

High-pressure processing to improve dairy product quality

W. Messens, Agricultural Research Centre, Ghent, Belgium and
J. Van Camp and K. Dewettinck, Ghent University, Belgium

15.1 Introduction: high-pressure principles and technologies

Although preliminary research into the application of HP to foods was carried out over a century ago, various HP-processed foods have only recently been launched, such as fruit preparations, fruit juices, rice cakes, and raw squid in Japan, fruit juices in France and guacamole in the USA (Smelt, 1998). This is principally due to the recent availability of suitable equipment and to the increasing consumer demand for minimally processed additive-free, shelf-stable products. So far, no HP-processed dairy products are known to be available on the market, although various studies have dealt with high-pressure (HP) processing of dairy products, including milk, yoghurt and cheese, as is described in this chapter.

First, an overview is given of the HP principle and equipment. The treatment of milk by HP is described in Section 15.2, including the effect on its nutritional properties. Effects on the endogenous microflora and the pathogenic bacteria are described in Section 15.3 and on the indigenous milk enzymes and pro-enzymes in Section 15.3.1. The effect of HP on the milk proteins is described in Section 15.4. Effects on other milk properties, including its appearance, viscosity and pH, are described in Section 15.5 and on its rennet coagulation properties in Section 15.5.1. Effects of HP on milk's cheese-making properties are described in Section 15.6 and on its yoghurt-making properties in Section 15.6.1. The treatment of cheeses by HP is described in Section 15.7, with the purpose of reducing the brining time (Section 15.7.1), preserving cheeses and/or destroying pathogenic microorganisms in cheese (Section 15.7.2) and accelerating the ripening (Section 15.7.3). Rheological property changes may also occur in

cheese when HP is applied, as described in Section 15.7.4. Future trends and research needed in this area are described in Section 15.8. Sources of further information on this topic are given in Section 15.9. Unless specified otherwise, 'milk' refers to 'bovine milk' in this chapter. Also, treatment temperature is near room temperature unless stated otherwise.

Two principles underlie the effect of HP. Firstly, the principle of Le Chatelier according to which any phenomenon (phase transition, chemical reactivity, change in molecular configuration, chemical reaction) accompanied by a decrease in volume (negative ΔV) will be enhanced by an increase in pressure and vice versa. Secondly, pressure is instantaneously and uniformly transmitted independently of the size and the geometry of the food. This is known as isostatic pressure (Heremans, 1982; Tauscher, 1995).

HP equipment generally consists of four main parts: a HP vessel and its closure, a pressure-generating system, a temperature-control device and a material-handling system. The most important part is the pressure vessel of which the wall thickness is determined by the maximum working pressure, the vessel diameter and the number of cycles the vessel is designed to perform. The pressure-generating system causes a slight rise in the temperature of the food, and makes the temperature-control device necessary for some applications (Mertens and Deplace, 1993). Non-liquid products are usually sealed in flexible packages before being placed in the HP vessel filled with water (sometimes mixed with a small amount of oil) that is used as a pressure-transmitting medium. Liquid products are placed directly in the HP vessel (Earnshaw, 1996). The former is referred to as indirect compression, the latter as direct compression. Next to batch operation, semi-continuous and continuous operations are used in food processing. In semi-continuous processing, several vessels are connected in series; while some are under constant pressure, others are being pressurised, loaded or unloaded. This minimises the operation time and allows energy recuperation. Continuous operation is suitable for liquid products, such as milk and fruit juices (Vardag *et al.*, 1995).

In the food industry, vessels with a volume of several thousand litres are in use. The typical operating pressures are in the range 100–500 MPa and holding times of 5–10 min (Myllymäki, 1996). Recent advances in equipment design now allow foods to be processed up to 900 MPa (Linton and Patterson, 2000).

15.2 The effects of high pressure on nutritional and other qualities in milk

The nutritional value of milk and dairy products is determined by the presence of both macronutrients (carbohydrates, proteins, lipids) and micronutrients (minerals, trace elements, vitamins). Since HP processing has only a limited influence on covalent bonds, it is expected that this technology will only marginally influence the nutritional characteristics of these food products in comparison to traditional thermal processing. Only in a limited number of

studies have the nutritional characteristics of pressure-treated milk and dairy products been evaluated. In some cases, the HP treatment was combined with moderate heating. Attention will be given here to the influence of pressure on Maillard browning reactions, selective hydrolysis and digestibility of milk proteins, and the stability of vitamins in milk during and after pressure treatment.

The effect of pressure on Maillard browning has been investigated in model systems. Hill *et al.* (1996) used an aqueous solution of glucose and lysine at different initial pH values (5.1–10.1) and temperatures (40–60°C). The Maillard reaction was followed by measuring the absorbance of the solutions post-pressurisation (600 MPa) at 420 nm. Compared to ambient pressure, the rate of Maillard browning at 50°C was retarded by pressure at pH 5.1 and 6.5 but enhanced at pH 8.0 and 10.1. The effect of pressure was negligible at pH 7.0–7.5. By gas chromatography and mass spectrometry, it was demonstrated that yields for volatile reaction products obtained from the reaction of glucose with lysine at pH 10.1 and 60°C were suppressed after pressurisation. It was suggested that under these conditions, pressure increased the rate of aldol condensation reactions (Hill *et al.*, 1999).

