, S.G., Wong, M., Pinder, D.N., and Hemar, Y. (2007) Effect of potato starch addition on the acid gelation of milk. *International Dairy Journal* **17**, 808–815.
- Ohmiya, K., Kajino, T., Shimizu, S., and Gekko, K. (1989) Effect of pressure on the association states of enzyme-treated caseins. *Agricultural and Biological Chemistry* **53**, 1–5.
- OpenCEL (2009) OpenCEL—changing the paradigm of processing wastewater. <http://www.opencel.com>. Accessed December 17, 2009.
- O'Reilly, C.E., O'Connor, P.M., Kelly, A.L., Beresford, T.P., and Murphy, P.M. (2000a) Use of hydrostatic pressure for inactivation of microbial contaminants in cheese. *Applied and Environmental Microbiology* **66**, 4890–4896.
- O'Reilly, C.E., O'Connor, P.M., Murphy, P.M., Kelly, A.L., and Beresford, T.P. (2000b) The effect of exposure to pressure of 50 MPa on Cheddar cheese ripening. *Innovative Food Science and Emerging Technologies* **1**, 109–117.
- Padiernos, C.A., Lim, S.Y., Swanson, B.G., Ross, C.F., and Clark, S. (2009) High hydrostatic pressure modification of whey protein concentrate for use in low-fat whipping cream improves foaming properties. *Journal of Dairy Science* **92**, 3049–3056.
- Pagan, R., Esplugas, S., Gongora-Nieto, M.M., Barbosa-Canovas, G.V., and Swanson, B.G. (1998) Inactivation of *Bacillus subtilis* spores using high intensity pulsed electric fields in combination with other food conservation technologies. *Food Science and Technology International* **4**, 33–44.
- Palacios, P., Burgos, J., Hoz, L., Sanz, B., and Ordoñez, J.A. (1991) Study of substances released by ultrasonic treatment from *Bacillus stearothermophilus* spores. *Journal of Applied Bacteriology* **71**, 445–451.
- Palmano, K.P., Patel, H.A., Carroll, T.J., Elgar, D.F., and Gonzalez-Martin, M.A. (2006) High pressure processing of metal ion lactoferrin. International Patent WO 2006/096073.
- Pandey, P.K. and Ramaswamy, H.S. (2004) Effect of high-pressure treatment of milk on lipase and glutamyl transferase activity. *Journal of Food Biochemistry* **28**, 449–462.
- Paquin, P. (1999) Technological properties of high pressure homogenizers: the effect of fat globules, milk proteins and polysaccharides. *International Dairy Journal* **9**, 329–335.
- Paquin, P., Lacasse, J., Subirade, M., and Turgeon, S. (2003) Continuous process of dynamic high-pressure homogenization for the denaturation of proteins. Patent U.S. 6511695-B1.

- Pardeshi, I.L., Pramod, R., Chhaya, R., and Srivastav, P.P. (2005) Application of ultrasonics in dairy industry. *Indian Dairyman* **57**, 59–64.
- Patel, H.A., Singh, H., Anema, S.G., and Creamer, L.K. (2006) Effects of heat and high hydrostatic pressure treatments on disulfide bonding interchanges among the proteins in skim milk. *Journal of Agricultural and Food Chemistry* **54**, 3409–3420.
- Patel, H.A., Carroll, T., and Kelly, A.L. (2008) Nonthermal preservation technologies for dairy applications. In: *Dairy Processing and Quality Assurance*, edited by R.C. Chandan, A. Kilara, and N.P. Shah, pp. 465–482. Hoboken, NJ: Wiley-Blackwell.
- Patist, A. and Bates, D. (2008) Ultrasonic innovations in the food industry: from the laboratory to commercial production. *Innovative Food Science and Emerging Technologies* **9**, 147–154.
- Patrignani, F., Iucci, L., Lanciotti, R., Vallicelli, M., Maina Mathara, J., Holzapfel, W.H., and Guerzoni, M.E. (2007) Effect of high-pressure homogenization, nonfat milk solids and milkfat on the technological performance of a functional strain for the production of probiotic fermented milks. *Journal of Dairy Science* **90**, 4513–4523.
- Patterson, M.F. (2005) Microbiology of pressure-treated foods. *Journal of Applied Microbiology* **98**, 1400–1409.
- Penas, E., Prestamo, G., Baeza, M.L., Martinez-Molero, M.I., and Gomez, R. (2006a) Effects of combined high pressure and enzymatic treatments on the hydrolysis and immunoreactivity of dairy whey proteins. *International Dairy Journal* **16**, 831–839.
- Penas, E., Restani, P., Ballabio, C., Prestamo, G., Fiocchi, A., and Gomez, R. (2006b) Evaluation of the residual antigenicity of dairy whey hydrolysates obtained by combination of enzymatic hydrolysis and high-pressure treatment. *Journal of Food Protection* **69**, 1707–1712.
- Penna, A.L.B., Subbarao, G., and Barbosa-Cánovas, G.V. (2007) High hydrostatic pressure processing on microstructure of probiotic low-fat yogurt. *Food Research International* **40**, 510–519.
- Pereda, J., Ferragut, V., Quevedo, J.M., Guamis, B., and Trujillo, A.J. (2007) Effects of ultra-high pressure homogenization on microbial and physicochemical shelf life of milk. *Journal of Dairy Science* **90**, 1081–1093.
- Picart, L., Thiebaud, M., Rene, M., Guiraud, J.P., Cheftel, J.C., and Dumay, E. (2006) Effects of high pressure homogenisation of raw bovine milk on alkaline phosphatase and microbial inactivation. A comparison with continuous short-time thermal treatments. *Journal of Dairy Research* **73**, 454–463.
- Pouliot, Y., Britten, M., and Latreille, B. (1990) Effect of high-pressure homogenization on a sterilized infant formula: microstructure and age gelation. *Food Structure* **9**, 1–8.
- Qin, B.-L., Chang, F.-J., Barbosa-Cánovas, G.V., and Swanson, B.G. (1995) Nonthermal inactivation of *Saccharomyces cerevisiae* in apple juice using pulsed electric fields. *Lebensmittel Wissenschaft und Technologie* **28**, 564–568.
- Rademacher, B. and Hinrichs, J. (2002) Ultra-high pressure technology for dairy products. *IDF Bulletin* **374**, 12–18.
- Rademacher, B. and Hinrichs, J. (2006) Effects of high pressure treatment on indigenous enzymes in bovine milk: reaction kinetics, inactivation and potential application. *International Dairy Journal* **16**, 655–661.
- Rademacher, B. and Kessler, H.G. (1997) High pressure inactivation of microorganisms and enzymes in milk and milk products. In: *High Pressure Bio-Science and Biotechnology*, edited by K. Heremans, pp. 291–293. Leuven, Belgium: Leuven University Press.
- Rademacher, B., Hinrichs, J., and Kessler, H.G. (1997) New dairy products at 1000 MPa. *DMZ-Lebensmittelindustrie-und-Milchwirtschaft* **117**, 1035–1039.
- Rademacher, B., Pfeiffer, B., and Kessler, H.G. (1998) Inactivation of microorganisms and enzymes in pressure-treated raw milk. In: *High Pressure Food Science, Bioscience and Chemistry*, edited by N.S. Isaacs, pp. 145–151. Cambridge: The Royal Society of Chemistry.
- Ramirez, R., Saraiva, J., Pérez, C., Lamela, J., and Torres, A. (2009) Reaction kinetics analysis of chemical changes in pressure-assisted thermal processing. *Food Engineering Review* **1**, 16–30.
- Raso, J.R., Condon, S., and Sala, F. (1998) Influence of temperature and pressure on the lethality of ultrasound. *Applied and Environmental Microbiology* **64**, 465–471.
- Rastogi, N.K. (2003) Application of high-intensity pulsed electrical fields in food processing. *Food Reviews International* **19**, 229–251.
- Riener, J., Noci, F., Cronin, D.A., Morgan, D.J., and Lyng, J.G. (2009a) Characterisation of volatile compounds generated in milk by high intensity ultrasound. *International Dairy Journal* **19**, 269–272.

- Riener, J., Noci, F., Cronin, D.A., Morgan, D.J., and Lyng, J.G. (2009b) The effect of thermosonication of milk on selected physicochemical and microstructural properties of yoghurt gels during fermentation. *Food Chemistry* **114**, 905–911.
- Riener, J., Noci, F., Cronin, D.A., Morgan, D.J., and Lyng, J.G. (2010) A comparison of selected quality characteristics of yoghurts prepared from thermosonicated and conventionally heated milks. *Food Chemistry* **119**, 1108–1113.
- Roach, A. and Harte, F. (2008) Disruption and sedimentation of casein micelles and casein micelle isolates under high-pressure homogenization. *Innovative Food Science and Emerging Technologies* **9**, 1–8.
- Ross, A.I.V., Griffiths, M.W., Mittal, G.S., and Deeth, H.C. (2003) Combining non-thermal technologies to control foodborne microorganisms. *International Journal of Food Microbiology* **89**, 125–138.
- Sala, F.J., Burgos, J., Condon, S., and Lopez, P. (1995) Effect of heat and ultrasound on microorganisms and enzymes. In: *New Methods of Food Preservation*, edited by G.W. Gould, pp. 177–204. New York: Blackie Academic and Professional.
- Saldo, J., McSweeney, P.L.H., Sendra, E., Kelly, A.L., and Guamis, B. (2002) Proteolysis of caprine cheese treated by high pressure to accelerate cheese ripening. *International Dairy Journal* **12**, 35–44.
- Sampedro, F., Rodrigo, M., Martinez, A., Rodrigo, D., and Barbosa-Cánovas, G.V. (2005) Quality and safety aspects of PEF application in milk and milk products. *Critical Reviews in Food Science and Nutrition* **45**, 25–47.
- Sánchez, E.S., Simal, S., Femenia, A., Benedito, J., and Rosselló, C. (2001a) Effect of acoustic brining on lipolysis and on sensory characteristics of Mahon cheese. *Journal of Food Science* **66**, 892–896.
- Sánchez, E.S., Simal, S., Femenia, A., and Llull, P.R.C. (2001b) Proteolysis of Mahon cheese as affected by acoustic assisted brining. *European Food Research and Technology* **212**, 147–152.
- Sandra, S. and Dalgleish, D.G. (2005) Effects of ultra-high-pressure homogenization and heating on structural properties of casein micelles in reconstituted skim milk powder. *International Dairy Journal* **15**, 1095–1104.
- Sandra, S. and Dalgleish, D.G. (2007) The effect of ultra high-pressure homogenization (UHPH) on rennet coagulation properties of unheated and heated fresh skimmed milk. *International Dairy Journal* **17**, 1043–1052.
- Sandra, S., Stanford, M.A., and Meunier Goddik, L. (2004) The use of high-pressure processing in the production of Queso Fresco cheese. *Journal of Food Science* **60**, FEP 153–FEP 158.
- Sanz, P., Palacios, P., Lopez, P., and Ordonez, J.A. (1985) Effect of ultrasonic waves on the heat resistance of *Bacillus stearothermophilus* spores. In: *Fundamental and Applied Aspects of Bacterial Spores*, edited by G.J. Dring, D.J. Ellars, and G.W. Gould. New York: Academic Press.
- Scherba, G., Weigel, R.M., and O'Brien, J.R. (1991) Quantitative assessment of the germicidal efficiency of ultrasonic energy. *Applied and Environmental Microbiology* **57**, 2079–2084.
- Schlager, L. and Gervais, A. (1970) Method for production of fresh cheese exhibiting little whey separation. West German Patent Application Number 1 909 199.
- Schneider, J., Baumgärtner, K.M., Feichtinger, J., Krüger, J., Muranyi, P., Schulz, A., Walker, M., Wunderlich, J., and Schmacher, U. (2005) Investigation of the practicality of low-pressure microwave plasmas in the sterilization of food packaging materials at industrial level. *Surface and Coatings Technology* **200**, 962–966.
- Scurrah, K.J., Robertson, R.E., Craven, H.M., Pearce, L.E., and Szabo, E.A. (2006) Inactivation of *Bacillus* spores in reconstituted skim milk by combined high pressure and heat treatment. *Journal of Applied Microbiology* **101**, 172–180.
- Sendra, E., Saldo, J., and Guamis, B. (1999) Goat's milk cheese accelerating ripening. Compositional indices. In: *Advantages in High Pressure Bioscience and Biotechnology*, edited by H. Ludwig, pp. 465–468. Heidelberg: Springer.
- Sener, N., Apar, D.K., and Ozbek, B. (2006) A modelling study on milk lactose hydrolysis and beta-galactosidase stability under sonication. *Process Biochemistry* **41**, 1493–1500.
- Senorans, F.J., Ibanez, E., and Cifuentes, A. (2003) New trends in food processing. *Critical Reviews in Food Science and Nutrition* **43**, 507–526.
- Sepulveda, D.R., Gongora-Nieto, M.M., Guerrero, J.A., and Barbosa-Cánovas, G.V. (2005) Production of extended-shelf life milk by processing pasteurized milk with pulsed electric fields. *Journal of Food Engineering* **67**, 81–86.
- Sepulveda, D.R., Gongora-Nieto, M.M., Guerrero, J.A., and Barbosa-Cánovas, G.V. (2009) Shelf life of whole milk processed by pulsed electric fields in combination with PEF-generated heat. *LWT-Food Science and Technology* **42**, 735–739.

- Sepulveda-Ahumada, D.R., Ortega-Rivas, E., and Barbosa-Cánovas, G.V. (2000) Quality aspects of cheddar cheese obtained with milk pasteurized by pulsed electric fields. *Food and Bioprocess Processing* **78**, 65–71.
- Serra, M., Trujillo, A.J., Quevedo, J.M., Guamis, B., and Ferragut, V. (2007) Acid coagulation properties and suitability for yogurt production of cows' milk treated by high-pressure homogenisation. *International Dairy Journal* **17**, 782–790.
- Serra, M., Trujillo, A.J., Pereda, J., Guamis, B., and Ferragut, V. (2008) Quantification of lipolysis and lipid oxidation during cold storage of yogurts produced from milk treated by ultra-high pressure homogenization. *Journal of Food Engineering* **89**, 99–104.
- Serrano, J., Velazquez, G., Lopetcharat, K., Ramírez, J.A., and Torres, J.A. (2004a) Effect of moderate pressure treatments on microstructure, texture, and sensory properties of stirred-curd cheddar shreds. *Journal of Dairy Science* **87**, 3172–3182.
- Serrano, J., Velazquez, G., Lopetcharat, K., Ramirez, J.A., and Torres, J.A. (2004b) Effect of moderate pressure treatments on microstructure, texture and sensory properties of stirred-curd cheddar shreds. *Journal of Dairy Science* **87**(10), 3172–3182.
- Serrano, J., Velazquez, G., Lopetcharat, K., Ramirez, J.A., and Torres, J.A. (2005) Moderately high hydrostatic pressure processing to reduce production costs of shredded cheese: microstructure, texture, and sensory properties of shredded milled curd Cheddar. *Journal of Food Science* **70**, S286–S293.
- Seyderhelm, I., Boguslawski, S., Michaelis, G., and Knorr, D. (1996) Pressure induced inactivation of selected food enzymes. *Journal of Food Science* **61**, 308–310.
- Shah, N.P., Tsangalis, D., Donkor, O.N., and Versteeg, C. (2008) Effect of high pressure treatment on viability of *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Streptococcus thermophilus* and *L. acidophilus* and the pH of fermented milk. *Milchwissenschaft* **63**, 11–14.
- Shamsi, K. (2010) *Effects of Pulsed Electric Field on Milk Properties*. Beau-Bassin, Mauritius: VDM Publishing House Ltd.
- Shamsi, K., Versteeg, C., Sherkat, F., and Wan, J. (2008) Alkaline phosphatase and microbial inactivation by pulsed electric field in bovine milk. *Innovative Food Science and Emerging Technologies* **9**, 217–223.
- Smith, K., Mittal, G.S., and Griffiths, M.W. (2002) Pasteurization of milk using pulsed electric field and antimicrobials. *Journal of Food Science* **67**, 2304–2308.
- Sobrinho-Lopez, A. and Martin-Belloso, O. (2008a) Enhancing the lethal effect of high-intensity pulsed electric field in milk by antimicrobial compounds as combined hurdles. *Journal of Dairy Science* **91**, 1759–1768.
- Sobrinho-Lopez, A. and Martin-Belloso, O. (2008b) Use of nisin and other bacteriocins for preservation of dairy products. *International Dairy Journal* **18**, 329–343.
- Soliva-Fortuny, R., Balasa, A., Knorr, D., and Martin-Belloso, O. (2009) Effects of pulsed electric fields on bioactive compounds in foods: a review. *Trends in Food Science and Technology* **20**, 544–566.
- Søløft-Jensen, J. and Hansen, F. (2005) New chemical and biochemical hurdles. In: *Emerging Technologies for Food Processing*, edited by D.-W. Sun, pp. 387–416. Amsterdam: Elsevier Academic Press.
- Stapelfeldt, H., Petersen, P.H., Kristiansen, K.R., Qvist, K.B., and Skibsted, L.H. (1996) Effect of hydrostatic pressure on the enzymic hydrolysis of β -lactoglobulin B by trypsin, thermolysin and pepsin. *Journal of Dairy Research* **63**, 111–118.
- Steeman, A. (2009) True innovation from process to packaging. *Packaging Digest*, October, 1–2.
- Stepaniak, L. (2004) Dairy enzymology. *International Journal of Dairy Technology* **57**, 153–171.
- Stewart, D.I., Kelly, A.L., Guinee, T.P., and Beresford, T.P. (2006) High pressure processing: review of application to cheese manufacture and ripening. *Australian Journal of Dairy Technology* **61**, 170–178.
- Su, Y., Zhang, Q.H., and Yin, Y. (1996) Inactivation of *Bacillus subtilis* spores using high voltage pulsed electric fields. Paper 26A-14 presented at 1996 IFT Annual Meeting, New Orleans.
- Sui, Q., Roginski, H., Williams, R.P.W., Versteeg, C., and Wan, J. (2010) Effect of ionic strength of pulsed electric field treatment medium on the physicochemical properties of lactoferrin. *Journal of Agricultural and Food Chemistry* **58**, 11725–11731.
- Sui, Q., Roginski, H., Williams, R.P.W., Versteeg, C., and Wan, J. (2011) Effect of pulsed electric field and thermal treatment on the physicochemical and functional properties of whey protein isolate. *International Dairy Journal* **21**, 206–213.
- Sun, D.-W., ed. (2005) *Emerging Technologies for Food Processing*. Amsterdam: Elsevier Academic Press.
- Taylor, M.J. and Richardson, T. (1980) Antioxidant activity of skim milk: effect of sonication. *Journal of Dairy Science* **63**, 1938–1942.

- Taylor, T.M., Roach, A., Black, D.G., Davidson, P.M., and Harte, F. (2007) Inactivation of *Escherichia coli* K-12 exposed to pressures in excess of 300 MPa in a high-pressure homogenizer. *Journal of Food Protection* **70**, 1007–1010.
- Terebiznik, M.R., Jagus, R.J., Cerrutti, P., de Huerdo, M.S., and Pilosof, A.M.R. (2000) Combined effect of nisin and pulsed electric fields on the inactivation of *Escherichia coli*. *Journal of Food Protection* **63**, 741–746.
- Tewari, G. (2007) High pressure processing of foods. In: *Advances in Thermal and Non-Thermal Food Preservation*, edited by G. Tewari and V.K. Juneja, pp. 203–239. Ames, IA: Blackwell Publishing.
- Tewari, G. and Juneja, V.K., eds. (2007) *Advances in Thermal and Non-Thermal Food Preservation*. Ames, IA: Blackwell Publishing.
- Thiebaud, M., Dumay, E., Picart, L., Guiraud, J.P., and Cheftel, J.C. (2003) High-pressure homogenisation of raw bovine milk. Effects on fat globule size distribution and microbial inactivation. *International Dairy Journal* **13**, 427–439.
- Thompson, A.K. and Singh, H. (2006) Preparation of liposomes from milk fat globule membrane phospholipids using a microfluidizer. *Journal of Dairy Science* **89**, 410–419.
- Ting, E.B., Balasubramaniam, V.M., and Raghubeer, E. (2002) Determining thermal effects in high-pressure processing. *Journal of Food Technology* **56**(2), 31–35.
- Toba, T., Hayasaka, I., Taguchi, S., and Adachi, S. (1990) A new method for manufacture of lactose-hydrolysed fermented milk. *Journal of the Science of Food and Agriculture* **52**, 403–407.
- Toepfl, S., Heinz, V., and Knorr, D. (2005) Overview of pulsed electric field processing for food. In: *Emerging Technologies for Food Processing*, edited by D.-W. Sun, pp. 69–97. Amsterdam: Elsevier Academic Press.
- Toepfl, S., Mathys, A., Heinz, V., and Knorr, D. (2006) Review: potential of high hydrostatic pressure and pulsed electric fields for energy efficient and environmentally friendly food processing. *Food Reviews International* **22**, 405–423.
- Torres, J.A. and Velazquez, G. (2005) Commercial opportunities and research challenges in the high pressure processing of foods. *Journal of Food Engineering* **67**, 95–112.
- Tovar-Hernandez, G., Peña, H.R., Velazquez, G., Ramirez, J.A., and Torres, J.A., (2005) Effect of combined thermal and high pressure processing on the microbial stability of milk during refrigerated storage. *IFT Annual Meeting*. New Orleans, Los Angeles, Abstract 54F-12.
- Trujillo, A.J., Ferragut, V., Gervilla, R., Capellas, M., and Guamis, B. (1997) High pressure effects on milk and milk products. *Recent Research Developments in Agricultural and Food Chemistry* **1**, 137–159.
- Trujillo, A.J., Capellas, M., Saldo, J., Gervilla, R., and Guamis, B. (2002) Applications of high-hydrostatic pressure on milk and dairy products: a review. *Innovative Food Science and Emerging Technologies* **3**, 295–307.
- Ugarte-Romero, E., Feng, H., and Martin, S.E. (2007) Inactivation of *Shigella boydii* 18 IDPH and *Listeria monocytogenes* Scott A with power ultrasound at different acoustic energy densities and temperatures. *Journal of Food Science* **72**, M103–M107.
- Ulusoy, B.H., Colak, H., and Hampikyan, H. (2007) The use of ultrasonic waves in food technology. *Research Journal of Biological Sciences* **2**, 491–497.
- van Loey, A., Verachtert, B., and Hendrikx, M. (2001) Effects of high electric fields pulses on enzymes. *Trends in Food Science and Technology* **12**, 94–102.
- Vannini, L., Lanciotti, R., Baldi, D., and Guerzoni, M.E. (2004) Interactions between high pressure homogenization and antimicrobial activity of lysozyme and lactoperoxidase. *International Journal of Food Microbiology* **94**, 123–135.
- van Opstal, I., Bagamboula, C.F., Vanmuysen, S.C.M., Wuytack, E.Y., and Michiels, C.W. (2004) Inactivation of *Bacillus cereus* spores in milk by mild pressure and heat treatments. *International Journal of Food Microbiology* **92**, 227–234.
- Vardag, T., Dierkes, H., and Körner, P. (1995) High pressure food processing. *Food Tech Europe* **2**, 106, 108–110.
- Vazquez-Landaverde, P.A., Torres, J.A., and Qian, M.C. (2006) Effect of high-pressure-moderate-temperature processing on the volatile profile of milk. *Journal of Agricultural and Food Chemistry* **54**, 9184–9192.
- Vega-Mercado, H., Powers, J.R., Barbosa-Cánovas, G.V., and Swanson, B.G. (1995) Plasmin inactivation with pulsed electric fields. *Journal of Food Science* **60**, 1143–1146.
- Vega-Mercado, H., Powers, J., Barbosa-Cánovas, G.V., Lueddecke, L., and Swanson, B.G. (2001) Change in susceptibility of proteins to proteolysis and the inactivation of an extracellular protease from

- Pseudomonas fluorescens* M3/6 when exposed to pulsed electric fields. In: *Pulsed Electric Fields in Food Processing. Fundamental Aspects and Applications*, edited by G.V. Barbosa-Cánovas and Q.H. Zhang, pp. 105–120. Lancaster, CA: Technomic Publishing Company Inc.
- Velez-Ruiz, J.F., Swanson, B.G., and Barbosa-Cánovas, G.V. (1998) Flow and viscoelastic properties of concentrated milk treated by high hydrostatic pressure. *Lebensmittel Wissenschaft und Technologie* **31**, 182–195.
- Vercet, A., Burgos, J., Crelier, S., and Lopez-Buesa, P. (2001) Inactivation of protease and lipase by ultrasound. *Innovative Food Science and Emerging Technologies* **2**, 139–150.
- Vercet, A., Burgos, J., Crelier, S., and Lopez-Buesa, P. (2002) Manothermosonication of heat resistant lipase and protease from *Pseudomonas fluorescens*: effect of pH and sonication parameters. *Journal of Dairy Research* **69**, 243–254.
- Versteeg, C. and Sanguansri, P. (2009) Developments in food processing technologies. *Food Science and Technology* **23**(3), 22–25.
- Viazis, S., Farkas, B.E., and Allen, J.C. (2007) Effects of high-pressure processing on immunoglobulin A and lysozyme activity in human milk. *Journal of Human Lactation* **23**, 253–261.
- Vilkhu, K., Mawson, R., Simons, L., and Bates, D. (2008) Applications and opportunities for ultrasound assisted extraction in the food industry—a review. *Innovative Food Science and Emerging Technologies* **9**, 161–169.
- Villamiel, M. and de Jong, P. (2000) Influence of high-intensity ultrasound and heat treatment in continuous flow on fat, proteins and native enzymes of milk. *Journal of Agricultural and Food Chemistry* **48**, 472–478.
- Villamiel, M., van Hamersveld, E.H., and de Jong, P. (1999) Review: effect of ultrasound processing on the quality of dairy products. *Milchwissenschaft* **54**, 69–73.
- Villamiel, M., Verdurmen, R., and de Jong, P. (2000) Degassing of milk by high-intensity ultrasound. *Milchwissenschaft* **55**, 123–125.
- Voigt, D.D., Stephan, S., and Kelly, A.L. (2007) The effect of high-pressure treatment of milk on Cheddar cheese manufacture and ripening. *International Dairy Federation, 2007World Dairy Summit*, Dublin, Ireland, September 29–October 4, 2007.
- Von Der Heide, R. (1961) Ultrasonic emulsification in processed cheese manufacture. *Deutsche Molkerei-Zeitung* **82**, 1662–1663.
- Wan, J., Mawson, R., Ashokkumar, M., Ronacher, K., Coventry, M.J., Roginski, H., and Versteeg, C. (2005) Emerging processing technologies for functional foods. *Australian Journal of Dairy Technology* **60**, 167–169.
- Wan, J., Shamsi, K., Sui, Q., Bermudez-Aquirre, D., Dunne, C.P., Barbosa-Cánovas, G., and Versteeg, C. (2008) Microbial safety and bioactive efficacy: effectiveness of pulsed electric field processing of dairy fluids. *American Dairy Science Association Meeting*, July 10, 2008, Indianapolis, Indiana.
- Wan, J., Coventry, M.J., Swiergon, P., Sanguansri, P., and Versteeg, C. (2009) Advances in innovative processing technologies for microbial inactivation and enhancement of food safety—pulsed electric field and low-temperature plasma. *Trends in Food Science and Technology* **20**, 414–424.
- Welti-Chanes, J., Lopez-Malo, A., Palou, E., Bermudez, D., Guerrero-Beltran, J.A., and Barbosa-Cánovas, G.V. (2005) Fundamentals and applications of high pressure processing of foods. In: *Novel Food Processing Technologies*, edited by G.V. Barbosa-Cánovas, M.S. Taipia, and M.P. Cano, pp. 157–181. Boca Raton, FL: CRC Press Inc.
- Whiteley, A.J. and Muir, D.D. (1996) Heat stability of homogenised concentrated milk: 1. Comparison of microfluidiser with a valve homogeniser. *Milchwissenschaft* **51**, 320–323.
- Wolfschoon-Pombo, A. (2001) Bactofugation. Current state of development and future prospects. *DMZ Lebensmittelindustrie und Milchwirtschaft* **122**, 620–624.
- Wouters, P.C., Alvarez, I., and Raso, J. (2001) Critical factors determining inactivation kinetics by pulsed electric field food processing. *Trends in Food Science and Technology* **12**, 112–121.
- Wu, H., Hulbert, G.J., and Mount, J.R. (2001) Effects of ultrasound on milk homogenization and fermentation with yogurt starter. *Innovative Food Science and Emerging Technologies* **1**, 211–218.
- Wuytack, E.Y., Diels, A.M.J., and Michiels, C.W. (2002) Bacterial inactivation by high-pressure homogenisation and high hydrostatic pressure. *International Journal of Food Microbiology* **77**, 205–212.
- Yeom, H.W., Evrendilek, G.A., Jin, Z.T., and Zhang, Q.H. (2004) Processing of yogurt-based products with pulsed electric fields: microbial, sensory and physical evaluations. *Journal of Food Processing and Preservation* **28**, 161–178.
- Yokoyama, H., Sawamura, N., and Motobayashi, N. (1992) Method for accelerating cheese ripening. European Patent application, EP 0 469 857 A1.

- Yu, L.J., Ngadi, M., and Raghavan, G.S.V. (2009) Effect of temperature and pulsed electric field treatment on rennet coagulation properties of milk. *Journal of Food Engineering* **95**, 115–118.
- Zamora, A., Ferragut, V., Jaramillo, P.D., Guamis, B., and Trujillo, A.J. (2007) Effects of ultra-high-pressure homogenization on the cheese-making properties of milk. *Journal of Dairy Science* **90**, 13–23.
- Zhang, H.P., Deligelsang, G.J., and Mu, Z.S. (1998) Denaturation of bovine milk IgG at high pressure and its stability. *Food Science China* **19**(4), 10–12.
- Zheng, L. and Sun, D.-W. (2006) Innovative applications of power ultrasound during food freezing processes—a review. *Trends in Food Science and Technology* **17**, 16–23.
- Zobrist, M.R., Huppertz, T., Uniacke, T., Fox, P.F., and Kelly, A.L. (2005) High-pressure-induced changes in rennet-coagulation properties of bovine milk. *International Dairy Journal* **15**, 655–662.
- Zulueta, A., Esteve, M.J., Frasquet, I., and Frigola, A. (2007) Fatty acid profile changes during orange juice-milk beverage processing by high-pulsed electric field. *European Journal of Lipid Science and Technology* **109**, 25–31.

7 Spray-Dried Dairy-Based Emulsions for the Delivery of Bioactives

M.A. Augustin and L. Sanguansri

7.1 INTRODUCTION

Consumers today are making food choices based on the effects that food have on their health. The link between diet and health has created the opportunity for development of functional foods for improving health and well-being. Functional foods have an effect on health that goes beyond simply nutrition.

Functional foods cover a wide range of products. Fortification of foods, which traditionally involves replacement of nutrient losses incurred during the processing, remains an effective strategy to alleviate nutritional deficiencies. New categories of functional foods and new foods that contain bioactive ingredients are also being developed with an increasing focus on bioavailability (Schneeman, 2004). Within the functional food category, there are also protective foods, aimed at prevention of chronic diseases.

Functional foods contain added bioactive ingredients, which have a physiological role in the body. The bioactives and functional ingredients of interest include a range of vitamins and minerals, healthy oils (omega-3 oils), and functional fatty acids, such as conjugated linoleic acid (CLA), proteins and peptides, and phytochemicals, such as carotene, lutein, tocopherol, and coenzyme Q₁₀. The role of vitamins and minerals in health is well established. However, foods containing bioactives that permit a health claim are of greatest interest and drive the marketing of functional food products, especially for newer bioactives. Health claims for bioactives in functional foods are allowed where there is substantial evidence of the effect *in vivo*. For example, there are health claims relating to increased folate intake and the prevention of neural tube defects in babies, calcium and the reduced risk of osteoporosis, omega-3 fatty acids (eicosapentaenoic acid, EPA and docosahexaenoic acid, and DHA) and the reduced risk of coronary heart disease, and plant sterols/stanols and reduced risk of coronary heart disease. In other cases, where benefits have been observed but there is not sufficient unequivocal evidence for a government health claim, there is still interest in delivery of the bioactives. For example, this is the case for probiotics, where there is a suggestion that their consumption have beneficial effects on gut health (Miles, 2007), and also for polyphenols, which have been implicated in reducing the risk of chronic diseases, such as cancer (Ruxton, 2008).

Functional foods provide the vehicle for increasing the level of bioactives in the diet, particularly if the foods used for delivery are part of the normal diet of the target population. Strategies to increase the level and bioavailability of bioactives in food may be based on breeding and farming practices for enhancement of levels inherent in food. Processing to alter the structure of foods containing bioactives may also be used to modulate the accessibility of the bioactive on ingestion. Alternatively, bioactives may be extracted from foods and made into new and novel ingredients that may be reincorporated into foods. However, once isolated from their natural food environment, bioactives often need to be stabilized and protected against degradation and converted into a convenient ingredient format for reincorporation into foods during manufacture of the final food product.

This chapter considers the rationale for protecting bioactive ingredients and discusses the development of ingredients containing bioactives for the functional food industry. The focus is on the science and technology that underpins the formulation and processing of spray-dried dairy-based emulsions for the delivery of bioactives. The properties of these dried ingredients and their application in functional food development is also covered.

7.2 CONSIDERATIONS FOR DELIVERY OF BIOACTIVES

As many bioactives are unstable when isolated from their natural environment, they require stabilization against degradation. Their incorporation into food should not affect the taste and texture of the final food product. In addition, they need to be able to exert their desired health benefits in the body after ingestion. To meet this requirement, the bioactives need to be protected from the environments until they are exposed to a trigger that releases the active at the target site in the body for their action.

Direct addition of bioactives to foods is possible in limited applications, where the specific bioactive is made stable in the environment it is exposed to prior to reaching its desired site of release. Where direct addition is not achievable without compromising the quality of the food, encapsulation of bioactives has been explored. By forming a protective barrier around the bioactive core, the bioactive may be shielded from factors that cause its degradation. Indeed, innovation in encapsulated bioactive ingredients has been driven by the growing functional food market (Frank, 2006; Anonymous, 2007).

There are a number of factors to consider during the development of microencapsulated bioactive ingredients, but it is the understanding of the functionality of the bioactive in the final food product that should drive the development of appropriate delivery strategies based on encapsulation (Table 7.1). It is essential that only food grade materials that are generally regarded as safe (GRAS) be used in the formulation of encapsulated bioactive ingredients to minimize if not avoid regulatory hurdles during commercialization (Table 7.2). Materials selected as encapsulants must have bland taste, good solubility in the aqueous phase, and good emulsifying and film forming properties. Good gelling properties are also desirable for some encapsulation systems.

Dairy proteins inherently possess all the material characteristics required of an encapsulant material without further modification or addition of emulsifiers. Dairy proteins have been widely used as an ingredient in the manufacture of spray-dried dairy and dairy-like emulsions because of their outstanding surface active and colloid stabilizing properties (Dickinson, 1997; Vega and Roos, 2006).

A number of processing technologies available in the food industry can be exploited for encapsulation of bioactive ingredients, including spray drying, fluid bed coating,

Table 7.1. Considerations for development of encapsulated bioactives.

Considerations	Bioactive core	Encapsulating materials	Formulate encapsulated ingredient	Encapsulation process	Encapsulated bioactive ingredient
Stages in development	Select Core	Screen materials and select from GRAS list Modify materials using food processing technologies if required	Design formulations considering bioactive load	Apply appropriate unit processes considering stability of the core	Develop specifications and applications
Development of ingredient	Understand physical and chemical properties: Structure Solubility Stability	Understand properties: Chemical structure Physical Stability functionality (e.g., gelling, solubility, and interfacial behavior)	Take into account: Ingredient interactions Stability Amenability to processing Triggers for release of core in intended application	Consider: Efficiency/consistency of process Effects of processing on bioactivity	Characterize bioactive ingredient and assess suitability for incorporation into foods Ingredient: Composition, Encapsulation efficiency Stability in various environments (e.g., moisture, heat, pH, shear, and enzymes)
Market issues	Work with supplier/user	Establish cost/availability	Compare with existing products and points of differentiation	Align with industry capability	Align with end user, work with nutritionists and food regulators

coacervation, and extrusion. Of these, spray drying is one of the most common methods used for converting liquid emulsions and dispersions into powders, as it is cost-effective, and if the appropriate formulation and process conditions are chosen, the desired properties of the encapsulated ingredient may be achieved (Gouin, 2004; Augustin and Sanguansri, 2008). Formulations with low viscosity at high total solids and good drying characteristics are desirable for conversion into powders by spray drying.

Maintaining the stability of the bioactive during the manufacture of the bioactive ingredient is important to ensure that the final product has the specified level of bioactives. In addition, the claimed level of the bioactive must be present at the end of the shelf life of both the ingredient, as well as in the final food product. The final food matrix selected as a delivery vehicle for the bioactive ingredient has an influence on the stability of the bioactive, as does the conditions under which the food product is stored. Overages of the

Table 7.2. Encapsulants used in the formulation of dairy based emulsions for delivery of bioactives into functional foods.

Main function	Ingredients
Emulsifier and film former	Sodium caseinate Whey protein isolates Whey protein concentrates Milk protein concentrate Skim milk powder Hydrolyzed casein and whey proteins
Stabilizer and film former	Alginates, carrageenan, gum acacia High methoxy pectin, low methoxy pectin Hydroxymethyl cellulose, carboxymethyl cellulose, methyl cellulose Chitosan
Excepients, matrix, and structure former	Lactose, fructose, galactose, glucose, maltose, sucrose, oligosaccharide, corn syrup solids, dried glucose syrup Hydrolyzed starch, maltodextrins, dextrins Native starch, resistant starch, modified starches

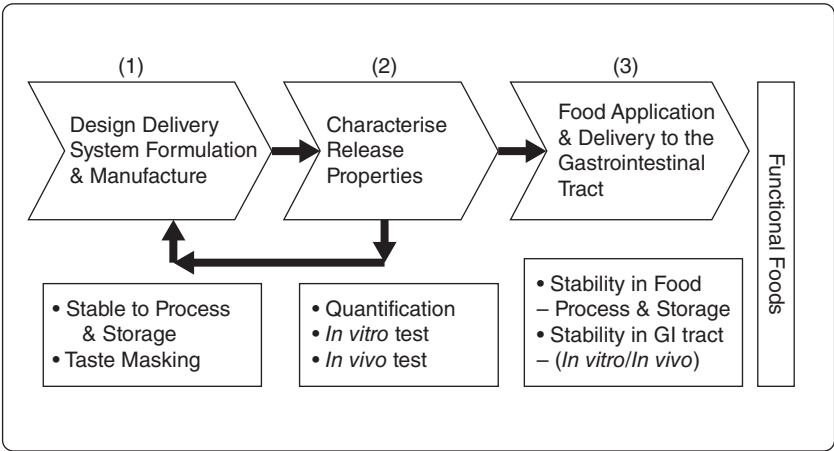


Figure 7.1 Approach for designing microcapsules for delivery of bioactives into functional foods.

bioactives may be required to compensate for losses during processing and storage of bioactive-containing products to comply with regulations.

The key stages of designing bioactive delivery systems for functional foods includes: (1) formulation and design of delivery systems; (2) characterization of microcapsules; and (3) final food product application trials (Figure 7.1). The successful delivery of bioactive ingredients into functional food products requires a multidisciplinary approach spanning disciplines including food chemistry, material science, food engineering, and nutrition. Understanding food regulations in the country of application is also essential.

7.3 SPRAY-DRIED DAIRY-BASED EMULSIONS

A spray-dried emulsion is a convenient and stable powder form obtained by spray drying liquid emulsions, containing the dissolved encapsulant ingredients in the aqueous phase. The encapsulant forms the interface and the matrix, protecting the oil or bioactive core

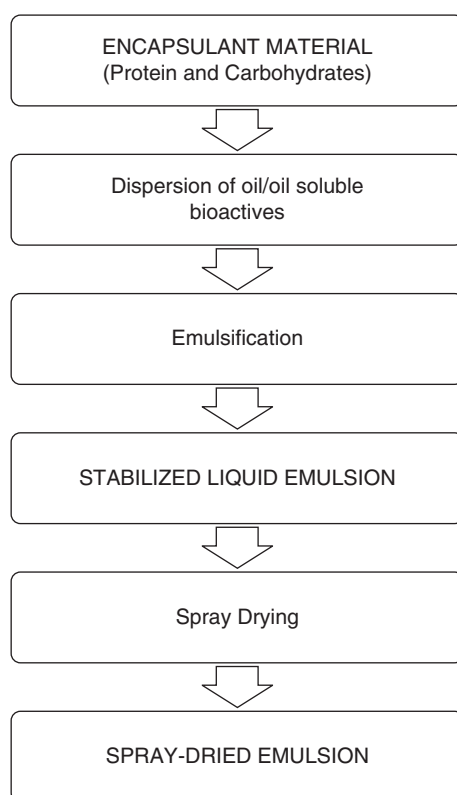


Figure 7.2 Process for manufacture of spray-dried emulsions containing an oil/oil soluble bioactive.

during and after drying. For encapsulation and delivery of oil-soluble/dispersible bioactives, the oil phase is used to carry the bioactive in the emulsion. When spray-dried emulsions are used as a delivery system for bioactive ingredients, they typically contain the bioactive core (e.g., oil, oil-soluble bioactive), an emulsifying material (e.g., low molecular weight surfactants, proteins, emulsifying gums, such as gum arabic, sugar beet pectin, and modified starch, such as octenyl succinate starch), and a matrix-forming material (e.g., sugars, maltodextrin, starch, and nonstarch polysaccharides). An outline of the process for manufacture of spray-dried emulsions is given in Figure 7.2. Where an oil-soluble bioactive is used, it is usually incorporated into the oil phase prior to emulsification.