More recently, Schwarzenbolz *et al.* (2000) demonstrated that Maillard-type reactions with ribose were increased under pressure. A solution of N^α -acetylarginine, N^α -acetyllysine and ribose in equimolar ratios at pH 7.4 was pressurised at 600 MPa for 2 h and at 60°C. Pressurisation resulted in a pressure-dependent increase of the pentosidine content, which is a fluorescent marker for the advanced Maillard reaction. This marker was also found in an enhanced yield in protein-bound form after pressure treatment of β -casein with ribose (ratio 1:10). Heating the β -casein/ribose solution for 2 h at 60°C did not result in a detectable amount of pentosidine, while the application of 600 MPa compared to 200 MPa was followed by a doubling of the amount of protein-bound pentosidine. However, by studying the effect of pressure (400 MPa, 30 min, 25°C) on raw milk, López-Fandino *et al.* (1996) could not detect furosine in the milk, indicating that no significant loss of available lysine occurred. As a consequence, although the work with model systems suggests an effect of HP on the Maillard reaction, work with dairy products does not directly confirm these trends, and more research will be required to investigate the influence of HP on the protein–carbohydrate interaction in milk and milk products.

One of the first papers demonstrating that HP can influence the preferential hydrolysis of milk proteins was by Hayashi *et al.* (1987). Casein was digested by thermolysin at both ambient and high pressure (200 MPa). On the contrary, β -lactoglobulin (β -Lg) was only selectively digested by the enzyme under pressure, while α -lactalbumin (α -La) was resistant to hydrolysis at both ambient and high pressure. The stability of α -La was attributed to its rigid structure, which is stabilised by four intramolecular disulphide bonds. β -Lg is more pressure-sensitive than α -La, and begins to denature around 200 MPa, thereby facilitating thermolysin digestion. Similarly, Okamoto *et al.* (1991) used pressures of 100–300 MPa to selectively eliminate β -Lg in milk whey by a 3-h

digestion with thermolysin at 30°C. Using pure β -Lg, maximum digestion (almost 60%) was obtained at 150 MPa. Using milk whey concentrate, the extent of β -Lg digestion was increased with increasing pressure, with less than 20% of β -Lg remaining post-pressurisation at 200 MPa. The difference in optimal pressure to digest β -Lg in the pure form and in the whey protein mixture (150 versus 200 MPa) might be related to the protective effects of the concentrated proteins in the whey concentrate on thermolysin and/or on its activity. The thermolysin digests had no binding affinity to five different types of anti- β -Lg monoclonal antibodies with distinct epitope specificity after treatment at 200 MPa, as evaluated by competitive ELISA.

Nakamura *et al.* (1993) investigated the use of HP as a pre-treatment of enzymatic hydrolysis to prepare hypoallergenic hydrolysates from whey protein concentrate (WPC) to apply in infant formulas. Hydrolysis of WPC with two proteases (papain W-40 from *Carica papaya* and proleather[®] from *Bacillus subtilis*) at 45°C for 6 h was investigated by HPLC. Compared to hydrolysis at ambient pressure, the pressure pre-treatment of WPC produced peptides with a narrower range of molecular weights. No difference between the different pressurisation conditions (200 MPa for 10 min, 400 MPa for 5 min, 600 MPa with no holding time) could be found, although the antigenicity of the protein hydrolysates as evaluated by ELISA decreased with the level of pressure applied. The preparation hydrolysed after compressing at 600 MPa resembled a hydrolysate treated at 60°C for 30 min. The authors concluded that HP before hydrolysis might be a promising procedure for the preparation of low antigenic WPC hydrolysates.

Van Willige and Fitzgerald (1995) used human digestive enzymes (trypsin and chymotrypsin) to evaluate the effect of pressure on the selective enzymatic hydrolysis at 37°C of different variants of β -Lg (A, B and AB). At ambient pressure, native β -Lg A was hydrolysed approximately three times faster than β -Lg B. At high pressures (100 and 300 MPa, for 0–180 min), the genetic variant associated differences in β -Lg hydrolysis disappeared. HP (<300 MPa, 30 min, 30°C) can also facilitate the hydrolysis of β -Lg B by pepsin and by trypsin, which was associated with the denaturation of the protein under pressure, as assessed with high-pressure fluorescence spectroscopy. The changes in conformation of the protein reversed slowly after decompression, whereby the enhanced digestibility of the protein was maintained at ambient pressure for several hours (Stapelfeldt *et al.*, 1996). All these studies clearly indicate that HP can selectively influence the digestion of milk proteins, which may find its application in altering digestibility and/or antigenicity of milk proteins.

Studies on the effect of pressure on vitamin retention in milk and milk products are limited. Sierra *et al.* (2000) found no loss in retention of vitamin B1 and B6 after pressure processing (400 MPa, 30 min) of raw milk. For vitamin B6, also the contents of pyridoxamine (36 μ g/l) and pyridoxal (291 μ g/l) did not change significantly.

15.3 The effects of high pressure on bacteria and enzymes

Heat treatments are still the most commonly used means of inactivating food spoilage and pathogenic bacteria in raw milk. Although efficient, thermal treatment may fail to destroy bacterial spores and may affect the appearance, taste and nutritional value of milk as well as its processing characteristics. Sensitivity to pressure varies greatly from one bacterial species to another, Gram-positive cells being generally more resistant than Gram-negative cells and spores more resistant than vegetative cells (Smelt, 1998).

Hite (1899) reported six decimal reductions in the microbial load of milk when subjected to 689 MPa for 10 min. Later, various studies have examined pressure inactivation of the indigenous microflora in milk. Combination of a treatment at 122 MPa and 13°C for 83 min was reported to result in a complete destruction of microbial vegetative cells in milk (Johnston, 1995). Mussa and Ramaswamy (1997) found first-order rate kinetics for inactivating microorganisms present in raw whole milk. Although higher pressures are more effective, treatment at a higher pressure for a shorter time or at a lower pressure for a longer time may bring about a given reduction in microbial count. At pressures above 350 MPa, complete destruction was observed after 10 min exposure. The shelf-life of milk treated at 350 MPa for 32 min was 18 days if stored at 5°C. Only a slight decrease in total aerobic count, but a pronounced reduction in psychrotrophic count, were found by treating raw whole milk at 200 MPa (López-Fandino *et al.*, 1996) which may resemble thermisation, a mild heat treatment of milk. Treatment of goat's milk for 15 min at 500 MPa was as efficient as pasteurisation in reducing the bacterial population of milk (Trujillo *et al.*, 1999b, Buffa *et al.*, 2001b). Garcia-Risco *et al.* (1998) did not observe a baroprotective effect of fat on microbial inactivation by HP. It was suggested that this effect could become noticeable only at fat levels higher than that of whole milk.

Lactococci inoculated into reconstituted skim milk were more sensitive than lactobacilli to pressures of 100–350 MPa. The treated cells exhibited lower acidification rates, even without affecting cell viability. HP increased the hydrolytic activity of lactococci and lactobacilli on the carboxyl-terminal fragment from β -casein, which contributes to bitterness in cheese, while the aminopeptidase or dipeptidase activity was not or only partly inhibited. It was suggested to apply HP to cheese milk to create an additional supply of enzymes with debittering properties (Casal and Gomez, 1999).

Various studies have been undertaken to assess the destruction of pathogenic bacteria in milk. A 3–4 log reduction of *Listeria innocua* by pressurisation at 200 MPa for five 1-min cycles (Kheadr *et al.*, 2002) and a 7–8 log reduction of *L. innocua* in ewe's milk by pressurisation at 450–500 MPa (10–15 min) were found (Gervilla *et al.*, 1997). The decimal reduction time (*D*-value) at 340 MPa of *L. monocytogenes* in ultra-high-temperature (UHT) treated milk was 13.2 min. In raw milk, the *D*-value was lower (9.3 min), which could be due to the presence of heat-labile antimicrobial compounds acting synergistically with

pressure to enhance inactivation (Styles *et al.*, 1991). The destruction by HP (100–500 MPa, 15 min) of various microorganisms inoculated in ovine milk was in the order *Pseudomonas fluorescens* > *Escherichia coli* ≥ *Listeria innocua* > *Lactobacillus helveticus* > *Staphylococcus aureus*. Pressurisations at temperatures below room temperature produced greater inactivation on *P. fluorescens*, *Listeria innocua* and *Lactobacillus helveticus*, whereas for *E. coli* and *S. aureus* the opposite was true. The fat content (0–50%) did not show a baroprotective effect (Gervilla *et al.*, 2000). For *S. aureus* in whole milk, the extent of inactivation increased with pressure (50–350 MPa) and treatment time (4–12 min) (Erkmen and Karatas, 1997).

A study by McClements *et al.* (2001) indicates the importance of the history of a bacterial culture prior to HP treatment and that bacterial spores require more severe treatments, probably in combination with other preservation techniques, to ensure inactivation. A more effective inactivation of bacterial contaminants can be achieved by combination of HP with heat or bacteriocins. This could improve the quality of minimally processed foods at lower pressure levels. This has been demonstrated for the destruction of *E. coli* and *S. aureus* using mild heating (up to 60°C) (Patterson and Kilpatrick, 1998), for the destruction of *S. aureus* and *L. innocua* using lacticin 3147 (Morgan *et al.*, 2000), and for the destruction of *E. coli* using mild heating (50°C) and lysozyme and nisin (García-Graells *et al.*, 1999). Dynamic high pressure (DHP), i.e. the use of consecutive, short pressure treatments interrupted by brief decompressions, has also been shown to be very effective for the destruction of *E. coli* (García-Graells *et al.*, 1999; Vachon *et al.*, 2002) and *Salmonella enteritidis* (Vachon *et al.*, 2002), offering a promising alternative for the cold pasteurisation of milk (Vachon *et al.*, 2002).