7.3.1 Formulation and preparation of emulsions

A range of ingredients, including low-molecular weight surfactants and surface-active biopolymers, may be used to stabilize the emulsion. Among some of the surface-active biopolymers are dairy proteins. These are commonly used in combination with carbohydrates. The proteins generally provide good film forming and emulsifying properties, while the carbohydrates impart good plasticizing properties. Together, the proteins and carbohydrates provide a supporting matrix and a strong flexible film with good barrier properties, isolating the oil core from the external environment surrounding the microcapsule. The selection of appropriate materials that can form a robust film around the oil droplets, which remains intact during processing and storage, is desirable.

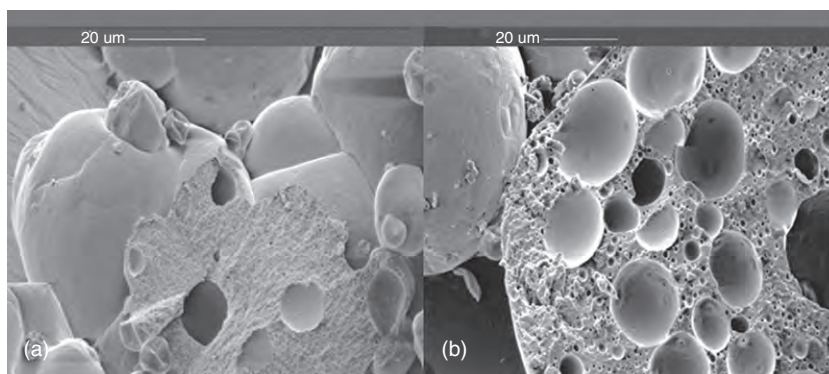


Figure 7.3 Scanning electron micrographs of 50% oil powder encapsulated within a heated casein-sugar matrix, showing powders made from emulsions homogenized at (a) 350/100 and (b) 180/80 bars (unpublished data).

Emulsion preparation involves the dispersion of the oil phase into very fine droplets, in which the emulsifying agent, such as the dairy proteins, provide protection or functionality at the oil–water interface to form stable oil-in-water (O/W) emulsions. Homogenization is generally used for dispersing the oil into fine droplets and forming the emulsion. Homogenization pressure can significantly influence the emulsion size and “free” (unencapsulated) fat of spray-dried emulsions (Rusli et al., 2006). The conditions used for homogenization can also have subsequent effects on powder structure (Figure 7.3). The emulsion formulation and other processing variables, such as temperature, total solids, pH, and viscosity, can influence the final emulsion properties and stability during processing or drying.

Drying the emulsion into thin films can be used to screen formulations before spray drying, by assessing the quality of the films and its ability to form flexible continuous film when dried. The presence of “free” fat droplets on the surface of the dried films is an indication of the encapsulating properties of the encapsulant ingredients.

Emulsions, whether in liquid or in dried forms, can serve as delivery vehicles for a range of bioactives. By changing the nature of the interfacial layer, different levels of protection of healthy oils and oil-soluble bioactives may be obtained, and the release of the bioactive may be controlled. Thus, emulsion-based systems are well suited to the delivery of oils and oil-soluble bioactives (McClements et al., 2007). Advances in emulsion technology can also provide the possibility of designing various types of emulsions for the delivery of bioactives, ranging from primary oil-in-water emulsions (O/W), water-in-oil emulsions (W/O), double emulsions (W/O/W or O/W/O), multilayered emulsions, solid-lipid particles O/W, and hydrogel particles containing oils (O/W/W) (McClements et al., 2007). Benichou et al. (2004) have also developed hybrids of natural biopolymers for encapsulation and slow release of bioactives in double emulsion systems.

When emulsions are converted to powders, the water soluble components of the bulk phase, particularly the carbohydrates and excess proteins, may also serve as carriers for bioactives.

7.3.2 Spray drying of emulsions

Spray drying of dairy fluids and emulsions has been practiced by the dairy industry for a long time. Various aspects of milk powder compositions and processing considerations

Table 7.3. List of milk powder and newer fortified milk powders.

Traditional milk fat-containing dairy-based powders	Fortified milk powders
Full-cream milk powder	Vitamin (A and D) fortified milk powder ^a
Cream powder	Calcium fortified milk powder ^b
Cheese powder	Omega-3 supplemented milk powder ^c
Sour cream powder	Conjugated-linolenic acid enriched milk powder ^d
Fat-filled milk powder	Insulin-like growth factor-I supplemented milk powder ^e

^aIndyk et al. (1996).^bWilliams et al. (2005).^cRamaprasad et al. (2006).^dRodríguez-Alcalá and Fontecha (2007).^eKang et al. (2006).

have been covered previously (Augustin and Clarke, 2008) and are not discussed here. Table 7.3 lists some of the traditional milk powders and interests in fortified milk powders.

Spray drying is still the most commonly used microencapsulation method in the food industry because it is efficient and cost effective. It uses equipment that is readily available in nearly all dairy plants and produces particles of reasonably good quality. The general process involves the atomization of stable emulsions into the drying chamber, which leads to evaporation of the water from the droplets and the formation of powder microcapsules. The microcapsules produced are generally water soluble or dispersible. Proper adjustment and control of the processing conditions enables the production of powders with good physical properties.

Spray-dried microencapsulated ingredients with tailored characteristics have been made possible by new dryer and atomizer designs. The new dryer designs allow drying of emulsions under much lower outlet temperatures (multistage dryers and filtermat dryers), longer residence time in the dryer (tall-form dryer), and therefore provide milder drying conditions, which can minimize the deterioration of heat-sensitive bioactives. New atomizer designs can also improve the properties of spray-dried emulsions, and much narrower particle size distributions of the powders can be achieved.

7.4 CASEIN AND WHEY PROTEIN-BASED SPRAY-DRIED EMULSIONS

Advances in fractionation of milk components have led to the development of various milk protein products, including caseins, whey protein concentrates and isolates, and milk protein concentrates. All these components may be used as emulsifiers in the formulation of stable milk protein-based emulsions. Factors that influence the formulation of milk protein emulsions are briefly considered below. The establishment of a stable milk protein-based emulsion is normally a prerequisite for the development of stable emulsions with added bioactives.

7.4.1 Factors affecting physical stability

The emulsifying properties of caseins and whey proteins have been exploited in the formulation of a range of O/W emulsion products. The protein molecules, being amphiphilic, rapidly adsorb on newly formed interfaces created during the dispersion of the fat into the

protein solution. On adsorption to the interfaces, the conformation of the adsorbed proteins may be altered. To form a stable emulsion, there must be sufficient protein emulsifier present to prevent destabilization of the emulsion due to flocculation or coalescence. The type of protein emulsifier and their state of aggregation affects encapsulation efficiency. The characteristics of the emulsion depend on the inherent interfacial properties of the protein and the homogenization conditions used (Dalgleish, 1996).

Emulsions with high levels of oils may be achieved with the use of milk proteins because of their excellent emulsifying properties. For the preparation of microencapsulated ingredients, it is desirable to have concentrated emulsions that are shelf stable. The shelf life of the emulsions may be extended by heat treatment (e.g., ultra high heat treatment), which destroys microorganisms. Alternatively, the emulsions may be converted into shelf-stable spray-dried emulsion products.

The requirements for providing the emulsions in stable liquid formats and as powdered products may differ. Where a high-heat or a UHT treatment is required, it is essential that the product withstands the heat treatment without excessive thickening or coagulation. When the emulsion is converted into powder, it is desirable to include sugars and various carbohydrates in the formulation. The level and type of carbohydrate used also influences encapsulation efficiency and the free-flowing properties of powders. Encapsulation efficiency is increased when an O/W emulsion is stabilized by a mixture of proteins and sugars compared with when proteins are used alone. Partial replacement of the whey protein by lactose improved encapsulation efficiency of the oil in spray-dried powders (Young et al., 1993). Decreasing the dextrose equivalent of the carbohydrate used in combination with the protein decreased microencapsulation efficiency at high oil : encapsulant ratios (Hogan et al., 2001). However, the type of carbohydrate used as a carrier to support the structure of the powder particles during drying can significantly affect the powder morphology and powder flow properties when used in combination with the same protein. For example, the use of maltodextrin (dextrose equivalent 24) in combination with whey protein concentrate in the formulation of 40% oil powders produced powders with better flow properties than those where the sucrose was used (Augustin et al., 2010).

Proteins can also be used in combination with emulsifying carbohydrates to stabilize emulsions. Under conditions where proteins and polysaccharides form water-soluble complexes, there can be synergistic effects on emulsion stabilization. The addition of xanthan gum to whey protein isolate increased surface hydrophobicity and surface activity (Benichou et al., 2007). This suggests that these types of complexes are likely to impart improved stability to O/W emulsions, and are therefore likely to be useful for preparation of spray-dried emulsions.

Another way to increase the stability of the emulsion and improve barrier properties of the interface is by the formation of multilayered O/W emulsions. These are formed by layer-by-layer adsorption of oppositely charged polyelectrolytes onto an existing oil droplet surface (McClements, 2005). Multilayer emulsions containing β -lactoglobulin with either pectin (Moreau et al., 2003) or carrageenan (Gu et al., 2004) were both found to be more stable to environmental stresses than conventional single layer emulsions.

7.4.2 Emulsions for delivery of bioactives

The suitability of liquid emulsion-based systems for the delivery of bioactives has been the subject of recent reviews (McClements et al., 2007; Garti and Yuli-Amar, 2008). For the delivery of healthy oils (e.g., fish oils), the oil is dispersed in the aqueous phase

Table 7.4. Types of emulsions that have been used for carrying bioactives.

Emulsion type	Uses
Oil-in-water emulsion	Suitable for carrying healthy oils (e.g., omega-3 oils) and oil-soluble bioactives (e.g., carotenes, tocopherols, and lutein)
Water-in-oil emulsion	Suitable for carrying water-soluble bioactives (e.g., vitamin C)
Double emulsions	Suitable for carrying both oil- and water-soluble bioactives
Multilayered oil-in-water emulsions	Similar to oil-in-water emulsions with greater stability to environmental stresses

containing protein, homogenized and dried. In the case of a lipophilic bioactive (e.g., lutein, carotene, and phytosterol), the bioactive component is dissolved in the oil phase prior to homogenization, with the aqueous phase containing the emulsifier. The emulsions are then spray dried to convert it from the liquid to the powdered state as outlined previously (Figure 7.2). A range of emulsions may be used for carrying bioactives (Table 7.4).

As mentioned previously, many healthy oils and lipophilic bioactives are susceptible to degradation when exposed to its surrounding environment and need to be protected. One strategy that has proved to be successful in protecting oxygen sensitive lipophilic bioactives is the development of emulsion-stabilizing materials with built-in antioxidant activities. This was demonstrated by the increased protection of fish oil powders against oxidation when heated protein—sugar mixtures (Maillard Reaction Products) were used as encapsulants in place of physical mixtures of proteins and sugars (Augustin et al., 2006).

Double emulsions can be useful for protecting water-soluble components and for controlling their release (Sabliov and Astete, 2008). An issue with the use of double emulsions is instability due to the coalescence and leakage of components from the inner water phase through the organic phase. This problem may in part be controlled by using higher polymer concentrations and higher viscosity of the inner phase, and by changing the hydrophilic emulsifier in contact with the bulk phase. The release of vitamin B₁₂ from the inner water phase of a water-in-oil-in-water (W/O/W) emulsion to the outer aqueous phase was decreased when the outer emulsifier was changed from caseinate to a caseinate—dextran complex (Fecher et al., 2007). It has been shown that the structure of double emulsions is preserved on drying (Edris and Bergenstahl, 2001). Taken together, this suggests that a double emulsion approach has the potential to deliver the component stabilized in the inner phase in a powder format.

When a bioactive, such as protein, is itself surface active, there are other factors to be considered. The encapsulation of an immunoglobulin (IgY) in a W/O/W emulsion using polyglyceryl condensed ricinolate (PGPR) as the primary emulsifier and a casein-dextran conjugate as the outside emulsifier resulted in a significant loss of activity of IgY on homogenization (Shimizu and Nakane, 1995). Multiple emulsification coupled with heat gelation has been used to prepare whey protein-based microcapsules containing IgY (Cho et al., 2005). These authors reported that the loss of IgY activity was dependent on the method used to prepare the emulsion. Both of these studies reported improved stability of the IgY to harsh environments once the protein was encapsulated.

Emulsion-based systems also lend themselves to the delivery of probiotics, which are dispersible but not soluble in the fat or aqueous phase. Probiotic bacteria were dispersed in an O/W emulsion stabilized by a heated casein-carbohydrate mixture and spray-dried. This process for encapsulating probiotics in an O/W emulsion system is shown in Figure 7.4. The encapsulated probiotic was protected during drying, storage, and in simulated

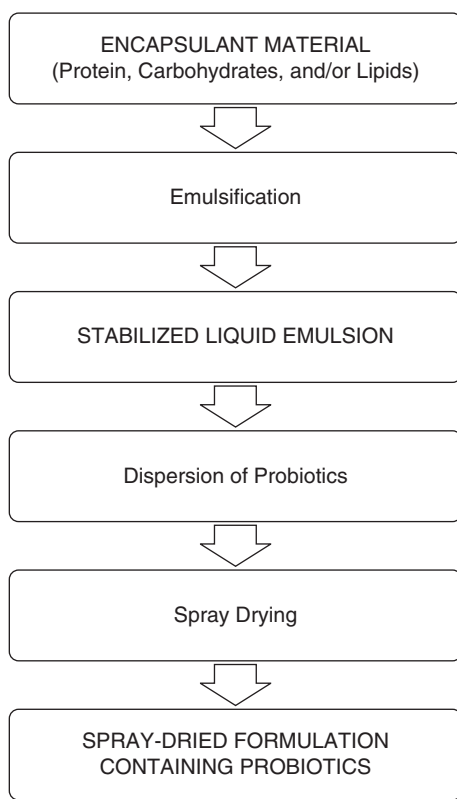


Figure 7.4 Process for encapsulation of probiotic bacteria in an oil-in-water emulsion system.

gastric conditions, and released on exposure to simulated intestinal fluid (Crittenden et al., 2006).

7.5 INCORPORATION OF BIOACTIVE INGREDIENTS INTO FUNCTIONAL FOODS

Physiologically active components can be added directly to food as an extract or concentrate if the bioactive is stable in itself and will not affect the shelf life and sensory acceptability of the final food product. Where direct addition to food is not possible because of stability or taste issues, the bioactive may be encapsulated prior to incorporation into the food product, in which the encapsulated bioactive is protected and isolated from the food environment. The stability of the bioactive and its possible interaction with other food components in the food matrix needs to be seriously considered during formulation, processing, and storage as this can influence the final food product's stability and acceptability (sensory properties). Encapsulation may also serve to mask undesirable tastes and flavors and improve the sensory properties of the final food product.

The drive to put bioactives into an increasing number of food systems and a diversity of food products places stringent demands on the encapsulated ingredients. It is also necessary to ensure the encapsulated bioactive is bioavailable. The matrix of the food product

will have a significant effect on the bioavailability of the bioactive (Garti and Yuli-Amar, 2008). In addition, the diet of the individual and genetic predisposition can also play a role in the efficacy of the bioactive. To obtain the evidence of bioavailability and prove a physiological effect, *in vivo* human trials are essential. The ultimate test of whether spray-dried emulsions are effective delivery vehicles for bioactives is whether it exerts its desired effect in humans. Establishing that the bioactive ingested has the desired health effect is a major challenge (Faulks and Southon, 2008).

7.6 CONCLUSION

As consumers continue to be interested in the health-enhancing role of physiologically active components in functional foods, food companies will continue to develop new food products to meet these requirements. Increasing numbers of consumers seek to optimize their health and well-being and reduce the risk or delay the onset of diseases by consuming functional food and drinks with some associated health benefits.

Encapsulated bioactive ingredients currently available in the marketplace are offered as: emulsions, dispersions, concentrates, pastes, spray-dried powders (from emulsions or dispersions), or as powder blends and premixes. Powder versions are often preferred by food companies, as they offer more stability, flexibility, and convenience. The incorporation of the bioactive should not compromise the sensory properties of the foods, and must be delivered to the appropriate site in the gastrointestinal tract for the bioactive to have its intended health benefit once the food is consumed.

Spray-dried dairy-based emulsions will continue to serve as the preferred format for delivery of bioactive ingredients because of their flexibility and convenience in a range in food applications.

REFERENCES

- Anonymous (2007) Encapsulation and the food industry. *Food Engineering & Ingredients* **32**(4), 31–33.
- Augustin, M.A. and Clarke, P.T. (2008) Dry milk products. In: *Dairy Processing and Quality Assurance*, edited by R.C. Chandan, A. Kilara, and N.P. Shah, pp. 319–336. Ames, IA: Wiley-Blackwell.
- Augustin, M.A. and Sanguansri, L. (2008) Encapsulation of bioactives. In: *Food Materials Science, Principles and Practice*, edited by J.M. Aguilera and P.J. Lillford, pp. 577–601. New York: Springer.
- Augustin, M.A., Sanguansri, L., and Bode, O. (2006) Maillard reaction products as encapsulants for fish oil powders. *Journal of Food Science* **71**(2), E25–E32.
- Augustin, M.A., Sanguansri, L., and Oliver, C.M. (2010) Functional properties of milk constituents: application for microencapsulation of oils in spray-dried emulsions—a minireview. *Dairy Science and Technology* **90**(2–3), 137–146. doi: 10.1051/dst/2009037.
- Benichou, A., Aserin, A., and Garti, N. (2004) Double emulsions stabilized with hybrids of natural polymers for entrapment and slow release of active matters. *Advances in Colloid and Interface Science* **108–109**, 29–41.
- Benichou, A., Aserin, A., Lutz, R., and Garti, N. (2007) Formation and characterization of amphiphilic conjugates of whey protein isolate (WPI)/xanthan to improve surface activity. *Food Hydrocolloids* **21**, 379–391.
- Cho, Y.-H., Lee, J.-J., Park, I.-B., Huh, C.-L., Baek, Y.-J., and Park, J. (2005) Protective effect of microencapsulation consisting of multiple emulsification and heat gelation process on immunoglobulin in yolk. *Journal of Food Science* **70**(2), E148–E151.
- Crittenden, R., Weerakkody, R., Sanguansri, L., and Augustin, M.A. (2006) Synbiotic microcapsules that enhance microbial viability during no refrigerated storage and gastrointestinal transit. *Applied and Environmental Microbiology* **72**, 2280–2282.

- Dalgleish, D.G. (1996) Conformations and structures of milk proteins adsorbed to oil—water interfaces. *Food Research International* **29**, 541–547.
- Dickinson, E. (1997) Properties of emulsions stabilized with milk proteins: overview of some recent developments. *Journal of Dairy Science* **80**, 2607–2619.
- Edris, A. and Bergenstahl, B. (2001) Encapsulation of orange oil in a spray dried double emulsion. *Die Nahrung* **45**, 133–137.
- Faulks, R.M. and Southon, S. (2008) Assessing the bioavailability of nutraceuticals. In: *Delivery and Controlled Release of Bioactives in Foods and Nutraceuticals*, edited by N. Garti, pp. 3–52. Cambridge: Woodhead Publishing Limited.
- Fecher, A., Knoth, A., Scherze, I., and Muschiolik, G. (2007) Stability and release properties of double-emulsions stabilised by caseinate-dextran conjugates. *Food Hydrocolloids* **21**, 943–952.
- Frank, P. (2006) Encapsulation: winning the shell game. *Prepared Foods* **175**(6), 97–104.
- Garti, N. and Yuli-Amar, I. (2008) Micro- and nano-emulsions for delivery of functional food ingredients. In: *Delivery and Controlled Release of Bioactives in Foods and Nutraceuticals*, edited by N. Garti, pp. 149–183. Cambridge: Woodhead Publishing Limited.
- Gouin, S. (2004) Microencapsulation: industrial appraisal of existing technologies and trends. *Trends in Food Science and Technology* **15**, 330–347.
- Gu, S.Y., Decker, E.A., and McClements, D.J. (2004) Influence of pH and κ -carrageenan concentration on physicochemical properties and stability of β -lactoglobulin-stabilized oil-in-water emulsions. *Journal of Agricultural and Food Chemistry* **52**, 3626–3632.
- Hogan, S.A., McNamee, B.F., O’Riordan, E.D., and O’Sullivan, M. (2001) Emulsification and microencapsulation properties of sodium caseinate/carbohydrate blends. *International Dairy Journal* **11**, 137–144.
- Indyk, H., Littlejohn, V., and Woollard, D.C. (1996) Stability of vitamin D3 during spray-drying of milk. *Food Chemistry* **57**, 283–286.
- Kang, S.H., Kim, J.U., Imm, J.Y., Oh, S., and Kim, S.H. (2006) The effects of dairy processes and storage on insulin-like growth factor-I (IGF-I) content in milk and in model IGF-I-fortified dairy products. *Journal of Dairy Science* **89**, 402–409.
- McClements, D.J. (2005) Theoretical analysis of factors affecting the formation and stability of multilayered colloidal dispersions. *Langmuir* **21**, 9777–9785.
- McClements, D.J., Decker, E.A., and Weiss, J. (2007) Emulsion-based delivery systems for lipophilic bioactive components. *Journal of Food Science* **72**(8), R109–R124.
- Miles, L. (2007) Are probiotics beneficial for health? *Nutrition Bulletin* **32**, 2–5.
- Moreau, L., Kim, H.-Y., Decker, E.A., and McClements, D.J. (2003) Production and characterization of oil-in-water emulsions containing droplets stabilized by β -lactoglobulin—pectin membranes. *Journal of Agricultural and Food Chemistry* **51**, 6612–6617.
- Ramaprasad, T.R., Baskaran, V., Debnath, S., and Lokesh, B. (2006) Shelf life of spray-dried milk formulations supplemented with n-3 fatty acids. *Journal of Food Processing and Preservation* **30**, 364–378.
- Rodríguez-Alcalá, L.M. and Fontecha, J. (2007) Fatty acid and conjugated linoleic acid (CLA) isomer composition of commercial CLA-fortified dairy products: evaluation after processing and storage. *Journal of Dairy Science* **90**, 2083–2090.
- Rusli, J.K., Sanguansri, L., and Augustin, M.A. (2006) Stabilization of oils by microencapsulation with heated protein-glucose syrup mixtures. *Journal of the American Oil Chemists’ Society* **83**, 965–972.
- Ruxton, C.H.S. (2008) Black tea and health. *Nutrition Bulletin* **33**, 91–101.
- Sabliov, C.M. and Astete, C.E. (2008) Encapsulation and controlled release of antioxidants and vitamins. In: *Delivery and Controlled Release of Bioactives in Foods and Nutraceuticals*, edited by N. Garti, pp. 297–330. Cambridge: Woodhead Publishing Limited.
- Schneeman, B. (2004) Emerging food technology and world health. *Journal of Food Science* **69**(4), CRH123–CRH126.
- Shimizu, M. and Nakane, Y. (1995) Encapsulation of biologically active proteins in a multiple emulsion. *Bioscience, Biotechnology, and Biochemistry* **59**, 492–496.
- Vega, C. and Roos, Y.H. (2006) Spray-dried dairy and dairy-like emulsions—compositional considerations. *Journal of Dairy Science* **89**, 383–401.
- Williams, R.P.W., D’Ath, L., and Augustin, M.A. (2005) Production of calcium-fortified milk powders using soluble calcium salts. *Le Lait* **85**, 369–381.
- Young, S.L., Sarda, X., and Rosenberg, M. (1993) Microencapsulating properties of whey proteins. I. Microencapsulation of anhydrous milk fat. *Journal of Dairy Science* **76**, 2868–2877.

8 Utilizing Dairy Protein Functionality in Food Microstructure Design

M. Golding

8.1 INTRODUCTION

Dairy proteins and their derivatives are found in a wide variety of food products that form part of our everyday diet. Consumer perception of milk proteins, as used in products such as ice cream, yogurt, and cheese, is generally one of superior quality based on a combination of sensory attributes and nutritional value provided for by the dairy proteins. However, many of these food products could not be manufactured without the functional role that milk proteins provide in creating the necessary microstructures that govern the material, and thus the sensory properties of these foods.

The history of milk protein as a functional ingredient in the production of foods can be traced back thousands of years (Ayto, 2002). The fermentation of milk with bacteria to form a semisolid food dates back to ~6000 BC. Consumption of yogurt by Neolithic people of the Near East almost certainly arose as a consequence of climate in those regions and an inability to provide adequate storage against the microflora responsible for fermentation. These people are also considered the earliest makers and consumers of cheese, the origin of which is believed to have occurred sometime between 8000 and 3000 BC. Milk coagulation most likely occurred accidentally as a consequence of milk storage in the internal organs of animals (e.g., stomach), thus providing one of the earliest examples of processed foods using enzymatic transformation. In this context, it seems remarkable that these foods have continued to be consumed through to the present day based on essentially the same fundamentals of processing.

While the initial formation of these foods may have occurred through circumstance or serendipity, we now possess a far greater understanding of the molecular interactions taking place in these systems, and how they contribute to the microstructural arrangement of these foods. In particular, the combination of six highly soluble major protein fractions: two globular (α -lactalbumin and β -lactoglobulin) and four disordered (α s1-, α s2-, β -, and κ -casein), provide a remarkably diverse range of functional behaviors (Fox, 2003). For food applications, dairy protein functionality can be used for rheological modification (viscosification and gelation), interfacial stabilization (for emulsions and foams), and other behaviors, such as film forming and water and oil binding. The ability to use milk protein functionality to enhance the properties of food products has long been recognized by

producers as a means for enhancing value through reduced costs, improved nutritional profile, and superior quality. This chapter will provide the reader with an understanding of the functional role of the milk protein system and how this system can be manipulated through processing, pH, ionic strength, and interactions with other ingredients, and thereby exploited in the formulation and structural design of foods.

8.2 CASEIN FUNCTIONALITY IN STRUCTURED FOODS

The arrangement of the individual casein proteins in milk as micelles has been widely studied since the 1950s when (Waugh and Hippel, 1956) first proposed a model based on calcium-casein interactions to describe the casein micelle suprastructure. Remarkably, a definitive model for the structural composition of the micelle remains elusive (Tunick, 2009). Even now, advances in characterization techniques have failed to fully quell the debate as to which of the currently proposed mechanisms is truly representative of the internal micellar structure (Fox and Brodtkorb, 2008).

The casein micelle system also presents as a biological curiosity (Kruif and Holt, 2003). Its nutritional function appears as much to do with the efficient delivery of calcium as it has to do with providing a source of amino acids. This functionality can be considered from a molecular perspective, particularly with regard to the primary structure of the caseins, which are characterized by a preponderance of proline residues, a pronounced lack of cysteine residues, and varying degrees of phosphorylated serine. The consequences of such a primary structure are that casein proteins present a relatively disordered secondary and tertiary structure with no observable denaturation and a pronounced ability to bind calcium. The biological assembly of caseins into associated micellar structures is a consequence of the intramolecular binding of colloidal calcium phosphate (CCP) to the phosphoserine rich α - and β - caseins (McMahon and Oommen, 2008). Micellar growth is ultimately limited by the formation of the phosphoserine deficient κ -casein shell on the surface of the micelle that inhibits further calcium bridging between protein molecules (Rollema et al., 1988; Rollema and Brinkhuis, 1989). Additionally, the exposure of the polar C-terminal on the surface may also limit further hydrophobic association. The micellar entity appears to be a means of encapsulating calcium within minimal risk of pathological calcification while maintaining it in a biologically available state.

The disordered structure of the caseins coupled with the relative lack of sulfated amino acids imparts a high thermal stability to the micelle, and the hairy coating of κ -casein prevents further addition of calcium ions without precipitation. The micelle itself is stabilized through a combination of steric and electrostatic repulsion, primarily provided by the κ -casein brush layer (Holt and Dalgleish, 1986). However, the structure of the surface brush layer is less tolerant to other changes in its environment, such as variations in pH (Holt and Horne, 1996) or addition of particular solvents, such as ethanol (Horne, 1984), resulting in coagulation or precipitation of the protein. Interactions between the micelles and other cosolutes can also affect solution properties of the casein protein system, with behaviors ranging from complexation type effects to phase separation due to thermodynamic incompatibility.

The casein proteins are also recognized for their high degree of amphiphilicity, allowing for effective adsorption and stabilization at interfaces (Dickinson, 2003b). This diverse spectrum of protein functional behaviors allows the casein proteins (mostly in the form of the self-assembled micellar suprastructure) to be used as structurants in a range of

applications depending on both processing conditions and the incorporation of different food components, resulting in an array of material and textural properties.

8.2.1 Acid coagulation

Modification of the casein micelle system to produce yogurt is one of the oldest examples of a structured food. Destabilization of the micellar system occurs as a consequence of acidification, resulting in the formation of an aggregated protein gel network. The microstructure of this protein particle network can be further modified through changes in thermal processing, controlling the kinetics of acidification, and the use of static high pressure processing to produce firmer, and better textured gels. Within the modern food industry, it is now also common practice to add additional hydrocolloids, most notably starch, gelatin, and/or pectin, as a means of improving the textural properties and physical stability of yogurts, particularly with respect to syneresis.

Acidification of milk in the pH range 5.5–4.5 results in a number of changes to the structural integrity of the casein micelle system, leading to the formation of a particle gel (Lucey and Singh, 2003). Initially, as pH is lowered to the range 5.5–5.0, the CCP within the micelle is solubilized. While interior calcium bridges within the micelle are seen as an important structural component in the assembly of the micelle, it is interesting to note that the removal of CCP through acidification, and at temperatures above 20°C, does not result in disintegration of the micelle (Dalglish and Law, 1988). This is due to the fact that hydrogen bonding and hydrophobic interactions also contribute to the structural integrity of the micelle. Evidence of this is given by the fact that at cooler temperatures, up to 40% of the interior micellar casein, most notably β -casein, is released from the micelle due to reduced hydrophobic interactions. While this release significantly increases the voluminosity of the micelle, there is actually little change to the hydrodynamic diameter of the micelle under these conditions (Dalglish and Law, 1988).

Coagulation of the micelle occurs when the pH is further lowered toward the isoelectric point (pI). It should be noted that while the pI of the four primary caseins cover the pH range 4.9–5.9, the pI of the casein micelle complex is defined as 4.6. Accordingly, as the pH of the system approaches 4.6, micellar stability is minimized as a consequence of charge neutralization, negating electrostatic repulsion, and a concurrent flattening of the hairy layer reducing steric repulsion (Heertje et al., 1985). However, formation of a gel structure also requires the formation of bonds between molecular species, and in the case of the casein micelle system, this is likely to be facilitated by electrostatic bridges. The effective zero net charge of the micelle at pH 4.6 is partly a consequence of charge balancing between cationic and anionic amino acids at this pH. It therefore stands to reason that these charged groups can interact with each other to form electrostatic bonds between particles (Roefs and Vanvliet, 1990). The importance of electrostatic interactions in the formation of the particle gel structure has been demonstrated by the addition of salt. The subsequent screening of charge by increasing ionic strength serves to reduce electrostatic interactions. In the case of the casein micelle system, addition of salt has been shown to result in a decrease to the gel strength of the particle network (Lucey et al., 1997b; Roefs and Vanvliet, 1990).

An acceptable yogurt texture requires the formation of a homogeneous, finely structured particle network. Simple addition of acid to milk to lower pH results in precipitation rather than gelation, due to the rapid formation of dense casein particle clusters. To achieve the requisite structure requires a uniform reduction in pH across the whole of the sample. A commonly used example of an acidulant that is able to provide a uniform pH drop is

glucono- δ -lactone (GDL), which hydrolyzes slowly in water to produce gluconic acid (Lucey et al., 1997a). In practice, GDL is not used in the manufacture of yogurt, partly as a result of sensory issues relating to taste. Rather, thermophilic bacterial starter cultures are used to directly ferment the lactose naturally present in milk to lactic acid. The gradual digestion of lactose provides a highly effective means of gradually lowering the pH to the right acidity, allowing the formation of a coarse, highly interconnected particulate network.

While the uniformity of acidification is necessary to achieve a percolated system, other factors, such as inoculation temperature and acidification kinetics, contribute to gel microstructure and material properties. A study of the effects of holding temperature on the rheological properties of acid caseinate gels prepared with GDL showed that increasing temperature resulted in more rapid hydrolysis of the GDL, with a corresponding faster onset of gelation (Takeuchi and Cunha, 2008). Interestingly, the elastic modulus of samples was seen to decrease with increasing temperature (Anema, 2008), indicating that gel strength was weakened by a faster rate of acidification (rheology was measured for all samples at a temperature of 5°C). A separate study examining the effects of GDL concentration on gelation of skim milk showed that increasing the level of acidulant also resulted in a more rapid onset of gelation (Horne, 2001), but in this case, faster gelation was accompanied by an increase in gel elastic modulus (when compared for equivalent pH). Faster gelation kinetics are associated with the formation of a more open structured fractal gel, while slower gelation produces more densely packed clusters.

Modern yogurt-making practices commonly employ a heating step of 90°C for 30 minutes prior to inoculation with cultures. This simple process modification is known to considerably improve the strength of the protein gel, and is generally recognized as providing a positive contribution to texture. A comparison of gel microstructure between set yogurts from unheated and heated milks shows the heated milk to possess a finer stranded and more filamentous structure than for an unheated sample. This change in structure is due to extensive binding of β -lactoglobulin (β -lg) to the micellar surface due to thermal denaturation and covalent binding of the β -lg to the κ -casein hairy layer. The bridging of β -lg to the surface of the micelle may reduce the number of potential binding sites between micelles, leading to the formation of a more fractal gel structure. Recent research by Xu et al. (2008) showed that even relatively short heating times can have a significant impact on the rheological properties of the acidified casein gel (Figure 8.1).

Another tool by which the structure and material properties of yogurt can be modified is through the use of static high pressure. The effects of high pressure treatment on the casein micelle structure are discussed in Section 8.2.3.

8.2.2 Enzymatic modification

Enzymes are present within almost all biological systems. From a food perspective, the presence of native enzymes in many raw materials, such as fruit, vegetables, and dairy products is associated with ripening and spoilage. Enzyme catalyzed transformations are used to modify the functional properties of food materials to achieve a number of different outcomes. Examples include protein hydrolysis for nutritional enhancement, and the hydrolysis of starch in the manufacture of maltodextrins, glucose, and fructose syrups. Enzymes, such as proteases and lipases, are naturally present within the milk system.

Deleterious reactions caused by indigenous enzymes, such as plasmin-catalyzed hydrolysis of milk proteins, are associated with a loss in quality of milk, including development of off-flavors, and have been considered as a potential mechanism for age gelation within

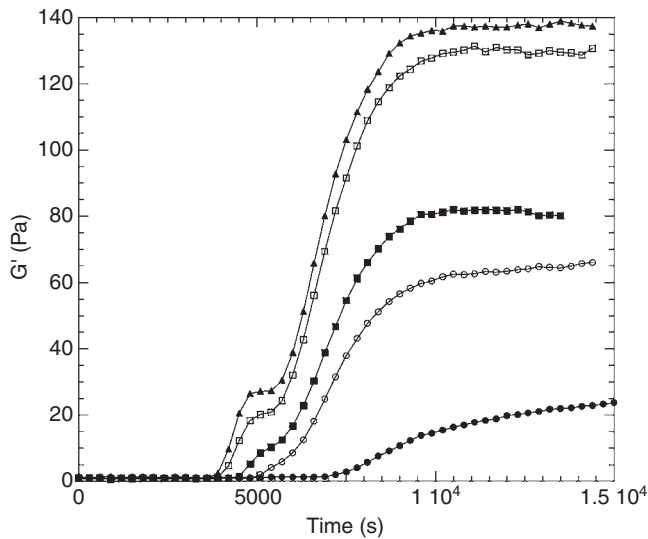


Figure 8.1 Elastic modulus (G') as a function of time for yogurt gels fermented at 45°C from bovine pasteurized skim milk. Gels were made from unheated milk (●) or milk heated for 1 minute, at 80°C (○), at 85°C (■), at 90°C (□), or at 95°C (▲). Reproduced with permission from Xu et al. (2008).

sterilized milk. However, enzymatic reactions can also be used in a favorable way. Such is the case with milk renneting, which has been an essential processing step in the production of cheese for thousands of years.

Rennet (chymosin) is a proteolytic enzyme possessing two aspartic acid residues at its active site. Chymosin is secreted in the lining of the abomasum of the calf, where its biological function is to coagulate the casein fraction within milk. It acts specifically on the κ -casein fraction with proteolysis taking place at the 105Phe–106Met bond (Hyslop, 2003), resulting in the formation of two distinct polypeptide fragments, namely para- κ -casein and the caseino- or glycomacropeptide (CMP or GMP). The casein micelle is stabilized through a brush coating rich in κ -casein on the surface of the micelle. The primary structure of κ -casein shows a degree of amphiphilicity associated with a hydrophilic C-terminal (residues 106–169) due to the presence of negatively charged and polar amino acids, as well as the glycosylation of a number of threonyl residues. In comparison, the N-terminal region (residues 1–105) has a pronounced hydrophobic character.

As part of the cheesemaking process, cleavage of surface κ -casein at the 105–106 bond has a pronounced effect on the structure and stability of the micelle. The C-terminal CMP, being hydrophilic, detaches from the surface of the micelle and is solubilized in the serum phase of the milk. The para- κ -casein N-terminal region remains associated with the micelle. Proteolysis of the surface κ -casein layer is typically followed by coagulation of the micellar structure, resulting in the formation of a weakly gelled particulate network. Coagulation occurs due to a loss of both steric and electrostatic repulsion as a consequence of removal of the negatively charged CMP (Fox and Mulvihill, 1990). This is coupled with an increased exposure of hydrophobic regions within the micelle and additional potential calcium binding sites, both of which promote the association of micelles into a particulate network. Formation of this gelled network into curd is the first stage in the structural development of cheese.

Further structural development occurs through drainage of the serum liquid, including whey proteins, lactose, and soluble minerals not associated with the coagulum. This increase in solids content serves to compress the casein particle network into a more interpenetrating, continuous structure, and appears to also result in exclusion and concentration of fat droplets into localized regions within the network (as fat droplets in cheese are not homogenized, they are not actively associated with the protein network) (Fox and Mulvihill, 1990; Everett and Auty, 2008). Such changes increase the strength and elasticity of the protein gel. Further ageing results in increasing proteolysis taking place from native and added enzymes that serve to further hydrolyze the protein structure. This proteolysis is an essential aspect of flavor development in cheese manufacture. From a structural perspective, visualization of aged cheese shows little indication of the initial micellar characteristics observed prior to renneting. The cheese emulsion is also prone to changes in stability during the ageing process, with aggregation of droplets leading to the formation of localized structures, including partially coalesced networks. This structural evolution is very much dependent on the initial composition and processing conditions of the cheese, and varies considerably between cheese types leading to distinct variations in material and sensory properties (Everett and Auty, 2008). However, all these later, subtle structural changes that take place are still dependent on the initial formation of the rennet catalyzed casein coagulum. Since this coagulum structure is the consequence of a very specific proteolytic pathway, variations in processing and composition can have marked effects on both the proteolysis step and the subsequent formation of the particle network. The preacidification of milk using starter cultures is a common feature of the modern cheesemaking process. It has been shown that rate of proteolysis and of coagulation increases as a consequence of lowering the pH (Vanhooydonk et al., 1986a,b). The increased rate of proteolysis may be a consequence of a lowering of charge repulsion between chymosin and κ -casein, since both are negatively charged at pH 6.8 (screening of charge through increasing ionic strength has also been shown to effect proteolytic efficiency). The observed increase in rate of coagulation may be a consequence of charge screening, allowing for closer contact between micelles even before proteolysis has taken place. The additional consequence of preacidification of cheese milk is to solubilize the CCP bridges within the micelle structure. How the changes in casein mineral balance affect proteolysis and coagulation is not well understood; however, the solubilization of CCP can affect the final calcium content within the cheese, which can have an important contribution to the final textural properties of the product.

The heat treatment of milk prior to rennet inoculation is also known to affect the renneting properties of the milk system. Increasing temperature above 75°C causes changes to the surface structure of the micelle. Denaturation of the β -Ig is accompanied by the formation of intermolecular covalent disulfide bonds. This can take place between β -Ig molecules or between β -Ig and κ -casein that has free cysteine residues within the para- κ -casein region of the molecule. Accordingly, sufficient heating can cause β -Ig to become bound to the surface of the casein micelle. For temperatures at which all native milk β -Ig is denatured, a 20% reduction in the rate of proteolysis is observed, while for less extreme thermal conditions, such that only 25% of β -Ig is denatured, there is only a marginal effect on the rate of proteolysis (Vanhooydonk et al., 1987).

For heat denatured systems, even partial association of β -Ig molecules with the surface of the casein micelle has a pronounced effect on coagulation time and subsequent rheological properties, with weaker gels being formed (Singh and Waungana, 2001). Given that the cysteine residues for κ -casein are located within the para- κ -casein region of the molecule,

it stands to reason that β -Ig bound to κ -casein during thermal treatment will remain with the micelle after chymosin proteolysis of the κ -casein, while the CMP will detach into solution. The bound β -Ig can provide additional micellar stabilization by reducing the surface hydrophobicity of the micelle and increasing surface charge, thereby enhancing repulsive interactions.

Coagulation behavior is also affected at lower temperatures. While decreasing temperature reduces the rate of proteolysis, it does not halt it. However, the ability of the proteolyzed micelle to undergo coagulation is compromised. This is considered to be primarily caused by a reduction in hydrophobic interactions with decreasing temperature. Most notably, at temperatures of $<8^{\circ}\text{C}$, it has been reported that the weakening of hydrophobic interactions allows β -casein, the most hydrophobic of the caseins, to dissociate from the micelle. Walstra (1990) has argued that the hairy layer thickness of the micelle is enhanced by internal β -casein dissociation, which then partly associates with the κ -casein on the outer edges of the micelle, providing an improved steric barrier to coagulation, as well as serving to reduce overall hydrophobic interactions between micelles. Curiously, when warmed, there is a degree of reassociation of the β -casein into the micelle. The increase in hydrophobic interactions occurring as the system is warmed is likewise sufficient to cause coagulation of the casein (Hyslop, 2003).

In addition to naturally derived chymosin, alternative sources of rennet are now widely used in cheesemaking, such as those produced using gene expression based on yeast or bacterial modification to produce molecularly identical chymosin fractions. This technology has seen extensive uptake, since the chymosin produced possesses equivalent functionality to that of calves' chymosin. The ability of molds to express proteolytic enzymes through a process of fermentation has also seen the growth of rennet-equivalent enzymes with the ability to provide appropriate coagulative properties.

Alternative proteolytic enzymes, derived from plant materials, are also able to coagulate milk to deliver an acceptable curd structure (Low et al., 2006). In such cases, proteolysis may occur at sites other than the κ -casein Phe-Met bond that is specific to rennet. This may lead to a greater overall degree of proteolysis, and while such effects are able to cause coagulation, the break-up of proteins into shorter chain polypeptide fragments is also known to be responsible for the development of negative flavor characteristics.

Rennet proteolysis is the most widely encountered enzymatic reaction for dairy systems. However, it is not the only way in which protein functional and material properties can be modified through enzymatic pathways. For a number of years, the anticoagulation enzyme transglutaminase has been considered as a potential means of delivering novel structures and attributes to many protein-based food systems. Transglutaminase catalyzes intermolecular covalent linkages between lysine and glutamine residues on adjacent protein molecules. The efficiency of the reaction is in part dependent on enzyme concentration and temperature of reaction (the optimal temperature for reaction being $\sim 40^{\circ}\text{C}$), but also the relative concentration and availability of lysine and glutamine residues within the protein structure (De Jong and Koppelman, 2002). For example, the casein and gelatin proteins, which possess open, disordered structures and relatively high levels of lysine and glutamine, are considered excellent substrates for intermolecular cross-linking by transglutaminase. For these systems, a minimum concentration of protein is still needed to provide sufficient molecular contact to take place to allow cross-linking to occur. Where this is the case, elastic gels can be formed, with gel strength increasing with protein concentration. The extent of cross-linking under these conditions can also be controlled through thermal inactivation of the enzyme, allowing for the material properties of the gel to be controlled.

As indicated, not all proteins are as susceptible to transglutaminase cross-linking as the caseins. A number of globular proteins, including β -lg, have been shown to be less effective substrates for transglutaminase cross-linking, at least while in the native state (Gauche et al., 2008). In comparing primary structures, it can be noted that β -lg possesses a similar number of both lysine and glutamine compared with β -casein. The reduced susceptibility of β -lg toward transglutaminase catalysis is attributed to the higher degree of secondary and tertiary structure of the native β -lg molecule, which lowers the number of Lys and Glu residues accessible for cross-linking. Denaturation and unfolding of the β -lg native structure, such as under high pressure, is able to expose these hidden residues to enzymatic catalysis. Cross-linking efficiency is therefore increased when the protein is in an unfolded state, such as when subjected to high pressure treatment. The use of transglutaminase in manufactured dairy based foods has yet to show significant commercial uptake, however a number of recent studies have begun to focus on the use of transglutaminase to provide further rheological control to structured milk protein systems, such as acid gels and process cheeses (Yuksel and Erdem, 2009; De Sa and Bordignon-Luiz, 2010; Jaros et al., 2010). While the enzyme has been cleared for use in foods as a processing aid by a number of regulatory bodies, it has yet to demonstrate widespread application in food systems.

8.2.3 High pressure

High pressure effects on the structure and stability of the casein and milk protein systems have been widely studied and reported (refer to Chapter 6). High pressure refers to the application of static pressures typically across the range 100–800 MPa. The use of high pressure processing in food applications is commonly used for microbial inactivation, but can also have certain consequence on the physicochemical properties of food ingredients, most notably protein and starches, by affecting specific intra- and intermolecular interactions. Increasing pressure decreases both hydrophobic and ionic interactions, while hydrogen bonds are less affected at low to moderate pressures, but are disrupted at sufficiently high pressures. In terms of protein molecular structure, pressure has no effect on primary structure, limited effects on secondary structure (except at very high pressures), but can cause significant changes to tertiary structure through disruption of hydrophobic and ionic bonds. High pressure can also affect quaternary structure through the facilitation of intermolecular disulfide interchange. From a molecular perspective, the casein proteins are insensitive to pressure, possessing open disordered structures, and a lack of sulfhydryl amino acids capable of intermolecular covalent bonding. However, the supramolecular structure of the casein micelle is affected by increasing pressure.

As has been discussed, the casein micelle structure is primarily stabilized through hydrophobic and calcium ion interactions. For applied pressures of <250 MPa, there is little effect on the casein micelle structure. At a pressure of 250–350 MPa, an increase in the mean hydrodynamic radius of the casein micelle system is observed from between 120–250% relative to untreated samples (Needs et al., 2000; Huppertz et al., 2004). This increase in size has been attributed to the binding of β -lg to the micelle surface either increasing individual micellar size, or alternatively promoting bridging between micelles, leading to the formation of small micellar clusters (Schrader and Buchheim, 1998). At pressures above 350 MPa, there is a pronounced decrease in the hydrodynamic radius of the casein system that is attributed to the solubilization of the calcium within the micelle coupled with a reduction in ionic and hydrophobic interactions. This causes a dissociation of the micellar structure. Holding time and the rate of decompression are also known to affect the

reassociation properties of the casein proteins once pressure is removed (Huppertz et al., 2006b).

For milk and relatively dilute protein solutions, the effects of static high pressure have little effect on the rheological properties of the system. However, with increasing protein concentration, changes in the micellar structure can lead to the formation of aggregated protein networks (Briscoe et al., 2002). The formation of such pressure-induced casein particle networks has also been shown to be particularly sensitive to the presence of sugars (Abbasi and Dickinson, 2001; Keenan et al., 2001), allowing protein gels to be formed at lower concentration relative to sugar-free controls. For skimmed milk powder (SMP) it was observed that a concentration of at least 25% was required for pressure induced gelation to occur based on a holding pressure of >400 MPa for at least 20 minutes (it should be noted that SMP contains $\sim 52\%$ lactose on a dry weight basis). However, when the sucrose content of the systems was increased to 30%, the concentration of SMP required to form a gel dropped to $<10\%$. The phase diagram for SMP-sucrose mixtures showing the concentration-dependent sol-gel transition behavior is shown in Figure 8.2.

From the phase diagram, it can be seen that at high levels of sugars ($>50\%$ total sugars), the system reverts to a sol state, irrespective of the concentration of SMP.

The textural properties of the gels formed as a consequence of high pressure treatment were found to be relatively weak and soft with an elastic modulus typically in the range of 10–500 Pa depending on concentration. The postulated mechanism for this effect is that the pressure applied to the systems must be sufficiently high to fully solubilize the calcium in the micelle, as well as causing micellar dissociation into smaller subunits, commonly termed submicelles. On pressure release, there is an ionic interaction between the casein subunits and calcium phosphate liberated from the micelles under pressure, leading to a reassociation between submicelles into larger aggregates. (Keenan et al., 2001) have shown that in the presence of sucrose, the submicellar reassociation results in the formation of finely structured percolating particle network in which submicelles appear linked together in extended chain structures that are kinetically trapped, thus forming a gel (Figure 8.3).

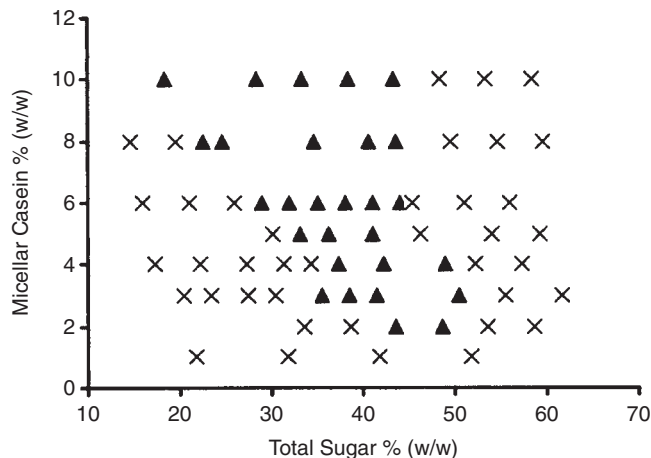


Figure 8.2 The sol-gel phase diagram for the SMP-sucrose system after high pressure treatment at 800 MPa for 20 minutes at 25°C. Micellar casein concentration is plotted against total sugars for the system (including lactose). x, sol state ($G' < G''$); ▲, gel state ($G' > G''$). Reproduced from Abbasi and Dickinson (2001) with permission.

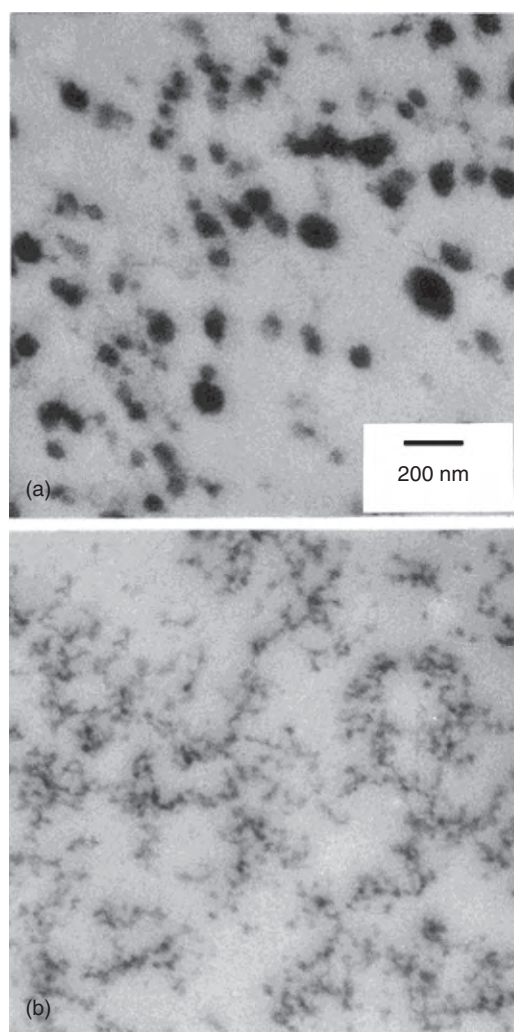


Figure 8.3 Transmission electron micrographs of 14.4% SMP/17.6% sucrose before (a) and after (b) treatment at 400 MPa for 40 minutes. Both micrographs are shown at the same magnification. Reproduced with permission from Keenan et al. (2001).

The fact that such a structure is formed rather than the more compact, micellar-like entities that form on pressure release in the absence of sugar is not well understood. This effect is thought to be either a consequence of higher calcium activity in the presence of sucrose, or a lowering of the solvent quality thus affecting the strength of the casein submicelle-calcium phosphate interactions (Keenan et al., 2001).

The effect of high pressure processing on other dairy systems, such as yogurts, has also been investigated. For such systems, the behavior of the casein micelle system is not only dependent on the particular conditions applied during pressurization, but also on additional aspects of formulation and processing associated with the yogurt-making process. Where milk was pressure treated prior to acidification, it was shown that pH for onset of gelation was higher than that of untreated milks, and that the rate of acidification was faster. For

such systems, it was observed that set yogurts showed less tendency toward syneresis, had higher gel strengths and contained a higher concentration of whey protein (Desobrybanon et al., 1994; Harte et al., 2002). The structure of set yogurts made with pressure-treated milk showed some distinct differences to that of acidified heat-treated milk. In the case of pressure treatment, it was shown that micelles were closely linked into particulate aggregates (Needs et al., 2000), in contrast to the heat-treated milks, which showed micelles linked into networks by filamentous bridges (assumed to be whey protein links).

In the case of stirred yogurt, the combined effects of temperature and pressure on skim milk prior to inoculation has been shown to have pronounced effects on the structural and material properties of the yogurt (Udabage et al., 2010). High pressure treatment (up to 400 MPa) at 25°C was shown to have little effect on yogurt viscosity in relation to a heat-treated control. Intriguingly, a combination of high pressure and elevated temperature was shown to decrease stirred yogurt viscosity compared with the heat-treated control. The authors proposed that the combination of high pressure (400 MPa) and temperature (90°C) resulted in the formation of larger micellar entities and separate complexes of whey protein and κ -casein. These larger micelle systems have been shown to be prone to precipitation, and this loss of stability is reflected in the formation of a weaker gel structure, as the micelles are less able to form a connective particulate network, hence the reduction in viscosity.

The effect of high pressure on microbiota, acidity, and texture has also been investigated after inoculation and acidification (Tanaka and Hatanaka, 1992; Reps et al., 2009). Pressures of 300 MPa and higher were found to prevent the lowering of pH for set yogurts over a 4-week storage period, relative to an unpressurized control. Pressure treatment was also found to affect the structure of the yogurt with increasing pressures, particularly above 300 MPa, leading to a loss of homogeneity and a more loose and flocculated texture. During storage, the high pressure-treated samples were also found to be more prone to syneresis. The work of Tanaka and Hatanaka (1992), for which the pressure ceiling was limited to 300 MPa, showed that at these pressures, the microbiological environment of the yogurt could be manipulated without any compromise in the stability or texture of the yogurt. It seems likely that for pressure treatments in excess of 300 MPa, there is a dissociation of the percolating yogurt particle gel network, leading to more localized aggregation of protein on pressure release with subsequent loss of structure.

In addition to acid milk gel systems, considerable attention has been given to the potential applicability of high pressure as part of the cheesemaking process (as reviewed by Huppertz et al., 2006a). Pretreatment of milk with high pressure has been shown to have a notable impact on coagulation properties, albeit with some inconsistencies in observed behavior. Early work on the pressurization of reconstituted skimmed milk showed that pressure treatment in the range 230–670 MPa resulted in a marked reduction in rennet coagulation time (Desobrybanon et al., 1994). However, while subsequent studies did confirm a reduction in coagulation time over the range 100–300 MPa, increasing pressure above 400 MPa either resulted in no change to coagulation time, or increased coagulation times relative to control (LopezFandino et al., 1996, 1997). Further studies on whey depleted solutions or in the presence of N-ethylmaleimide, a reagent which blocks sulfhydryl interchange, showed that rennet coagulation time was reduced when there was no association of whey with the micellar surface—an observation consistent with studies on the coagulation time of milks processed at elevated temperatures (Needs et al., 2000). As with thermal treatment, high pressure-induced covalent bridging of β -lg to the surface of casein micelles (which typically takes place at pressures in excess of 250 MPa) is

considered inhibitory to both chymosin proteolysis of κ -casein and subsequent aggregation of the micelles after κ -casein hydrolysis (Huppertz et al., 2006a).

Conversely, increasing pressure treatment in the range 100–600 MPa applied to milks after heat treatment (90°C, 10 minutes) results in a pronounced reduction in rennet coagulation time, such that a pressure of 600 MPa held for 30 minutes decreases coagulation time by about 50% relative to untreated milks (Huppertz et al., 2005). An implication from this study is that pressure-assisted dissociation of casein micelles exposes internal κ -casein which is then available for hydrolysis on (partial) reassociation of the micelles after pressure release. This argument may also hold true in the case of pressure treatment prior to thermal treatment on the basis that micellar dissociation after β -lg interaction with surface κ -casein will still enable the liberation of κ -casein from the interior of micelles. Supporting data are inconclusive, as it was shown that there were no changes in the release of GMP as affected by high pressure (Needs et al., 2000). This observation indicates an equivalent availability of κ -casein for hydrolysis (even though it must be assumed that a proportion of micellar surface κ -casein undergoes covalent bridging with β -lg). However, an earlier study (LopezFandino et al., 1997) showed that at a pressure of 400 MPa with a holding time of 30 minutes, there was a reduction in the release of GMP in milk (at a pressure of 200 MPa hydrolysis was not affected).

As discussed in Section 8.2.2, rennet coagulation proceeds via a two-step mechanism: κ -casein hydrolysis and paracasein micellar association leading to gel formation. The effects of pressure treatment on this second stage again show variation depending on the degree of pressure applied. Rate of gelation has been shown to increase with pressure, reaching a maximum in the range 250–400 MPa (LopezFandino et al., 1996). Likewise, an increase in gel firmness was observed in this pressure range. Such behavior may seem counter-intuitive given the observations made previously, which indicated that pressure treatment either did not affect, or increased rennet coagulation time at increasingly high pressures. An explanation for this effect may be related to other phenomenological changes to the micelle system, which may contribute to the coagulation behavior of the protein. Particularly relevant is the solubilization of calcium from the interior of the micelle, which may facilitate cross-linking of the micelles through electrostatic interactions as pressure is released.

One final consequence of pressure treating milk as part of the cheese-making process is that the pressure-induced covalent bridging of β -lg to κ -casein can increase the amount of whey protein retained in the curd structure. Applied pressures of 600–800 MPa have been shown to increase curd yield by up to 25% through retention of β -lg that would otherwise be released from the curd as serum protein. While increasing the protein content of cheese in this way is seen as desirable, a disadvantage is that the modified curd structure is more resistant to syneresis. The increased propensity of the cheese to retain moisture through this mechanism reduces processing efficiency (Huppertz et al., 2004).

The use of high pressure in dairy manufacturing is still limited in scope, primarily due to the cost prohibitive nature of the process, relative to potential benefits arising from the use of the technology. However, processing costs associated with the use of high pressure have been progressively decreasing as the technology gains more widespread application across the food industry and also benefits from engineering advances. Such cost reductions may allow for the use of high pressure in dairy manufacturing, where its ability to manipulate protein structure may show positive benefits for reducing ingredient costs, improving naturalness, as well as serving to provide effective product safety through microbiological inactivation.

8.2.4 Mixed biopolymer effects

The interaction of milk proteins with other ingredients in food formulations plays an important role in the design of many structured foods. Of particular scientific and commercial interest has been the relationship between (casein) milk proteins and polysaccharides. This is a complex area, and to provide a full assessment on the nature of the interactions between proteins and other biopolymers would be beyond the scope of this chapter. Rather, an abbreviated summary of the different types of interaction will be given together with examples of applications in food structure design.

For the casein/skimmed milk system, we can broadly define interactions between protein and polysaccharides as associative, dissociative (thermodynamic incompatibility), or cosoluble noninteracting. Each of these will be considered in turn. Associative interactions arise through the formation of specific bonds between the micelle structure and a cooperative biopolymer. Generally, the most common form of interaction is through charge complexation, (i.e., formation of electrostatic bonds from species with opposing charge). However, molecules can also interact through formation of hydrogen bond or hydrophobic effects. For protein/polysaccharide systems, the formation of covalent bonds is rarely encountered, except through specific molecular mechanisms such as the Maillard reaction and product (Augustin et al., 2006).

8.2.4.1 Charge complexation

For the casein system, electrostatic complexation of the protein with a biopolymer system is very much dependent on additional factors, such as pH, ionic strength, and the relative concentrations of the complexing macromolecule (Ye, 2008). At neutral pH, the net surface charge of the casein micelle has been reported as -26 mV , which confers the micelle with a degree of electrostatic stabilization. At this pH, there is a greater contribution to the charge of the micelle from anionic amino acids relative to cationic species. However, with decreasing pH, there is increasing protonation of charged groups on amino acids. For example, anionic carboxylate (COO^-) groups are protonated to carboxylic acid groups (COOH), and amine groups (NH_2) become increasingly protonated to ammonium cations (NH_3^+). For the casein system, the lowering of pH results in the reduction of negative charge associated with the micelle, to the point at which the positively and negatively charged residues are in balance, the so-called isoelectric point (pI).

As mentioned earlier, while all four major casein fractions have slightly different pIs in the range 4.9–5.5, the pI of the micellar entity is generally considered to occur at a pH of 4.6, at which point the net charge of the protein is effectively zero. The slightly lower pI of the casein micelle is attributed to the presence of CCP within the micellar structure, which skews the pI to a more acidic pH. As the pH is lowered, the pI of the protein becomes progressively more cationic in nature (Doublier et al., 2000). At neutral pH or under mildly acidic conditions, most polysaccharide systems are either charge neutral (e.g., guar and locust bean gum) or polyanionic, due to the presence of carboxyl or sulfated charge groups (e.g., pectin, alginate, and carrageenan). The exception is the positively charged chitosan. Nonionic polysaccharides do not participate in electrostatic complexation with proteins, although they may have other structural interactions which will be discussed shortly. For anionic polysaccharide systems, it would certainly be expected that electrostatic interactions would occur at a pH below that pI, such that cationically charged proteins will complex with the anionic polysaccharide (assuming the

polysaccharide still carries a negative charge at the pH of interest). It should be noted that the resulting structure of the casein-polysaccharide complex and its behavior in solution is very much dependent on other factors, such as concentration of both species of biopolymer, ionic strength, relative charge density (of both biopolymer systems), and processing conditions (Ye, 2008).

Generally, it can be expected that electrostatic interactions will be enhanced as the difference in net charge of the opposing species increases in magnitude. In this respect, changes in relative pH can enhance and decrease the strength of interactions, while increasing ionic strength is usually associated with a reduction in interaction behavior as a consequence of charge screening between groups of opposing charge. The nature and position of the charged groups can also have an impact on the interaction potential between polysaccharides and casein protein. For example, anionic sulfated groups, found on polysaccharides such as carrageenan, are known to interact more strongly with cationic groups (Kato, 1996). The propensity for electrostatic complexation to take place is also affected by the relative numbers and accessibility of charged groups. Polysaccharides can show variability in the degree of charged residues present on the backbone of the molecule.

Modifications to the structure of the polysaccharide can also be made to vary the degree of molecular charge as for low and high methoxylated pectins. In the case of proteins, the availability of cationic residues may also be affected by the secondary, tertiary, and quaternary structures of the proteins. While the disordered structure of the caseins would appear to make them good substrates for electrostatic complexation, it is worth remembering that for the casein micelle, accessibility of binding sites is primarily limited to the surface structures of the micelle, and accordingly, electrostatic linking results in the formation of particulate networks.

In terms of practical applications, charge complexation between micellar casein and polysaccharides at pH around or below the pI has been used in the stabilization of acid milk drinks as a means of preventing the protein from precipitating out (Laurent and Boulenger, 2003; Jensen et al., 2010). This is commonly achieved through the addition of high methoxy (HM) pectin. The pectin backbone consists mainly of galacturonic acid and galacturonic acid methyl ester units forming linear polysaccharide chains (homogalacturonan) and is normally classified according to its degree of esterification (DE). For HM pectins, generally >50% of the carboxyl groups occur as methyl esters (with the remaining carboxyl groups predominantly taking the acid form at neutral pH). Accordingly, HM pectins are less charge sensitive than LM pectins. For example, LM pectins can form intermolecular complexes through formation of electrostatic Ca^{2+} bridges, whereas HM pectins are insensitive to the presence of calcium. For HM pectin casein mixtures, at neutral pH, there is no interaction between the polysaccharide and the protein. However, acid milk drinks are generally manufactured to a pH range of 3.4–4.6, a point at which the protein is prone to precipitation. As this pH range is below that of the pI of the micelle, there is a charge interaction between the carboxylate groups on the pectin and the protonated amino acid groups on the protein. The effect on micellar stability is, however, very much dependent on the relative concentration of the polysaccharide. Low concentration of pectin addition can cause aggregation of casein micelles due to a bridging mechanism. Where there is insufficient polysaccharide to adequately coat the surface, pectin molecules can form bonds between micelles. At intermediate concentrations, the surface of each micelle will be sufficiently coated with pectin so as to prevent any bridging from taking place between separate micelles. Over this concentration range, it has been observed that casein micelles

are stabilized under acidic conditions. This phenomenon is attributed to the extension of nonbound regions of the hydrophilic pectin molecule into the aqueous phase, thereby providing a steric contribution to the stability of the micelle and preventing precipitation of the protein. Intriguingly, if the pectin concentration is taken too high, the micellar system again becomes destabilized, in this case through a depletion mechanism caused by excess unadsorbed pectin in the aqueous phase. This also causes the micelles to become aggregated, resulting in phase separation (sedimentation).

As noted, at $\text{pH} < \text{pI}$ for the casein system many anionic polysaccharides are able to form electrostatic complexes with the protein. In addition to the specific interaction with pectin used in the manufacture of acid milk drinks, other product applications make use of this effect (Tromp et al. 2004).

For $\text{pH} > \text{pI}$, the net charge of the micelle becomes increasingly negative, and would therefore be expected to negate electrostatic complexation with anionic polysaccharides as a consequence of charge repulsion. While this is indeed true for a number of anionic polysaccharides, there are particular examples for which the casein micelle is able to interact with specific anionic polysaccharides through electrostatic attraction between localized groups of opposing charge. A particular example of this effect is the interaction between micellar casein and κ -carrageenan at neutral pH (Spagnuolo et al., 2005).

While at neutral conditions the net charge of the micelle is appreciably negative, it is important to consider that there will still be cationic amino acids, notably arginine, lysine, and histidine, located on the polypeptide providing a positive charge contribution to the protein. Above pI , the charge on these groups is usually screened out by neighboring anionic residues. However, within the primary structure of κ -casein, there is a band between residues 21 and 100 that contains seven cationic amino acid groups but only one anionic group. Accordingly, there is a localized cationic charge in this region of the protein that is not compromised by surrounding anionic groups. This structural peculiarity allows κ -carrageenan to form a specific electrostatic complex with κ -casein present on the surface of the casein micelles (Figure 8.4).

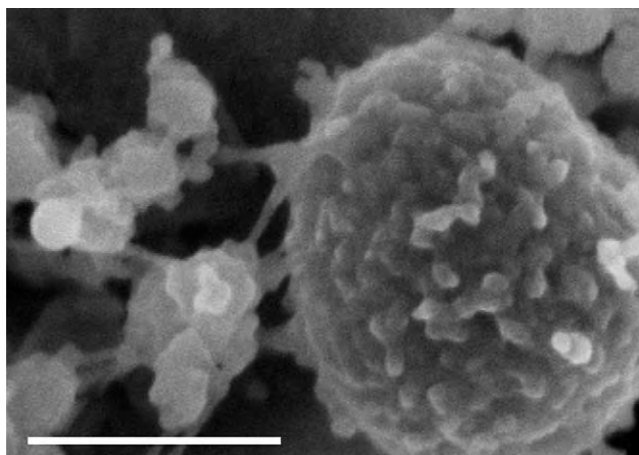


Figure 8.4 Field emission scanning electron micrograph showing interaction of casein micelle with κ -carrageenan. Bar = 200 nm. Reproduced with permission from (Spagnuolo et al., 2005).

The fact that this complex occurs for κ -carrageenan and not other anionic polysaccharides may be due to the fact that the sulfate groups on the carrageenan polysaccharide backbone are able to form stronger electrostatic bonds than carboxylate groups, allowing for a more effective charge association with the localized cationic groups on the κ -casein molecule.

This particular biopolymer interaction is employed in food applications, providing stability and texture to neutral dairy protein based products. As with the interaction with HM pectin, there is a strong concentration dependence that greatly influences the structural and material properties of the complexed systems. Even at very low concentration of added κ -carrageenan (typically in the region of 0.02%), the complexation of the polysaccharide with the protein results in the formation of a weak thixotropic gel capable of suspending particles. This effect is commonly used in chocolate milkshakes for improving the stability of cocoa particles against sedimentation. The slight thickening effect produced at these low concentrations is sufficient to enhance the mouthfeel of neutral dairy drinks, providing a richer texture. Higher concentrations of κ -carrageenan (>0.2%) form an inter-penetrating cross-linked gel with casein micelles, even at relatively low protein concentrations. The greater bond strength between protein and polysaccharide for this system results in the formation of gels with a higher degree of elasticity than most other variants of micelle particle gels. Milk gels formed using κ -carrageenan are commonly used in dairy dessert products to provide texture.

Another example of charge complexation at neutral pH is given by the interaction between chitosan and casein. Chitosan is produced by the deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans and cell walls of fungi. It is a linear polysaccharide composed of randomly distributed β -(1-4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit). As pH is decreased, these amino groups become protonated, making the polysaccharide increasingly polycationic. Even for $\text{pH} > \text{pI}$ of the casein system, the proteins are able to participate in electrostatic complexation. This behavior has been shown in a study by Ausar et al. (2001) that examined the effects of chitosan addition on the stability of the casein system. Chitosan fractions (0.2%) of differing molecular weight were added to whole and skimmed milks and the structure evaluated using an optical microscope. All chitosan fractions, irrespective of molecular weight, were found to interact with the casein protein in milk. However, there were pronounced differences in the nature of the particle aggregates depending on the molecular weight of the polysaccharide (Figure 8.5). Addition of high molecular weight chitosan (>600 kDa) leads to the formation of densely packed large aggregates, resulting in a high degree of precipitation due to multiple binding of micelles to chitosan chains. As the molecular weight of the chitosan is reduced, the structure of the protein network becomes less densely packed, resulting in the formation of finely stranded particulate networks with fewer dense clusters. For low molecular weight chitosan (~80 kDa), the structure of the network resembles the particulate network formed as a consequence of renneting or acid gelation.

For chitosan oligosaccharides, the aggregated protein-polysaccharide structure is even finer, with thinly stranded particular structures forming a percolating network. The reduction in the number of cationic charge groups present on the lower molecular weight chitosan would limit availability for micellar bridging to the polysaccharide backbone, resulting in a less densely packed structure.

The ability to regulate structure in this way has implications for the design of food systems where material properties are defined by the formation of particulate networks.

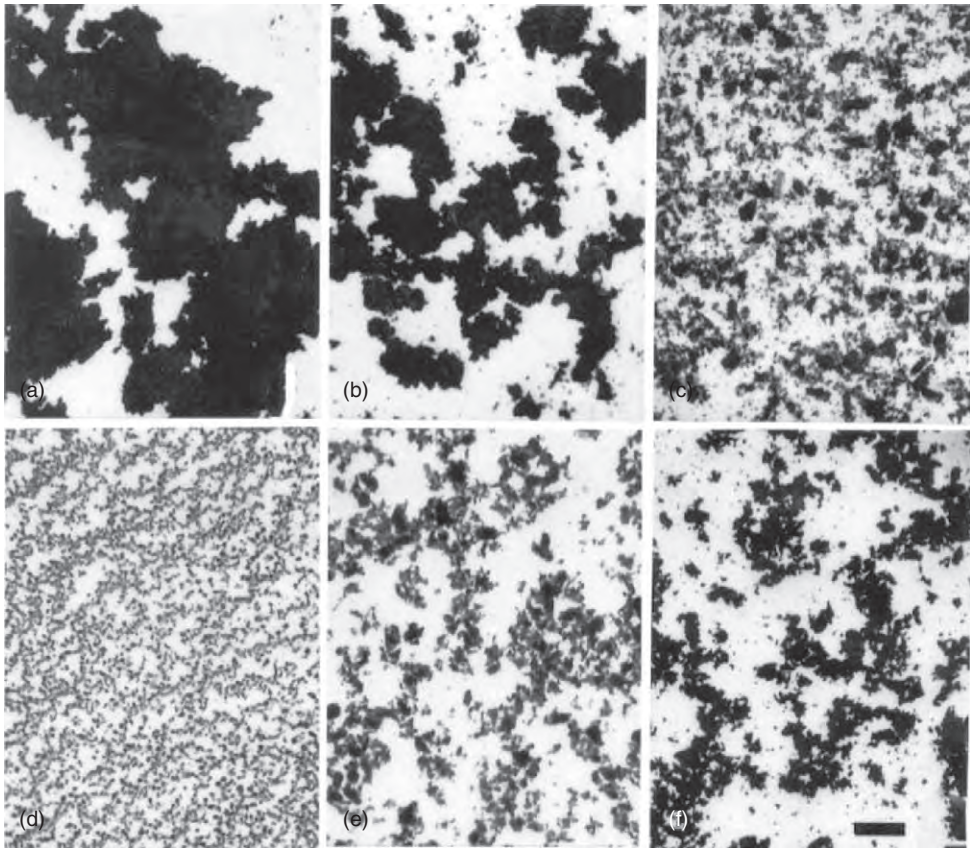


Figure 8.5 Microscopic analysis of casein micelles aggregates. Samples of milk casein aggregates obtained with (A) high molecular weight chitosan (MWC), (B) medium MWC, (C) low MWC, (D) chitosan oligosaccharides, (E) rennet, and (F) acid were analyzed and photographed in a light microscope at 50 \times . Bar: 200 μ m. Reproduced with permission from Ausar et al. (2001).

However, the specific use of chitosan in products remains limited through international regulation (chitosan does not yet have an e-number), as well as more specific issues, such as a tendency to impart astringency in foods. Nonetheless, studies have been carried out on the potential uses of chitosan in food applications. A particular example is the use of chitosan to isolate β -lg (Casal et al., 2006) and glycosylated caseinomacropeptide from whey (Casal et al., 2005). As discussed earlier, rennet proteolysis of casein liberates the hydrophilic C-terminal polypeptide (CMP). In cheese manufacturing, the CMP fragments are excluded from the curd along with the whey proteins. The C-terminal CMP has a high incidence of anionic amino acid residues that are able to bind to the polycationic chitosan. Intriguingly, glycosylated CMP has a higher affinity for binding to chitosan in comparison with nonglycosylated CMP, allowing for some degree of fractionation between the two macropeptide variants (Casal et al., 2005).

A similar approach has been investigated for the selective fractionation of β -lg from total whey protein. The effects of chitosan addition on the cheese-making process have also been investigated. In the manufacture of Apulia (a spreadable Italian cheese) it was shown that chitosan addition resulted in an improvement to the rheological and sensorial

properties of the product, while having little effect on cheese microflora (Gammariello et al., 2008). Another separate study was carried out on the addition of chitosan to low fat cheese, where it was seen to have no significant effects on rheological properties, microbial counts, and microstructure relative to control. Given that chitosan is reported as having both antimicrobial effects and the ability to complex with enzymes, it may appear unusual that there is such little effect (observed from both studies) of chitosan on the microflora during the cheesemaking process. A study by (Ausar et al., 2002) shows that order of addition is important here. Addition of chitosan to milk prior to inoculation appears to result in preferential binding of chitosan to the surface of the casein micelles, thereby reducing the ability of chitosan to inactivate microorganisms. In contrast, addition of chitosan to the microbial broth prior to addition to milk was shown to have an inhibitory effect on subsequent bacterial growth.

8.2.4.2 *Thermodynamic incompatibility*

Where no charge interaction is able to take place between milk protein and polysaccharide, a dissociative relationship between the two phases may occur as a consequence of thermodynamic incompatibility. The limited cosolubility that is commonly observed for biopolymer mixtures of proteins and polysaccharides is caused by an excluded volume effect that exists for systems with disparate structures in solution (such effects can also occur for structurally dissimilar polysaccharides, for mixtures of native and denatured proteins, and for different classes of protein). Excluded volume occurs based on the principle that the hydrated volume of one molecular component of a thermodynamically incompatible system will diminish the volume available for each subsequent molecule, as well as for molecules of the opposing species. As molecules compete for solution space, such excluded volume effects lead to repulsive interactions between macromolecules, leading to phase separation for structurally dissimilar biopolymers. The nature and magnitude of such effects are very much dependent on the structural (dis)similarity between the two biopolymers, concentration, solvent effects (ionic strength, pH), and other factors affecting hydration properties, such as temperature.

For casein protein systems, where there is usually considerable structural dissimilarity between the micelles and added polysaccharides, phase separation is commonly observed (Hemar et al., 2001). For $\text{pH} > \text{pI}$, both neutral polysaccharides (e.g., locust bean gum, guar gum, and agar) and anionic polysaccharides (alginates, carrageenans) can cause phase separation. For $\text{pH} < \text{pI}$, nonionic polysaccharides can still have such effects, but for anionic polysaccharides, the situation is more complex due to electrostatic interactions with the protein, as discussed earlier. Regions of thermodynamic incompatibility based on the relative concentration of both biopolymers can be mapped out using a phase diagram, such as that for casein-sodium alginate (Figure 8.6). The curved line is known as the cloud point line, or bimodal. For concentrations of protein and polysaccharide that fall below this line, the system will exist as a single phase. Above the line, phase separation into separate protein and polysaccharide rich domains will occur. The “tie-lines” that run across the bimodal show the final relative concentration of each biopolymer and their phase-separated state for any combination of biopolymer concentration that falls on that particular tie line. The relative concentration of biopolymer in the phase separated state is given by tracking the tie line back to the bimodal.

While there are variations in the concentrations required to induce phase separation for different biopolymers, most systems will display phase separation behavior above a

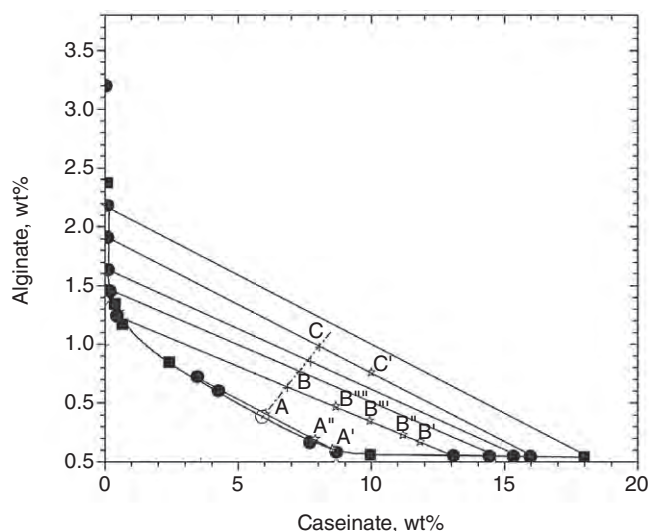


Figure 8.6 Isothermal phase diagram of water-sodium caseinate-sodium alginate system at 23°C, pH 7.0, $I = 0.002$. ●, Composition of coexisting phases; ■, other points on the phase diagram; ○, critical point. Reproduced with permission from Antonov and Moldenaers (2011).

combined polymer concentration of ~4%. For the casein system, the effectiveness of different anionic polysaccharides in inducing phase separation has been studied, decreasing in the order: pectin > carboxymethyl cellulose > sodium alginate > gum arabic > dextran sulfate (Morris, 1990).

The kinetics of biopolymer phase separation can result in some interesting structural events. Depending on relative concentration, one biopolymer can be dominant forming a continuous phase in which the less dominant biopolymer forms a dispersed phase through a mechanism of spinodal decomposition. Gradual ripening/demixing of this dispersed phase will lead to increasing size of particle gradually leading to phase separation. By adjusting the concentration of both biopolymers, it is possible to “invert” this water-in-water type emulsion system, such that a protein continuous system becomes dispersed and vice versa for the polysaccharide phase. At an intermediate concentration, it is even possible to create a bicontinuous system. Many of these biopolymer systems are able to form gels, and depending on the degree of phase separation, it is possible to fix either the continuous phase or the dispersed phase to kinetically trap the structures, leading to some interesting rheological properties.

Studies on the phase behavior of casein/milk proteins with guar gum (Bourriot et al., 1999b; Antonov et al., 2007), iota-carrageenan (Garnier et al., 2003), κ -carrageenan (Bourriot et al., 1999a), sodium alginate (Antonov and Moldenaers, 2011), amylopectin (de Bont, van Kempen et al. 2002), locust bean gum (Schorsch et al., 1999, 2000), and xanthan (Hemar et al., 2001) have all been reported.

The ability to structure water-in-water systems is also affected by solvent conditions (pH and ionic strength). For example, in the case of caseinate/pectin mixtures, water-in-water emulsions can be formed for $\text{pH} > 6$ (Figure 8.7). The rate of demixing between the two phases was retarded at higher viscosities, achieved by either increasing pectin concentration, or through lowering of temperature. Interestingly, lowering the pH

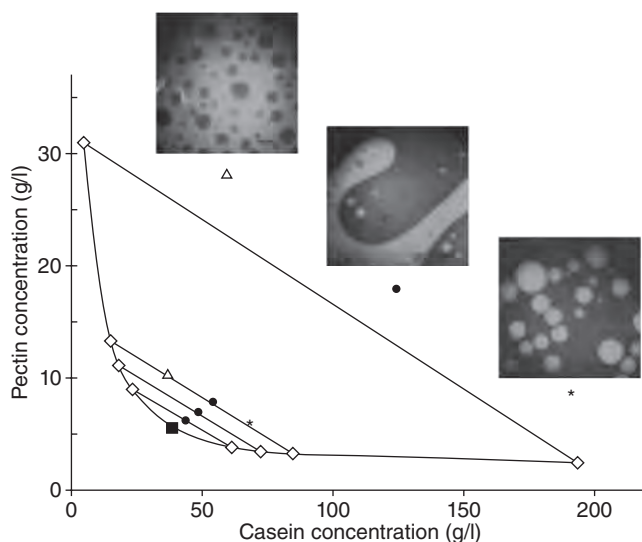


Figure 8.7 Phase diagram of pectin/casein mixtures at pH 7 in 0.01 M phosphate buffer: diamonds, binodal points; circles, initial concentrations; square, critical point; thick full curve, binodal; thin full line, tie lines. Images show mixtures with different volumes of polymer phases for the same tie line: upper, 75% pectin/25% caseinate; middle, 50% pectin/50% caseinate; lower, 25% pectin/75% caseinate. Bright areas correspond to the pectin-rich phase. Reproduced with permission from Rediguieri, de Freitas et al. (2007).

during water-in-water emulsion formation allows the formation of caseinate-pectin microparticles of $\sim 3\mu\text{m}$ in diameter. Once formed, these microparticles were able to maintain size and were insensitive to the presence of salts (Rediguieri, de Freitas et al. 2007).