15.3.1 Effect on indigenous milk enzymes and pro-enzymes in milk

Because of the relative economic significance of the various enzymes in milk, their stability to HP has been investigated. In a buffer system, almost no reduction in catalase activity was found at 600 MPa (Trujillo *et al.*, 1997). Lactoperoxidase in raw milk is resistant to pressure of 400 MPa (López-Fandino *et al.*, 1996) up to 700 MPa combined with temperatures between 20°C and 65°C. Application of pressure even exerted a protective effect (Ludikhuyze *et al.*, 2001). Trujillo *et al.* (1999b) found that pasteurised and HP-treated goat's milk (500 MPa, 15 min) had different lipase activity. Alkaline phosphatase, used for evaluating the effectiveness of pasteurisation, was still active in raw milk samples treated at 400 MPa for 20–60 min (Johnston, 1995; López-Fandino *et al.*, 1996). Almost complete inactivation was obtained above 700 MPa (Johnston, 1995). Mussa and Ramaswamy (1997) have shown that alkaline phosphatase cannot be used as an indicator of the effectiveness of milk pressurisation. The barostability of lysozyme in milk has not yet been studied. However, since its three-dimensional structure is similar to that of α -La, lysozyme could be pressure resistant (Trujillo *et al.*, 1997). The proteolysis by

plasmin in milk kept post-pressurisation at 37°C for 48 h has been studied. López-Fandino *et al.* (1996) found no effect of pressures between 100 and 400 MPa, while Scollard *et al.* (2000) found little effect for milk treated at 50 MPa, but at 300–500 MPa proteolysis was increased, and above 500 MPa, proteolysis was less than that of raw milk. It was suggested that the increase of proteolysis in milk (at 300–500 MPa) could be due to changes in micelle structure facilitating increased availability of substrate bonds to plasmin (Scollard *et al.*, 2000). HP treatments at higher temperatures increased plasmin inactivation, which reached 86.5% at 60°C. Pressurisation at 40–60°C compared to 25°C reduced the proteolytic activity and improved the organoleptic properties of milk, and could be used to produce milk of good sensory properties with an increased shelf-life (Garcia-Risco *et al.*, 2000).

15.4 The effects of high pressure on milk proteins

15.4.1 Casein micelles in milk

HP treatment causes changes to casein micelle size of skim milk (Needs *et al.*, 2000b) and reconstituted skim milk (Desobry-Banon *et al.*, 1994; Gaucheron *et al.*, 1997). At 200 MPa, partial disintegration of casein micelles occurred and at ≥ 400 MPa the disintegration was complete (Desobry-Banon *et al.*, 1994; Gaucheron *et al.*, 1997; Needs *et al.*, 2000b). The turbidity decreased as a result of treatments up to 300 MPa; applying higher pressures up to 600 MPa caused little further decrease in turbidity (Needs *et al.*, 2000b). The size of micelles in milk also depends on the temperature during treatment (Gaucheron *et al.*, 1997) and the heat treatment of the milk before HP treatment (Schrader *et al.*, 1997). The casein micelles in raw or pasteurised skim milk are more sensitive to pressure than the heat-induced casein–whey protein complexes in UHT-treated milk (Schrader *et al.*, 1997). Also, subsequent heating of skim milk at 30°C post-pressurisation restored the original size distribution (Shibauchi *et al.*, 1992). The ionic calcium concentration was unaltered (Johnston *et al.*, 1992; De La Fuente *et al.*, 1999) or slightly increased (López-Fandino *et al.*, 1998) by HP treatment while levels of total serum calcium and phosphorus were both increased to a similar extent by HP treatment of skim milk (Shibauchi *et al.*, 1992), reconstituted skim milk (Desobry-Banon *et al.*, 1994) and raw whole milk (López-Fandino *et al.*, 1998). It is believed that this additional non-ionic calcium and phosphorus are released by the fragmentation of the micelle structure by pressure (Johnston, 1995). Schrader and Buchheim (1998) suggested that these changes in casein micelles in milk by HP are due to three discrete processes:

- Irreversible HP-induced dissolution of heat-precipitated colloidal calcium phosphate (CCP) formed by severe heat treatment of milk
- HP-induced partial dissolution of indigenous CCP, resulting in largely reversible disintegration of casein micelles or casein–whey protein aggregates
- HP-induced denaturation of whey proteins.

Pressurisation increased the levels of non-sedimentable casein in raw milk (Desobry-Banon *et al.*, 1994; López-Fandino *et al.*, 1998). The dissociation of individual caseins was in the order $\beta > \kappa > \alpha_{s1} > \alpha_{s2}$, corresponding to the ester-phosphate content. This indicates that the caseins more tightly bound to the CCP dissociated to a lesser extent (López-Fandino *et al.*, 1998). Also, HP possibly resulted in the formation of large fragments (possibly containing denatured β -Lg) and/or some reaggregation (Johnston *et al.*, 1992). Indeed, β -Lg in skim milk was found to be denatured above 200 MPa and reaggregated to casein micelles or submicelles (Schrader *et al.*, 1997; López-Fandino *et al.*, 1998; Schrader and Buchheim, 1998). The surface hydrophobicity increased post-pressurisation, particularly at higher pressures and greater treatment times. This effect persisted for 8 days at 5°C, and could have resulted from micellar casein fragmentation and/or from unfolding of individual casein chains (Johnston *et al.*, 1992).