In terms of applications, mixed milk protein and polysaccharide components can be found in many foods, notably ice creams, yogurts, cream cheeses, milkshakes, and other dairy-based foods. The phase behavior in these more compositionally complex systems is not always well understood. However, the contribution of mixed biopolymer systems is increasingly being considered as a tool to control microstructure and material properties of foods. For example, the addition of polysaccharides to ice cream mixes is common formulation practice for improving quality by enhancing texture, reducing ice crystallization and improving melt stability. In the frozen structure, the relative compositions of protein and polysaccharide are concentrated due to ice formation, and any structures in the remaining water phase are kinetically trapped, preventing any phase separation from occurring through thermodynamic incompatibility.

However, prior to freezing, ice cream mixes may be prone to instability due to the presence of protein and polysaccharide at concentrations where phase separation can occur. A recent study by Vega and Goff (2005) investigated phase separation in soft-serve ice cream mixes containing milk protein and locust bean gum. For concentrations typically used in the manufacture of soft-serve ice cream, phase separation was observed in the mix during a 21-day storage period. Interestingly, the addition of a second polysaccharide, namely κ -carrageenan, resulted in an interaction with the casein, appearing to allow greater mixing of the protein into the serum phase, inhibiting serum separation. The additional stability

imparted to the system as a consequence of κ -carrageenan in this case was not thought to be due to the formation of a weak gel.

The protective effects of added polysaccharides on the ice recrystallization process are reported as being enhanced as a consequence of phase separation. A report by Regand and Goff (2002) showed that thermodynamic incompatibility between biopolymers (locust bean gum, carboxymethyl cellulose, xanthan gum, and gelatin) promoted localized high concentrations of milk proteins that were located at the ice crystal interface. It was speculated that these were able to provide enhanced water-holding capacity that improved the stabilizer effect. The extent of phase separation was seen to be directly proportional to ice crystal growth inhibition. It was suggested that water-holding by the stabilizer and proteins caused a reduction in the kinetics of the ice recrystallization (Regand and Goff, 2002). Further information relating to the effects of polysaccharide stabilizers on ice crystallization and recrystallization in ice cream is provided by Adapa et al. (2000) in a review article.

The effects of polysaccharide addition have also been assessed in other food formats. For example, a study by Jana et al. (2008) investigated the addition of xanthan, carrageenan, or locust bean gum to casein-based Mozzarella analogs and their effects on material and product properties. Intriguingly, polysaccharide addition did not appear to greatly influence the (measured) textural profile of modified cheeses relative to controls, although carrageenan and xanthan were able to more effectively incorporate oil into the casein matrix, reducing the amount of oil exudation during baking. Sensory analysis showed that xanthan gum addition provided a more acceptable texture. From a structural perspective, it is difficult to ascertain what effect the addition of the various polysaccharides is having. The fact that texture analysis showed few differences would suggest that the protein network dominates the structure, and that under these conditions, the mixture of protein and polysaccharide is less prone to phase separation. In separate studies, the addition of pectin (Macku et al., 2008) and carrageenan (Cernikova et al., 2008) to processed cheese has been evaluated. However, for these more acidic systems, interactions between proteins and polysaccharides may be associative rather than dissociative.

One final product area where mixed biopolymer interaction has been used to influence the structural and material properties of the product is in the development of low-fat spreads (Chronakis and Kasapis, 1995). For example, addition of maltodextrin to low fat oil-in-water spread emulsions has been investigated (Chronakis, 1997). The system was designed such that steric exclusion between the milk protein and the maltodextrin would lead to the creation of a discrete maltodextrin phase that would be able to act as a fat mimetic. Addition of increasing amounts of maltodextrin was seen to result in an increase to the elastic nature of the model water continuous spread systems. Indeed, the ability to produce and fix polysaccharide droplet structures through phase separation in milk protein systems is seen as a potential means of controlling texture and achieving (partial) fat replacement in a broad range of soft solid products. Biopolymer addition (LM pectin and alginate) has also been investigated in the formulation of oil continuous low-fat spreads (Madsen, 2000). It was observed that the addition of the polysaccharides to the dispersed water phase had an influence on a number of product parameters, including the stability, appearance, texture, droplet size distribution, and, accordingly, microbiological stability of the spread, and the melting profile showed an effect on meltdown properties in the mouth and flavor release. The specific rheological aspects of the water phase that appeared to dominate product properties were attributed to specific interactions between the milk protein and the polysaccharides, most likely due to a mechanism of phase separation.

8.3 APPLICATIONS OF WHEY PROTEIN STRUCTURING IN FOODS

The higher concentration of casein proteins in milk relative to the whey proteins is reflected in the fact that the primary structuring component in most milk-based foods, such as yogurt and cheese, is the casein micelle. However, as has been noted, interactions between micelles and whey proteins can have important consequences for the material properties of such products. In particular, the covalent binding of β -lg to κ -casein on the micellar surface, as a consequence of thermal processing, is attributed to the enhancement of yogurt rheological properties and texture, while conversely reducing the effectiveness of rennet coagulation.

Recently, the benefits of concentrated and isolated whey proteins as functional and nutritional ingredients has gained increasing recognition (de Wit, 1998; Jayaprakasha and Brueckner, 1999; Mulvihill and Ennis, 2003). The amino acid composition of whey protein is considered nutritionally superior to that of casein and many other protein sources. Accordingly, where whey was once considered essentially a waste product of cheese manufacture, it is now seen as a high value commodity, particularly as an ingredient and fortifying agent in protein-dense products, such as nutrition/meal bars and beverages.

The functional properties of isolated whey protein tend to focus on two particular aspects, pH stability and thermal stability. Unlike casein proteins, a lowering of pH toward the isoelectric point does not result in precipitation of whey protein. Accordingly, acid-based beverages, such as fruit drinks or carbonated soft drinks, which are typically produced at a pH of ~ 3 , can be fortified with whey protein while maintaining good protein solubility and stability. Additionally, the whey protein in such products has a relatively bland flavor (Ozer and Kirmaci, 2010).

The thermal properties of whey protein are also recognized as providing an important textural contribution to many food products. Both β -lg and α -lactalbumin (α -la) are globular proteins and are susceptible to denaturation under a range of applied stresses, such as thermal, high pressure, or treatment with denaturing chemicals, such as urea. For food applications, thermal treatments are most commonly used to induce denaturation (Singh and Havea, 2003). In the case of β -lg, the initial denaturation temperature is specified at 78°C based on differential scanning calorimetry (DSC) analysis, while for α -la, the onset of denaturation occurs at a slightly lower temperature, 62°C (Dewit and Klarenbeek, 1984; Kinsella and Whitehead, 1989). Denaturation results in unfolding of the native protein structure, revealing much of the internal structure. The exposure of interior residues, most notably hydrophobic regions of the molecule, can enhance the functional properties of the protein, particularly in relation to its amphiphilicity. Unfolded globular proteins have been shown to exhibit improved foaming and emulsification properties. However, such improvements are very much dependent on limiting the extent of protein aggregation that is also a consequence of denaturation. For systems where extensive aggregation is able to take place, the reduction in solubility comprises the ability of the protein molecules to act as amphiphiles, with a subsequent loss of interfacial functionality.

Protein aggregation is, however, an important mechanistic aspect of the formation of whey protein gels. The aggregation is a consequence of the exposure of internal thiol groups that are then able to participate in intermolecular disulfide covalent cross-linking. Under the right conditions, the formation of highly cross-linked aggregation results in whey protein gelation. First, a minimum concentration of protein is required to create a sufficiently percolated structure to support formulation of a gel. Even ignoring the effects of the pH and ionic environment, the concentration of whey protein required to form a gel

shows variation depending on the source of the protein. For whey protein concentrates, a minimum concentration of 7.5% is required to form a rigid gel (100°C, 10 minutes), as reported by Zirbel and Kinsella (1988), while Matsudomi et al. (1991) reported that a protein concentration of 14% was required for gelation to occur (90°C, 15 minutes, pH 7). The apparent difference in protein concentration may be attributed to other compositional variations between whey protein concentrates and isolates, particularly in relation to the balance of lactose and mineral content.

The minimum protein concentration required for gelation to take place is strongly influenced by electrostatics. At neutral pH and in the absence of added salts, the whey proteins carry a net negative charge. Electrostatic repulsion between molecules therefore requires a higher level of protein to provide sufficient molecular interaction to enable disulfide bridges to form. Screening of the protein charge by adjusting pH closer to the isoelectric point or through the addition of salt reduces the charge repulsion between protein molecules, facilitating intermolecular disulfide bond formation at lower protein concentrations.

Gel strength is also affected by both protein concentration and aqueous environment. Not surprisingly, gel firmness increases with increasing protein content. With regards to pH and ionic strength, gel strength is reduced in situations where protein repulsion is either maximized or minimized. Under conditions where electrostatic repulsion is enhanced, gel strength decreases due to a reduction in protein–protein interactions. However, minimization of protein interactions also results in a reduction in gel strength due to the formation of densely packed aggregates rather than finely stranded structures. A balance between protein–protein and protein–solvent interactions is therefore required to optimize gel strength (Havea, 2003). The ability to modulate gel rheology in this way is an important consideration in formulating foods dependent upon whey gelation. As such, whey protein gels are used in a number of food product areas to provide texture modification or ingredient replacement. In particular, whey protein gels are used in comminuted meat products as a water binder and texturizer, as well as assisting in the stabilization of meat emulsions. Typical products include luncheon meats, frankfurters and sausages. Whey proteins are also used to enhance texture in bakery products, providing an equivalent functionality to egg proteins, thereby allowing replacement of egg from such products (Mulvihill and Ennis, 2003).

More recently, the specific use of microparticulated whey protein has seen application as a fat replacer in many different food systems, particularly dairy products such as cheeses, yogurts, and spreads (Tamime et al., 1995; Muir et al., 1999; Sandoval-Castilla et al., 2004) (Figure 8.8).

Microparticulation of whey protein is achieved by combining heating and shearing of the protein under controlled pH conditions (Singer and Dunn, 1990; Cheftel and Dumay, 1993). For example, whey protein particles of between 0.1 and 3 μm have been prepared by shearing a whey solution at a rate in excess of 5000/min across a range of temperatures (80–120°C from 3 seconds to 15 minutes) and pH 3.7–4.2. Once formed, the microparticulated proteins can be incorporated into a range of food products, and remain relatively unchanged by any further processing. The gel strength and size of the particulates produced provide a textural perception not dissimilar to that of fat droplets. They are therefore used as a fat mimetic for lowering or replacing the fat content in emulsion-based foods. While the use of microparticulated whey proteins provides adequate textural substitution for fat in such systems, it is unable to mimic the flavor profile that is imparted by fat in such products (Muir et al., 1999).

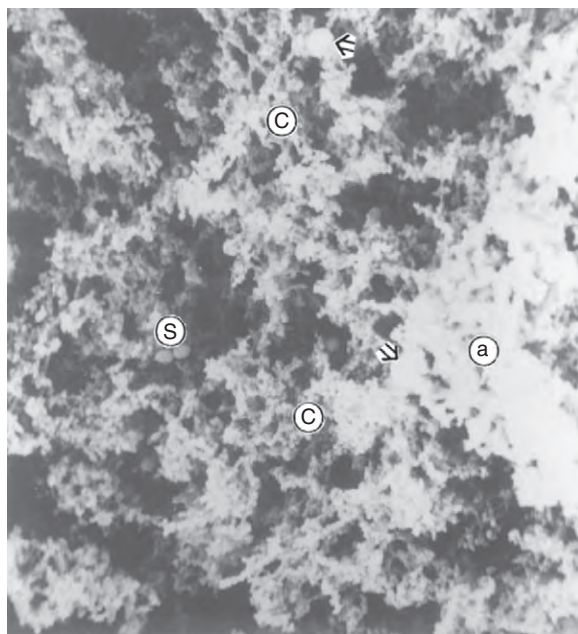


Figure 8.8 Microparticulated whey protein incorporated into set yogurt microstructure. Network consists of casein micelles chains (c), with microparticulated whey protein (arrows) and few fused casein micelles aggregates (a) (magnification is 5400 \times) scale bar = 1 μ m. (Sandoval-Castilla et al., 2004).

8.4 MILK PROTEINS AS EMULSIFIERS

Both casein and whey protein fractions are known to possess excellent emulsifying ability (Dickinson, 2003b), and are accordingly used to both stabilize and structure emulsions in a wide array of food systems, from simple milk to ice creams, yogurts, spreads, cream liqueurs, and processed cheeses.

The highly effective emulsifying properties of casein are attributed to the disordered structures on the individual proteins coupled with the portioning of hydrophobic and hydrophilic amino acids into well-defined regions within the primary structure. For β - and κ -casein in particular, the molecules behave almost as classic amphiphiles, with each protein possessing a distinct hydrophilic domain (the *N*-terminal in the case of β -casein and the *C*-terminal in the case of κ -casein) and hydrophobic domain. In the case of β -casein, the molecule is sufficiently disordered and amphiphilic so as to form micelles in solution above a well-defined critical micelle concentration (CMC) (Leclerc and Calmettes, 1997). The α_s -caseins are generally more hydrophilic and do not possess archetypal headgroup/tailgroup arrangements in the same manner as β - and κ -casein (Dickinson and Matsumura, 1994). For α_{s1} -casein, the primary structure is characterized by three distinct hydrophobic regions: residues 1–44, 90–113, and 132–199. Of the two hydrophilic domains located between these hydrophobic sections, the domain encompassing residues 45–89 is the most highly charged, comprising 7 of the 8 phosphoserines on the molecule. The α_{s2} -casein fraction is characterized by two distinctive hydrophobic regions: residues 160–207 located at the *C*-terminal end of the molecule, and a central region from residues 90–120. The

remainder of the molecule is predominantly hydrophilic with three separate clusters sharing the 11 phosphoserine amino acids.

The partitioning of hydrophobic and hydrophilic domains affects the structural arrangement of the casein proteins when adsorbed to an oil–water interface. Mostly, these can be based on the context of the “train–loop–tail model,” which applies primarily to flexible (synthetic) homo- and copolymers, but can be adapted to disordered proteins, such as the caseins (Horne, 1999). The “train–loop–tail model” implies that depending on localized composition, segments of a biopolymer chain can reside in close contact with the interface (in the form of trains), or can extend into (either of) the solvent phases in the form of loops (for segments within the body of the molecule) or tails (for terminal segments). Taking β -casein at the oil–water interface as an example, highly hydrophilic regions of the molecule, notably the N-terminal sequence, form a tail structure extending 5 nm into the aqueous phase at pH 7 (Dickinson, 2003b). The remainder of the molecule, being predominantly hydrophobic in nature, forms a mixture of train structures bound closely to the interface coupled with highly hydrophobic regions that are solvated into the oil phase in the form of additional loops and tails (Dalgleish and Leaver, 1991; Atkinson et al., 1995).

In contrast to the disordered casein proteins, the globular whey proteins adopt a different structural model at the air–water interface. In solution, these proteins have a higher degree of secondary and tertiary structure that serves to minimize the exposure of hydrophobic amino acid residues to the aqueous environment. Generally, the native structure is retained on initial adsorption but undergoes time-dependent unfolding, allowing buried hydrophobic residue to be exposed to the oil–water interface (Corredig and Dalgleish, 1995; Fang and Dalgleish, 1997). The degree of unfolding and interaction between adsorbed protein molecules is very much dependent on other factors, such as temperature.

The differences in adsorption behavior and structure between the casein and whey protein variants is manifest in a number of other interfacial properties (Atkinson et al., 1995), such as surface coverage (whey proteins generally have lower equivalent surface coverage than casein proteins, and micelles in particular are notable for providing a particularly thick interfacial layer, although this can change with time), surface rheology, and surface charge. In spite of these differences, at neutral pH and low ionic strength, both casein and whey proteins can confer excellent stability to emulsions through electrostatic and steric stabilization. Unfortunately, most foods do not exist under such ideal conditions, and the stability of milk protein-based emulsions can be readily compromised by a number of different factors, including pH, ionic strength, the presence of divalent cations, and thermal and high pressure processing. However, natural serendipity coupled with the creativity of food technologists have exploited the instability of dairy protein emulsions under such conditions to create diversely structured emulsion systems through mechanisms such as flocculation. This in turn can impart significant changes to the material and organoleptic properties of a product. Accordingly, the choice of a particular milk protein system in relation to the other constituents and processing pathway is an important consideration in the design of food products comprising dairy proteins.

A good example is cream liqueurs (Muir, 1989). These are neutral alcoholic beverages typically containing 16% milkfat, 3.3% casein protein (in the form of sodium caseinate), 19% added sugar, and 14% alcohol. The emulsion is typically prepared at 45–55°C to ensure that the fat is fully molten, otherwise the system will not homogenize effectively. The product is typically expected to have a shelf life in excess of a year. To achieve this, the emulsion droplets need to be sufficiently small and noninteracting so as not to display significant creaming over this timeframe. In this respect, sodium caseinate has been found

to be far superior to any other protein system considered for this particular application. Under the high pressure homogenization conditions used, the protein is able to produce droplets of mean particle size distribution in the range of 0.2–0.6 μm (Heffernan et al., 2009). At pH close to neutral, the casein proteins provide excellent charge and steric stabilization, thereby minimizing any interactions between droplets. Maintaining stable droplets in this size range is sufficient to provide an extended shelf life to the product with no apparent loss of overall product stability or quality. However, it should be noted that additional considerations regarding formulation need to be made to ensure effective emulsion stabilization is maintained.

Emulsion stability may be compromised simply through varying the concentration of the protein (Dickinson et al., 1997; Dickinson and Golding, 1998). Studies have shown that caseinate-stabilized emulsions occupy a relatively small concentration window of stability. At protein concentrations below those required to provide adequate surface coverage on droplets (typically less than a third of saturation coverage), emulsions are prone to a bridging mechanism as individual protein molecules can become surface bound to more than one droplet, leading to droplet aggregation.

At a caseinate concentration in excess of that required for full surface coverage, non-adsorbed caseinate (submicelles) can lead to flocculation through a depletion mechanism in which protein submicelles are excluded from droplets. Both bridging and depletion mechanisms will result in significantly reduced stability for this product (Figure 8.9).

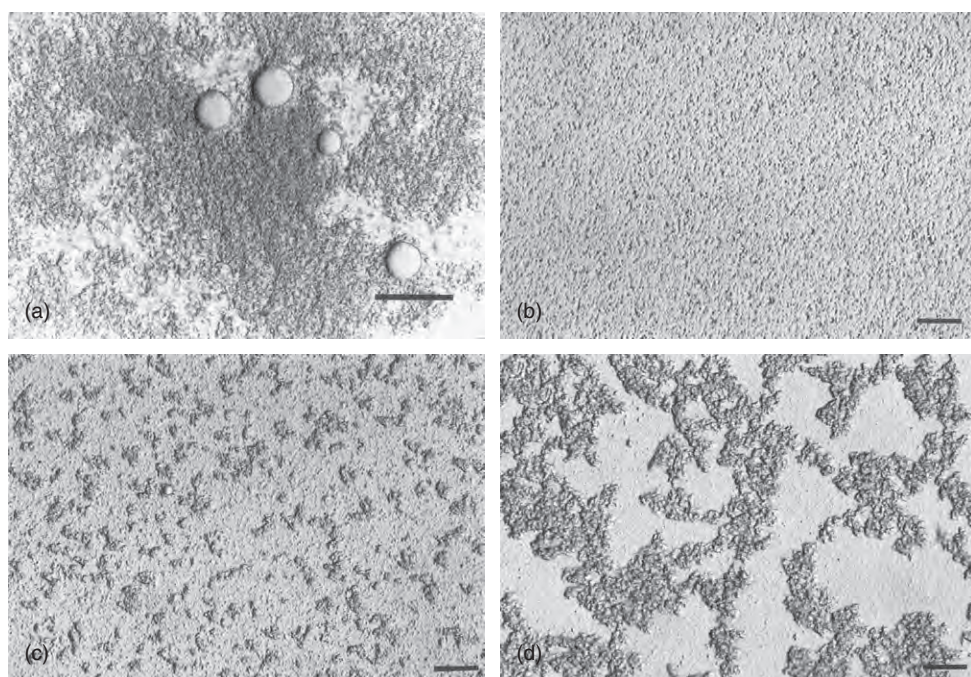


Figure 8.9 Micrographs depicting stability behaviors for caseinate stabilized depending on protein concentration. Figure (A)—below saturation coverage bridging flocculation and coalescence observed; (B)—saturation coverage, stable emulsion; (C and D) extend of depletion flocculation shown to correlate with increasing unbound caseinate concentration. Reproduced with permission from Dickinson et al. (1997).

The relative content of ethanol is also a consideration in this product. Both protein and emulsion phases are tolerant of alcohol addition at the usual content of 14% for such beverages. In fact, alcohol content can be increased to as much as 25% while maintaining acceptable stability (Banks and Muir, 1985).

However, increasing alcohol concentration above these levels leads to a combination of protein precipitation and droplet aggregation (believed to be due to a flattening of the interfacial protein layer), resulting in rapid, visible coagulation of the beverage. This effect can be easily demonstrated by mixing a cream liqueur with a spirit of higher alcoholic content. Another particular formulation issue that was noted to have a particular effect on long-term emulsion stability was the presence of residual calcium from the cream and caseinate sources. Calcium levels were believed to be sufficiently high ($>10\text{mM}$) so as to cause electrostatic bridging between caseinate stabilized droplets, leading to rapid creaming and phase separation. To combat this effect, a sequestrant, usually sodium citrate, is incorporated into commercial cream liqueur formulations to prevent calcium-induced flocculation from taking place (Banks et al., 1981).

Casein as an emulsifier is also known for its heat tolerance during processing. Due to the disordered structure of the individual proteins and the lack of cysteine residues, the caseins do not display denaturation or aggregation characteristics typical of most globular proteins. The casein micelle itself is susceptible to some changes on heating above 70°C , mainly due to binding of serum whey protein to the micelle surface (Smits and Vanbrouwershaven, 1980). In the particular case of sodium caseinate, a product depleted of whey protein, the thermal stability of the system is even higher, such that the system can be heated to 120°C for up to 20 minutes with no significant changes in structure being observed, although some changes in functional properties have been observed at longer treatment times (Guo et al., 1996). For most milk protein systems based on casein: whey ratios found in whole milk, the higher concentration of casein protein relative to whey (typically 80:20) ensures that from an emulsion perspective, the interface is predominantly coated with casein proteins. Most processes generating neutral emulsions using dairy proteins, such as homogenized milks and (nondairy) creams, ice cream, and yogurts will involve a heating step to ensure microbial inactivation. For relatively short heat treatments, from pasteurization conditions through to sterilization, the functionality of the caseins is not significantly altered, and emulsions with a high degree of stability can usually be prepared. Emulsion stability can be optimized against thermal processing by using whey-depleted protein fractions, such as sodium caseinate.

Whey proteins, as noted earlier, are less heat tolerant. Unfolding of the native structure exposes the free thiol groups that are accordingly able to participate in the formation of intermolecular disulfide bridges. This holds true for interfacial β -lg molecules on the surface of droplets, which can form associations with surrounding molecules, both on the surface (leading to a strengthening of the interfacial layer), but also to surface proteins on neighboring droplets, and any nonadsorbed protein present in the aqueous phase. Emulsions stabilized purely by whey protein will therefore be prone to varying degrees of aggregation on heating above 78°C , the extent of which will be dependent on a number of factors, including protein concentration, oil phase volume, specific temperature and holding time, variations in both pH and ionic environment, and the presence of other functional components, such as surfactants (Chen and Dickinson, 1998; Chen et al., 2000; Kerstens et al., 2006). For milk systems, while the dominance of casein at the interface mostly prevents droplet aggregation from taking place, extreme thermal treatments, such as retort sterilization, can result in association taking place even for relatively low concentrations of whey

protein bound to the interface. This can also be an issue for UHT-sterilized evaporated milks, where the relative concentrations of protein and fat are increased due to the removal of water, and therefore the system is more prone to aggregation (Schmidt et al., 1971). However, it should be noted that the strength of droplet aggregates formed through covalent interactions may show resistance to homogenization, and thus even a second homogenization step may not serve to return the postheat-treated emulsion to its original droplet size.

While these examples of droplet aggregation highlight some of the technical issues relating to the formation of stable emulsion systems, the deliberate destabilization of emulsions through heat-induced aggregation can be used to enhance the structural and material properties of a number of products. Heat-treated emulsions stabilized with whey protein can form emulsion-based gel systems. The particular structure of the emulsion gel is very much dependent on the relative concentration of both protein and oil phase volume (as well as ionic strength and pH).

Systems for which the oil content is high in relation to the protein in the aqueous phase are more likely to form particulate type gel systems, in which droplets are either connected to each other directly through interfacial covalent bridges, or through the formation of localized protein bridges. In contrast, high concentrations of protein relative to the oil phase volume are more likely to result in continuous protein networks in which the emulsions are embedded as active filler particles (i.e., interfacial protein will be bound to protein in the continuous phase) (Dickinson and Chen, 1999). Depending on the type of structures formed, systems with quite different gel strength can be produced. By and large, covalent gels of this type tend to result in the formation of elastic textures, with fracture properties ranging from brittle to springy depending on formulation and process. Such gelling systems are of interest in food systems, most notably in comminuted meat products, such as frankfurters, where whey proteins are used to emulsify fat, which then contributes to the texture of the finished product through formation of a gelled network during subsequent cooking (Correia and Mittal, 2000). Another application area where this approach can be useful is in the manufacture of pet foods.

Other forms of droplet interaction, such as through loss of steric and charge repulsion, electrostatic bridging, and depletion flocculation, can be used to create emulsion droplet networks and gels. Again, the formation of a structured emulsion can provide a valuable contribution to the material properties of the product, thereby improving texture. For casein-/milk-based emulsion systems, loss of electrostatic stability and a flattening of the interfacial layer upon lowering of pH can cause droplet aggregation through localized charge interactions. For products such as yogurt, the protein stabilized interface of emulsion droplets can become cross-linked into the protein network (Barrantes et al., 1996; Houze et al., 2005). Because the fat content of yogurt is generally low, these droplets act more as independent active filler particles that may result in some reinforcement of the protein network and slightly higher viscosities. There is also a pronounced effect on texture with increasing fat levels.

The presence of emulsion droplets as active filler particles (i.e., the droplet interface can directly interact with molecules solubilized in the continuous phase) provides a structural component in higher fat products, such as processed cheeses (Heertje and Lewis, 1993; Mistry and Anderson, 1993). For regular full fat processed cheeses (typically 22–25% fat), fat droplets are seen to be interspersed in a diffuse protein matrix. This structure is achieved through the addition of emulsifying salts, which sequester calcium, thereby improving the solubility of the protein in the product and allowing this protein to act as an emulsifier for the fat in the system. Without emulsifying salts, the protein remains predominantly as

precipitated aggregates, and there is no subsequent means for stabilizing the fat in the system. With the addition of emulsifying salts, the presence of protein-stabilized fat droplets binding to the protein in continuous phase with the system improves firmness while inhibiting the overall elasticity generated by the continuous protein network. Comparison with low fat analogues of processed cheeses shows the protein network dominating texture resulting in a more elastic, gel-like mouthfeel (Johnson et al., 2009).

For other acid products, such as cream cheese, the behavior of the emulsion system has a pronounced contribution to the texture of the product. For cream cheeses, the addition of starter cultures serves to lower the pH of the system by conversion of lactose to lactic acid. The microstructure of cream cheese has been characterized as a heterogeneous structure populated by clusters of protein-coated fat globules (Monteiro et al., 2009). Protein aggregates are shown to act as bridges between localized regions of flocculated fat, resulting in the formation of a mixed protein-emulsion droplet network. In terms of texture, the characteristic spreadability and smooth texture of cream cheese is attributed to the disruption of the protein structures by fat clusters, preventing excessive elasticity while providing additional firmness to the overall structure. Given that the acidic environment is the mechanism by which both continuous phase protein and emulsion droplets are caused to aggregate, it is not surprising that variations in the pH of the system will have an effect on structure and material properties. As indicated earlier in the chapter, the isoelectric point of whole casein occurs at pH 4.6, and it is at this pH that stability of both aqueous phase and interfacial protein will be at a minimum, resulting in both formation of protein and emulsion aggregates. Control of formulation and processing conditions is particularly important for maintaining an acceptable texture. The formation of densely clustered protein aggregates, for example, occurring through excessive heat treatment or inappropriate acidification, can result in a grainy rather than a smooth texture (Sainani et al., 2004).

Cream cheese firmness is strongly influenced by the pH. Experimentally, the pH of cream cheese can be varied to either stronger or less acidic conditions after manufacture by exposing the finished cheese to an atmosphere of a volatile base or acid, such as ammonia or acetic acid, respectively. Based on this approach, Aliste and Kindstedt (2005) showed that increasing pH from an initial value of about 4.6–6.3 (using ammonia vapor) resulted in a pronounced decrease in firmness, most likely due to a loss of cohesiveness of the protein network.

Other forms of droplet aggregation can be induced depending on formulation conditions. Electrostatic flocculation can be induced for casein-based emulsion systems, particularly those stabilized by sodium caseinate, through the addition of ionic calcium, which leads to the formation of calcium bridges. Addition of calcium to milk protein-based emulsions is often seen as a technical issue, due to potentially unwanted precipitation of the emulsion. However, under controlled conditions, this approach can be used to modify rheological properties of emulsions. Electrostatic bridging of milk protein-stabilized emulsions by (anionic) polysaccharides does not readily occur at neutral pH for the same reasons that milk proteins and polysaccharides do not directly interact. At $\text{pH} < 4.6$, bridging between protein and anionic polysaccharides can take place as the interfacial protein layer carries a net positive charge. Again, as with protein-polysaccharide systems, the resulting structure is dependent on the relative concentration of both emulsion and polysaccharide components. Complexation between proteinaceous interfaces and anionic biopolymers can lead to bridging flocculation of emulsions at low concentrations. Increasing the concentration of the biopolymer can lead to stabilization of emulsion droplets through layering of the biopolymer at the protein interface, increasing the interfacial layer thickness and producing

additional steric or charge stabilization to the droplets. Further increases in biopolymer concentration can result in increasing amounts of the biopolymer in the aqueous phase, which can again lead to destabilization of the emulsion through depletion flocculation (Dickinson, 2003a; Cho and McClements, 2009).

The formation of layer-by-layer complexes at (milk protein based) emulsion interfaces through aspects such as pH control is currently of particular interest (Surh et al., 2006; Paliandre et al., 2007; Katsuda et al., 2008). Mixed multilayers of this type have potential benefits for a number of food applications. These include the ability to reduce the rate of oxidation of emulsion systems containing labile polyunsaturated oils, thereby maintaining the nutritional benefits of these systems for longer periods. Also of interest is the ability to use interfacial layering to control the rate/extent of emulsion digestion. The formation of multilayer interfaces responsive to the gastrointestinal environment is seen as presenting options for controlled digestion of fat, which may have benefits for managing energy intake, or achieving targeted release of lipid-based nutrients in the gut.

Addition of both anionic and nonionic polysaccharides can often lead to depletion flocculation of emulsion systems beyond a certain polysaccharide concentration (Cao et al., 1990; Dickinson, 1996). Depletion effects arise due to size exclusion of polysaccharide molecules from the intervening space between neighboring droplets. This leads to an osmotic pressure gradient, causing an attractive interaction between droplets. Depletion interactions occur homogeneously within a sample, and while such interactions are generally weak and reversible, they can result in extensive aggregation of emulsion droplets, leading to rapid separation of emulsion systems. However, depletion effects can also be effective for the rheological modification of an emulsion system (Dickinson et al., 1995; Moschakis et al., 2006). Extensive depletion interactions can lead to the formation of highly flocculated droplet networks that exhibit considerably higher viscosities relative to stable, unflocculated emulsion systems of equivalent fat content. Without additional stabilization, such fat networks can also be prone to separation, either through rapid creaming or syneresis (depending on relative fat content). However, the presence of the polysaccharide, in addition to causing the depletion behavior, can be used to stabilize the emulsion through either increasing continuous phase viscosity or by imparting a yield stress which serves to either reduce the rate of separation or immobilizes the droplet network. In this way, the combination of aqueous phase viscosity and viscosity achieved through the formation of the emulsion network can greatly enhance the thickness of the system as a whole. This approach can be used for many emulsion-based foods as a means of enhancing overall texture, particularly in the case of low fat systems, such as reduced oil dressings (Parker et al., 1995). This approach is likewise applicable to dairy protein-stabilized emulsion systems, and again could be beneficial in improving the microstructure and material properties of low fat spreads, yogurts, and cream cheese-type products (Everett and McLeod, 2005; Girard and Schaffer-Lequart, 2007).

8.5 MILK PROTEINS AS FOAMING AGENTS

The amphiphilic nature of milk proteins enables them to adsorb to air–water interfaces, where they serve to lower surface tension and provide a rheological contribution to the interface. In this respect, both casein and whey proteins are able to form and stabilize foam systems (Borcherding et al., 2009), although there are some pronounced differences in the surface properties of the two systems (Marinova et al., 2009). The disordered, block

copolymer characteristics of the casein proteins allow them to readily adsorb to surfaces, effectively lowering surface tension. However, there is very little interaction between casein protein molecules at the interface due to a combination of limited interfacial unfolding, lack of free sulfhydryl amino acid residues, and charge repulsion between hydrophilic segments of the molecules. Accordingly, at neutral pH and low ionic strength, the surface rheology of molecular caseins is actually quite weak compared with many other food protein systems, and while under these conditions the individual caseins all present a high degree of foamability, the foam (Patino et al., 2001) stability of the caseins is relatively poor. However, it should also be noted that for milk-based foams, casein is predominantly in the form of micelles. The structural arrangement of caseins within micelles presents a hydrophilic surface layer, and it has been shown that the casein micelle system is relatively ineffective in adsorbing to bubble surfaces. Studies have shown that for aerated milk systems, the surface layer of bubbles is primarily composed of whey proteins and monomeric caseins (Borcherding et al., 2008).

Native whey proteins, in particular β -lg, are also able to effectively adsorb to the air–water interface, lowering surface tension (Patino et al., 2001). The globular structure of the whey proteins makes them initially less amphiphilic; however, on adsorption to the air–water interface, the protein can undergo a degree of structural unfolding to promote exposure of internal hydrophobic groups to the interfacial layer. The unfolding of secondary and tertiary protein structures leads to greater interaction between molecules at the adsorbed layer, with the resulting protein surface network having a greater surface elasticity as a consequence (Patino et al., 2001). The higher film strength of the whey protein interface imparts good foam stability, particularly with respect to coalescence, and accordingly, whey proteins are considered excellent foaming agents. The surface properties of both casein and whey protein films can be influenced by a range of additional factors, including temperature, pH, ionic environment, and additional formulation aspects (e.g., competitive adsorption, binding of other biopolymers, and the influence of enzymes) (Marinova et al., 2009).

The surface properties of milk proteins see them widely applied as foam stabilizers in aerated foods. Particular food applications include ice cream, whipped cream, mousse, and foamed beverages, such as cappuccino. Of these, ice cream is perhaps the most commercially significant. Ice cream is a frozen foam, typically containing 50% air by volume. In the case of ice creams manufactured without the use of added emulsifiers, the air phase is stabilized by milk proteins during the freezing process, when air is introduced under pressure (Zhang and Goff, 2004a,b). On extrusion, the resulting pressure drop and warming of the ice cream before it can be introduced into a hardening freezer results in an increase in bubble size distribution. For the milk protein stabilized foam, bubble size distribution ranges from 50 to 200 μm in the final product. The addition of emulsifiers promotes bubble stabilization through the adsorption of fat globules to the bubble surface in a form of Pickering stabilization. This interfacial coating provides a more effective form of foam stabilization relative to the protein-coated surface alone. Accordingly, the inclusion of emulsifiers is able to result in a foam structure with a finer bubble size distribution than for protein (50–100 μm) (Zhang and Goff, 2004a). The fact that a finer air structure can be related to improved product quality is one of the reasons why emulsifiers are often included in ice cream formulations.

For whipped creams, the mechanism of foam stabilization is similar to that observed for ice cream, with extensive adsorption of fat globules to the air–water interface providing effective bubble stability. This adsorption is also coupled with extensive partial coalescence

between fat droplets in the serum phase, which provides structural rigidity to the foam. While final foam stabilization of whipping is achieved through attachment of fat to the surface of the bubbles, during the early stages of whipping the incorporated air is stabilized by milk protein. In fact, it appears that the ability of fat droplets to subsequently adsorb to the protein stabilized bubble surface is dependent on the nature of bubble surface rheology imparted by different milk protein fractions. An interesting study by Besner and Kessler (1998) on the effects of milk protein type on whipping properties of nonhomogenized creams showed that substitution of the native milk protein system with specific milk protein fractions resulted in considerable variation in the whipping time required to produce the final structure. Relative to the native protein composition in whipping cream, replacement with undenatured whey protein was found to result in a pronounced increase in whipping time. This increase is considered to be a consequence of whey protein producing a highly viscoelastic interface acting as a barrier to fat globule adsorption. In contrast, the use of microparticulated whey, which did not readily adsorb to the air–water interface, was found to result in a greatly reduced whipping time. Similar results were achieved when increased levels of casein protein were substituted, again reflecting the fact that the casein protein was less effective at providing a viscoelastic interface relative to whey protein.

Recently, Allen et al. (2006) have investigated the use of acidification to improve the foaming properties of homogenized whipping creams. For these systems, the stabilization of fat droplets by protein inhibits the ability of the fat droplets to adsorb to the bubble surface (as opposed to the phospholipid interface present for nonhomogenized fat droplets). Consequently, homogenized creams are ineffective at providing an appropriate whipped structure delivering the characteristic sensory properties associated with whipping creams. The research by Allen et al. (2006) showed that the whipping properties of a 30 vol% oil-in-water emulsion system stabilized with sodium caseinate could be improved if the emulsion was aerated while undergoing gradual acidification. It was observed while the initial foam structure was stabilized with caseinate protein, acidification resulted in the aggregation of fat droplets surrounding bubbles, providing structural stabilization to the foam. Bubble stability was also considered to be improved by protein cross-linking on the surface, and by attachment of fat droplets to the protein network at the air–water interface. While foam stability was greatly improved, significant differences relating to the rheology of this system compared with a nonhomogenized whipping cream were observed. The natural dairy foam was observed to be considerably firmer than the foam produced by the sodium caseinate emulsion. The variation in rheological behavior was attributed to different structuring mechanisms of the fat droplets (flocculation in the case of the caseinate emulsion, partial coalescence in the case of the natural dairy foam), and by the absence of a solid fat component in the case of the caseinate-based emulsion.

8.6 CONCLUSIONS

Milk proteins have been shown to be a constituent part of many diverse food systems and applications. While milk protein addition may be considered to enhance the quality of foods from a nutritional perspective, or through a direct organoleptic contribution in terms of adding “dairy notes” to a product, it has clearly been demonstrated that milk proteins also play a highly diverse functional role in the structuring of many different food products. The particular molecular and macromolecular properties of both casein and whey proteins can be manipulated through processing (thermal and high pressure treatments), biochemical

response (enzymatic modification), and solution properties (pH, ionic strength, and interaction with other ingredients) to create a remarkable array of protein-based food microstructures. These structures are able to impart highly controlled and desirable textures to products, as well as potentially improving stability. The dairy system is also notable for the molecular amphiphilicity of its protein fractions, allowing both casein and whey proteins (and their derivatives) to be used in the stabilization and structural design of both emulsion and foam-based foods. The structuring of milk proteins as a means of controlling the material and textural properties of foods is one of the earliest examples of food microstructure design. The application of milk protein functionality continues to play an important role in the manufacture of many food products within the contemporary food industry. Ongoing research and development into the structure-function properties of these systems continues to provide new opportunities for the enhancement of foods.

REFERENCES

- Abbasi, S. and Dickinson, E. (2001) Influence of sugars on high-pressure induced gelation of skim milk dispersions. *Food Hydrocolloids* **15**, 315–319.
- Adapa, S., Schmidt, K.A., Jeon, I.J., Herald, T.J., and Flores, R.A. (2000) Mechanisms of ice crystallization and recrystallization in ice cream: a review. *Food Reviews International* **16**, 259–271.
- Aliste, M.A. and Kindstedt, P.S. (2005) Effect of increasing pH on texture of full and reduced-fat cream cheese. *Australian Journal of Dairy Technology* **60**, 225–230.
- Allen, K.E., Dickinson, E., and Murray, B. (2006) Acidified sodium caseinate emulsion foams containing liquid fat: a comparison with whipped cream. *LWT-Food Science and Technology* **39**, 225–234.
- Anema, S.G. (2008) Effect of temperature and rate of acidification on the rheological properties of acid skim milk gels. *Journal of Food Processing and Preservation* **32**, 1016–1033.
- Antonov, Y., Lefebvre, J., and Doublier, J.L. (2007) Phase separation in aqueous casein-guar gum systems. *Polymer Bulletin* **58**, 723–730.
- Antonov, Y.A. and Moldenaers, P. (2011) Structure formation and phase-separation behaviour of aqueous casein-alginate emulsions in the presence of strong polyelectrolyte. *Food Hydrocolloids* **25**, 350–360.
- Atkinson, P.J., Dickinson, E., Horne, D.S., and Richardson, R.M. (1995) Neutron reflectivity of adsorbed protein films. In: *Proteins at Interfaces II—Fundamentals and Applications*, edited by T.A. Horbett and J.L. Brash, pp. 311–320. Washington, DC: American Chemical Society.
- Augustin, M.A., Sanguansri, L., and Bode, O. (2006) Maillard reaction products as encapsulants for fish oil powders. *Journal of Food Science* **71**, E25–E32.
- Ausar, S.F., Bianco, I.D., Badini, R.G., Castagna, L.F., Modesti, N.M., Landa, C.A., and Beltramo, D.M. (2001) Characterization of casein micelle precipitation by chitosans. *Journal of Dairy Science* **84**, 361–369.
- Ausar, S.F., Passalacqua, N., Castagna, L.F., Bianco, I.D., and Beltramo, D.M. (2002) Growth of milk fermentative bacteria in the presence of chitosan for potential use in cheese making. *International Dairy Journal* **12**, 899–906.
- Ayto, J. (2002) *An A to Z of Food and Drink*. Oxford: Oxford University Press.
- Banks, W. and Muir, D.D. (1985) Effect of alcohol content on emulsion stability of cream liqueurs. *Food Chemistry* **18**, 139–152.
- Banks, W., Muir, D.D., and Wilson, A.G. (1981) Extension of the shelf-life of cream-based liqueurs at high ambient-temperatures. *Journal of Food Technology* **16**, 587–595.
- Barrantes, E., Tamime, A.Y., Sword, A.M., Muir, D.D., and Kalab, M. (1996) The manufacture of set-type natural yoghurt containing different oils .2. Rheological properties and microstructure. *International Dairy Journal* **6**, 827–837.
- Besner, H. and Kessler, H.G. (1998) Interfacial interaction during the foaming of nonhomogenized cream. *Milchwissenschaft-Milk Science International* **53**, 682–686.
- Borcherding, K., Lorenzen, P.C., Hoffmann, W., and Schrader, K. (2008) Effect of foaming temperature and varying time/temperature-conditions of pre-heating on the foaming properties of skimmed milk. *International Dairy Journal* **18**, 349–358.