15.4.2 Other milk proteins

HP treatment of raw milk led to an increased denaturation of β -Lg by increasing pressures above 100 MPa (López-Fandino *et al.*, 1996; López-Fandino and Olano, 1998b; Arias *et al.*, 2000; Garcia-Risco *et al.*, 2000; Scollard *et al.*, 2000). Above 400 MPa, relatively little further denaturation occurred (Scollard *et al.*, 2000). The level of denaturation of β -Lg is influenced by heating the milk before pressurisation (Gaucheron *et al.*, 1997; Needs *et al.*, 2000a), by the temperature during HP treatment (López-Fandino and Olano, 1998b; Garcia-Risco *et al.*, 2000), by the pH of the milk during treatment (Arias *et al.*, 2000) and by the species the milk is derived from (Felipe *et al.*, 1997; López-Fandino and Olano, 1998a). Compared to β -Lg, α -La is much more resistant to denaturation by HP. It appeared to be completely resistant to pressures up to 500 MPa (López-Fandino *et al.*, 1996; Felipe *et al.*, 1997; Gaucheron *et al.*, 1997; López-Fandino and Olano, 1998a, 1998b; Garcia-Risco *et al.*, 2000; Needs *et al.*, 2000a). The higher number of disulphide bonds in α -La (four compared to two in β -Lg) (Gaucheron *et al.*, 1997) and the lack of a free sulphhydryl group in α -La (López-Fandino *et al.*, 1996) giving it a more rigid molecular structure than β -Lg, may explain these differences in pressure sensitivity. The conformation of bovine serum albumin (BSA) remains fairly stable at 400 MPa; which may be explained by the high number of disulphide bonds (17) stabilising the three-dimensional structure of BSA (López-Fandino *et al.*, 1996). Immunoglobulins are resistant to pressures up to 300 MPa (Felipe *et al.*, 1997).

15.5 The effects on other properties of milk

Some studies did not observe changes in the pH values of skim milk post-pressurisation (Johnston *et al.*, 1992; López-Fandino *et al.*, 1996). In later studies, an increase in the pH value by HP treatment of raw and UHT-treated skim milk (Schrader *et al.*, 1997; Schrader and Buchheim, 1998) and caprine

milk (De La Fuente *et al.*, 1999) was found. The pH increase was higher when the milk was treated at higher pressures or lower temperatures and when UHT-treated milk was pressure-treated compared to raw and pasteurised milk (Schrader and Buchheim, 1998). According to Schrader and Buchheim (1998), this pH shift is caused by the pressure-induced partial dissociation of the micellar CCP. In UHT-treated milk, also a pressure-induced dissociation of the heat-induced insoluble calcium phosphate takes place. The first pH shift is reversible while the latter is irreversible.

The disintegration of casein micelles into small fragments leads to a reduction of the milk's lightness (*L*)-values (Johnston *et al.*, 1992; Shibauchi *et al.*, 1992; Desobry-Banon *et al.*, 1994). This leads to a change in appearance: from 400 MPa onwards, the skim milk became almost transparent (Johnston *et al.*, 1992; Shibauchi *et al.*, 1992; Desobry-Banon *et al.*, 1994; Gaucheron *et al.*, 1997). The *L*-value of whole raw milk was only slightly decreased by HP treatment (Mussa and Ramaswamy, 1997).

HP treatment increased the dynamic viscosity of skim milk (Shibauchi *et al.*, 1992) and reconstituted skim milk (Desobry-Banon *et al.*, 1994), depending on the pressure intensity and to lesser extent on the treatment time (Shibauchi *et al.*, 1992). The changes started at 200 MPa and levelled off at 400 MPa. An increase in viscosity of 19% and 38% was observed for reconstituted skim milk at 430 MPa and skim milk at 500 MPa, respectively. For pressure-treated (400 MPa for 20 min) whole raw milk, a 21% increase in viscosity was found (Mussa and Ramaswamy, 1997). According to Desobry-Banon *et al.* (1994), the viscosity increase can be explained by the disintegration of the casein particles into smaller ones leading to an increased fraction of casein micelles in the total volume.

15.5.1 Effect on the rennet coagulation properties of milk

The time necessary for rennet coagulation (RCT) of milk is affected by the pressure intensity, the duration of the treatment at certain pressures, the temperature of the treatment, the pH of the milk and the milk species. At pressures up to 200 MPa, most studies agree that the RCT of bovine, ovine and caprine milk decreases (Desobry-Banon *et al.*, 1994; López-Fandino *et al.*, 1996; López-Fandino and Olano, 1998a; Arias *et al.*, 2000; Needs *et al.*, 2000b). Desobry-Banon *et al.* (1994) suggested that this is caused by the disruption of casein micelles by HP, giving them an increased specific surface area that increases the probability of interparticle collision. Processing of milk at higher pressures increased the RCT again to reach values that were close to those of untreated milk (López-Fandino *et al.*, 1996; López-Fandino and Olano, 1998a; Arias *et al.*, 2000; Needs *et al.*, 2000b; Buffa *et al.*, 2001d) or that were higher (Needs *et al.*, 2000b). The disruption of casein micelles appeared to be complete at treatments at and above 400 MPa. Whey proteins, particularly β -Lg, were denatured at increasing pressure, and the denatured β -Lg was incorporated into the gels. Its presence interfered with the secondary aggregation phase and

reduced the overall rate of coagulation (Needs *et al.*, 2000b). One study reported that the RCT of milk treated at pressures above 200 MPa remained similar to that treated at 200 MPa (Desobry-Banon *et al.*, 1994). Treatments of ovine and caprine milk at 400 MPa did not considerably lengthen the RCT (López-Fandino and Olano, 1998a), while treatment of goat's milk at 500 MPa increased the RCT (Buffa *et al.*, 2001d). The duration of the treatment at 400 MPa, but not at 200 MPa, also affected the RCT: a 10 min treatment reduced the RCT, while longer times increased the RCT (López-Fandino *et al.*, 1996). The combined use of HP with temperatures of at least 40°C delayed RCT (López-Fandino and Olano, 1998b). Acidification of milk before pressurisation decreased the RCT, while alkalinisation increased the RCT (Arias *et al.*, 2000).

The rennet coagulation process was investigated under pressure at 30°C. Initial proteolysis by chymosin (primary phase) was hardly affected up to 130 MPa, while the coagulation time (secondary phase) was approximately nine times higher than at ambient pressure (Ohmiya *et al.*, 1987). López-Fandino and Olano (1998b) reported that HP treatment reduced the rate of release of caseinomacropeptide (CMP) from κ -casein at 200–300 MPa by rendering κ -casein less susceptible to the action of chymosin, possibly because of interactions between denatured β -Lg and κ -casein.

Curd firmness was increased by treatment of milk for 30 min at 300 MPa, but not at 100, 200 or 400 MPa. The firmness was also dependent on the duration of the treatment (López-Fandino *et al.*, 1996). However, Needs *et al.* (2000b) found that skim milk treated at 200–600 MPa yielded higher curd firmness values than untreated milk. The microstructure of these curds differed. Curds produced from pressure-treated (600 MPa) milk contained dense networks of fine strands, which were continuous over much bigger distances than in curds produced from untreated milk, where the strands were coarser with large interstitial spaces. Pandey *et al.* (2000) found an increase in the gel strength of the rennet curds with a decrease in pressure level (200–400 MPa), temperature (3–21°C) and holding time (10–110 min). Molina *et al.* (2000) observed that the curd firmness of milk with reduced fat content treated at 400 MPa for 15 min was higher than that of equivalent untreated milk. For goat's milk, a treatment at 400–500 MPa improved the curd's consistency (López-Fandino and Olano, 1998a, Buffa *et al.*, 2001d).

15.6 The effects on cheese and yoghurt-making properties of milk

The wet curd yield was increased by up to 20% by pressurisation of milk at 300–400 MPa for 30 min compared to untreated milk. In addition, the loss of proteins in the whey was reduced. Lower levels of β -Lg (not of α -La) were found in the rennet whey of pressurised milks, indicating that at least part of the denatured β -Lg was retained in the curd (López-Fandino *et al.*, 1996; López-Fandino and Olano, 1998b; Arias *et al.*, 2000; Molina *et al.*, 2000; Needs *et al.*, 2000b).

Syneresis from the curds was similar to control samples when the milk was treated at up to 400 MPa, but was reduced by 600 MPa (Needs *et al.*, 2000b). Pandey *et al.* (2000), however, found a decrease in water-holding capacity of the rennet curds with a decrease in pressure (200–400 MPa), temperature (3–21°C) and holding time (10–110 min). The combined application of 400 MPa and 40°C was found to increase the curd yield compared to 20°C (López-Fandino and Olano, 1998b). With increasing pH of milk, the wet curd yield and the moisture retention in the curd increased for samples treated at 400 MPa (Arias *et al.*, 2000). Wet curd yield of caprine milk was also increased above 300 MPa, while it was increased above 200 MPa for ovine milk (López-Fandino and Olano, 1998a).

Cheddar cheeses made from pressurised milk retained more moisture and protein than cheeses made from pasteurised milk. They produced 7% higher yields using 586 MPa for three 1-min cycles (Drake *et al.*, 1997) and 4% higher yields using 200 MPa for five 1-min cycles (Kheadr *et al.*, 2002). Pressurisation of semi-skim milk (400 MPa, 15 min) increased the yield of reduced-fat cheese by 9% (Molina *et al.*, 2000). In cheese-making experiments with pressurised goat's milk (500 MPa, 15 min) increased yields were also found (Trujillo *et al.*, 1999b). The higher yields are due to retention of β -Lg and, especially, moisture. The latter is explained by the less close reaggregation of the casein micelles and fat globules post-pressurisation, allowing more moisture to be entrapped in the cheese. The presence of whey proteins can also facilitate increased moisture binding in the cheese (Drake *et al.*, 1997) which may have adverse effects as well. The goat's milk and reduced-fat cheeses made from HP-treated milk had higher levels of salt (Trujillo *et al.*, 1999a; Molina *et al.*, 2000), which may be due to the effect of the moisture content on the rate of salt absorbed by cheese.