- Borchering, K., Chlörenzen, P., and Hoffmann, W. (2009) Effect of protein content, casein-whey protein ratio and pH value on the foaming properties of skimmed milk. *International Journal of Dairy Technology* **62**, 161–169.
- Bourriot, S., Garnier, C., and Doublier, J.L. (1999a) Micellar-casein-kappa-carrageenan mixtures. I. Phase separation and ultrastructure. *Carbohydrate Polymers* **40**, 145–157.
- Bourriot, S., Garnier, C., and Doublier, J.L. (1999b) Phase separation, rheology and microstructure of micellar casein-guar gum mixtures. *Food Hydrocolloids* **13**, 43–49.
- Briscoe, B.J., Luckham, P.F., and Staeritz, K.U. (2002) Gelation of milk protein concentrates induced by moderate hydrostatic pressures. *High Pressure Research* **22**, 633–637.
- Cao, Y., Dickinson, E., and Wedlock, D.J. (1990) Creaming and flocculation in emulsions containing polysaccharide. *Food Hydrocolloids* **4**, 185–195.
- Casal, E., Corzo, N., Moreno, F.J., and Olano, A. (2005) Selective recovery of glycosylated caseinmacropeptide with chitosan. *Journal of Agricultural and Food Chemistry* **53**, 1201–1204.
- Casal, E., Montilla, A., Moreno, F.J., Olano, A., and Corzo, N. (2006) Use of chitosan for selective removal of beta-lactoglobulin from whey. *Journal of Dairy Science* **89**, 1384–1389.
- Cernikova, M., Bunka, F., Pavlinek, V., Brezina, P., Hrabec, J., and Valasek, P. (2008) Effect of carrageenan type on viscoelastic properties of processed cheese. *Food Hydrocolloids* **22**, 1054–1061.
- Cheftel, J.C. and Dumay, E. (1993) Microcoagulation of proteins for development of creaminess. *Food Reviews International* **9**, 473–502.
- Chen, J.S. and Dickinson, E. (1998) Viscoelastic properties of heat-set whey protein emulsion gels. *Journal of Texture Studies* **29**, 285–304.
- Chen, J.S., Dickinson, E., Langton, M., and Hermansson, A.M. (2000) Mechanical properties and microstructure of heat-set whey protein emulsion gels: effect of emulsifiers. *Lebensmittel-Wissenschaft Und Technologie-Food Science and Technology* **33**, 299–307.
- Cho, Y.H. and McClements, D.J. (2009) Theoretical Stability Maps for Guiding Preparation of Emulsions Stabilized by Protein-Polysaccharide Interfacial Complexes. *Langmuir* **25**, 6649–6657.
- Chronakis, I.S. (1997) Structural-functional and water-holding studies of biopolymers in low fat content spreads. *Food Science and Technology-Lebensmittel-Wissenschaft & Technologie* **30**, 36–44.
- Chronakis, I.S. and Kasapis, S. (1995) A rheological study on the application of carbohydrate-protein incompatibility to the development of low fat commercial spreads. *Carbohydrate Polymers* **28**, 367–373.
- Corredig, M. and Dalgleish, D.G. (1995) A differential microcalorimetric study of whey proteins and their behavior in oil-in-water emulsion. *Colloids and Surfaces. B, Biointerfaces* **4**, 411–422.
- Correia, L.R. and Mittal, G.S. (2000) Functional properties of some meat emulsion extenders. *International Journal of Food Properties* **3**, 353–361.
- Dalgleish, D.G. and Law, A.J.R. (1988) pH-induced dissociation of bovine casein micelles. I. Analysis of liberated caseins. *Journal of Dairy Research* **55**, 529–538.
- Dalgleish, D.G. and Leaver, J. (1991) The possible conformations of milk-proteins adsorbed on oil-water interfaces. *Journal of Colloid and Interface Science* **141**, 288–294.
- de Bont, P.W., van Kempen, G.M.P., and Vreeker, R. (2002) Phase separation in milk protein and amylopectin mixtures. *Food Hydrocolloids* **16**, 127–138.
- De Jong, G.A.H. and Koppelman, S.J. (2002) Transglutaminase catalyzed reactions: impact on food applications. *Journal of Food Science* **67**, 2798–2806.
- De Sa, E.M.F. and Bordignon-Luiz, M.T. (2010) The effect of transglutaminase on the properties of milk gels and processed cheese. *International Journal of Dairy Technology* **63**, 243–251.
- de Wit, J.N. (1998) Nutritional and functional characteristics of whey proteins in food products. *Journal of Dairy Science* **81**, 597–608.
- Desobrybanon, S., Richard, F., and Hardy, J. (1994) Study of acid and rennet coagulation of high pressurized milk. *Journal of Dairy Science* **77**, 3267–3274.
- Dewit, J.N. and Klarenbeek, G. (1984) Effects of various heat-treatments on structure and solubility of whey proteins. *Journal of Dairy Science* **67**, 2701–2710.
- Dickinson, E. (1996) Biopolymer interactions in emulsion systems: influences on creaming, flocculation, and rheology. In: *Macromolecular Interactions in Food Technology*, edited by N. Parris, A. Kato, L.K. Creamer, and J. Pearce, pp. 197–207. Washington, DC: American Chemical Society.
- Dickinson, E. (2003a) Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids* **17**, 25–39.

- Dickinson, E. (2003b) Interfacial emulsifying and foaming properties of milk proteins. In: *Advanced Dairy Chemistry—Proteins Part B*, edited by P. Fox and P. McSweeney. New York: Kluwer Academic Plenum Publishers.
- Dickinson, E. and Chen, J.S. (1999) Heat-set whey protein emulsion gels: role of active and inactive filler particles. *Journal of Dispersion Science and Technology* **20**, 197–213.
- Dickinson, E. and Golding, M. (1998) Influence of alcohol on stability of oil-in-water emulsions containing sodium caseinate. *Journal of Colloid and Interface Science* **197**, 133–141.
- Dickinson, E. and Matsumura, Y. (1994) Proteins at liquid interfaces: role of the molten globule state. *Colloids and Surfaces. B, Biointerfaces* **3**, 1–17.
- Dickinson, E., Goller, M.I., and Wedlock, D.J. (1995) Osmotic-pressure, creaming, and rheology of emulsions containing nonionic polysaccharide. *Journal of Colloid and Interface Science* **172**, 192–202.
- Dickinson, E., Golding, M., and Povey, M.J.W. (1997) Creaming and flocculation of oil-in-water emulsions containing sodium caseinate. *Journal of Colloid and Interface Science* **185**, 515–529.
- Doublier, J.L., Garnier, C., Renard, D., and Sanchez, C. (2000) Protein-polysaccharide interactions. *Current Opinion in Colloid & Interface Science* **5**, 202–214.
- Everett, D.W. and Auty, M.A.E. (2008) Cheese structure and current methods of analysis. *International Dairy Journal* **18**, 759–773.
- Everett, D.W. and McLeod, R.E. (2005) Interactions of polysaccharide stabilisers with casein aggregates in stirred skim-milk yoghurt. *International Dairy Journal* **15**, 1175–1183.
- Fang, Y. and Dalgleish, D.G. (1997) Conformation of beta-lactoglobulin studied by FTIR: effect of pH, temperature, and adsorption to the oil-water interface. *Journal of Colloid and Interface Science* **196**, 292–298.
- Fox, P. (2003) Milk proteins: general and historical aspects. In: *Advanced Dairy Chemistry—Proteins*, edited by P. Fox and P. McSweeney. New York: Kluwer Academic Plenum Publishers.
- Fox, P. and Brodtkorb, A. (2008) The casein micelle: historical aspects, current concepts and significance. *International Dairy Journal* **18**, 677–684.
- Fox, P. and Mulvihill, D. (1990) Casein. In: *Food Gels*, edited by P. Harris. London: Elsevier Applied Science. London
- Gammariello, D., Chillo, S., Mastromatteo, M., Di Giulio, S., Attanasio, M., and Del Nobile, M.A. (2008) Effect of chitosan on the rheological and sensorial characteristics of apulia spreadable cheese. *Journal of Dairy Science* **91**, 4155–4163.
- Garnier, C., Michon, C., Durand, S., Cuvelier, G., Doublier, J.L., and Launay, B. (2003). Iota-carrageenan/casein micelles interactions: evidence at different scales. *Colloids and Surfaces B-Biointerfaces* **31**(1–4), 177–184.
- Gauche, C., Vieira, J.T.C., Ogliari, P.J., and Bordignon-Luiz, M.T. (2008) Crosslinking of milk whey proteins by transglutaminase. *Process Biochemistry* **43**, 788–794.
- Girard, M. and Schaffer-Lequart, C. (2007) Gelation of skim milk containing anionic exopolysaccharides and recovery of texture after shearing. *Food Hydrocolloids* **21**, 1031–1040.
- Guo, M.R., Fox, P.F., Flynn, A., and Kindstedt, P.S. (1996) Heat-induced modifications of the functional properties of sodium caseinate. *International Dairy Journal* **6**, 473–483.
- Harte, E., Amonte, M., Luedecke, L., Swanson, B.G., and Barbosa-Canovas, G.V. (2002) Yield stress and microstructure of set yogurt made from high hydrostatic pressure-treated full fat milk. *Journal of Food Science* **67**, 2245–2250.
- Havea, H.S.P. (2003) Thermal denaturation, aggregation and gelation of whey proteins. In: *Advanced Dairy Chemistry—Proteins Part B*, edited by P. Fox and P. McSweeney. New York: Kluwer Academic Plenum Publishers.
- Heertje, I. and Lewis, D.F. (1993) Structure and function of food-products—a review. *Food Structure* **12**, 343–364.
- Heertje, I., Visser, J., and Smits, P. (1985) Structure formation in acid milk gels. *Food Microstructure* **4**, 267–277.
- Heffernan, S.P., Kelly, A.L., and Mulvihill, D.M. (2009) High-pressure-homogenised cream liqueurs: emulsification and stabilization efficiency. *Journal of Food Engineering* **95**, 525–531.
- Hemar, Y., Tamehana, M., Munro, P.A., and Singh, H. (2001) Viscosity, microstructure and phase behavior of aqueous mixtures of commercial milk protein products and xanthan gum. *Food Hydrocolloids* **15**, 565–574.
- Holt, C. and Dalgleish, D. (1986) Electrophoretic and hydrodynamic properties of bovine casein micelles interpreted in terms of particles with an outer hairy layer. *Journal of Colloid and Interface Science* **114**, 513–524.

- Holt, C. and Horne, D. (1996) The hairy casein micelle: evolution of the concept and implications for dairy processing. *Netherlands Milk and Dairy Journal* **50**, 1–27.
- Horne, D.S. (1984) Steric effects in the coagulation of casein micelles by ethanol. *Biopolymers* **23**, 989–993.
- Horne, D.S. (1999) *Caseins: Interfacial Layer Properties and Their Influence on Emulsion Stability*. Amsterdam: Elsevier Science.
- Horne, D.S. (2001) Factors influencing acid-induced gelation of skim milk. In: *Food Colloids—Fundamentals of Formulation*, edited by E. Dickinson and R. Miller, pp. 345–351. Cambridge: Royal Society of Chemistry.
- Houze, G., Cases, E., Colas, B., and Cayot, P. (2005) Viscoelastic properties of acid milk gel as affected by fat nature at low level. *International Dairy Journal* **15**, 1006–1016.
- Huppertz, T., Fox, P.F., and Kelly, A.L. (2004) Properties of casein micelles in high pressure-treated bovine milk. *Food Chemistry* **87**, 103–110.
- Huppertz, T., Hinz, K., Zobrist, M., Uniacke, T., Kelly, A.L., and Fox, P.F. (2005) Effects of high-pressure treatment on the rennet coagulation and cheese-making properties of heated milk. *Innovative Food Science & Emerging Technologies* **6**, 279–285.
- Huppertz, T., Kelly, A.L., and de Kruif, C.G. (2006a) Disruption and reassociation of casein micelles under high pressure. *Journal of Dairy Research* **73**, 294–298.
- Huppertz, T., Smiddy, M.A., Upadhyay, V.K., and Kelly, A.L. (2006b) High-pressure-induced changes in bovine milk: a review. *International Journal of Dairy Technology* **59**, 58–66.
- Hyslop, D. (2003) Enzymatic coagulation of milk. In: *Advanced Dairy Chemistry—Proteins Part B*, edited by P. Fox and P. McSweeney. New York: Kluwer Academic Plenum Publishers.
- Jana, A.H., Pinto, S., and Solanky, M.J. (2008) Quality of casein based Mozzarella cheese analogue as influenced by stabilizers. *Journal of Food Science and Technology-Mysore* **45**, 454–456.
- Jaros, D., Jacob, M., Otto, C., and Rohm, H. (2010) Excessive cross-linking of caseins by microbial transglutaminase and its impact on physical properties of acidified milk gels. *International Dairy Journal* **20**, 321–327.
- Jayaprakasha, H.M. and Brueckner, H. (1999) Whey protein concentrate: a potential functional ingredient for food industry. *Journal of Food Science and Technology-Mysore* **36**, 189–204.
- Jensen, S., Rolin, C., and Ipsen, R. (2010) Stabilisation of acidified skimmed milk with HM pectin. *Food Hydrocolloids* **24**, 291–299.
- Johnson, M.E., Kapoor, R., McMahon, D.J., McCoy, D.R., and Narasimmon, R.G. (2009) Reduction of sodium and fat levels in natural and processed cheeses: scientific and technological aspects. *Comprehensive Reviews in Food Science and Food Safety* **8**, 252–268.
- Kato, A. (1996) Functional protein-polysaccharide conjugates. *Comments on Agriculture and Food Chemistry* **3**, 139–153.
- Katsuda, M.S., McClements, D.J., Miglioranza, L.H.S., and Decker, E.A. (2008) Physical and oxidative stability of fish oil-in-water emulsions stabilized with beta-lactoglobulin and pectin. *Journal of Agricultural and Food Chemistry* **56**, 5926–5931.
- Keenan, R.D., Young, D.J., Tier, C.M., Jones, A.D., and Underdown, J. (2001) Mechanism of pressure-induced gelation of milk. *Journal of Agricultural and Food Chemistry* **49**, 3394–3402.
- Kerstens, S., Mugnier, C., Murray, B.S., and Dickinson, E. (2006) Influence of ionic surfactants on the microstructure of heat-set beta-lactoglobulin-stabilized emulsion gels. *Food Biophysics* **1**, 133–143.
- Kinsella, J. and Whitehead, D. (1989) Proteins in whey: chemical, physical and functional properties. *Advances in Food and Nutrition Research* **33**, 343–438.
- Kruif, C.D. and Holt, C. (2003) Casein micelle structure, functions and interactions. In: *Advanced Dairy Chemistry—Proteins Part A*, edited by P. Fox and P. McSweeney. New York: Kluwer Academic Plenum Publishers.
- Laurent, M.A. and Boulenguer, P. (2003) Stabilization mechanism of acid dairy drinks (ADD) induced by pectin. *Food Hydrocolloids* **17**, 445–454.
- Leclerc, E. and Calmettes, P. (1997) Interactions in micellar solutions of beta-casein. *Physical Review Letters* **78**, 150–153.
- LopezFandino, R., Carrascosa, A.V., and Olano, A. (1996) The effects of high pressure on whey protein denaturation and cheese-making properties of raw milk. *Journal of Dairy Science* **79**, 929–936.
- LopezFandino, R., Ramos, M., and Olano, A. (1997) Rennet coagulation of milk subjected to high pressures. *Journal of Agricultural and Food Chemistry* **45**, 3233–3237.
- Low, Y.H., Agboola, S., Zhao, H., and Lim, M.Y. (2006) Clotting and proteolytic properties of plant coagulants in regular and ultrafiltered bovine skim milk. *International Dairy Journal* **16**, 335–343.

- Lucey, J.A. and Singh, H. (2003) Acid coagulation of milk. In: *Advanced Dairy Chemistry—Proteins Part B*, edited by P. Fox and P. McSweeney. New York: Kluwer Academic Plenum Publishers.
- Lucey, J.A., van Vliet, T., Grolle, K., Geurts, T., and Walstra, P. (1997a) Properties of acid casein gels made by acidification with glucono-delta-lactone. 2. Syneresis, permeability and microstructural properties. *International Dairy Journal* **7**, 389–397.
- Lucey, J.A., van Vliet, T., Grolle, K., Geurts, T., and Walstra, P. (1997b) Properties of acid casein gels made by acidification with glucono-delta-lactone. 1. Rheological properties. *International Dairy Journal* **7**, 381–388.
- Macku, I., Bunka, F., Pavlinek, V., Leciabiva, P., and Hrabec, J. (2008) The effect of pectin concentration on viscoelastic and sensory properties of processed cheese. *International Journal of Food Science and Technology* **43**, 1663–1670.
- Madsen, F. (2000) Substitution of gelatine in low-fat spread: a rheological characterisation. In: *Gums and Stabilisers for the Food Industry 10*, edited by P.A. Williams and G.O. Phillips, pp. 411–420. Cambridge: Royal Society of Chemistry.
- Marinova, K.G., Basheva, E.S., Nenova, B., Temelska, M., Mirarefi, A.Y., Campbell, B., and Ivanov, I.B. (2009) Physico-chemical factors controlling the foamability and foam stability of milk proteins: sodium caseinate and whey protein concentrates. *Food Hydrocolloids* **23**, 1864–1876.
- Matsudomi, N., Rector, D., and Kinsella, J.E. (1991) Gelation of bovine serum-albumin and beta-lactoglobulin—effects of pH, salts and thiol reagents. *Food Chemistry* **40**, 55–69.
- McMahon, D. and Oommen, B. (2008) Supramolecular structure of the casein micelle. *Journal of Dairy Science* **91**, 1709–1721.
- Mistry, V.V. and Anderson, D.L. (1993) Composition and microstructure of commercial full-fat and low-fat cheeses. *Food Structure* **12**, 259–266.
- Monteiro, R.R., Tavares, D.Q., Kindstedt, P.S., and Gigante, M.L. (2009) Effect of pH on microstructure and characteristics of cream cheese. *Journal of Food Science* **74**, C112–C117.
- Morris, E. (1990) Mixed biopolymer gels. In: *Food Gels*, edited by P. Harris. London: Elsevier Applied Science.
- Moschakis, T., Murray, B.S., and Dickinson, E. (2006) Particle tracking using confocal microscopy to probe the microrheology in a phase-separating emulsion containing nonadsorbing polysaccharide. *Langmuir* **22**, 4710–4719.
- Muir, D.D. (1989) Cream liqueurs. *Journal of the Society of Dairy Technology* **42**, 31–31.
- Muir, D.D., Tamime, A.Y., Shenana, M.E., and Dawood, A.H. (1999) Processed cheese analogues incorporating fat-substitutes—I. Composition, microbiological quality and flavour changes during storage at 5 degrees C. *Food Science and Technology-Lebensmittel-Wissenschaft & Technologie* **32**, 41–49.
- Mulvihill, D.M. and Ennis, M. (2003) Functional milk proteins: production and utilization. In: *Advanced Dairy Chemistry—Proteins Part B*, edited by P.F. Fox and P. McSweeney. New York: Kluwer Academic Plenum Publishers.
- Needs, E.C., Stenning, R.A., Gill, A.L., Ferragut, V., and Rich, G.T. (2000) High-pressure treatment of milk: effects on casein micelle structure and on enzymic coagulation. *Journal of Dairy Research* **67**, 31–42.
- Ozer, B.H. and Kirmaci, H.A. (2010) Functional milks and dairy beverages. *International Journal of Dairy Technology* **63**, 1–15.
- Paliandre, S., Decker, E.A., and McClements, D.J. (2007) Improvement of stability of oil-in-water emulsions containing caseinate-coated droplets by addition of sodium alginate. *Journal of Food Science* **72**, E518–E524.
- Parker, A., Gunning, P.A., Ng, K., and Robins, M.M. (1995) How does xanthan stabilise salad dressing? *Food Hydrocolloids* **9**, 333–342.
- Patino, J.M.R., Sanchez, C.C., Nino, M.R.R., and Fernandez, M.C. (2001) Structural and dynamic properties of milk proteins spread at the air-water interface. *Journal of Colloid and Interface Science* **242**, 141–151.
- Redigueri, C.F., de Freitas, O., Lettinga, M.P., and Tuinier, R. (2007) Thermodynamic incompatibility and complex formation in pectin/caseinate mixtures. *Biomacromolecules* **8**, 3345–3354.
- Regand, A. and Goff, H.D. (2002) Effect of biopolymers on structure and ice recrystallization in dynamically frozen ice cream model systems. *Journal of Dairy Science* **85**, 2722–2732.
- Reps, A., Jankowska, A., and Winiewska, K. (2009) The effect of high pressure on selected properties of yoghurt. *High Pressure Research* **29**, 33–37.
- Roefs, S. and Vanvliet, T. (1990) Structure of acid casein gels .2. Dynamic measurements and type of interaction forces. *Colloids and Surfaces* **50**, 161–175.

- Rollema, H. and Brinkhuis, J. (1989) A H-NMR study of bovine casein micelles; influence of pH, temperature and calcium ions on micellar structure. *Journal of Dairy Research* **56**, 417–425.
- Rollema, H., Brinkhuis, J., and Vreeman, H. (1988) H-NMR studies of bovine k-casein and casein micelles. *Netherlands Milk and Dairy Journal* **42**, 233–248.
- Sainani, M.R., Vyas, H.K., and Tong, P.S. (2004) Characterization of particles in cream cheese. *Journal of Dairy Science* **87**, 2854–2863.
- Sandoval-Castilla, O., Lobato-Calleros, C., Aguirre-Mandujano, E., and Vernon-Carter, E.J. (2004) Microstructure and texture of yogurt as influenced by fat replacers. *International Dairy Journal* **14**, 151–159.
- Schmidt, D.G., Buchheim, W., and Koops, J. (1971) Electron-microscopical study of fat-protein complexes in evaporated milk, using freeze-etching technique. *Netherlands Milk and Dairy Journal-Nederlands-Nederlands Melk En Zuiveltijdschrift* **25**, 200.
- Schorsch, C., Jones, M.G., and Norton, I.T. (1999) Thermodynamic incompatibility and microstructure of milk protein locust bean gum sucrose systems. *Food Hydrocolloids* **13**, 89–99.
- Schorsch, C., Jones, M.G., and Norton, I.T. (2000) Effect of sucrose on milk protein, LBG and their interactions. In: *Gums and Stabilisers for the Food Industry 10*, edited by P.A. Williams and G.O. Phillips, pp. 292–302. Cambridge: Royal Society of Chemistry.
- Schrader, K. and Buchheim, W. (1998) High pressure effects on the colloidal calcium phosphate and the structural integrity of micellar casein in milk—II. Kinetics of the casein micelle disintegration and protein interactions in milk. *Kieler Milchwirtschaftliche Forschungsberichte* **50**, 79–88.
- Singer, N.S. and Dunn, J.M. (1990) Protein microparticulation—the principle and the process. *Journal of the American College of Nutrition* **9**, 388–397.
- Singh, H. and Havea, P. (2003). Thermal denaturation, aggregation and gelation of whey proteins. In: *Advanced Dairy Chemistry—Proteins Part B*, edited by P.F. Fox and P. McSweeney. New York: Kluwer Academic Plenum Publishers..
- Singh, H. and Waungana, A. (2001) Influence of heat treatment of milk on cheesemaking properties. *International Dairy Journal* **11**, 543–551.
- Smits, P. and Vanbrouwershaven, J.H. (1980) Heat-induced association of beta-lactoglobulin and casein micelles. *Journal of Dairy Research* **47**, 313–325.
- Spagnuolo, P.A., Dalgleish, D.G., Goff, H.D., and Morris, E.R. (2005) Kappa-carrageenan interactions in systems containing casein micelles and polysaccharide stabilizers. *Food Hydrocolloids* **19**, 371–377.
- Surh, J., Decker, E.A., and McClements, D.J. (2006) Influence of pH and pectin type on properties and stability of sodium-caseinate stabilized oil-in-water emulsions. *Food Hydrocolloids* **20**, 607–618.
- Takeuchi, K.P. and Cunha, R.L. (2008) Influence of ageing time on sodium caseinate gelation induced by glucono-delta-lactone at different temperatures. *Dairy Science & Technology* **88**, 667–681.
- Tamime, A.Y., Kalab, M., Muir, D.D., and Barrantes, E. (1995) The microstructure of set-style, natural yogurt made by substituting microparticulate whey protein for milk fat. *Journal of the Society of Dairy Technology* **48**, 107–111.
- Tanaka, T. and Hatanaka, K. (1992) Application of hydrostatic-pressure to yogurt to prevent its after-acidification. *Journal of the Japanese Society for Food Science and Technology-Nippon Shokuhin Kagaku Kogaku Kaishi* **39**, 173–177.
- Tromp, R.H., de Kruif, C.G., van Eijk, M., and Rolin, C. (2004) On the mechanism of stabilisation of acidified milk drinks by pectin. *Food Hydrocolloids* **18**, 565–572.
- Tunick, M. (2009) Dairy innovations over the past 100 years. *Journal of Agricultural and Food Chemistry* **57**, 8093–8097.
- Udabage, P., Augustin, M.A., Versteeg, C., Puvanenthiran, A., Yoo, J.A., Allen, N., McKinnon, I., Smiddy, M., and Kelly, A.L. (2010) Properties of low-fat stirred yoghurts made from high-pressure-processed skim milk. *Innovative Food Science & Emerging Technologies* **11**, 32–38.
- Vanhooydonk, A.C.M., Boerrigter, I.J., and Hagedoorn, H.G. (1986a) pH-induced physicochemical changes of casein micelles in milk and their effect on renneting .2. Effect of pH on renneting of milk. *Netherlands Milk and Dairy Journal* **40**, 297–313.
- Vanhooydonk, A.C.M., Hagedoorn, H.G., and Boerrigter, I.J. (1986b) pH-induced physicochemical changes of casein micelles in milk and their effect on renneting .1. Effect of acidification on physicochemical properties. *Netherlands Milk and Dairy Journal* **40**, 281–296.
- Vanhooydonk, A.C.M., Dekoster, P.G., and Boerrigter, I.J. (1987) The renneting properties of heated milk. *Netherlands Milk and Dairy Journal* **41**, 3–18.
- Vega, C. and Goff, H.D. (2005) Phase separation in soft-serve ice cream mixes: rheology and microstructure. *International Dairy Journal* **15**, 249–254.

- Walstra, P. (1990) On the stability of casein micelles. *Journal of Dairy Science* **73**, 1965–1979.
- Waugh, D. and Hippel, P.V. (1956) k-Casein and the stabilisation of casein micelles. *Journal of the American Chemical Society* **78**, 45–167.
- Xu, Z.M., Emmanouelidou, D.G., Raphaelides, S.N., and Antoniou, K.D. (2008) Effects of heating temperature and fat content on the structure development of set yogurt. *Journal of Food Engineering* **85**, 590–597.
- Ye, A.Q. (2008) Complexation between milk proteins and polysaccharides via electrostatic interaction: principles and applications—a review. *International Journal of Food Science and Technology* **43**, 406–415.
- Yuksel, Z. and Erdem, Y.K. (2009) The influence of transglutaminase treatment on functional properties of set yoghurt. *International Journal of Dairy Technology* **63**, 86–97.
- Zhang, Z. and Goff, H.D. (2004a) *Studying the Composition of the Air Interface in Aqueous Milk Protein Foam and Ice Cream*. Brussels: International Dairy Federation.
- Zhang, Z. and Goff, H.D. (2004b) Protein distribution at air interfaces in dairy foams and ice cream as affected by casein dissociation and emulsifiers. *International Dairy Journal* **14**, 647–657.
- Zirbel, F. and Kinsella, J.E. (1988) Factors affecting the rheological properties of gels made from whey-protein isolated. *Milchwissenschaft-Milk Science International* **43**, 691–694.

9 Probiotics and Prebiotics

D.Y. Ying and C. Gantenbein-Demarchi

9.1 INTRODUCTION

As soon as he is born, man becomes the habitat of a very rich microbial flora. The skin, the mucous membranes and the gastrointestinal contents become stocked with such a flora, but very small number of these microorganisms have up to the present been recognised or described . . . For long it was thought that in healthy individuals all these micro-organisms were inoffensive and sometimes even useful.

Metchnikoff (1907)

For centuries, humans have benefited from traditional fermented foods as part of their diet. Many of these foods are primary sources of naturally occurring probiotic bacteria. There have been beneficial effects associated with the consumption of these foods for thousands of years. In developed countries, the past century has seen a changing of consumer behavior. There is a growing trend back to healthier foods and a growing interest in foods with documented health benefits. This newfound popularity of functional foods has been one of the key factors responsible for an increase in the market of products containing probiotic and/or prebiotic ingredients.

Dairy-based products, such as yogurt, and high-fiber products are common foods on the market. Various *Lactobacillus* spp. and *Bifidobacterium* spp. are the most frequently used probiotic cultures. In recent years, an increasing number of categories of products that included pro- and/or prebiotics, as well as new probiotic strains, have contributed to the growth of foods and beverages with enhanced digestive health aspects. In 2008, there were 232 new pro- or prebiotic products introduced worldwide and an increase in the number of new products was expected in 2009 and beyond. The number of probiotic and prebiotic product launches in Japan was higher than in any other country, with the United States placed second but gaining in importance as a market for probiotics and prebiotics (Anon, 2009).

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9.2 DEFINITIONS

Various definitions for functional foods have been proposed by different authorities, researchers, and industries. However, a legislative definition of the term functional foods has yet to be established in most countries. According to Shah (2001), functional foods are defined as foods “that contain some health-promoting component(s) beyond traditional nutrients.” In the consensus document of the European Commission Concerted Action on Functional Food Science in Europe (FUFOSE), it is stated that “a food can be regarded as ‘functional’ if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease” (Anon, 1999). Other terms for functional foods, such as designer foods, medicinal foods, nutraceuticals, therapeutic foods, superfoods, foodiceuticals, and medifoods, are also used (Shah, 2001). The U.S. Federal Food, Drug and Cosmetic Act (FFDCA) introduced some definitions for the differentiation between a food and a drug to help the industry determine the safety and labeling requirements of their products (Ross, 2000). While the first generation of functional foods were foods that were supplemented with components such as vitamins, which have recognized health attributes, nowadays, there is interest in a wider range of functional components, such as probiotics and prebiotics, with potential positive effects on the gut microbiota composition.

The term “probiotics,” first used by Lilly and Stillwell (1965), derives from Greek words and means “for life” (Vasiljevic and Shah, 2008). The most frequently cited definition comes from Fuller (1992), who defines probiotics as “a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance.” Other definitions for probiotics are listed in the overview of Vasiljevic and Shah (2008) and Sanders (2009). According to Shortt (1999), the definition of Fuller (1992) implicates the importance of viable cells and restricts probiotic activity to the gut and to animals. However, the definition of Havenaar and Huis in’t Veld (1992) includes the potential application of probiotics to microbial communities at other sites. The definition considers that probiotics are “a mono- or mixed-culture of life microorganisms, which when applied to man or animal, affects beneficially the host by improving the properties of the indigenous microflora.” An EC-supported group of European scientists suggested that a probiotic for human nutrition should be defined as a “live microbial food ingredient that is beneficial to health” (Salminen et al., 1998a). This definition incorporates the results of research on the benefits of probiotic effects mediated from dead microbes, for example, probiotic effects on immune parameters. One of the most recent definitions follows recommendations of a FAO/WHO working group and describes probiotics as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO, 2002). As there is no legal definition for the term probiotic, the key criteria for a product labeled “probiotic” is that it should contain microbes that are alive and that the microbes should be defined, properly named, and be safe (Sanders, 2009).

The definition of prebiotics was introduced by Gibson and Roberfroid (1995) as “non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health.”

Synbiotics are products with a combined probiotic and prebiotic effect. The combined effects contribute in a synergistic manner to the health of the consumer by improving the

survival and/or implantation of the probiotic in the intestinal tract. The definition of synbiotics is a “mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare” (Gibson and Roberfroid, 1995). Schrezenmeir and de Vrese (2001) claim that the term synbiotics should only be used with a combination of probiotics and prebiotics, where the prebiotic compound directly favors the growth and survival of the probiotic, as has been shown in the combination of oligofructose and bifidobacteria.

9.3 PROBIOTICS

9.3.1 Historical aspects

The health benefits of probiotics became the subject of a specific study at the beginning of the last century when Metchnikoff postulated the “longevity without aging” theory, suggesting the consumption of lactobacilli fermented milk led to a better and prolonged life (Metchnikoff, 1907). Metchnikoff claimed that toxin-producing bacteria, normally present in the intestine, were inhibited and displaced by lactic acid bacteria (LAB) and their products, which were found in sour milks. He attributed the good health and long life of the Bulgarians to their large consumption of fermented milk, called yahourth (Lourens-Hattingh and Viljoen, 2001). In the same time period, another researcher of the Pasteur Institute in France, Tissier, found similar beneficial effects with bifidobacteria, isolated from the stools of breast-fed infants (O’Sullivan et al., 1992). Shortt (1999) provides a thorough overview of further historical perspectives on the concept of probiotics, commencing with the first documented consumption of cultured dairy products, which were mentioned in the Bible and the sacred books of Hinduism. Danone was established by Isaak Carasso in 1919, enthused by the microbiological research results at the Pasteur Institute and reported benefits of Balkan yogurt.

In 1948, the randomized controlled trial was first introduced as a benchmark in clinical research. The first successful therapy results with *Lactobacillus acidophilus* were published by Gordon and colleagues in the late 1950s. However, overenthusiasm, limited knowledge of the gut flora, as well as poorly designed and controlled clinical studies that did not follow the randomized controlled trial concept resulted in products with inappropriately described bacterial species. The scientific basis was questioned, resulting in skepticism around the whole concept of probiotics (Shortt, 1999).

The interrelationship between intestinal microorganisms and the beneficial role of probiotics and prebiotics in the mammalian gastrointestinal tract has only recently been demonstrated due to new techniques and improved methodology, in particular metagenomics and metabolomics (Saulnier et al., 2009). In parallel to the advances in clinical research, developments in the assessment and improvement of the stability of probiotic bacteria have also taken place. It was recognized that probiotic research should be established on a scientific basis, and there should be a “general agreement regarding the key selection criteria for probiotic bacteria for use in human foods.” These include that probiotics should be of human origin, nonpathogenic, acid and bile tolerant, show good survivability during processing, have good shelf life, and show evidence of beneficial health effects (Lee and Salminen, 1995; Shortt, 1999; Champagne et al., 2005).

9.3.2 Overview of important probiotic strains

Lactic acid bacteria (LAB) are usually described as Gram-positive microorganisms that are devoid of cytochromes. These bacteria favor anaerobic conditions but are aerotolerant. They are fastidious, acid-tolerant and strictly fermentative, producing lactic acid as a main product (Stiles and Holzapfel, 1997). The most important genera include: *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, and *Bifidobacterium*. They are divided into two major phylogenetic branches based on their GC (guanine–cytosine) pair content and carbohydrate metabolism. Bifidobacteria, having a relatively high GC pair content, belong to the *Actinomycetes* branch, while other genera with lower GC pair content belong to the *Clostridium* branch (Vasiljevic and Shah, 2008). According to their carbohydrate metabolism, LAB are divided into two groups: homofermentative LAB (*Lactococcus*, *Pediococcus*, *Enterococcus*, *Streptococcus*, and some lactobacilli), with lactic acid as the primary and generally sole metabolite, and heterofermentative LAB (*Leuconostoc*, *Weissella*, and some lactobacilli), with lactate, CO₂, ethanol, or acetate as metabolites.

Detailed descriptions of the different genera of food-related LAB and their new taxonomic classification have been published (Stiles and Holzapfel, 1997). New results based upon modern molecular techniques have resulted in significant changes and reorganization of the taxonomic classification of LAB. Genomic analysis was conducted on the widely used probiotic bacterium *Lactobacillus rhamnosus* GG, comparing its 3Mbp genome sequence with the similarly sized genome of *L. rhamnosus* LC705, an adjunct starter culture exhibiting reduced binding to mucous membranes. Both genomes demonstrated high sequence identity and synteny. However, for both strains, genomic islands, 5 in GG and 4 in LC705, punctuated the colinearity. These islands were predicted to contain a significant number of strain-specific genes, including those coding for bacteriophage components, sugar metabolism and transport, and exopolysaccharide biosynthesis. It was discovered that strain-specific genes encoding mucous-binding pili were present in one of the GG islands while being absent in the LC705 islands. This finding explained the ability of GG strains to persist in the human gut longer than LC705 strains (Kankainen et al., 2009). Most probiotic cultures used for commercial applications belong to the genera *Lactobacillus* and *Bifidobacterium* and some of them are listed in Tables 9.1 and 9.2. However, some species of the genera *Lactococcus* and *Enterococcus*, and even *Propionibacterium* and the yeast *Saccharomyces*, are considered to have health-promoting effects (Sanders and Huis in't Veld, 1999).

Other *Lactobacilli* strains known as probiotics but with less commercial applications are *Lactobacillus amylovorus*, *Lactobacillus crispatus*, *Lactobacillus gasseri*, and *Lactobacillus gallinarum* (only used for animals) (Holzapfel and Schillinger, 2002).

Another group of microorganisms known as enterococci also have special implications in the dairy industry. They occur as nonstarter LAB (NSLAB) in a variety of cheeses, especially artisan cheese. Despite their controversial utilization as starter culture due to opportunistic pathogenicity (Vasiljevic and Shah, 2008), enterococci play an acknowledged role in the development of sensory characteristics during the ripening of many cheeses, and have also been used as a component of cheese starter cultures. This positive influence of enterococci seems to be due to specific biochemical traits, such as lipolytic activity, citrate utilization, and the production of aromatic volatile compounds. Enterococci of dairy origin have also been reported to produce bacteriocins (enterocins) that are inhibitory against food spoilage or pathogenic bacteria, that is, *Listeria monocytogenes*,

Table 9.1. Commercially used probiotic *Lactobacilli* strains.^a

Species	Strains
<i>Lactobacillus acidophilus</i>	LA-1/LA-5 (Chr. Hansen); NCFM (Danisco/Rhodla); DDS-1 (Nebraska Cultures), SBT-20621 (Snow Brand Milk Products Co.); R0052 (Institute Rosell); LAFTI® L10 (DSM Food Specialties); LB (Lacteol Laboratory); La (Danisco)
<i>Lactobacillus acidophilus Johnsonii</i>	La1 (Lj1/NCC90) (Nestle)
<i>Lactobacillus delbrueckii ssp. bulgaricus</i>	Lb12/LBY27 (Chr. Hansen); 2038 (Meiji Milk Products)
<i>Lactobacillus lactis</i>	L1A (Essum AB)
<i>Lactobacillus casei</i>	DN-114 001 (Immunitas) (Danone); Shirota (Yakult); CRL 431 Gilliland (La-Mo) (Chr. Hansen); F19 (Arla Foods)
<i>Lactobacillus plantarum</i>	299V (Probi AB); 271 (Probi AB); Lp01;
<i>Lactobacillus rhamnosus</i>	GG1 (Valio Dairy); GR-1 (Urex Biotech); LB21 (Essum AB); 271 (Probi AB); R0011 (Institute Rosell); HN001 (DR20) (Fonetera)
<i>Lactobacillus reuteri</i>	SD2112/MM2 (BioGaia); Protectis ATCC 55730 (BioGaia); ATCC PTA 5289 (BioGaia)
<i>Lactobacillus paracasei</i>	CRL 431 (Chr. Hansen); Lpc (Danisco); LAFTI® L26 (DSM Food Specialties); F19 (Medipharm)
<i>Lactobacillus fermentum</i>	RC-14 (Urex Biotech); ME-3 (Tallina Piimatööstuse AS, Estonia)
<i>Lactobacillus helveticus</i>	B02
<i>Lactobacillus salivarius</i>	UCC118 (University College Cork)

^aAdapted from Sanders and Huis in't Veld (1999), Shah (2004), Shah (2007), Prado et al. (2008), Siro et al. (2008), Vasiljevic and Shah (2008), and Saulnier et al. (2009).