Sensory and microbiological analysis of Cheddar cheeses indicated no differences between pasteurised and pressurised milk cheeses, but the latter had a pasty and weak texture (Drake *et al.*, 1997) or were more firm, elastic and cohesive and less brittle than the first (Kheadr *et al.*, 2002). Pressurisation of reduced-fat milk, however, improved cheese texture (Molina *et al.*, 2000). Goat's milk cheeses made from HP-treated milk had higher pH and salt, matured more quickly (higher extent of proteolysis) and developed stronger flavours than cheeses made from pasteurised milk (Trujillo *et al.*, 1999a). Cheeses made from HP-treated goat's milk were firmer and less fracturable than those made from pasteurised milk, but differences became less notable towards the end of ripening (Buffa *et al.*, 2001c). Cheeses made from HP-treated goat's milk showed a similar level of lipolysis to cheeses made from raw milk, whereas the level of lipolysis in cheese made from pasteurised milk was lower. This behaviour could be explained by heat-sensitive but pressure-resistant characteristics of the milk lipase. No differences in the sensorial attributes between cheeses were found (Buffa *et al.*, 2001a). Goat's cheese made from pressurised milk had similar microbiological characteristics to pasteurised milk cheeses (Buffa *et al.*, 2001b).

15.6.1 Effect on the yoghurt-making properties of milk

Acid-set gels obtained using glucono- δ -lactone (GDL) from HP-treated skim milk showed an improved rigidity and gel breaking strength, a greater resistance to syneresis and an increased index of protein hydration with increasing pressure and treatment time (Johnston *et al.*, 1993). Coagulation started at a higher pH and yielded a stronger gel than untreated milk (Desobry-Banon *et al.*, 1994). Also, solvation of the pellets was higher. These changes were in accordance with micelle disruption into small clusters or aggregates (Famelart *et al.*, 1997).

The microstructure and rheological properties of set yoghurts made from skim milk subjected to heat and pressure (600 MPa, 15 min), fortified by addition of whey proteins, were very different. Pressure-treated milk yoghurt had a much higher storage modulus but yielded more readily to large deformation than the heated milk yoghurt (Needs *et al.*, 2000a). In stirred yoghurt, hydrodynamic properties and viscosity were improved when the milk was treated for 1 h in the 100–600 MPa range (Johnston *et al.*, 1994). The firmness of yoghurt made from HP-treated ewe's milk (200–500 MPa, 10–55°C, 15 min) increased with increasing pressure, and an additional increase in firmness was observed at 55°C. The level of syneresis increased during storage for yoghurts made from pasteurised or heat-treated milk, but not from HP-treated milk (Ferragut *et al.*, 2000).

The acidification of milk with GDL under pressure (50–200 MPa) was examined by Schwertfeger and Buchheim (1999). Coagulation of samples treated at higher pressures and/or for longer times occurred. The structures of these aggregates differed distinctly from the homogeneous gel formed by acidification of milk at ambient pressure; a fine-stranded, coherent coagulum was formed at 200 MPa for 20 min.

HP may also induce the gelation of milk concentrates at low temperature and neutral pH in the absence of any coagulating enzyme or gelling agent (Kumeno *et al.*, 1993; Vélez-Ruiz *et al.*, 1998).

15.7 High-pressure treatment of cheese

HP treatments have been applied to cheese to assess whether the brining time could be shortened, which could reduce processing time and improve cheese quality (Morris *et al.*, 1985). The preservation and destruction of pathogenic microorganisms in fresh cheese by HP has been the object of some studies. To compensate for the considerable cost associated with cheese storage, the use of the HP technology to accelerate cheese ripening was evaluated. Other changes, such as rheological property changes, also occur in cheese when HP is applied. Some of these changes may be advantageous; others may be drawbacks of the method.

15.7.1 High-pressure brining of cheese

Neither salt uptake nor salt diffusion was accelerated by brining of Gouda cheese (Messens *et al.*, 1999a) and Manchego-type cheese (Pavia *et al.*, 2000) under HP, but the water loss of Gouda cheese was reduced by brining at pressures above 200–300 MPa (Messens *et al.*, 1999a). This pressure-brining of Gouda cheese at 300 MPa for 30 min, however, disrupted the paracasein micelle structure, yielding more proteins, particularly β -casein and peptides in the cheese serum. The hydrolysis of β -casein by plasmin is accelerated by HP brining as indicated by UREA-PAGE (Messens *et al.*, 1998).