Table 9.2. Commercially used probiotic *Bifidobacteria* strains.^a

Species	Strains
<i>Bifidobacterium animalis ssp. Lactis</i>	Bb12 (Chr. Hansen)
<i>Bifidobacterium lactis</i>	LAFTI® B94 (DSM Food Specialties); HOWARU™/BI (Danisco); HN019 (DR10) (Fonterra); Bb-02; B94 (DSM Food Specialties)
<i>Bifidobacterium longum</i>	SBT-29281 (Snow Brand Milk Products Co. Ltd.); BB536 (Morinaga Milk Industry Co. Ltd); UCC 35642 (University College Cork)
<i>Bifidobacterium breve</i>	Yakult (Yakult)
<i>Bifidobacterium animalis</i>	DN-173010 (Bioactiva, ActiRegularis®) (Danone)
<i>Bifidobacterium bifidus</i>	Bb-11
<i>Bifidobacterium essensis</i>	Bioactiva (Danone)
<i>Bifidobacterium infantis</i>	Shirota; Immunitas; 744; 01
<i>Bifidobacterium adolescentis</i>	ATCC 15703; 94-BIM
<i>Bifidobacterium laterosporus</i>	CRL 431

^aAdapted from Sanders and Huis in't Veld (1999), Shah (2004), Shah (2007), Prado et al. (2008), Siro et al. (2008), Vasiljevic and Shah (2008), and Saulnier et al. (2009).

Staphylococcus aureus, *Vibrio cholerae*, *Clostridium* spp., and *Bacillus* spp. (Giraffa, 2003). There are recognized probiotic strains of streptococci such as *Streptococcus thermophilus* STY-31 (Chr. Hansen) or *Lactococcus lactis* L1A (Essum AB). The latter has been used in combination with *Leuconostoc mesenteroides* in a Swedish fermented milk product called filmjölök with less acidity than yogurt, but containing diacetyl as a typical flavor compound.

9.3.3 Health benefits

One of the key selection criteria for probiotic bacteria for use in human foods is the evidence of one or more beneficial health effects. At present, a wide variety of different probiotic strains are used, but the main strains with beneficial characteristics are members of the genera *Lactobacillus* and *Bifidobacterium* (Shah, 2007). Several reviews report a number of health benefits. Some of these benefits are well established, while others still require further study. It is essential to know that “health benefits imparted by probiotic bacteria are strain specific, and not species- or genus-specific” (Shah, 2007). It is also important for the understanding of probiotics that “no strain will provide all proposed benefits, not even strains of the same species, and not all strains of the same species will be effective against defined health conditions” (Shah, 2007).

In order to understand the function of probiotics, it is important to comprehend the complexity of the gastrointestinal tract (GIT) and the composition and the effects of colonic bacteria on the health of the host. Holzapfel et al. (1998), Holzapfel and Schillinger (2002) and Isolauri et al. (2004) provide thorough overviews and descriptions of colonic bacteria and the range of concentrations of such bacteria in the human colon. The gut flora, representing more than 400 species, is predominantly strictly anaerobic, comprising a wide variety of different genera or groups, such as *Clostridia*, *Bacteroides*, *Desulfovibrios*, and *Methanobrevibacter*, but also strains of LAB, such as *Bifidobacterium*, *Lactobacillus*, or *Streptococcus*. Further molecular studies revealed the dominance of *Bacteroides*, comprising up to 37% of the total bacterial flora (Kolida et al., 2006; Walker et al., 2006). From the time of birth until adulthood, humans exhibit considerable changes in their intestinal microflora (Ishibashi and Shimamura, 1993). Immediately after birth, colonization by many bacteria begins. Within one to two days, coliforms, enterococci, clostridia, and lactobacilli can be found, followed by bifidobacteria, which will dominate the flora in concentrations up to 10^{10} – 10^{11} cfu/g feces after 5 days in breastfed infants. Lactococci, enterococci, and lactobacilli can be detected only at 1% of the flora, and other strains are normally absent. During childhood, gradual changes occur in the intestinal flora, resulting in reduced and altered *Bifidobacterium* strains, but these strains remain the third most common genus in the gastrointestinal tract. The flora is dominated by *Bacteroides* (86%), followed by *Eubacterium*. Until middle age, the gut microflora is rather stable, whereas nearing old age, there is a further decline in *Bifidobacterium* and an increase in certain kinds of harmful bacteria, such as *Clostridium perfringens*. It has to be noted, however, that the intestinal flora varies from segment to segment, and depends on the diet, genetic background, and physiological state of the host, as well as on microbial factors and interactions (Holzapfel et al., 1998; Holzapfel and Schillinger, 2002). Any disturbances in the balance of the intestinal flora due to age or extrinsic factors, such as stress or bacterial contamination, can result in infectious diseases. Intestinal bacteria can be classified into three categories: harmful, beneficial, and neutral with respect to human health. *Bifidobacterium* and *Lactobacillus* are considered as the beneficial bacteria. However, *Escherichia coli*, *Clostridium*, *Proteus*, and certain types of *Bacteroides* can be harmful (Ishibashi and Shimamura, 1993).

The *Bifidobacteria* strains present in probiotic functional food supplements play a role in managing adherence and colonization of the host intestinal mucosa, thereby preventing disease (Walker and Duffy, 1998). Adherence properties are essential for the balance of intestinal microflora, intestinal bacterial enzyme activity, and stabilization of intestinal permeability (Katayama et al., 1997). Adhesion to intestinal cells can be nonspecific, where adhesion is based on biochemical factors, or specific, where adhesion depends on adhesion

molecules on the surface of adherent bacteria and receptor molecules on epithelial cells (Bernet et al., 1993). The mechanisms behind the disease prevention role of probiotics include (1) strengthening of the gut mucosal barrier; (2) gut microflora modification; (3) adherence to intestinal mucosa with an ability to prevent adherence of pathogen proliferation; (4) modification of dietary proteins by the intestinal microflora; (5) modification of bacterial enzyme activity; and (6) influence on gut mucosal permeability (Goldin and Gorbach, 1984).

Many reviews document the main health benefits of a wide variety of *Lactobacillus* and *Bifidobacterium* species and/or strains (Ouwehand and Salminen, 1998; Salminen et al., 1998a; Scheinbach, 1998; Walker and Duffy, 1998; Ziemer and Gibson, 1998; Dunne et al., 1999; Sanders and Huis in't Veld, 1999; Saarela et al., 2000, 2002; Lourens-Hattingh and Viljoen, 2001; Marteau, 2001; Holzapfel and Schillinger, 2002; Isolauri et al., 2004; Kolida et al., 2006; Walker et al., 2006; Shah, 2007; Vasiljevic and Shah, 2008). The main health claims include: antimicrobial activity and preventing gastrointestinal infections, effectiveness against diarrhea, improvement in lactose metabolism, anticarcinogenic and antimutagenic properties, reduction in serum cholesterol, prevention of *Helicobacter pylori* infection, reduction in inflammatory bowel disease and immune system stimulation, allergy prevention, antihypertensive effect, urogenital infection prevention, and hepatic encephalopathy prevention.

Health claims associated with probiotic food products are strictly regulated by the Natural Health Product Directorate (NHPD) in Canada, the Food and Drug Administration (FDA) in the United States, and the European Food Safety Authority (EFSA) in the European Union. According to NHPD and FDA, health claims for probiotic products are allowed, whereas under EU regulations, any health claim referring to prevention, treatment, or cure of a human disease for a food product is prohibited (Kolida et al., 2006).

9.3.4 Safety assessments of probiotics

The long history of human exposure and consumption of LAB has led to the conclusion that they are generally safe. There has been a focus placed on their possible role as a probiotic with health benefits. However, recently some human infections (e.g., endocarditis, bacteraemia, or urinary tract infections) caused by LAB were reported. In most of these cases, the source of infection came from commensal LAB flora rather than ingested bacteria, and occurred with patients already having some underlying disease or predisposed condition. With the notable exception of the enterococci, LAB can be considered less opportunistically pathogenic than a number of other members of the commensal microflora. Members of the genera *Lactococcus* and *Lactobacillus* are most commonly given the “generally recognized as safe” (GRAS) status, while members of the genera *Streptococcus* and *Enterococcus* and some other genera of LAB contain some opportunistic pathogens (Salminen et al., 1998b).

The *Enterococcus* genus is not considered GRAS, and safety assessment for enterococci remains controversial. While often very useful in cheese technology, some of the isolates of the genus *Enterococcus* can be opportunistic pathogens for humans. The review by Ogier and Serror (2008) summarizes the positive and negative traits that illustrate the controversial nature of enterococci. They propose “a case-by-case evaluation of each potential technological strain” before the use of enterococci in fermented food products. The positive traits of enterococci, outlined by Moreno et al. (2006), include (1) bacteriocin production; (2) citrate metabolism; and (3) proteolysis and lipolysis. These allow many enterococci

strains to function as probiotics. On the other hand, negative traits of enterococci include (1) cytolytic activity; (2) antibiotic resistance; and (3) virulence factors, such as aggregation substances, gelatinase, and extracellular surface proteins, which present the potential for enterococci to behave as opportunistic pathogens (Moreno et al., 2006).

Meile et al. (2008) explored the safety aspects of specific strains of *Propionibacteria*, used in starter cultures in hard-cheese ripening, and *Bifidobacteria*, used in fermented dairy products and milk powder. They state that the explored strains are generally safe for use in the dairy industry and for human consumption, with the exception of the single occasionally pathogenic species, *Bifidobacterium dentium*, which is implicated in dental and other infections. The potential for tetracycline resistance *tet(W)* gene was also found in certain *Bifidobacterium* strains, although this presents no evidence for a potential health hazard.

New procedures have been established to assess the safety of new strains, particularly those genetically modified strains where their safety has yet to be demonstrated (Adams, 1999). The safety factors that must be considered for the assessment of LAB include: (1) intrinsic properties, such as adhesion factors, antibiotic resistance, plasmid existence, and transfer potential; (2) metabolic products and toxicity; (3) mucosal effects such as invasion potential and degradation; (4) dose response effects; (5) clinical assessment of potential side effects; and (6) surveillance of large populations using epidemiological studies following the introduction of new strains (Adams, 1999; Salminen et al., 1996 and, 1998b).

An example of a recent probiotics safety assessment is presented in the study by Dekker et al. (2009), where a double-blind placebo-controlled clinical trial was conducted on human infants aged 0–2 years and their mothers from 35 weeks gestation, using two probiotic bacterial strains (*L. rhamnosus* HN001 and *Bifidobacterium animalis* subsp. *lactis* HN019). Dekker and his colleagues argued that the relatively immature state of the neonatal gut and gut-associated immune system presented the perfect environment to demonstrate the safety of probiotic strains, particularly since the probiotics would be for use as ingredients in infant formulae. The findings suggested that there were no statistically significant differences between treatment groups for incidence of adverse events, morphometric data, wheeze, and antibiotic use over the treatment period, allowing the authors to conclude that the two “probiotic strains H001 and H019 were safe and well tolerated in infants, and did not affect normal growth” (Dekker et al., 2009).

9.3.5 Consumer acceptance and product overview

The demand for functional foods has been increasing in recent decades due to increasing awareness that food is not only intended to satisfy hunger and provide the necessary nutrients for life, but can also provide a positive contribution to consumer physical and mental well-being. Examples include: improving the general conditions of the body, decreasing the risk of some diseases (e.g., cholesterol lowering products), and even curing some illnesses. First promoted by the Japanese in 1984, the awareness of the functional food concept quickly developed in Europe and the United States and presented as a commercial opportunity. Nowadays, functional foods represent a key segment of the food industry.

According to Dairy Council Digest Archives (Anon, 2009), the demand for functional foods, such as foods containing pro- and prebiotics, is driven by several factors. These include (1) advances in science, technologies, and processing techniques; (2) improved and more widespread communication with consumers; (3) efforts for healthcare cost reduction through disease prevention; (4) adaptation of government regulations for food marketing and labeling; (5) an increasingly ageing population (over 65 years old) and their particular

interest in functional foods to improve health; and (6) growing health awareness by the consumer.

Consumer acceptance is the key factor in the successful marketing of functional foods, and therefore anticipating consumer reaction is of critical importance to the success of the industry. Numerous studies measuring U.S. and European consumer reaction toward functional foods have been conducted, as cited in the review by Siro et al. (2008). Their findings suggested that sociodemographic characteristics, cognitive, and attitudinal factors emerged as potential determinants of consumer acceptance and choice of functional foods. Furthermore, consumer studies found that although Americans seemed overwhelmingly aware and accepting of functional foods, they still had lower frequencies of healthy food consumption. In Europe, consumers appeared to be far more critical and suspicious of new food products and the food production process, which they felt had become increasingly secret. Surveys reported that functional foods were at risk of being regarded as a food category, which was not embraced as enthusiastically by the consumer as the food industry had hoped for (Jonas and Beckmann, 1998). Most studies showed that consumer acceptance of functional foods is far from being unconditional, with one of the main conditions for acceptance being taste, as well as the trustworthiness of health claims. Several studies also identified the typical functional food customer as being female, well educated, in a higher income class and older than 55. All studies consistently identified females as the most likely users or buyers of functional foods (Siro et al., 2008).

9.3.6 Probiotic dairy products and the world market

Probiotic cultures have been exploited extensively by the dairy industry as a tool for the development of novel functional products. Shirota's isolation of *Lactobacillus casei* and its successful incorporation into the fermented dairy product called "Yakult" initiated the foundation of the same company in 1935 (Yakult Central Institute for Microbiological Research, 1998). Probiotic milk products have represented the biggest probiotic market, with LAB (mainly *Lactobacilli* and *Bifidobacteria*) being the most studied and used bacteria. However, extensive research and development in recent times have resulted in a great number of new dairy products. In 2004, it was estimated that more than 70 probiotic-containing products have been marketed in the world (Shah, 2004). Since then this list has been continually expanding. Today, probiotics represent the major and still growing segment and comprise approximately 65% of the functional food world market (Holzapfel and Schillinger, 2002).

As of 2003, functional foods had a market share of around 2–3% in the U.S. food market, estimated to be worth ~USD5 billion. The global market is estimated to be between USD33 billion and USD60 billion, with the United States representing the largest market, followed by Europe and Japan. The main European markets of dairy probiotics are Scandinavia, the Netherlands, Switzerland, Croatia, and Estonia; while Greece, France, and Spain can be considered developing markets (Mäkinen-Aakula, 2006; as cited in Siro et al., 2008).

Recently, the functional foods market has been dominated by gut health products, in particular dairy probiotics, which constitute more than half of the functional food sales (Siro et al., 2008). In 2007, there were more than 350 probiotic products worldwide. Some examples are Actimel and Activia from Danone, France, Gefilus from Valio, Finland, AB milk products or Cultura from Denmark, ProViva from Skane mejerier, Sweden, Kyr from Italy, Soytreant from Lifeway in the United States, Yakult from Japan, Vitality, Bifidus milk, Bioghurt, and Biogarde from Germany, Biokys from Slovakia, Acidophilus milk or Philus

from Sweden, Evolus from Finland, and Aktifit, Symbalance, or LC1 from Switzerland (Lourens-Hattingh and Viljoen, 2001; Siro et al., 2008). Other U.S. or worldwide marketed products include Good Start Natural®, BiogaiaAB Reuteri Drops, DanActive™ Dairy Drink, Danimals® Yogurt, BiogaiaAB Probiotic LifeTop Cap, or Yo-Plus™ Yogurt (Saulnier et al., 2009). Many more products are listed in the above cited reviews and in the review by Prado et al. (2008). An overview of FOSHU approved LAB-containing products like Meiji Bulgaria Yoghurt LB81, Bifidus Plain Yoghurt, Takanashi Drink Yoghurt Onaka-He-GG, Yukijirushi Nachure, and a wide variety of Yakult products is given in the review by Shortt (1999).

9.3.7 Other probiotic food products and recent developments

Probiotic strains can be found in some traditional fermented products such as fermented vegetable products (e.g., kimchi, sauerkraut, or tempeh) or traditionally fermented fish or cereals (Rivera-Espinoza and Gallardo-Navarro, 2010). In recent years, an increasing number of carriers for probiotics have been examined, including edible spreads (Charteris et al., 2002) and meat (Arihara et al., 1998). Probiotic organisms are also available in products, such as infant formula, fruit juices, or ice-cream, and even in desserts like chocolate mousse (Alles et al., 2004; Aragon-Alegro et al., 2007; Homayouni et al., 2008; Vasiljevic and Shah, 2008; Cruz et al., 2009).

Since fruit and vegetables already contain beneficial nutrients (minerals, vitamins, dietary fibers, and antioxidants), and lack the dairy allergens, such as lactose, they are ideal substrates for probiotics. Due to the high tolerance of the probiotic strains to acidic environments, fruit and vegetable products have a promising “synbiotic” potential. Remaining issues, such as sensory aspects or LAB survival during the final pasteurization, must be investigated.

Throughout the history of human nutrition, various fermented cereal products have been created, but only recently have the probiotic properties of some products become known. Cereals, due to their water-soluble fiber, oligosaccharides, and resistant starch content, are considered to fulfill the prebiotic concept. Integrating probiotics into cereals, promising synbiotic products can be launched. Rivera-Espinoza and Gallardo-Navarro (2010) and Prado et al. (2008) confirm in their reviews the actual status and the challenges associated with fruit-, vegetable-, or cereal-based probiotic products.

Meat, primarily various dry sausages that have not undergone heating processes, can be considered as an ideal product carrying probiotics due to their probiotic protecting features, such as providing adequate nutrients, a raised pH, and low oxygen content. Some of the meat and fish products containing suitable probiotic strains are discussed by Rivera-Espinoza and Gallardo-Navarro (2010).

9.3.8 Guidelines for the evaluation of probiotics for food use

Various reviews outline criteria or guidelines for microorganisms to be characterized as probiotics. Some of the most important criteria include (1) human origin; (2) GRAS status; (3) long-term activity and longevity; (3) high resistance to gastric acidity and bile acid toxicity; (4) good adherence to human intestinal cells and intestinal mucins; (5) production of antimicrobial substances against gut pathogens; (6) safety in food and during clinical use in immunocompromised individuals, and (7) efficacy and safety in humans (Kolida et al., 2006). It is apparent that no probiotic product can fulfill all of these criteria; products

often do not contain the concentration of live cells indicated on the labeling, or they lack one or more of the other criteria. It is recognized that “viability and purity of probiotic preparations is of the utmost importance for both the functionality and the safety of probiotic products” (Kolida et al., 2006). In order to have an important influence on the intestinal milieu, probiotics should exhibit good adhesion to the human intestinal wall. Therefore, new probiotics should be obtained from adhesive bacteria from the human gut rather than from feces or planktonic flora. The “birth” of a new probiotic strain, as described by Del Piano et al. (2006), starts with withdrawal of the bacteria by parentoscopic brushing, isolation, identification, typing and biotype selection of the strain, determination of the resistance of the strain to gastric juice, bile and pancreatic secretion, safety evaluation, resistance and stability to freeze-drying, microencapsulation, *in vitro* studies, and finally animal and human clinical investigations.

In 2002, guidelines for the evaluation and minimal requirements of probiotic status were introduced by the WHO (FAO/WHO, 2002). They include a description of standard methods for clinical evaluations targeting Phase 1 (safety), Phase 2 (efficacy), Phase 3 (effectiveness), and Phase 4 (surveillance), and can be summarized as follows (Walker et al., 2006):

- a. *Taxonomy*. Strain identification by phenotyping and genotyping methods and named in accordance with the International Code of Nomenclature.
- b. *Health benefit*. Labeling of the product with the minimal daily amount required in order to confer specific health benefit(s). Proof of health benefit(s) via randomized double-blind, well-controlled studies showing statistical significance.
- c. *Safety aspects and functionality*. By *in vitro* and/or animal studies. New strains also have to be tested for their safety with phase 1 human studies and at least one double-blind placebo-controlled phase 2 clinical trial.
- d. *Labeling*. Correct and relevant information including the genus, species, and strain, the minimum number of bacteria to be used, the viable concentration present at the end of the product shelf life, as well as information on storage conditions.

9.4 PREBIOTICS

Prebiotics are nondigestible food components that selectively stimulate certain bacterial groups resident in the colon, such as bifidobacteria and lactobacilli, which are considered beneficial to the human host. The prebiotic substances derived from whey/lactose include lactitol, lactulose, lactosucrose, and galactooligosaccharides. A wide range of indigestible but fermentable dietary carbohydrates that stimulate the growth of probiotic bacteria are also regarded as prebiotics (Holzapfel and Schillinger, 2002).

“In order for a food ingredient to be classified as a prebiotic, it must (1) be neither hydrolysed nor absorbed in the upper part of the gastrointestinal tract; (2) be a selective substrate for one or a limited number of beneficial bacteria commensal to the colon, which are stimulated to grow and/or are metabolically activated; (3) consequently, be able to alter the colonic flora in favour of a healthier composition; and (4) induce luminal or systemic effects that are beneficial to the host health” (Gibson and Roberfroid, 1995). Certain carbohydrates are classified as colonic foods—foods not absorbed in the upper gastrointestinal tract or hydrolyzed by the human digestive system, enter the colon and serve “as substrates for the endogenous colonic bacteria, thus indirectly providing the host with energy,

Table 9.3. Classification of certain carbohydrates as colonic foods and prebiotics.^a

Carbohydrate	Colonic food	Prebiotic
Resistant starch		
Nonstarch polysaccharides	Yes	No
Plant cell wall polysaccharides	Yes	No
Hemicelluloses	Yes	No
Pectins	Yes	No
Nondigestible oligosaccharides		
Fructooligosaccharides	Yes	Yes
Galactooligosaccharides	Yes	Yes
Soybean oligosaccharides	Yes	?
Glucooligosaccharides	?	No

^aAdapted from Gibson and Roberfroid (1995), and Roberfroid (2007).

?, Status not yet defined.

metabolic substrates and essential micronutrients.” Table 9.3 classifies carbohydrates as colonic foods and/or prebiotics (Gibson and Roberfroid, 1995; Roberfroid, 2007).

Prebiotics exist in short-chain and long-chain forms, which indicate how many links are in each molecule. Short-chain prebiotics like fructooligosaccharides (FOS) have between 2 and 8 links. These are quickly fermented in the right side of the colon to nourish the good bacteria there. These short-chain carbohydrates (SCC) or low-digestible carbohydrates (LDC) provide interesting possibilities for inclusion into conventional food products for their “bifidogenic” effects (Holzapfel and Schillinger, 2002). Long-chain prebiotics like inulin have from 9 to 64 links. Long-chain prebiotics persist through to the left colon and are fermented there. The fructans, inulin, and FOS are the most commonly used prebiotics in Europe and the United States, largely owing to their economy of manufacture. Galactooligosaccharides are also on the market in the EU and the United States, but have yet to become as widely used as the fructans (Rastall and Maitin, 2002). It is important that these substances reach the cecum, where they are fermented and well tolerated; however, dose-related undesirable effects due to osmotic potential and/or excessive fermentation may occur (Holzapfel and Schillinger, 2002).

Recently, improved techniques for analysis of the gut microflora, new food manufacturing biotechnologies, and increased understanding of the metabolism of prebiotic inulin and oligosaccharides by probiotics are facilitating the ability of the food industry to rationally develop prebiotics for specific functional properties and health outcomes (Wang, 2009). The investigations of prebiotics have shown that fermentability of fructans in batch culture experiments has a preferential stimulating effect on health promoting *Bifidobacteria* while limiting the growth of populations of potential pathogens (Walker and Duffy, 1998).

In vitro studies showed that several of the commercially available carbohydrates and compounds were able to enhance the growth of probiotic cultures *Lactobacillus acidophilus*, *B. animalis lactis*, and *Lactobacillus casei* *in vitro*, as shown by measurement of optical density levels over a period of 48 hours. Of the compounds used in the *L. acidophilus* cultures, soybean oligosaccharide (SOS) produced maximal growth, followed by FOS and inulin; of the compounds used in *B. animalis lactis* cultures, SOS produced maximal growth, followed by raffinose and FOS; of the compounds used in *L. casei* cultures, FOS produced maximum growth, followed by inulin and SOS (Su et al., 2007).

One of the methods used to describe the extent to which prebiotics supported selective growth of lactobacilli and bifidobacteria has been to establish a quantitative score using a

prebiotic activity assay. This is based on the comparison of relative changes in biomass between certain probiotic species and a mixture of enteric strains on cultures containing prebiotics (1%) when incubated (anaerobically) over 24 hours. Scores can then be calculated for prebiotic activity based on the biomass data and can provide a basis for the evaluation of combinations of probiotic ingredients for applications as synbiotics in dairy and other foods (Huebner et al., 2007).

Human milk has also been found to contain components with bifidogenic effects. The important growth-promoting bifidus factors in human milk are the human milk oligosaccharides, which function to (1) stimulate the growth of bifidobacteria in the colon of breastfed infants (prebiotic function); and (2) act as soluble receptor analogues for pathogens and thereby directly inhibits certain pathogenic microorganisms. This prompted developers of infant milk formulas to focus on those bioactive components present in human milk. Following subsequent research, many nondigestible oligosaccharides were added to infant milk formulas in an attempt to promote the favorable bifidogenic effects (Alles et al., 2004).

Cereals also represent an alternative for the production of prebiotic functional foods, as they contain (1) water-soluble fiber, such as β -glucan and arabinoxylan; and (2) indigestible oligosaccharides, such as galacto- and fructooligosaccharides and resistant starch, which have been suggested to benefit the gut microbial composition and activities by selectively stimulating the growth of a number of colonic bacteria. The multiple beneficial effects of cereals have led to the design of novel cereal foods or cereal ingredients that target specific populations. The main parameters to be considered are (1) the composition and processing of cereal grains; (2) the substrate formulation; (3) the growth capability and productivity of the starter culture; (4) the stability of the probiotic strain during storage; (5) the organoleptic properties; and (6) the nutritional value of the final product. In addition, processing techniques, such as milling, sieving, and debranning, or pearling, could be used to separate specific fractions of fiber from different cereal varieties or cereal by-products. Finally, the constituents in cereal, for example, starch, can be used as microencapsulation materials for probiotics (Charalampopoulos et al., 2002).

9.5 CHALLENGES AND APPROACHES FOR PROBIOTIC INGREDIENTS

The success of worldwide research and development activities for new probiotic foods depends on several factors. In addition to the costs and the marketing challenges, at least seven questions must be addressed (adapted from Champagne et al., 2005). These questions include: (1) the type or form of probiotic to be selected; (2) the amount to be added to have a beneficial effect; (3) toxicity issues, if any; (4) viability of probiotic bacteria during processing; (5) determination of the concentration of the active compound (strains/cell population); (6) stability of the probiotics during storage; and (7) changes in sensory properties during supplementation.

In the following sections, only a few of the challenges (e.g., strain selection, technological hurdles, or methodology to prove health effects) are outlined. Current new and innovative approaches in the field of probiotics include prestressing by acidity or heat stress as described by Girgis et al. (2003), Maus and Ingham (2003), and Vasiljevic and Shah (2008). Other research activities concentrate on the influences of growth conditions and

fermentation technologies (Lacroix and Yildirim, 2007) or protective agents, such as prebiotics (Capela et al., 2006; Oliveira et al., 2009) on the survival of probiotics.

9.5.1 Strain selection

The overall key and desirable criteria for the selection of probiotics in commercial applications are summarized by Klaenhammer and Kullen (1999), Saarela et al. (2000), Holzapfel and Schillinger (2002), Champagne et al. (2005), and Vasiljevic and Shah (2008). They consider general, technical, functional, and beneficial aspects, as shown in Table 9.4. The safety and nonpathogenicity of a new strain are considered of major importance.

In addition, each potential probiotic strain should be assessed and documented individually. Only well-defined strains should be used in trials, and, if possible, all human studies should be randomized, double blind, and placebo controlled. Confirmation of the results

Table 9.4. Key criteria for the selection of probiotic strains.^a

Key criteria	Property
Appropriateness and safety	Origin (natural inhabitant of the species targeted: human origin for human probiotics) Generally regarded as safe (GRAS) status (pathogenicity and infectivity, virulence factors: toxicity, metabolic activity, and intrinsic properties, e.g., antibiotic resistance) Accurate taxonomic identification Assessment and documentation independently of each potential strain
Technological suitability and competitiveness	Genetically stable Desired viability during processing and storage Good sensory properties Phage resistance Resistant to lysozyme and phenolic compounds Low sensitivity to oxygen Ease of large-scale production and concentration to high cell densities Good stability during culture preparation Stability during processing Good stability during storage (i.e., in dried or frozen form) and delivery Growth in food matrices for example, milk Inexpensive cultivation Low activity below 15°C Ability to compete with the normal microflora, including the same or closely related species
Functional performance	Tolerance to gastric acid and bile Resistance to proteases and digestive enzymes Adhesion to mucosal surface Survival and colonization <i>in vivo</i> Validated and documented health effects
Desirable physiological activity	Able to exert one or more clinically documented health benefits, such as: <ul style="list-style-type: none"> • Immunomodulation • Antagonistic activity • Cholesterol metabolism • Lactose metabolism • Antimutagenic and anticarcinogenic properties

^aAdapted from Klaenhammer and Kullen (1999), Saarela et al. (2000), Holzapfel and Schillinger (2002), Champagne et al. (2005), and Vasiljevic and Shah (2008).

and preferably publication of results in peer-reviewed journals are required (Salminen et al., 1996).

9.5.2 Understanding the probiotic mechanism

Although the efficacy of probiotics has been demonstrated for various health problems, the complexity of the microbial community presents a challenge for the understanding of molecular processes underlying host–microbe interactions. Moreover, the list of health claims made for prebiotics is much longer than the list of probiotic effects, for which actual clinical evidence is present. According to the guidelines for the evaluation of probiotics for food use, introduced in 2002 by WHO, functionality and safety have to be demonstrated by laborious and costly *in vitro* and *in vivo* tests. Sanders and Huis in't Veld (1999) describe several approaches for the *in vitro* testing of the strains. An example is the dynamic model of TNO Nutrition and Food Research at Zeist in the Netherlands that helps to simulate all major constituents of the human gastrointestinal system: from peristaltic movement, physiological pH curves, gastrointestinal secretion, intestinal absorption of nutrients and water, physiological gastric emptying, intestinal transport patterns, to high efficiency water absorption in the colon. Despite the advantages of these dynamic models, the high cost and the sophisticated equipment required represent major disadvantages. A simple set of *in vitro* tests for the screening of possible probiotic strains, as described by Vinderola et al. (2008), help to determine some parameters of technological, biological, and probiotic importance as a basis for future and more complex studies for probiotic candidates.

Although numerous attempts have been made to define the cellular targets for probiotic action, the molecular basis for much of their action remains unknown. The molecular structures implicated in probiotic function have yet to be characterized. With the use of genome analysis tools, such as DNA arrays and real-time PCR, the molecular bases of those “beneficial effects” can be explored, and bacterial genetic traits can be linked to the probiotic function. Furthermore, knowledge of this genomic data has applications in gene cloning, allowing the most suitable strains of probiotic bacterial to be tailored for specific host requirements (Schiffrin and Blum, 2001).

The development of different models with simplified human intestinal microbiota, as recently reviewed by Wohlgemuth et al. (2010), help to study the mechanisms and influences of probiotics on human health. Recent progress in the retrieval and analysis of huge amounts of sequence data has created the opportunity to completely analyze the genomes of all members of the intestinal microbiota. The initiation of the Human Microbiome Project will be the basis for realizing this ambitious metagenomic approach and for gaining insight into the human microbiome and the influences of probiotics on the human health status. Aside from improving the credibility of the probiotic concept, new findings will be the scientific basis for the development for “tailor-made strategies” for the prevention of various diseases (Wohlgemuth et al., 2010).

9.5.3 Survival of probiotics

Probiotics are viable organisms that are susceptible to various external factors, like temperature, pH, water activity, osmotic pressure, and the presence of oxygen (Anal and Singh, 2007). The stability of these probiotic bacteria is also influenced by their genus, species, strain biotype, and composition of the other active ingredients (Del Piano et al., 2006). The poor survivability of the probiotic bacteria when exposed to the external environment has

been well documented (Ross et al., 2005). The survival of such organisms in the gastrointestinal tract is even more questionable (Kailasapathy, 2002), because they have to survive the high acid conditions of the stomach and the presence of enzymes and bile salts in the small intestine. Stability during storage is a critical requirement for the probiotic food product, as consumers demand that the product purchased contains sufficient viable probiotic cells at the time of consumption. Numerous technological operations, as well as challenging food matrices, influence the functionality of probiotics and their stability during processing and storage. Despite controversy regarding the viability of strains, it may still be a requirement that high viability is maintained during storage. The review by Lourens-Hattingh and Viljoen (2001) focuses on the concept of “therapeutic minimum” levels and the importance of the survival of probiotic microorganisms in food products. The “therapeutic minimum” is explained as the adequate number of viable cells needed to be consumed regularly for the transfer of the “probiotic” effect to consumers. It is therefore imperative to ensure the survival of these bacteria in the carrier food and in the GI tract. Approximately 10^6 – 10^8 cfu/g of intestinal content is required in the GI tract to significantly affect the environment of the gut. However, increasing data on bacterial populations suggest that the addition level will vary depending on the strain and the desired health effect (Champagne et al., 2005).

To achieve high probiotic concentrations in the carrier food, either high concentrations of the bacteria must be added to the final product, or there must be acceptable growth during the manufacturing process. Probiotics are typically added to fermented dairy and other products, where starters are mostly necessary for technical purposes. LAB occupy a central role in the functionalities of starter cultures, initiating the rapid acidification of the raw material, and accelerating and steering the fermentation process to produce a fermented food. By producing a variety of organic compounds, such as acetic acid, ethanol, aroma compounds, and several important enzymes, LAB are able to enhance the shelf life, improve texture and microbial safety, and contribute positively to the sensory profile of the end product (Leroy and De Vuyst, 2004).

While in most cases high numbers of probiotics are important to achieve the claimed health effects, it is important to consider growth or survival dependency of species and strain selection and physiology, type of matrix, and its properties (e.g., pH, carbon, nitrogen, mineral and oxygen content, water activity, and buffering capacity) or processing steps, as shown in the overview of Champagne et al. (2005) and Rivera-Espinoza and Gallardo-Navarro (2010). Low viability of probiotic bacteria is a major problem even in a wide range of fermented dairy products, including yogurts, soft, semi-hard, and hard cheeses, ice cream, and frozen fermented dairy desserts (Desmond et al., 2005). Several influencing factors have been reported. These include titratable acidity, pH, hydrogen peroxide, dissolved oxygen content, storage temperature, species and strains of associative fermented dairy product organisms, concentration of lactic and acetic acids, and even whey protein concentration (Dave and Shah, 1997). Because the ability of probiotics to survive during processing and storage are not linked, it is necessary to specifically examine factors such as pH level or oxygen that affect the shelf life of probiotics. Generally fermented products have an acidic pH, but continuous acidification during cold storage may lead to “over-acidification,” a detrimental effect on probiotics because of their sensitivity to an acidic environment. Oxygen levels also affect the storage of probiotic food products. This is firstly as a result of direct toxicity to the oxygen-sensitive LAB cells, which may die due to the intracellular production of hydrogen peroxide. Secondly, oxygen levels may affect probiotics indirectly, because certain cultures, particularly *Lactobacillus delbrueckii*, may

have a synergistic inhibitory effect on bifidobacteria through the excretion of peroxide in the medium (Champagne et al., 2005). The recent findings of Kurtmann et al. (2009a,b) show that products of oxidation reactions and Maillard reactions interact with the bacteria, and the storage stability of freeze-dried lactobacilli is decreased. Further studies are necessary to identify the influences of the different reaction products on the bacterial cell wall and possible damage of specific bacterial cell components.

The selection of appropriate probiotic cultures should also consider sensorial properties like taste and flavor, as well as technological (product stability and viscosity) aspects of the probiotic products during processing and storage. The reviews by Ross et al. (2005) and Mattila-Sandholm et al. (2002) explore the many technological challenges in the development of probiotic food products. They highlight that from a commercial point of view, the technological suitability of the strains is just as important as the clinical evidence supporting their health-promoting activity. Most human intestinal isolates that are obligate anaerobes grow poorly outside the human gut. As a result, the large-scale cultivation and storage of probiotic lactobacilli and bifidobacteria presents a major technological hurdle to their exploitation.

9.5.4 Microencapsulation of probiotics

Since probiotic bacteria need to be viable and stable in order to provide beneficial effects to the host, they are required to withstand processing conditions and also be viable in sufficient numbers during storage (Del Piano et al., 2006). Hence, the presence of a physical barrier to protect the probiotic, increasing its survival rate during drying and storage, and in turn increasing its efficacy, is desirable.

Microencapsulation has been shown to provide an effective barrier that protects cells from degradation through interaction with the external environment and from various stresses present during production and/or storage, enhancing the viability of probiotics and improving their shelf life. Microencapsulation is a process by which the functional core ingredients (in this case, the probiotic bacteria) are surrounded by a coating or a matrix of encapsulant materials to produce microcapsules in the micrometer to millimeter size range. The encapsulant materials can offer an effective barrier against oxygen, heat, and other external stresses during processing and storage. Microencapsulation can also protect probiotics against low pH and proteases during gastric transit until their release in the intestine. The production of small capsules with suitable sensory properties and the use of materials that are inexpensive, stable, and of food grade is also desirable (Crittenden et al., 2006).

The most common processing methods used in microencapsulation include spray drying, spray cooling, extrusion, fluidized bed drying, coacervation, and freeze drying. Typical commercial probiotics are supplied in freeze dried form. Spray drying is an economical alternative method due to its low cost compared with freeze drying (Santivarangkna et al., 2007; Meng et al., 2008). However, high mortality may be a result of simultaneous dehydration and thermal inactivation of the microorganisms.

In order to sustain probiotic viability during the freeze-drying process, cryoprotectants are used. These carrier materials interact with the cell membrane lipids and proteins. Water molecules are effectively replaced so that the physiological activities of the probiotic bacteria are regained when they became rehydrated (Chavez and Ledebor, 2007). One of the major factors causing loss of viability in probiotic bacteria during long-term storage is cell membrane lipid and protein oxidation. Thus, in order to maintain probiotic viability during storage, carrier materials have to be able to act as an oxygen barrier and also preferably

possess antioxidative properties (Kurtmann et al., 2009b). It is also necessary for the encapsulant matrix to remain in a glassy state at storage temperature and water activity, although glass transition temperature (T_g) cannot be regarded as an absolute threshold of bacteria stability during storage (Higl et al., 2007). The significantly higher viscosity of materials when in the glassy state and the arrested molecular mobility contribute to an improved storage stability of the probiotic bacteria compared with that achieved with the matrix in a rubbery state.

A wide range of proteins (e.g., casein, whey protein, skim milk, soy protein, and gelatin), carbohydrates (starch, sucrose, trehalose, lactose, cellulose, alginate, gum acacia, carrageenan, pectin, etc.), and lipids have been used as food grade encapsulants for probiotics produced using various microencapsulation technologies (Teixeira et al., 1995; Gardiner et al., 2000; Conrad et al., 2000; Desmond et al., 2002; Picot and Lacroix, 2004; Crittenden et al., 2006; Chavez and Ledebøer, 2007; Lahtinen et al., 2007; Santivarangkna et al., 2008; Ding and Shah, 2009). However, an encapsulation matrix using food grade, low-cost materials, and manufacturing process to meet all the requirements for probiotics has yet to be achieved.

The ideal microencapsulant materials should possess the following features: (1) ability to interact with cell membrane lipids and proteins to replace water molecules to protect cell viability during the drying process; (2) a high glass transition temperature (T_g) so that the microcapsule matrix will retain the glassy state at nonrefrigerated storage conditions and in intermediate to high humidity environments; (3) high oxygen barrier and/or antioxidative properties (e.g., radical and oxygen scavenger, chelating power); and (4) resistance to low pH, protease, and bile salt to protect the bacteria during gastrointestinal transit, and colonic release.

Many studies involving different encapsulants and processing methods have reported that microencapsulation protects the probiotic from external stresses, and that survival rates were enhanced compared with that of the free cells (Kailasapathy, 2002; Desmond et al., 2005; Crittenden et al., 2006; Anal and Singh, 2007; Champagne and Fustier, 2007; Chavez and Ledebøer, 2007; Ding and Shah, 2009). There have been many recent advances in microencapsulation of probiotics for industrial applications and target delivery, in addition to new developments and techniques for enhancing their viability during fermentation, processing, and utilization in commercial products (Carvalho et al., 2004; Anal and Singh, 2007; Meng et al., 2008; Vasiljevic and Shah, 2008). However, maintaining the long-term viability of probiotics remains a significant challenge in most food applications, for example, in products with high water activity and in products stored under ambient conditions prior to retail sale.

REFERENCES

- Adams, M.R. (1999) Safety of industrial lactic acid bacteria. *Journal of Biotechnology* **68**, 171–178.
- Alles, M.S., Scholtens, P.A.M.J., and Bindels, J.G. (2004) Current trends in the composition of infant milk formulas. *Current Paediatrics* **14**, 51–63.
- Anal, A.K. and Singh, H. (2007) Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. *Trends in Food Science and Technology* **18**, 240–251.
- Anon (1999) Scientific concepts of functional foods in Europe. Consensus document. *British Journal of Nutrition* **81**(Suppl. 1), S1–S27.
- Anon (2009) Dairy council digest archives. *Functional Foods: An Overview*. <http://www.aarkstore.com/reports/Boosting-Immunity-Through-Digestion-The-Relation-Among-Probiotics-Prebiotics-and-Digestive-Enzymes-31224.html>. Accessed April 5, 2011.

- Aragon-Alegro, L.C., Alegro, J.H.A., Cardarelli, H.R., Chiu, M.C., and Saad, S.M.I. (2007) Potentially probiotic and synbiotic chocolate mousse. *LWT-Food Science and Technology* **40**, 669–675.
- Arihara, K., Ota, H., Itoh, M., Kondo, Y., Sameshima, T., Yamanaka, H., Akimoto, M., Kanai, S., and Miki, T. (1998) *Lactobacillus acidophilus* group lactic acid bacteria applied to meat fermentation. *Journal of Food Science* **63**, 544–547.
- Bernet, M.F., Brassart, D., Meeser, J.R., and Servin, A. (1993) Adhesion of human *Bifidobacteria* strains to cultured human intestinal epithelial cells and inhibition of enteropathogen-cell interactions. *Applied Environmental Microbiology* **59**, 4121–4128.
- Capela, P., Hay, T.K.C., and Shah, N.P. (2006) Effect of cryoprotectants, prebiotics and microencapsulation on survival of probiotic organisms in yoghurt and freeze-dried yoghurt. *Food Research International* **39**, 203–211.
- Carvalho, A.S., Silva, J., Ho, P., Teixeira, P., Malcata, F.X., and Gibbs, P. (2004) Relevant factors for the preparation of freeze-dried lactic acid bacteria. *International Dairy Journal* **14**, 835–847.
- Champagne, C.F. and Fustier, P. (2007) Microencapsulation for the improved delivery of bioactive compounds into foods. *Current Opinion in Biotechnology* **18**, 184–190.
- Champagne, C.P., Gardner, N.J., and Roy, D. (2005) Challenges in the addition of probiotic cultures to foods. *Critical Review in Food Science and Nutrition* **45**, 61–84.
- Charalampopoulos, D., Wang, R., Pandiella, S.S., and Webb, C. (2002) Application of cereals and cereal components in functional foods: a review. *International Journal of Food Microbiology* **79**, 131–141.
- Charteris, W.P., Kelly, P.M., Morelli, L., and Collins, J.K. (2002) Edible table (bio)spread containing potentially probiotic *Lactobacillus* and *Bifidobacterium* species. *International Journal of Dairy Technology* **55**, 44–56.
- Chavez, B.E. and Ledebor, A.M. (2007) Drying of probiotics: optimization and process to enhance storage survival. *Drying Technology* **25**(7), 1193–1201.
- Conrad, P.B., Miller, D.P., Cielenski, P.R., and de Pablo, J.J. (2000) Stabilization and preservation of *Lactobacillus acidophilus* in saccharide matrices. *Cryobiology* **41**, 17–24.
- Crittenden, R., Weerakkody, R., Sanguansri, L., and Augustin, M.A. (2006) Synbiotic microcapsules that enhance microbial viability during nonrefrigerated storage and gastrointestinal transit. *Applied and Environmental Microbiology* **72**, 2280–2282.
- Cruz, A.G., Antunes, A.E.C., Sousa, A.L.O.P., Faria, J.A.F., and Saad, S.M.I. (2009) Ice-cream as a probiotic food carrier. *Food Research International* **42**, 1233–1239.
- Dave, R.I. and Shah, N.P. (1997) Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. *International Dairy Journal* **7**, 31–41.
- Dekker, J.W., Wickens, K., Black, P.N., Stanley, T.V., Mitchell, E.A., Fitzharris, P., Tannock, G.W., Purdie, G., and Crane, J. (2009) Safety aspects of probiotic bacterial strains *Lactobacillus rhamnosus* HN001 and *Bifidobacterium animalis* subsp. *lactis* HN019 in human infants aged 0–2 years. *International Dairy Journal* **19**, 149–154.
- Del Piano, M., Morelli, L., Strozzi, G.P., Allesina, S., Barba, M., Deidda, F., Lorenzini, P., Ballaré, M., Montino, F., Orsello, M., Sartori, M., Garelo, E., Carmagnola, S., Pagliarulo, M., and Capurso, L. (2006) Probiotics: from research to consumer. *Digestive and Liver Disease* **38**(Suppl. 2), 248–255.
- Desmond, C., Ross, R.P., O'Callaghan, E., Fitzgerald, G., and Stanton, C. (2002) Improved survival of *Lactobacillus paracasei* NFB3 338 in spray-dried powders containing gum acacia. *Journal of Applied Microbiology* **93**, 1003–1011.
- Desmond, C.B., Corcoran, M., Coakley, M., Fitzgerald, G.F., Ross, R.P., and Stanton, C. (2005) Development of dairy-based functional foods containing probiotics, and prebiotics. *Australian Journal of Dairy Technology* **60**, 121–126.
- Ding, W.K. and Shah, N.P. (2009) Effect of various encapsulating materials on the stability of probiotic bacteria. *Journal of Food Science* **74**(2), M100–M107.
- Dunne, C., Murphy, L., Flynn, S., O'Mahony, L., O'Halloran, S., Feeney, M., Morrissey, D., Thornton, G., Fitzgerald, G., Daly, C., Kiely, B., Quigley, E.M.M., O'Sullivan, G.C., Shanahan, F., and Kevin, J. (1999) Probiotics: from myth to reality. Demonstration of functionality in animal models of disease and in human clinical trials. *Antonie van Leeuwenhoek* **76**, 279–292.
- FAO/WHO (2002) Guidelines for the evaluation of probiotics in food. Report of a joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food. London, Ontario, Canada.
- Fuller, R. (1992) History and development of probiotics. In: *Probiotics, the Scientific Basis*, edited by R. Fuller, pp. 1–8. London: Chapman & Hall.
- Gardiner, G.E., O'Sullivan, E., Kelly, J., Auty, M.A.E., Fitzgerald, G.F., Collins, J.K., Ross, R.P., and Stanton, C. (2000) Comparative survival rates of human-derived probiotic *Lactobacillus paracasei* and

- L. salivarius strains during heat treatment and spray drying. *Applied and Environmental Microbiology* **66**(6), 2605–2612.
- Gibson, G.R. and Roberfroid, M.B. (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. Critical Review. *Journal of Nutrition* **125**, 1401–1412.
- Giraffa, G. (2003) Functionality of enterococci in dairy products. *International Journal of Food Microbiology* **88**, 215–222.
- Girgis, H.S., Smith, J., Luchansky, J.B., and Klaenhammer, T.R. (2003) Stress adaptation of lactic acid bacteria. In: *Microbial Stress Adaptation and Food Safety*, edited by A.E. Yousef and V.K. Juneja. Boca Raton, FL: CRC Press.
- Goldin, B. and Gorbach, S.L. (1984) The effect of milk and *Lactobacillus* feeding on human intestinal bacterial enzyme activity. *American Journal of Clinical Nutrition* **33**, 15–18.
- Havenaar, R. and Huis in't Veld, J.H.J. (1992) Probiotics: a general view. In: *Lactic Acid Bacteria in Health and Disease*, Vol. 1. edited by B.J.B. Wood. Amsterdam: Elsevier Applied Science Publishers.
- Higl, B., Kurtmann, L., Carlsen, C.U., Ratjen, J., Forst, P., Skibsted, L.H., Kulozik, U., and Risbo, J. (2007) Impact of water activity, temperature, and physical state on the storage stability of *Lactobacillus paracasei* ssp. *Paracasei* freeze-dried in a lactose matrix. *Biotechnology Progress* **23**, 794–800.
- Holzapfel, W.H., Haberer, P., Snel, J., Schillinger, U., and Huis in't Veld, J.H.J. (1998) Overview of gut flora and probiotics. *International Journal of Food Microbiology* **41**, 85–101.
- Holzapfel, W.H. and Schillinger, U. (2002) Introduction to pre- and probiotics. *Food Research International* **35**, 109–116.
- Homayouni, A., Azizi, A., Ehsani, M.R., Yarmand, M.S., and Razavi, S.H. (2008) Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. *Food Chemistry* **111**, 50–55.
- Huebner, J., Wehling, R.L., and Hutkins, R.W. (2007) Functional activity of commercial prebiotics. *International Dairy Journal* **17**, 770–775.
- Ishibashi, N. and Shimamura, S. (1993) Bifidobacteria: research and development in Japan. *Food Technology* **47**(6), 129–134.
- Isolauri, E., Salminen, S., and Ouwehand, A.C. (2004) Probiotics. *Best Practice & Research Clinical Gastroenterology* **18**, 299–313.
- Jonas, M.S. and Beckmann, S.C. (1998) Functional foods: consumer perceptions in Denmark and England. MAPP Working Paper No. 55.
- Kailasapathy, K. (2002) Microencapsulation of probiotic bacteria: technology and potential applications. *Current Issues Intestinal Microbiology* **3**, 39–48.
- Kankainen, M., Paulin, L., Tynkkynen, S., von Ossowski, I., Reunanen, J., Parpanen, P., Satokari, R., Vesterlund, S., Hendrickx, A.P.A., Lebeer, S., De Keersmaecker, S.C.J., Vanderleyden, J., Hämäläinen, T., Laukkanen, S., Salovuori, N., Ritari, J., Alatalo, E., Korpela, R., Mattila-Sandholm, T., Lassig, A., Hatakka, K., Kinnunen, K.T., Karjalainen, H., Saxelin, M., Laakso, K., Sarakka, A., Palva, A., Salusjärvi, T., Auvinen, P., and de Vos, W. (2009) Comparative genomic analysis of *Lactobacillus rhamnosus* GG reveals pili containing a human-mucus binding protein. *Proceedings of the National Academy of Science* **106**, 17193–17198.
- Katayama, M., Xu, D., Specian, R., and Deitch, E. (1997) Role of bacterial adherence and the mucus barrier on bacterial translocation: effects of protein malnutrition and endotoxin in rats. *Annals of Surgery* **225**(3), 317–326.
- Klaenhammer, T.R. and Kullen, M.J. (1999) Selection and design of probiotics. *International Journal of Food Microbiology* **50**, 45–57.
- Kolida, S., Saulnier, D.M., and Gibson, G.R. (2006) Gastrointestinal microflora: probiotics. *Advances in Applied Microbiology* **59**, 187–219.
- Kurtmann, L., Skibsted, L.H., and Carlsen, C.U. (2009a) Browning of freeze-dried probiotic bacteria cultures in relation to loss of viability during storage. *Journal of Agricultural and Food Chemistry* **57**, 6736–6741.
- Kurtmann, L., Carlsen, C.U., Risbo, J., and Skibsted, L.H. (2009b) Storage stability of freeze-dried *Lactobacillus acidophilus* (La-5) in relation to water activity and presence of oxygen and ascorbate. *Cryobiology* **58**, 175–180.
- Lacroix, C. and Yildirim, S. (2007) Fermentation technologies for the production of probiotics with high viability and functionality. *Current Opinion in Biotechnology* **18**, 176–183.
- Lahtinen, S.J., Ouwehand, A.C., Salminen, S.J., Forsell, P., and Myllärinen, P. (2007) Effect of starch- and lipid-based encapsulation on the culturability of two *Bifidobacterium longum* strains. *Letter of Applied Microbiology* **44**, 500–505.

- Lee, Y.-K. and Salminen, S. (1995) The coming age of probiotics. *Trends in Food Science & Technology* **6**, 241–245.
- Leroy, F. and De Vuyst, L. (2004) Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science & Technology* **15**, 67–78.
- Lilly, D. and Stillwell, R.H. (1965) Probiotics: growth-promoting factors produced by microorganisms. *Science* **147**, 747–748.
- Lourens-Hattingh, A. and Viljoen, B.C. (2001) Yogurt as probiotic carrier food. *International Dairy Journal* **11**, 1–17.
- Marteau, P. (2001) Prebiotics and probiotics for gastrointestinal health. *Clinical Nutrition* **20**(Suppl. 1), 41–45.
- Mattila-Sandholm, T., Myllärinen, P., Crittenden, R., Mogensen, G., Fondén, R., and Saarela, M. (2002) Technological challenges for future probiotic foods. *International Dairy Journal* **12**, 173–182.
- Maus, J.E. and Ingham, S.C. (2003) Employment of stressful conditions during culture production to enhance subsequent cold- and acid-tolerance of bifidobacteria. *Journal of Applied Microbiology* **95**, 146–154.
- Mäkinen-Aakula, M. (2006) Trends in functional foods dairy market. In *Proceedings of the third functional food net meeting*, Liverpool, UK.
- Meile, L., Le Blay, G., and Thierry, A. (2008) Safety assessment of dairy microorganisms. *International Journal of Food Microbiology* **126**, 316–320.
- Meng, X.C., Stanton, C., Fitzgerald, G.F., Daly, C., and Ross, R.P. (2008) Anhydrobiotics: the challenges of drying probiotic cultures. *Food Chemistry* **106**, 1406–1416.
- Metchnikoff, L.E. (1907) *The Prolongation of Life: Optimistic Studies* (1907 Edition Reprinted in 2004). New York: Springer Publishing Company.
- Moreno, M.R.F., Sarantionopoulos, P., Tsakalidou, E., and De Vuyst, L. (2006) The role and application of enterococci in food and health. *International Journal of Food Microbiology* **106**, 1–24.
- O'Sullivan, M.G., Thornton, G., O'Sullivan, G.C., and Collins, J.K. (1992) Probiotic bacteria: myth or reality? *Trends in Food Science & Technology* **3**, 309–314.
- Ogier, J.C. and Serror, P. (2008) Safety assessment of dairy microorganisms: the Enterococcus genus. *International Journal of Food Microbiology* **126**, 291–301.
- Oliveira, R.P.S., Florence, A.C.R., Silva, R.C., Perego, P., Converti, A., Gioielli, L.A., and Oliveira, M.N. (2009) Effect of different prebiotics on the fermentation kinetics, probiotic survival and fatty acids profiles in nonfat symbiotic fermented milk. *International Journal of Food Microbiology* **128**, 467–472.
- Ouweland, A.C. and Salminen, S.J. (1998) Review: the health effects of cultured milk products with viable and non-viable bacteria. *International Dairy Journal* **8**, 749–758.
- Picot, A. and Lacroix, C. (2004) Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. *International Dairy Journal* **14**, 505–515.
- Prado, F.C., Parada, J.L., Pandey, A., and Socol, C.R. (2008) Review: trends in non-dairy probiotic beverages. *Food Research International* **41**, 111–123.
- Rastall, R.A. and Maitin, V. (2002) Prebiotics and synbiotics: towards the next generation. *Current Opinion in Biotechnology* **13**, 490–496.
- Rivera-Espinoza, Y. and Gallardo-Navarro, Y. (2010) Non-dairy probiotic products. *Food Microbiology* **27**, 1–11.
- Roberfroid, M. (2007) Prebiotics: the concept revisited. *The Journal of Nutrition* **137**, 830S–827S.
- Ross, R.P., Desmond, C., Fitzgerald, G.F., and Stanton, C. (2005) Overcoming the technological hurdles in the development of probiotic foods. *Journal of Applied Microbiology* **98**, 1410–1417.
- Ross, S. (2000) Functional foods: the Food and Drug Administration perspective. *American Journal of Clinical Nutrition* **71**(Suppl.), 1735S–1738S.
- Saarela, M., Mogensen, G., Fondén, R., Mättö, J., and Mattila-Sandholm, T. (2000) Probiotic bacteria: safety, functional and technological properties. *Journal of Biotechnology* **84**, 197–215.
- Saarela, M., Lähteenmäki, L., Crittenden, R., Salminen, S., and Mattila-Sandholm, T. (2002) Gut bacteria and health foods—the European perspective. *International Journal of Food Microbiology* **78**, 99–117.
- Salminen, S., Isolauri, E., and Salminen, E. (1996) Clinical uses of probiotics for stabilizing the gut mucosal barrier: successful strains and future challenges. *Antonie van Leeuwenhoek* **70**, 347–358.
- Salminen, S., Bouley, C., Boutron-Ruault, M.-C., Cummings, J., Franck, A., Gibson, G., Isolauri, E., Moreau, M.C., Roberfroid, M., and Rowland, I. (1998a) Functional food science and gastrointestinal physiology and function. *British Journal of Nutrition* **80**, 147–171.

- Salminen, S., von Wright, A., Morelli, L., Marteau, P., Brassart, D., de Vos, W., Fondén, R., Saxelin, M., Collins, K., Mogensen, G., Birkeland, S.E., and Mattila-Sandholm, T. (1998b) Demonstration of safety of probiotics—a review. *International Journal of Food Microbiology* **44**, 93–106.
- Sanders, M.E. (2009) How do we know when something calls “probiotic” is really a probiotic? A guideline for consumers and health care professionals. *Functional Food Review* **1**, 3–12.
- Sanders, M.E. and Huis in’t Veld, J. (1999) Bringing a probiotic-containing functional food to the market: microbiological, product, regulatory and labelling issues. *Antonie van Leeuwenhoek* **76**, 293–315.
- Santivarangkna, C., Kulozik, U., and Foerst, P. (2007) Alternative drying process for the industrial preservation of lactic acid starter cultures. *Biotechnology Progress* **23**, 302–315.
- Santivarangkna, C., Higl, B., and Foerst, P. (2008) Protection mechanisms of sugars during different stages of preparation process of dried lactic acid starter cultures. *Food Microbiology* **25**, 429–441.
- Saulnier, D.M.-A., Spinler, J.K., Gibson, G.R., and Versalovic, J. (2009) Mechanisms of probiosis and prebiosis: considerations for enhanced functional foods. *Current Opinion in Biotechnology* **20**, 135–141.
- Scheinbach, S. (1998) Probiotics: functionality and commercial status. *Biotechnology Advances* **16**(3), 581–608.
- Schiffirin, E.J. and Blum, S. (2001) Food processing: probiotic microorganisms for beneficial foods. *Current Opinion in Biotechnology* **12**, 499–502.
- Schrezenmeir, J. and de Vrese, M. (2001) Probiotics, prebiotics, and synbiotics—approaching definition. *American Journal of Clinical Nutrition* **73**(Suppl.), 361S–364S.
- Shah, N.P. (2001) Functional foods, probiotics and prebiotics. *Food Technology* **55**, 46–51.
- Shah, N.P. (2004) Probiotics and prebiotics. *Agro Food Industry Hi-tech* **15**, 13–16.
- Shah, N.P. (2007) Functional cultures and health benefits. Review. *International Dairy Journal* **17**, 1262–1277.
- Shortt, C. (1999) The probiotic century: historical and current perspectives. *Trends in Food Science & Technology* **10**, 411–417.
- Siro, I., Kapolna, E., Kapolna, B., and Lugasi, A. (2008) Functional food. Product development, marketing and consumer acceptance—a review. *Appetite* **51**, 456–467.
- Stiles, M.E. and Holzapfel, W.H. (1997) Lactic acid bacteria of foods and their current taxonomy. Review article. *International Journal of Food Microbiology* **36**, 1–29.
- Su, P., Henriksson, A., and Mitchell, H. (2007) Selected prebiotics support the growth of probiotic monocultures *in vitro*. *Food Microbiology* **13**, 134–139.
- Teixeira, P.C., Castro, M.H., Malcata, F.X., and Kirby, R.M. (1995) Survival of *Lactobacillus delbrueckii* ssp. *Bulgaricus* following spray-drying. *Journal of Dairy Science* **78**, 1025–1031.
- Vasiljevic, T. and Shah, N.P. (2008) Probiotics—from Metchnikoff to bioactives. Review. *International Dairy Journal* **18**, 714–728.
- Vinderola, G., Capellini, B., Villarreal, F., Suarez, V., Quiberoni, A., and Reinheimer, J. (2008) Usefulness of a set of simple *in vitro* tests for the screening and identification of probiotic candidate strains for dairy use. *LWT—Food Science and Technology* **41**, 1678–1688.
- Walker, W.A. and Duffy, L.C. (1998) Diet and bacterial colonization: role of probiotics and prebiotics. *The Journal of Nutritional Biochemistry* **9**, 668–675.
- Walker, W.A., Goulet, O., Morelli, L., and Antoine, J.M. (2006) Progress in the science of probiotics: from cellular microbiology and applied immunology to clinical nutrition. *European Journal of Nutrition* **45**(Suppl. 1), I/1–I/18.
- Wang, Y. (2009) Prebiotics: present and future in food science and technology. *Food Research International* **42**, 8–12.
- Wohlgemuth, S., Loh, G., and Blaut, M. (2010) Recent developments and perspectives in the investigation of probiotic effects. *International Journal of Medical Microbiology* **300**, 3–10.
- Yakult Central Institute for Microbiological Research (1998) *Lactobacillus casei* strain *Shirota*. Tokyo: Yakult Honsha Company Ltd.
- Ziemer, C.J. and Gibson, G.R. (1998) An Overview of probiotics, prebiotics and synbiotics in the functional food concept: Perspectives and future strategies. *International Dairy Journal* **8**, 473–479.

10 Dairy Ingredient Safety: The No Compromise Area

D. Eddy and A. Astin

10.1 INTRODUCTION

Modern approaches to dairy product and ingredient development will not be successful without assured safety. This chapter addresses issues of the microbiological sciences of microbial ecology, risk management, and hazard control in the processing and application of dairy products and ingredients. The importance of scientifically based dairy food safety is highlighted, particularly with moves toward emerging processing technologies, many of which are nonthermal and will require validation of safety.

And no one pours new wine into old wineskins. If he does, the wine will burst the skins, and both the wine and the wineskins will be ruined. No, he pours new wine into new wineskins.

Mark 2:22 (New International Version)

Long ago, it was recognized that old systems can have trouble in dealing with new approaches, and as indicated by the passage above, the blending of the two just does not work. Innovative products and processes can be successfully harvested from science and safely incorporated into the fabric of industry if the system that holds them together is engineered to be fit for purpose. There is a new system of regulation now appearing in various parts of the world that heralds a new approach to delivering a successful outcome that does not build on the old prescriptive ways. This new system only requires that the specified outcome be achieved—how that happens is up to the industry and the regulators to determine. But it must be based on sound science and provide all of the evidence necessary to assure the outcome. It must let go of the past and embrace the future in a fresh and relevant manner. This new system aims to reduce the red tape that binds and places unwarranted requirements on industry in order to be able to achieve those defined outcomes.

Regulation and the achievement of identified health outcomes needs to embrace science and capture the vision of the industry. It needs to work in partnership with the industry from the outset rather than remain distant. But that does not mean that regulation should forgo its independence and protection of the consumer. Regulators need to develop the role of partners with the industry before they can effectively fulfill their responsibility as

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protectors of the consumer. They need to guide the industry into a safe future rather than stand against its creativity.

10.2 BACKGROUND

Dairy products constitute one of the key elements of the recognised essential food groups. The composition and quality of the constituents of milk are a rich source of nutrition for the consumer, as well as the reason that microbes grow so well in this medium. It is therefore essential that manufacturers of dairy-based products are in control of all stages of the process to ensure finished product safety at the point of consumption.

There is a long history for the use of the basic dairy product types, such as milk, butter, yogurt, and cheese. However, as other chapters of this book will reveal, there is an ever-increasing range of products that can be derived from dairy constituents. As time progresses and technologies continue to develop, novel dairy products will emerge that require unique processing characteristics and have demanding shelf-life requirements.

It is essential therefore that manufacturers and regulators work together to ensure that the consumer and the industry are both protected from the damaging impact of food safety incidents and product recalls.

10.3 DAIRY DEVELOPMENTS

Farmers and nomads have kept milk-producing animals for millennia. Whether these animals were goats, sheep, cows, buffaloes, horses, yaks, or camels, milk and dairy products have been a staple part of the diet across many countries.

Fermentation processes have been used for millennia to create and preserve foods. Bread, beer and fermented milk products such as yogurt and cheese curd have been produced throughout the centuries (Shaw, 2005). It is difficult to comprehend that a variety of traditional fermented dairy products had been created before microorganisms were discovered and the concept of germ theory had been developed. Originally, the fermentation process was initiated by natural organisms present in the milk and by the action of enzymes in the storage pouch. Subculturing from one batch into the next became an unlikely advancement in process development and in achieving a degree of product consistency.

However, the likelihood of dairy products causing death and disease remained very real due to the frequent contamination of milk from the surrounding environment or diseases carried by the animal. Two aspects of dairy product management would have reduced the impact of any adverse health incidents—the daily sourcing of fresh milk straight from the animals and the intrinsic immune defense system that consumers would have built up against pathogenic organisms by consuming a wide variety of contaminated food products over a period of time. The absence of what is currently termed “food miles” and the need for extended use-by dates that are applied to current-day products would have also assisted in minimizing adverse health incidents.

The mechanical separation of milk in the latter part of the nineteenth century resulted in the utilization of the cream fraction, with the skimmed milk often being used as feed to pigs and other animals. The conversion of this cream into butter, where the aqueous phase was dispersed as discrete droplets throughout the continuous fat phase of the product, also reduced the likelihood of pathogenic bacteria growing enough to cause illness and disease

in the consumer. Salting of the butter enabled an increased control over potential growth of pathogens in the discrete water droplets within the product.

It was in this same period that Louis Pasteur demonstrated that microorganisms were responsible for the deterioration of food products like milk and wine. From the start of the twentieth century, governments around the world responded to outbreaks of milk-borne disease and introduced pasteurization as a legal requirement for dairy product processing. This single requisite step in the process resulted in a great leap forward in the protection of the consumer against disease.

However, an increase in the adoption of new technology throughout the latter part of the nineteenth and early part of the twentieth centuries added a new dimension to the range and versatility of dairy products and the use of the skim portion of milk. Centrifugal separation, mechanical evaporation, and roller drying introduced a capability not previously seen in the larger-scale producers of dairy product manufacture. The scale and duration of milk processing was extended and with it came an increased requirement to be able to effectively clean this equipment.

Over the course of the twentieth century, processes were developed to take the manufacture of dairy products to a new level. The technological know-how for larger scale batches were at first introduced and then followed the automation of batch processing into a continuous production methodology. Effective pasteurization processes ensured that products could be consistently manufactured without adverse health effects, although post-pasteurization contamination required continuing diligence in good manufacturing practices on the part of the manufacturer.

By the 1960s and 1970s, manufacturing technology was increasing in pace and became the focus of several areas of scientific endeavor. Ultra heat treatment (UHT) and separation technologies started to appear. The components of milk—fat, protein, minerals, and lactose—were all experiencing scientific activity, and both filtration and ion exchange separation technologies were being developed and applied.

During the 1990s and as a result of increased interest in some heat sensitive components of milk, especially the proteins and peptides, a major thrust of the scientific work began looking at alternative mechanisms to the heat treatment processes that were being applied. These efforts aimed to provide an acceptable kill step for pathogenic microorganisms at the same time as retaining the functional attributes of the native protein and peptide fractions.

Of course, the real issue here is a defining of the term acceptable. The time—temperature relationship that is used to define what constitutes pasteurization, provides a specific rate of kill for the known dairy pathogens. The difficulty will be for scientists and research technologists to successfully argue that the reduced rate of kill for a new process should be considered acceptable. Will the regulators and standards development authorities require equivalence to be actually equivalent in every way to the current pasteurization process?

Interest continues therefore with other types of novel unit processes and there is now a consistent amount of research work occurring to determine how any new processing systems can be utilized to provide value-added, nutritional, or functional products.

Most recently, work has been investigating the use of processes, such as high hydrostatic pressure processing, pulsed electric field, ultrasonic and cool plasma microwave. Unlike the filtration or separation studies, each of these processes aims to reduce the reliance on heat treatment processes to control the presence of pathogenic microorganisms. In their own individual way, they are being studied to determine if and where they may have

Table 10.1. Microbiological processing hurdles.**Main microbiological hurdles**

Heat treatment (e.g., pasteurization)
Chilling/refrigeration
Low water activity (A_w)
pH/acidification
Low redox potential
Preservatives
• Chemical (e.g., salt/nitrite)
• Natural (e.g., smoking and bioactives)
Atmosphere (oxygen/ CO_2/N_2)
High hydrostatic pressure processing
Competitive microflora

potential use within the food industry. Moreover, depending on how the selected constituent is affected by the nonheat treatment process and the stage at which this control step is applied, the process may potentially deliver significant benefits to the safety and functionality of the end product.

From a microbiological standpoint, the use of membrane processes to concentrate the dairy fractions also concentrates any microbiological contaminants that may also be present. Furthermore, because many of these products are protein-based, microbial reduction is made more difficult after the concentration process has been undertaken due to the increase in viscosity. Therefore, heat treatment applied early when the component is in a diluted condition has been the only way of including a microbiological kill step in the process. All subsequent processing relies on good manufacturing practices to prevent contamination of the product.

With these emerging technologies that do not utilize heat to provide a microbial kill step, it is likely that a combination of some heat and other supportive “cool” kill step will provide better microbial and functional protection to the finished product.

10.4 PROCESSING REQUIREMENTS

Preservation techniques for the processing of milk have been developing and adapting for millennia with fermentation (acidification), salting, and drying being the main mechanisms used to deliver extended shelf stability. A number of milk and dairy product preservation factors are listed in Table 10.1. These preservation factors represent the introduction of *hurdle technology* to microbial growth capability. These hurdles can be applied either simultaneously or sequentially in an effort to limit the potential for microbial growth in any given environment. Leistner and Gorris (1995) provide an excellent review on hurdle technology, where individual microbiological barriers can be used to provide an adequate level of protection by the end of a process.

10.5 HAZARDS AND RISKS

Risk analysis incorporates the areas of risk assessment and risk management into a framework of risk communication. It is essential to be able to assess that if a hazard is present, whether it could become a real risk to the health of the consumer if maintained in the

food under the expected or adverse storage conditions. This brings into focus two issues—knowing what hazards exist or are emerging within the environment and being able to adequately assess whether they pose a real risk to the consumer.

The dairy industry knows about microbiological, chemical, toxicological, and physical hazards, and is vigilant in scanning the environment for any new or emerging hazards. Risk managers need to be able to set the standards that will enable elimination or control of risks. They need to define the questions risk assessors need to answer in order to adequately provide a means to address any potential risk.

New products and processes are a source for novel hazards to arise. With a changing population that have increasing levels of allergenic sensitivities, as well as a greater life expectancy for the young, the elderly and the immunocompromised, new products and processes may well be the means by which an unknown hazard or risk can arise.

It is therefore beneficial if the development of new products or processes includes an evaluation of whether any new hazards or risks are possible, and then include appropriate representation by authorities and agencies to assist in determining whether special conditions need to be addressed. The inclusion of this evaluation early in the development of a new product or process can assist in long delays to the market if problems arise later in the implementation phase. Authorities and agencies need to partner with the industry in order to protect both consumer and industry from potential problems that may be unforeseen in the development and implementation stages.

10.6 REGULATION

As outlined above, to fulfill their responsibilities to the government and to consumers, regulatory agencies and authorities tend to be risk averse. This should be clearly distinguished from being hazard averse. The mere presence of a microbiological hazard does not necessarily constitute a risk to the consumer. The environmental conditions and numbers of viable bacteria present will play a role in whether a risk to food safety is present. Regulations have developed over time to take account of what has been known or discovered to be beneficial to the safety and quality of food. As additional information and knowledge is gained, more comprehensive and detailed regulations and requirements have resulted. Subsequently, regulations tend to be prescriptive in nature—expressing a set of actions that must be undertaken for a desired outcome to be achieved. Herein lies a major problem. There are always alternatives to what has been prescribed, and more than one way to achieve an outcome.

More proactive regulators and standards-setting bodies are moving toward outcomes-based systems where the required outcome is clearly articulated, but the means by which that outcome must be achieved is determined by the individual or company. The “Code of Hygienic Practice for Milk and Milk Products” (2004) is a good example. This codex document specifies overarching principles that apply to the production, processing, and handling of all milk and milk products. These principles are:

- From raw material production to the point of consumption, dairy products produced under this code should be subject to a combination of control measures, and these control measures should be shown to achieve the appropriate level of public health protection.
- Good hygienic practices should be applied throughout the food chain so that milk and milk products are safe and suitable for their intended use.

- Wherever appropriate, hygienic practices for milk and milk products should be implemented within the context of HACCP as described in the Annex to the Recommended International Code of Practice—General Principles of Food Hygiene.
- Control measures should be validated as effective.

In the end, regulators are accountable for ensuring that industry achieves acceptable levels of food safety, and as long as this goal is achieved, they have been successful in their task. Layering prescriptive requirements upon industry to achieve an end result becomes burdensome and steeped in red tape and bureaucracy.

As innovation in both product and process development leads the dairy industry into the future, both regulators and industry need to realize that “equivalence” in procedures does not, and cannot, be equivalent.

The dairy industry will continue to move into the future with an ongoing requirement that all improvements made to the production and processing of foods be based on sound science. Other contributors to this book cover the range of developments that are occurring in the area of dairy product innovation.

The intent of many developments is to create greater efficiencies, more active and functional products, better nutritional properties, or more effective delivery systems for food nutrients. The capability of undertaking something different in order to achieve something unique will always be a driver for scientists and developers to present innovation to the consumer.

In all of this activity, the position of a food regulator will remain simple—to ensure that the product is safe to the consumer. No more and no less. The question is how far beyond simply achieving safety do the current regulations extend? What levels of microbial reduction would be required? And will alternative processes that deliver defined microbial reductions be accepted as delivering equivalence?

Acceptability will depend on the body of scientific evidence that is available to support the alternative processes. At the core of this will be an approved validation process. Often the new technology is left unsupported by a lack of evidence that can be used to substantiate the validation claims.

Whether a producer or manufacturer is able to achieve something unique does not warrant the justification for allowing it. Scientists and developers need to ensure that whatever aspects of the production and/or manufacturing process they are changing to make the resultant product or process beneficial, they have not unintentionally introduced hazards that may present a real risk to the consumer. With changing processes and advancing scientific knowledge, innovation needs to be constantly aware of the potential for an increase in risk.

In the past, the techniques used for extending the shelf stability of dairy products have provided hurdles that limit the capability for the growth of microorganisms. These hurdles impact differently on different microorganisms, and may need to be reviewed as microbial evolution produces strains that have an elevated resistance to the impact of these hurdles. With constant environmental pressures, microorganisms have shown their capability to survive and grow in the presence of preservation hurdles.

10.7 MICROBIOLOGICAL ECOLOGY

Microorganisms associated with milk and dairy products have existed for thousands of years. The microorganisms capable of being transmitted through dairy products are

Table 10.2. Microbiological pathogens in dairy products.**Hazard (pathogen) identification**

Aeromonas spp.
Bacillus cereus
Brucella spp.
Campylobacter jejuni/coli
Clostridium spp.
Coxiella burnetii
Corynebacterium ulcerans
Cryptosporidium
Enterobacter sakazakii
Pathogenic Escherichia coli
Listeria monocytogenes
Mycobacterium bovis
Mycobacterium avium subsp. *paratuberculosis*
Salmonella spp.
Shigella spp.
Staphylococcus aureus
Streptococcus spp.
Yersinia enterocolitica

Extract from "A Risk Profile of Dairy Products in Australia." Used with permission from Food Standards Australia New Zealand.

represented in Table 10.2 ("A Risk Profile," 2004). In addition to these potential hazardous microorganisms (pathogens), a greater number of spoilage organisms can be found to reduce the quality of a product. It should not be the responsibility of a regulator or food safety standards agency to require manufacturers to comply with levels of overall microbial numbers, although this may be a good indicator of effective HACCP and good manufacturing practices. Food quality is ultimately a competitive attribute within the industry, and a product's microbial specifications should remain the responsibility of the manufacturer and their customers.

10.8 FOOD SAFETY PROGRAMS AND FOOD SAFETY OBJECTIVES

During the 1960s, the National Aeronautics and Space Administration (NASA) and Pillsbury developed the hazard analysis critical control point (HACCP) concept for the production of foods destined for the NASA space program. This concept has spread to the wider industry and been used internationally as the basis of safety for food production and processing systems. The move away from quality control (product clearance based on endpoint product testing) to quality assurance (in process control measures to assure conformance to predetermined acceptance criteria) has been a great step forward in preempting problems and eliminating them before they occur.

Not only is HACCP fundamental to a food safety program, but so is the establishment of good manufacturing practices (GMP) within the production or processing facility. In combination, HACCP and GMP collectively provide adequate control over the environment and production/manufacturing process to ensure that potential food safety hazards are effectively managed. Continual review of the food safety program is necessary to establish that the controls that are in place (HACCP or GMP) are both individually and collectively

valid in providing an effective barrier against a constantly changing external, and increasingly internal, microbiological environment.

The determination of an endpoint level of microbiological safety and quality traditionally has been applied at the completion of processing. Manufacturers sampled and tested their finished products to determine the microbiological loading and ascertain whether any detectable pathogens were present. If pathogens were present above the maximum level of a specified Food Standard or product specification, measured in organisms per unit quantity of product, then the batch of product would be rejected. This quality control methodology relied on appropriate procedures for sampling and analytical methodology to determine whether products are “in or out” of a predetermined safety or quality specification.

A paradigm shift is emerging that has begun to change the way Standards are being interpreted and applied. Rather than use the end of the manufacturing process as the point at which safety and quality attributes are measured, a manufacturing *performance objective*, the point of consumption has been determined to be a better position to represent any risk to the consumer and to provide an association with levels of health in the consuming public.

To be assured that product quality and safety is still present at the time when the product is consumed, a new term *food safety objective* has been created. The point of consumption is the “line in the sand” from which product and process attributes are measured—both backwards through distribution, storage, processing, and production, and forwards to assess any impact to public health statistics. From this view, the microbiological assessments within the industry can be directly related to health incidents or requirements of any particular global, regional, or local area. Cole (2004) provides an excellent overview of the food safety objective concept.

Governments around the world constantly measure rates at which diseases and health incidents occur. In this way, outbreaks can be quickly identified. In 1985 a nurse responsible for monitoring neonatal infections at a large Californian hospital noticed an increase in cases of listeriosis. Normally, around three to five cases would occur in a year, but at this time, five cases had occurred within a period of 2 weeks. The notification and subsequent investigation resulted in one of the first attributions of listeriosis to being a foodborne illness.

Since that period the prevalence of listeriosis has been reported by a number of countries. The International Commission on Microbiological Specifications for Foods (ICMSF) (2002) indicated in “Microorganisms in Foods 7” that the range in the 11 countries for which data was reported was between 0.1 and 1.3 cases per 100,000 persons. For a country that has a high rate of incidence to declare a need to reduce its rate, then that health objective (i.e., down to 0.05 cases per 100,000), can be translated into a lower food safety objective, and consequently lower performance objective.

10.9 EMERGING PROCESSING TECHNOLOGIES

There is no question that this same concept for controlling current food safety risks will equally apply to innovative products or use of emerging technologies.

There is a paradigm shift that needs to be adopted by both regulators and industry. Regulators need to become less prescriptive in demanding a process that industry needs to follow and more willing to accept outcomes that can demonstrate that overall there is an equal or diminished risk to food safety. Providers of innovative products or processes need

to ensure that any evidence supporting their claim that this new technology does not constitute an unacceptable risk to food safety is based on sound science and emerges from a credible source. In the end, it will be vital that assurances around the identification and control of potential hazards and risk mitigation strategies have been provided, accepted, and then incorporated into manufacturing food safety programs. It should be understood that providing these assurances may not be straightforward or within the capability of smaller businesses, and as such, it will be essential that companies developing these new technologies or manufacturers wishing to adopt innovative systems are willing to undertake the necessary justification and validation work.

For example, effective pasteurization at a given time and temperature relationship will provide a certain log kill for different pathogenic organisms. This rate of kill will likely be different for high hydrostatic pressure or pulsed electric field processing. Simply being different in its overall outcome has not yet been established to be better or worse; it is simply different.

In each particular food environment pathogens will have a greater or lesser likelihood for survival and growth. The impact on the survival and growth of likely pathogens in that environment should form the basis upon which the rate of kill is deemed acceptable or not for that process. To be continuously compared with equivalence to pasteurization does not mean that an innovative process must be equivalent. Circumstances may enable an acceptable level of risk to be achieved by a process in a given product that is not equivalent to a pasteurization step.

A more likely outcome will be the combined use of existing and emerging technologies. This is being evidenced in the application of reduced heat treatment processes in conjunction with high hydrostatic pressure processing technology. This combination achieves a successful log reduction in pathogens while providing functional benefits to the finished product, above what is currently achievable with existing technologies. It is expected that other combinations of food processing technologies will develop in the same way. When the outcome can be demonstrated that safe food will be produced, then there needs to be an acceptance of these technologies by food standards agencies and regulators.

Certainly, these new technologies will need to establish their own validation criteria and be able to achieve the desired outcome.

Recognition and acceptance of the results of the validation process will be fundamental in gaining approval by regulators and standards agencies to utilize innovative products or processes.

10.10 VALIDATION AND VERIFICATION

There are a number of definitions for validation, depending on the context in which it is used. In principle, validation of a process comes back to the basics of “*proving (with facts, figures and objective data) that what you intend to do (process to apply), WILL achieve the required outcome.*” It is implicit in this definition that the proof is reproducible by an independent assessor. As circumstances change or microbes evolve, the validity of the process may need to be reconfirmed. For instance, methicillin was once effective against *Staphylococcus aureus* infection, but with the acquisition of genes that provide resistance to this particular antimicrobial drug, this treatment method is no longer valid. Similarly, the incorporation of nisin into food at traditional levels where it had once been shown to

be effective may no longer constitute a sufficient hurdle that will ensure the prevention of outgrowth of all Gram-positive sporing organisms. So while the process can be validated, shown to be effective at this point in time, constant awareness of the changing nature of infectious organisms and infectious disease warrants continuous review of these validated processes.

The validation of a process is an essential first step in assuring that the food safety program is maintaining control over the outcomes of the production process. The next requirement is verification, and it needs to be able to confirm to a third party that the food safety program is followed every time the process is undertaken. To ensure that food safety controls are occurring, regulators require that manufacturers provide records to show the program is being followed—they *verify* that “*what is required and agreed to be done is actually being done.*”

It should be a relatively easy procedure to verify compliance with a food safety program. A producer or manufacturer needs to be able to show, by producing records, that they are following the requirements as agreed in the food safety program. While this may at first appear easy, the complexity of manufacturing processes and the individual stages of control and management throughout a production or manufacturing process may mean that extensive record keeping is needed to be able to provide verification to a third party.

10.11 CONCLUSION

The discovery of microbial existence in the seventeenth century followed by the understanding of the impact of heat treatment by Louis Pasteur gave rise to an additional method by which disease incidents could be controlled within the dairy food industry. The introduction and acceptance of pasteurization as a process was made over a number of years in various places around the world. In many cases, severe outbreaks of disease, such as tuberculosis, that were attributed to the consumption of contaminated dairy products, were the catalyst for governments to introduce legislation requiring mandatory pasteurization of milk.

The incidence of disease outbreaks has reduced dramatically since this period as a direct result of pasteurization. As a result, there is a growing view that the current generation of manufacturers and consumers are becoming complacent about the potential for dairy products to harbor and transmit serious diseases. It is the role and responsibility of regulators and standards agencies to maintain an awareness in the industry of the capability for dairy products to become contaminated if adequate food safety systems are not in place and maintained.

The successful development of any new dairy product or process is not only dependent on the maintenance of a safe product, but also on the resultant quality, taste, appearance, nutritional value, and functionality. Nonpathogenic microorganisms also have the capability to negatively impact these characteristics, which will in turn reduce the acceptability of the finished product to the consumer.

The dairy industry worldwide has always been at the forefront of innovation and product development. This has enabled the continued acceptance and relevance of dairy to the consumer. As a source of high value nutrients, dairy products are being introduced to more cultures each year. It is essential for consumer health and international trade that the dairy industry continues to do all that it can to assure the safety of its products in an ever-changing world.

REFERENCES

- A Risk Profile of Dairy Products in Australia (2007) Food Standards Australia New Zealand. p. iv. http://www.foodstandards.gov.au/_srcfiles/P296%20Dairy%20PPPS%20FAR%20Attach%20%20FINAL%20-%20mr.pdf. Accessed August 27, 2008.
- Code of Hygienic Practice for Milk and Milk Products (2004) CAC/RCP 57. Codex Alimentarius.
- Cole, M. (2004) Food safety objectives—concept and current status. *Mitteilungen aus Lebensmitteluntersuchung und Hygiene* **95**, 13–20. http://www.cdc.gov/print.do?url=http%3A//www.cdc.gov/nczved/dfbmd/disease_listing/listeriosis_gi.html. Accessed June 7, 2008.
- ICMSF (2002) Evaluating risks and establishing food safety objectives. *Microorganisms in Foods 7: Microbiological Testing in Food Safety Management*. Springer Science+Business Media LLC, p. 24.
- Leistner, L. and Gorris, L.G.M. (1995) Food Preservation by hurdle technology. *Trends in Food Science & Technology* **6**, 41–46.
- Shaw, I. (2005) *Is It Safe to Eat? Enjoy Eating and Minimize Food Risks*. Berlin: Springer.

11 Market Acceptance of Dairy Ingredients: What Consumers Are Thinking and Demanding

B. Davis and B. Katz

11.1 BACKGROUND: RECOGNITION AND RELEVANCE ARE DRIVERS OF CONSUMER ACCEPTANCE

From the consumer perspective, raising interest levels in dairy ingredients, or in any ingredient for that matter, requires two things—recognition and relevance. Consumers are less likely to trust things that they do not recognize or understand. In the absence of awareness, an ingredient needs to be tied to familiar foods or beverages that the consumer knows and appreciates. The link of omega-3 fatty acids to fish is a good example of this. Ten years ago, omega-3 fatty acids were not as widely known, but their link to fish has always been clear; this provided acceptability at the consumer level. Consumers are more likely to have confidence in food itself than in an ingredient found in food. This is demonstrated by the following: only 30% of shoppers in the United States take specialty ingredient supplements on a regular basis, but 88% of shoppers agree that it is important to eat foods that are naturally rich sources of key vitamins and minerals (1).

There are certain ingredients, such as calcium, that can stand on their own merits based on awareness. The majority of shoppers in most countries place calcium high on their list of ingredients of interest because they are well aware of calcium and its requirement in the diet. Typically, while consumer interest in functional ingredients is growing, the depth of average consumer knowledge remains relatively low for most ingredients. But regardless of their actual knowledge of different types of calcium or the mechanism by which it works, consumers recognize calcium as an ingredient with a valued benefit because of high levels of awareness and its association with dairy. They understand that calcium gives you strong bones and teeth and can help prevent osteoporosis in later life, all important things that are relevant, desired benefits.

However, the majority of dairy ingredients, such as *conjugated linoleic acid (CLA)*, α -lactalbumin and lactoferrin are not well known to the typical shopper. Until adequate levels of awareness are created, linking ingredients to known foods or beverages can help aid in the growth of knowledge. As an illustration, data from the U.S. HealthFocus® Trend Report (HealthFocus International, 2009) showed that 17% more shoppers were very interested in “fiber for cholesterol lowering benefits” than “beta-glucan for lower cholesterol.” The general term “fiber” is more widely known, and, therefore, more widely accepted.

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Similarly, it is critical to link unfamiliar dairy ingredients to their familiar source, that is, milk, in order to boost awareness and acceptance.

In the case of dairy ingredients, probiotics are probably the best known example of the importance of consumer awareness. Figure 11.1 shows a TrendSense™ (2009) chart from Sloan Trends, Inc. (Escondido, CA) which indicates when probiotics reached the level of awareness necessary among consumers for a commercial launch (“commercialization phase”). This figure, as well as Figure 11.2 (Innova Market Insights, 2009), also indicates the substantial increase in probiotic launches that followed once the commercialization entry point was met. According to Innova Market Insights that tracks product launches,

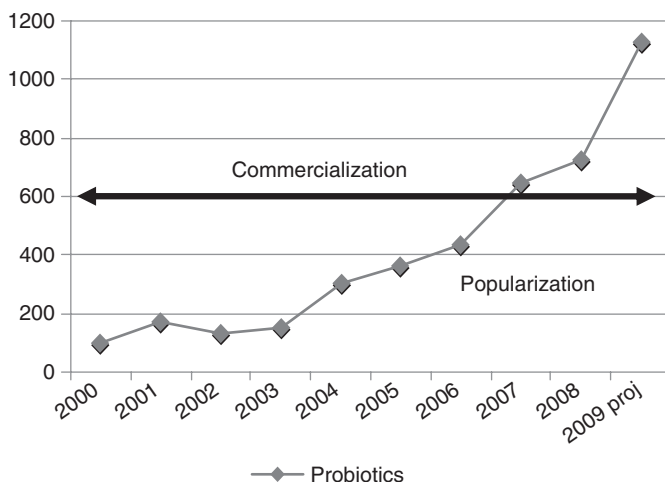


Figure 11.1 TrendSense™ commercialization threshold for consumer awareness of probiotics. Source: Sloan Trends, Inc. 2009.

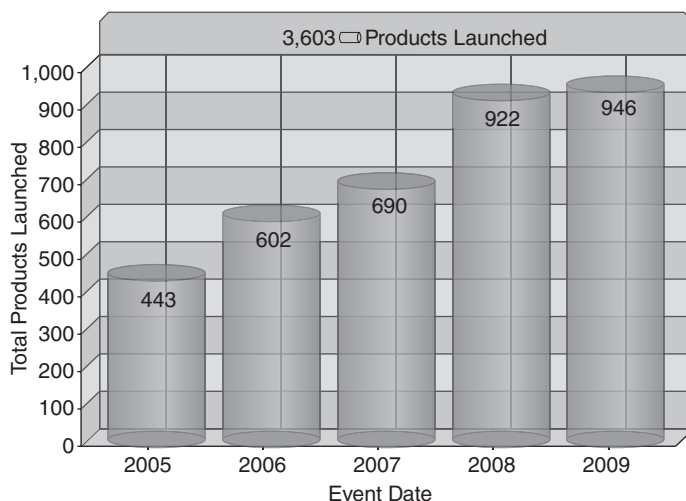


Figure 11.2 Probiotic product launches. Source: Innova Market Insights, 2009.

claims and clinical studies on a global basis, 922 product launches with probiotics were made in 2008 once the commercialization threshold was met. This represents a 25% increase over the previous year. Comparatively, as is illustrated in Figure 11.3, the awareness levels for CLA and whey protein are below the consumer awareness threshold for commercialization. So, while products with these ingredients may have opportunity in niche or specialty markets, their success in the mass market will be limited until greater awareness is developed.

The second requirement to an effective launch is relevance. Ingredients, whether naturally occurring or added during processing, are of greater interest when they deliver a benefit for which the consumer perceives a need. Success is not based simply on adding the ingredient, regardless of how sound the science is. Success is more likely to come from understanding and communicating how an ingredient or product delivers on a consumer need *from the perspective of the consumer*. So the focus of the communication to shoppers always needs to be on the benefit of the ingredient, not the ingredient itself.

The significance of recognition and relevance is observed in the following example from the recent HealthFocus International Trend Report (HealthFocus International, 2009). When shoppers were asked to indicate their interest in a list of ingredients and ingredient—benefit combinations, fewer shoppers said they were extremely interested in whey protein, an unfamiliar ingredient, than in protein. This is probably because they do not understand the source or benefit of whey protein. Considerably more shoppers were interested in protein and interest increased further when protein was associated with a benefit of importance (Table 11.1). Therefore, the key to success in functional foods and beverages is to build and communicate messaging based on proven science for ingredients that are recognizable, acceptable, and meet the benefits of most interest to shoppers.

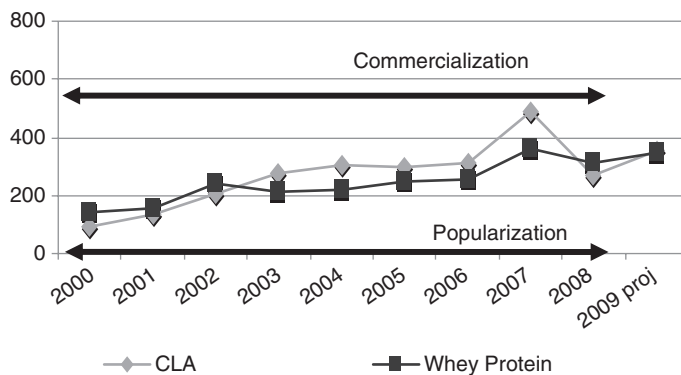


Figure 11.3 Commercialization threshold for consumer awareness of CLA and whey protein.
Source: Sloan Trends, Inc. 2009.

Table 11.1. U.S. shopper interest is directly related to awareness and benefits of interest.

U.S. shoppers extremely interested in	(%)
Whey protein	22
Protein	31
Protein and weight management	36

Source: HealthFocus® International U.S. Trend Report 2009.

Table 11.2. Top food and beverage choices for functional benefits.

Benefit	Top 3 food/beverage delivery vehicles
Promotes good sleep	Milk , fruit/vegetable juices, soup
Helps relieve stress	Milk , fruit/vegetable juices, breakfast cereal/ energydrinks/soup
Gives me longer lasting energy	Milk , fruit/vegetable juices, breakfast cereal/energydrinks
Improves my memory and concentration	Fruit/vegetables juices, milk , energy drinks
Helps me manage my weight	Fruit/vegetables juices, yogurt, milk
Helps me look good	Fruit/vegetable juices, milk, yogurt

Source: HealthFocus® International 2008 Global Trend Study.
Boldface indicates dairy foods/beverages.

Milk and dairy represent familiar foods that shoppers are willing to accept as having functional benefits. In a 2008, 18-country survey (U.S. HealthFocus Trend Report, 2008) shoppers were asked to choose which foods they would be interested in purchasing if they could provide one of six benefits. The benefits were: promotes good sleep, helps relieve stress, gives me longer lasting energy, improves my memory and concentration, helps me manage my weight, and helps me look good. Of the 15 food options, 3 were dairy (ice cream, yoghurt and milk), and these dairy options consistently were chosen by a majority of shoppers as top choices for delivery of functional benefits (Table 11.2). Globally, a majority of shoppers choose milk as an option for all benefits, and milk was the top choice if it could aid in sleep, stress relief, or longer-lasting energy. These data demonstrate the potential for dairy foods and ingredients.

11.2 SHOPPER INTEREST IN SELECT FUNCTIONAL BENEFITS

With this backdrop in mind, shopper interest and awareness of benefits will be examined in the context of dairy ingredients. Benefits are the focus; however, dairy ingredients with the potential to deliver on these benefits are discussed as appropriate. The benefit areas that will be explored include:

1. Cognitive performance
2. Weight management
3. Lifestyle concerns (depression, stress, and sleep)
4. Gastrointestinal health

11.2.1 Cognitive performance

According to the HealthFocus International Trend Reports (HealthFocus International, 2008, 2009), lack of mental sharpness or focus was one of the top health concerns in the Americas, ranking closely with high cholesterol and high blood pressure. Globally, this concern is growing, though it is currently lower in Asia and Europe than in Latin America and North America. Shoppers in Latin America register similar concern for lack of mental sharpness, as do shoppers in the United States. Of even greater concern for shoppers in the United States than a lack of mental sharpness, is maintaining mental sharpness as they age. Concerns about mental sharpness in the United States were higher among women, fairly consistent across income groups, and, as expected, higher in older age groups. However,

Table 11.3. U.S. shopper interest in cognitive benefits.

Extremely interested in food or beverage if it could deliver the following benefit	(%)
Helps improve concentration	35
Helps improve memory	40
Promotes mental alertness	42
Helps retain mental sharpness with aging	48

Source: HealthFocus® International U.S. Trend Report 2009.

this is not to suggest younger shoppers were unconcerned about their mental acuity; 59% of 18- to 29-year-old shoppers are extremely or very concerned about mental sharpness (HealthFocus International, 2009) (Table 11.3).

This concern about mental sharpness extends beyond their own health, as 47% of shoppers in international markets were extremely or very concerned about mental development for their children. In fact, this is among the top five concerns parents express for their children's health. Similarly, 54% of U.S. shoppers were extremely or very concerned about their child's mental development, making this a top 10 concern among U.S. parents (HealthFocus International, 2009).

The link between nutrition and mental performance is greater outside of the United States, where shoppers in many markets were more likely to report taking action. The number of shoppers who said they were choosing foods to improve their mental performance was 37% in the countries outside the United States, a number that has been fairly high for the last 5 years. Asian shoppers are driving this trend as they are far more likely (40%) to take action than in Europe (27%) or Latin America (22%). In the United States, the number is growing much more quickly, but is still lower at 27% of shoppers.

In the United States, consumer interest seems to be primarily in maintaining their ability to function the same as they get older. Their concern is less about measurable functional outcomes like improving concentration or memory. People are not expecting to raise their IQs; they simply want to maintain their "mental status quo" as they age. The difference between the number of shoppers who are very interested in foods or beverages that help improve concentration versus those interested in foods and beverages that maintain mental sharpness is quite significant (35% vs. 48%). However, note that in the youngest shoppers 18–29, as many are interested in improving concentration (46%) as are interested in retaining mental sharpness with age (46%) (Table 11.3).

Although there are limited data linking milk with cognitive function, research from investigators in the United Kingdom suggest the dairy ingredient, vitamin D, may be associated with improved cognitive function in older men (Lee et al., 2009). A systematic review of published literature on this topic was conducted in 2009, demonstrating inconclusive results, possibly due to differences in methodology and types of cognitive tasks and subjects studied in the various investigations (Annweiler et al., 2009). Additional research is required to determine whether vitamin D plays a role in cognitive function, and, if so, which areas of function (memory, processing, acuity, etc.) it influences, who would most benefit from supplementation with this vitamin, and the dose required for efficacy.

11.2.2 Weight management

Weight management is another topic of great interest to shoppers. Because this subject is both complex and emotional, when developing and marketing functional food products

directed toward this benefit, it is critical to understand consumer attitudes and underlying motivations. Most shoppers express a desire to lose weight, especially those in the Americas and Europe. In the Americas, over 50% of shoppers report that they want to lose weight, while in Europe, this number is around 44% and is driven by French and Italian shoppers. However, for a variety of reasons, shoppers are not taking action on their desire to lose weight, so the number reporting they are actually dieting is far lower than the number who say they want to lose weight. Over 40% of shoppers in the Americas say that losing weight is a “consistent struggle.” A sedentary lifestyle is the top reason most shoppers give for not losing weight, and for the majority of shoppers, the reasons they give for wanting to lose weight are more emotional than related to wellness.

In fact, for most shoppers, weight is not necessarily equated to health, but rather to self-esteem and appearance. Only 10% of shoppers in the United States and 1% in other countries give weight loss as their primary reason for eating healthy foods. Forty-five percent of shoppers in the United States think they can be overweight and still be healthy, and this number has been growing. This is an indicator that while weight is clearly in the shopper consciousness, their primary reasons for concern are not the medical consequences of overweight and obesity. But this is not due to a lack of concern over weight-related health issues. Far more shoppers are concerned about health conditions directly caused by obesity, such as heart disease and diabetes, than are concerned about overweight/obesity itself. As described below, the relationship between excess weight and increased risk of these medical conditions appears not to be as top of mind to consumers who want to lose weight. So when communicating with shoppers on the benefits of weight loss, it is important to remember that while they may have an understanding of the medical need to be at their correct weight and be concerned about these medical conditions, this may not be a motivating factor for weight loss.

In a recent U.S. HealthFocus Trend Report (HealthFocus International, 2009), considerable data were collected to gain an understanding of weight management attitudes among shoppers. When asked why they wanted to lose weight, over 50% of U.S. shoppers said they were simply tired of being heavier than they wanted to be and 45% said they wanted to fit into clothes they already owned. In contrast, only 15% said it was because they had seen people close to them get sick with weight being a contributing factor to their illness. The emotional and appearance-related factors seem to be greater drivers than the medical reasons. And other priorities take precedence over taking action to lose weight. In addition, while many want to lose weight, most are not on diets. However, current weight does appear to be a motivating factor for weight loss. Those shoppers with a body mass index (BMI) indicating they were obese were the most likely to be on a diet. Whereas 51% of obese shoppers reported being on a diet, only 40% of shoppers in the overweight range said they were dieting. But even when obese, only half of people are actually on diets.

Further complicating weight management are several other factors. For example, the act of eating is often for reasons other than sustenance. Shoppers admit to eating for social reasons (47%) or because they are bored (43%). In addition, lack of consistency plays a significant role in diet and health. Forty-eight percent of shoppers say that they go through periods where they pay attention to both their diet and exercise and periods where they pay attention to neither.

The impact of these drivers for functional foods and weight management is that it is important to understand the psyche of the shopper when communicating about the ingredients for this complex benefit. Many shoppers are interested in options that fit into a diet that may not be consistently healthy. Most people are not on a diet that requires an ongoing

effort to restrict certain foods and overall intake. Rather, they report being very inconsistent in their efforts, but do, in fact, want to make an effort. Providing products that target shopper motivations for weight management and fit into their lifestyles will be welcomed.

Due to its vast public health implication, as well as intense interest from the general public, weight management approaches of functional foods, including dairy foods, have been the subject of considerable scientific research (Kovacs and Mela, 2006; Van Loan, 2009). Numerous investigations have been conducted to define the role of dairy foods and calcium in weight management. Van Loan (2009) reviewed the literature in this area and concluded observational studies show that an inverse relationship exists between dairy or calcium intake and body weight, body fat, or BMI. Further, although randomized clinical trials (RCTs) have shown inconsistent results, they tend to provide evidence of greater weight loss by overweight or obese individuals who have incorporated increased dairy or calcium intakes along with weight loss diets. Biological plausibility of the effect of calcium from dairy or supplements on weight loss has been investigated, and a recent meta-analysis demonstrated that dietary calcium has the potential to increase fecal fat excretion (Christensen et al., 2009). Further research efforts will be directed toward understanding whether this fat loss plays a relevant role in weight management.

Other dairy ingredients being investigated for their role in weight management include CLAs that are found in milk and milk products, among other sources. There appears to be discrepancy between results from animal studies that show a role for these fatty acids in weight management and human studies that do not consistently show weight or fat loss effects (Plourde et al., 2008). However, as reviewed by Benjamin and Spener (2009), there are data showing biological activities of various CLA isomers that are consistent with weight management effects. Further defining which CLA isomers are effective as well as appropriate doses for efficacy is the subject of continued research with these dairy ingredients.

11.2.3 Lifestyle concerns

Issues, including depression, sleep problems, tiredness/lack of energy, and stress can significantly impact daily life. Substantial numbers of shoppers are concerned about and afflicted by these conditions. As is described below, many shoppers express interest in using functional food products to help manage these problems.

Depression is a growing concern in Western cultures including the United States, Australia, and Europe. In the United States, concern about depression has increased from 36% of shoppers in 2000 to 41% in 2009. In Europe, the number of shoppers greatly concerned about depression has also substantially increased over a 5-year period, from 23% in 2003 to 30% in 2008. However, other regions, such as Asia and Latin America, do not show an increase in concern. There has been a steady increase in depression as a concern among younger shoppers in the U.S. HealthFocus Trend Report (HealthFocus International, 2009). In the United States, considerably more of the youngest shoppers (52%) are concerned about depression. Twenty-one percent of shoppers on average say that depression affects them; this number increases to 25% of the youngest shoppers.

Stress is another area where sharp increases in concern were observed globally. According to the HealthFocus Global Trend Study (HealthFocus International, 2008), in 2003, 28% of study respondents reported high concern over stress, but this number jumped to 38% in 2008. Considerably more shoppers (a greater than 10 percentage point increase)

reported concern about stress in the Asia-Pacific, Asian, and European regions. Whereas levels of concern about stress remained relatively constant over the past decade at 48% in the United States and 50% in Latin America, this represents a much greater proportion of shoppers than in any other region (HealthFocus International, 2008, 2009). Similar to the increased concern over stress, those who reported being afflicted by stress has risen dramatically as well, with nearly one-third (29%) of shoppers globally saying so in 2008 compared with 18% in 2003. Even in Latin America, there was a significant rise in the number of people who said they were stressed in 2008 (31%) compared with 2003 (22%). Globally, substantially fewer people are saying their stress levels can be handled by them than 5 years ago (73% in 2003 vs. 57% in 2008). With this much stress and concern about it, it is not surprising that 62% of respondents globally express great interest in foods that claim the benefit of “reduced feelings of stress.” Milk is the food chosen most often to be a delivery vehicle for the benefit “helps to relieve stress,” with 62% of shoppers making this dairy product their top choice (Table 11.2).

Sleep problems and tiredness/lack of energy are also lifestyle issues that impact shoppers to a significant degree. From a global perspective, sleep problems afflict nearly one-quarter and is of concern to 38% of shoppers. However, in Australia, where one-third of shoppers report problems with sleep, an equal number of people report concern about this issue. In most regions studied, the number of people concerned with sleep problems roughly equals the number concerned about tiredness/lack of energy. Again, Australia is an outlier with substantially more people reported being concerned about tiredness/lack of energy (48%) than are concerned with sleep problems (35%).

11.2.4 Gastrointestinal health

Gastrointestinal or digestive health is of growing concern globally, but especially in Asia, Latin America, and Europe. Although concern outweighs the number of people affected by gastrointestinal problems, there is a substantial rise in the number of shoppers who reported being affected by digestive issues in 2008 compared with 2003 (see Table 11.4 and Figure 11.4). Concern by U.S. shoppers increased from 31% in 2004 to 41% in 2008, with reported incidence increasing from 18% to 24% during that same time period.

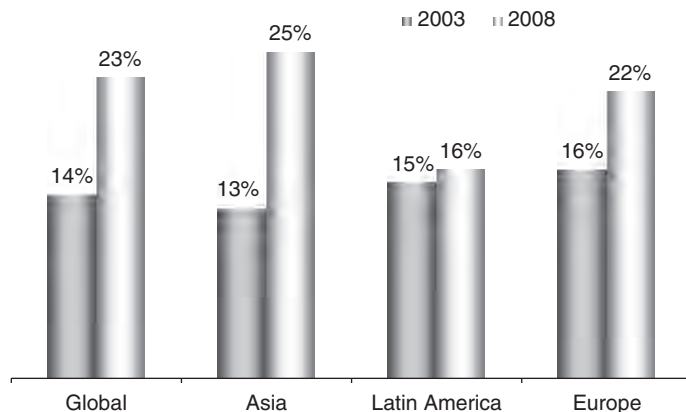


Figure 11.4 Reported incidence of gastrointestinal/digestive health problems.

Source: HealthFocus® International 2008 Global Trend Study.

Table 11.4. Concern about gastrointestinal/digestive health problems.

	2003	2008
Global	34%	39% ^a
Asia	22%	41% ^a
Latin America	56%	49% ^a
Europe	22%	27% ^a

Source: HealthFocus® International 2008 Global Trend Study.

^aRepresents statistically significant increase versus 2003.

A majority of shoppers (62%) expressed interest in the food benefit of “improving digestion” and, aligned with their increased concern, 73% of shoppers in Latin America report this interest. In the United States, almost half (45%) believe that dietary changes can help them treat or avoid gastrointestinal/digestive health problems. And globally, it is probiotics that shoppers are increasingly aware of; in 2003, only 29% reported being aware of the relationship between probiotics and digestive health, but in 2008, this number rose to 42% of shoppers. In the United States, 65% of total shoppers acknowledged interest in probiotics and digestive health. This relationship was of particular significance to shoppers aged 18–29 with 75% reporting high interest.

This increased consumer interest correlates well with the product launch data shown in Figures 11.1 and 11.2. According to Innova Market Insights (2009), 33% of the probiotic products launched in 2009 were accompanied by gastrointestinal/digestive health claims, whereas only 5% of products had immune health claims. Dairy products accounted for 93% of these 2009 launches.

11.3 CONCLUDING REMARKS

Successful commercialization of functional dairy foods requires consumer recognition and relevance. Understanding consumer knowledge of an ingredient is critical to determining how to communicate it. In certain instances, where ingredients are well known, such as in the case of calcium or probiotics, the ingredient can stand alone. However, when sufficient consumer knowledge does not exist, linking ingredients to known food sources and benefits may help to bridge the awareness gap.

Identifying benefits of interest to the shopper is equally as important when determining how to launch functional products. The past decade has seen increased consumer concern, and to some degree, increased reported incidence, in various health conditions. Shoppers also acknowledge greater awareness of and interest in using foods to manage these conditions, making this an ideal time to communicate the functional benefits of new food products. However, it is imperative to determine the specific benefit consumers are seeking, as was demonstrated above for cognition. U.S. shoppers were looking to maintain their current level of mental sharpness with age rather than improve mental acuity. Successful product claims match the needs shoppers are interested in. Understanding consumer demographics will also aid in targeting the most receptive audiences for functional foods and benefits.

REFERENCES

- Annweiler, C., Allali, G., Allain, P., Bridenbaugh, S., Schott, A.-M., Kressig, R.W., and Beauchet, O. (2009) Vitamin D and cognitive performance in adults: a systematic review. *European Journal of Neurology* **16**, 1083–1089.
- Benjamin, S. and Spener, F. (2009) Conjugated linoleic acids as functional food: an insight into their health benefits. *Nutrition and Metabolism* **6**, 36.
- Christensen, R., Lorenzen, J.K., Svith, C.R., Bartels, E.M., Melanson, E.L., Saris, W.H., Tremblay, A., and Astrup, A. (2009) Effect of calcium from dairy and dietary supplements on faecal fat excretion: a meta-analysis of randomized controlled trials. *Obesity Reviews* **10**, 475–486.
- HealthFocus® International (2008) Global Trend Study.
- HealthFocus® International (2009) The 2009 U.S. HealthFocus® Trend Report.
- Innova Market Insights (2009) <http://www.innovadatabase.com>.
- Kovacs, E.M. and Mela, D.J. (2006) Metabolically active functional food ingredients for weight control. *Obesity Reviews* **7**, 59–78.
- Lee, D.M., Tajar, A., Ulubaev, A., Pendleton, N., O'Neill, T.W., O'Connor, D.B., Bartfai, G., Boonen, S., Bouillon, R., Casanueva, F.F., Finn, J.D., Forti, G., Giwercman, A., Han, T.S., Huhtaniemi, I.T., Kula, K., Lean, M.E.J., Punab, M., Silman, A.J., Vanderschueren, D., and Wu, F.C.W. (2009) Association between 25-hydroxyvitamin D levels and cognitive performance in middle-aged and older European men. *Journal of Neurology, Neurosurgery and Psychiatry* **80**, 722–729.
- Plourde, M., Jew, S., Cunnane, S.C., and Jones, P.J. (2008) Conjugated linoleic acids: why the discrepancy between animal and human studies? *Nutrition Reviews* **66**, 415–421.
- TrendSense™ (2009) Sloan Trends, Inc.
- Van Loan, M. (2009) The role of dairy foods and dietary calcium in weight management. *Journal of the American College of Nutrition* **28**(Suppl. 1), 120S–129S.

12 The Future of Dairy Ingredients: Critical Considerations That Will Underpin Future Success

P.S. Tong and G.W. Smithers

12.1 INTRODUCTION

Over the years, dairy ingredients have been used in food systems because they provide desirable sensory properties, physical properties, and/or nutritional traits in a convenient, wholesome, and economical way. These key attributes, which are made available in a wide array of dairy ingredients in large amounts, make them desirable to food formulators and ultimately to food consumers. Emerging science, which improves our understanding of the chemistry of dairy components and the beneficial role that dairy ingredients play in health and wellness, will fuel future demand for existing and new generations of dairy ingredients and dairy ingredient applications.

12.2 EVOLUTION OF DAIRY INGREDIENTS

While past history is not always a good indicator of what the future may hold, a historical perspective can shed some light that can help us understand how some parts of the dairy ingredients industry may change or stay close to the status quo as we move forward. The evolution of dairy ingredients has reflected changing market (consumers and processors) interests, needs, and technology innovations over the years. In addition, government policies have influenced milk supplies, prices, and investments in innovation, and thereby influenced dairy ingredient marketplace behavior. For example, environmental considerations, technological advances, marketplace expansion, and renewed interest in bioactivity have transformed whey from an expensive waste stream into a series of highly valued ingredients (Smithers, 2008). The value and usefulness of these ingredients has paralleled developments in cost-effective technology for their isolation together with the receptiveness and sophistication of the marketplace (Figure 12.1). There is no reason to believe that the future of the dairy ingredients sector will not continue to be influenced by such forces in the foreseeable future. However, the pace at which market forces facilitate changes in dairy ingredients will likely accelerate as communications technology improves our ability to observe and understand market changes and allows a faster marketplace response. Hence,

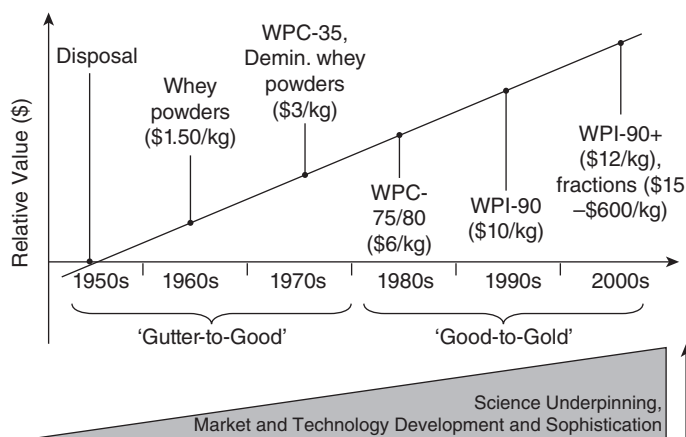


Figure 12.1 “League” of whey protein and peptide products illustrating the parallel development of higher value and functional ingredients with enhancements in science understanding, and with improvements in market and technology development and sophistication (reproduced with permission of Elsevier from Smithers, 2008).

having a good perspective on the overall direction of dairy ingredients will enable individuals to distinguish fleeting fads from truly sustainable dairy ingredient market opportunities (Mellentin, 2003).

12.2.1 “First-generation” dairy ingredients

Because milk is highly perishable, development of early dairy ingredients was motivated by dehydration as a means of preservation. Early dairy ingredients were produced primarily as commodity products by removal of water to create preserved dairy ingredients (whole milk powders, nonfat dry milk, whey powder, and buttermilk powder). Since fresh milk supplies often exceeded the milk producing region’s demand, drying of milk supplies into powders that could be rehydrated allowed these regions to economically transport dairy commodities over long distances to new markets where milk was not as plentiful. Such ingredients could then be either reconstituted to their original concentration and consumed as liquid milk or used as concentrated sources of dairy solids in other dairy and food formulations. Such products and applications will continue to be important uses of significant quantities of dairy ingredients because there will continue to be regions of the world that have limited ability to produce enough fresh milk for the population in their region due to insufficient farmland, weather, or lack of other resources. Imported dairy ingredients will also continue to serve as a source of milk solids in regions of the world that are transitioning to self-sufficiency of milk supply (Tong, 2003). As more science drives increased recognition by nutritional professionals, governments, and consumers concerning the importance of consuming dairy foods for human health and wellness, the demand for basic dairy commodities will only increase in the future.

Further, food safety issues that can heighten consumer perceptions of the safety of a region’s milk supply can also drive sustained demand for imported dry dairy ingredients. The recent public health issues surrounding melamine contamination of the Chinese milk supply and the subsequent strong demand for imported dairy solids in China is one key example.

12.2.2 “Second-generation” dairy ingredients

Innovations in separation and purification science, and their practical application in dairy fluid processing, resulted in the next generation of dairy ingredients. Major dairy fluids, such as skim milk, whey, and whey permeate, were separated, fractionated, and/or purified to produce crude milk and whey fractions, such as milk protein concentrate, whey protein concentrate, lactose, milk protein isolate, whey protein isolate, demineralized whey, caseinate (e.g., sodium), permeate powder, mineral fractions, and butter and anhydrous milkfat. The optimization of continuous cross-flow (tangential) membrane filtration has allowed the dairy industry to modify the ratios of the major dairy components in dairy fluids and/or to further concentrate components of interest economically into viable food ingredients (Tong, 2007). For example, sweet whey from cheese manufacturing with relatively low protein content was ultrafiltered to concentrate the protein sufficiently to make it an economically viable alternative to skim milk powder, and with further concentration, to make a highly functional whey protein concentrate powder. With additional knowledge from industrial and academic research concerning the chemistry, structure, and behavior of milk proteins, the industry was able to more specifically tailor functionality of concentrated dairy protein streams for enhanced functionality, including such traits as heat stability, gel strength, emulsification, solubility, or viscosity, and/or to provide more specific nutritional benefits (Mulvihill and Ennis, 2003).

12.2.3 “Third-generation” dairy ingredients

Most recently, more “specialty” ingredients have been produced using additional purification and isolation processes to take crude fractions and purify individual milk components, such as individual milk proteins (e.g., beta-lactoglobulin, alpha-lactalbumin, lactoferrin, glycomacropeptide, and others) (Figure 12.1). Additionally, fractions were further modified using either chemical or enzymatic methods to produce specialty ingredients with specific functional properties, such as tagatose, bioactive peptide fractions (e.g., growth factors), lactitol, and lactobionic acid. Unfortunately, the extra costs associated with purification processes and the challenges of finding practical ways to utilize the remaining streams from which such compounds were isolated represent some challenges to commercial exploitation. Nonetheless, for some specialized food applications, such purified proteins and other milk compounds enable food manufacturers to better customize their nutritional profiles, health claims, and/or other food properties. These innovations now open up the opportunity to tailor the composition of a dairy food for specific nutritional and functional properties by blending various fat, carbohydrate, protein, and mineral components in limitless combinations. As a better understanding of what stabilizes and destabilizes such systems is established, and what combinations are optimal for specific nutritional or functional targets, the demand for these specialty ingredients will grow (German et al., 2003).

12.3 NEXT GENERATION OF DAIRY INGREDIENTS

As the consumers’ experience and knowledge of food quality and the nutritional implications of food consumption grow, the expectations of what dairy ingredients must provide will grow. Additionally, growing concerns about food safety and food production practices on the environment will influence how dairy ingredients are manufactured, marketed, and utilized.

12.3.1 Verifiable dairy food quality and safety

Because consumers increasingly want to know more about the food they eat, where it has come from, and how it has been processed, food processors are expecting their ingredient suppliers to be able to provide verification of origin so that traceability becomes a reality and so-called “farm-to-fork” communication and promotion can be used. Advances in analytical capabilities to facilitate better identification of sources of milk supplies used in dairy ingredient manufacture could facilitate this traceability approach. Further, interest in greater diligence to ensure the safety of dairy ingredients remains strong. Rapid and more sensitive detection techniques and chemical or microbiological fingerprinting of dairy ingredients and milk supplies could result. Such capabilities will likely lead to new and substantiated ingredient attributes that will be a part of the next generation of dairy ingredients.

12.3.2 Optimal nutritional and functional performance

More specific and predictable functional performance in simple food systems will continue to expand. Growing interest and developments in nanotechnology and microencapsulation will create new opportunities for dairy ingredients to deliver nutrients and other food components. The latest tools of biotechnology and molecular biology will lead to a better understanding of milk and its benefits to human health, such as stimulating intestinal health, improving immune function, optimal absorption of nutrients, binding or removing toxins and pathogens, and mediating inflammation (Ward et al., 2004). Amino acids, peptides, minor proteins, oligosaccharides, and glycolipids may become dairy-derived ingredients (Madureira et al., 2010; Dewettinck et al., 2008). Preserving the bioactivity of these milk components will require new processing technologies that can provide preservation without thermal denaturation. Recent reports suggest that high pressure, low temperature pasteurization using supercritical gases like carbon dioxide are effective in killing microorganisms with minimal heat damage (Spilimbergo, 2011). Such approaches may also result in improved flavors and other sensory properties that are altered by current thermal-based processing methods. Changes in the structural properties of whey proteins and caseins due to high pressure processing could lead to modified functional properties of value in food applications (Huppertz et al., 2006).

12.3.3 Sustainability, environment, and animal welfare

Approaches to document the carbon footprint of dairy producing practices will impact dairy ingredient production costs and their perception in relation to sustainable environmental practices. Recent studies suggest that life cycle assessment provides useful information on which dairy practices result in a more sustainable dairy industry (Thomassen et al., 2008). Additionally, the welfare of dairy cattle will likely be considered as another measure of dairy ingredient quality (Keyserlink et al., 2009).

12.4 CONCLUSIONS

Dairy ingredients must continue to deliver taste, food function, and basic nutrition. However, next-generation dairy ingredients will be smarter in how they deliver nutrition and

functionality. Additionally, such ingredients will also need to reflect the consumers growing interest in and concerns about food safety, animal welfare, sustainability, and the environment.

REFERENCES

- Dewettinck, K., Rombaut, R., Thienpont, N., Le, T.T., Messens, K., and Van Camp, J. (2008) Nutritional and technological aspects of milk fat globule membrane material. *International Dairy Journal* **18**, 436–457.
- German, J.B., Morgan, C.J., and Ward, R.E. (2003) Milk: a model for nutrition in the 21st century. *Australian Journal of Dairy Technology* **58**, 49–54.
- Huppertz, T., Fox, P.F., de Kruf, K.G., and Kelly, A.L. (2006) High pressure-induced changes in bovine milk proteins: a review. *Biochimica et Biophysica Acta* **1764**, 593–598.
- Keyserlink, M.A.G., Rushen, J., de Passille, A.M., and Weary, D.M. (2009) Invited review: the welfare of dairy cattle—key concepts and the role of science. *Journal of Dairy Science* **92**, 4101–4011.
- Madureira, A.R., Tavares, T., Gomes, A.M.P., Pintado, M.E., and Malcata, F.X. (2010) Physiological properties of bioactive peptides obtained from whey proteins. *Journal of Dairy Science* **93**, 437–455.
- Mellentin, J.C. (2003) Five Strategies to enter the market. *Australian Journal of Dairy Technology* **58**, 169–174.
- Mulvihill, D.M. and Ennis, M.P. (2003) Functional milk proteins: production and utilization. In: *Advanced Dairy Chemistry, Volume 1—Proteins*, 3rd ed, edited by P.F. Fox and P.L.H. McSweeney, pp. 1175–1228. New York: Kluwer Academic/Plenum Publishers.
- Smithers, G.W. (2008) Whey and whey proteins-From gutter-to-gold. *International Dairy Journal* **18**, 695–704.
- Spilimbergo, S. (2011) Milk pasteurization at low temperature under N₂O pressure. *Journal of Food Engineering* **105**, 193–195.
- Thomassen, M.A., van Calker, K.J., Smits, M.C.J., Lepema, G.L., and de Boer, I.J.M. (2008) Life cycle assessment of conventional and organic milk production in the Netherlands. *Agricultural Systems* **96**, 95–107.
- Tong, P.S. (2003) Recombined and reconstituted products. In: *Encyclopedia of Dairy Sciences*, edited by H. Roginski, J.W. Fuquay, and P.F. Fox, pp. 2401–2404. San Diego, CA: Academic Press.
- Tong, P.S. (2007) An evolution of technology, products and applications. *New Food* **3**, 38–39.
- Ward, R.E., Watzke, H.J., Jimenez-Flores, R., and German, J.B. (2004) Bioguided processing: a paradigm change in food production. *Food Technology* **58**, 44–48.

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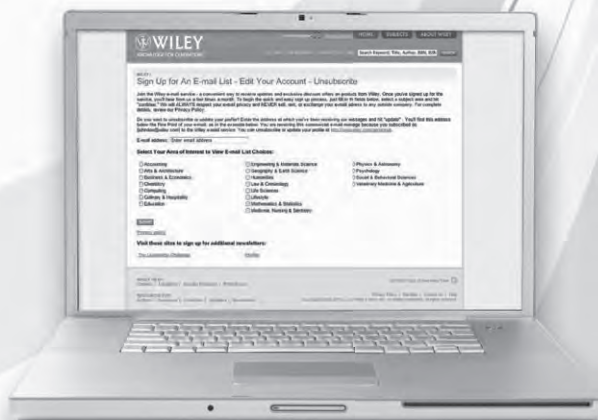
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