15.7.2 Elimination of microorganisms in cheese by high pressure

The refrigerated shelf-life of pasteurised goat's milk cheese can be extended by HP treatment (400–500 MPa for 5–15 min) since any bacterial growth during refrigerated storage was observed (Capellas *et al.*, 1996). HP can also be used to inactivate *Listeria monocytogenes* in goat's milk cheeses manufactured from strongly contaminated raw milk without any modifications on its organoleptic characteristics (Gallot-Lavallee, 1998). The combination of 400–500 MPa with mild heat (50°C) gave a reduction of *Staphylococcus carnosus* inoculated in fresh goat's milk cheese. The combination of 500 MPa and nisin was the most effective treatment. Inactivation of *Bacillus subtilis* spores inoculated in fresh cheese can be achieved by germination followed by vegetative cells inactivation (Capellas *et al.*, 2000).

The effectiveness of HP treatment to inactivate microbial contaminants in Cheddar cheese (slurry) has been assessed by O'Reilly *et al.* (2000a). The relative sensitivities of the isolates to HP were *Penicillium roqueforti* > *Escherichia coli* > *S. aureus*. The organisms were more sensitive to pressure in cheese than in slurry. In addition to cell death, the presence of sub-lethally injured cells in slurries post-pressurisation was demonstrated (O'Reilly *et al.*, 2000a). Pressurisation at 500 MPa for 15 min reduced the numbers of *L. monocytogenes* in Gouda, Edamski and Poslaski cheese by approximately 6 log units. The indigenous microflora was less pressure sensitive than *L. monocytogenes* (Szczański *et al.*, 1997). Pressurisation at 200 MPa caused approximately a 2 log reduction of the numbers of *L. monocytogenes* and *E. coli* O157:H7 in smear-ripened cheese manufactured using raw milk. From 300 MPa onwards, pressurisation resulted in complete inactivation of both pathogens (O'Reilly *et al.*, 2001). HP treatment (340 and 544 MPa for 10–30 min) of Swiss cheese slurries reduced the microbial population during prolonged storage at 30°C for up to 5 days (Jin *et al.*, 1996).

15.7.3 Accelerated ripening of cheese by high pressure

Accelerated cheese ripening by HP (3 days at 50 MPa) has been demonstrated for the first time in a patent by Yokoyama *et al.* (1992). This yielded a Cheddar cheese with a taste comparable to that of a matured commercial cheese. Using

the same pressure conditions, O'Reilly *et al.* (2000b) also observed an increase in the levels of proteolysis of immature Cheddar cheese but the differences became smaller during ripening. The degradation of α_{s1} -casein was enhanced by HP resulting in an accumulation of α_{s1} -I-casein. The enhancement of Cheddar cheese ripening was far less pronounced than in the patent, which could be attributed to the higher level of starter bacteria added to the cheese milk in the latter than conventionally. In both studies, the enhancement of proteolysis by HP could be partly due to a temperature effect, since cheeses were treated at a higher temperature than conventionally used for Cheddar cheese storage. For goat's milk cheese, Sendra *et al.* (1999) found that the enhanced proteolysis by exposure to 50 MPa for 3 days at 25°C compared to 3 days at 14°C and ambient pressure was due more to the elevated storage temperature than to the pressure level applied.

Yokoyama *et al.* (1992) also described the addition of lipase and protease to Parmesan-type cheese curd at salting, followed by treatment at 50 MPa for 3 days. This resulted in a Parmesan-type cheese equivalent to a commercial cheese. Pressure-treated Gouda cheese (14°C) obtained a higher pH than the untreated equivalent, particularly shortly post-pressurisation (Messens *et al.*, 1999b). Various indexes of cheese proteolysis (Reps *et al.*, 1998; Messens *et al.*, 1999b) and the SDS-PAGE profiles (Messens *et al.*, 1999b) were similar in treated and untreated cheeses. Hence, the proteolysis by chymosin and plasmin, and the proteinase/peptidase system of the starter, were not influenced by HP, although pressures above 225 MPa decreased starter bacterial growth (Messens *et al.*, 1999b). Butyric acid and acetoin were found in lower concentrations after ripening of the pressurized Gouda cheeses (Butz *et al.*, 2000). Pressurisation for 3 days at 50 MPa did not lead to an enhancement of proteolysis (Messens *et al.*, 1999b). Other semi-hard cheeses with a composition different from that of Gouda cheese, e.g. Saint-Paulin (higher water content), and Loo Light (lower fat content) also showed no increased proteolysis after treatment at 50 MPa for 8 h (Messens, 2000).

HP treatment of white mould-ripened cheeses, such as Camembert, led to an increase in its proteolysis (Reps *et al.*, 1998; Messens *et al.*, 2001), depending on the pressure and the maturity of the cheese. For 10-day-old Camembert, the highest degree of proteolysis was observed when 50 MPa for 2 h was applied (Reps *et al.*, 1998). Treatment at 50 MPa for 8 h enhanced the levels of pH 4.6-SN (soluble nitrogen) and trichloroacetic acid soluble nitrogen (TCA-SN) near the rind. Messens *et al.* (2001) suggested that this could have resulted from the increased pH caused by HP treatment leading to a higher action of the metalloproteinase of *Penicillium camemberti* (Messens *et al.*, 2001).

Coryneform bacteria, especially *Brevibacterium linens*, dominate the surface microflora of smear-ripened cheeses, such as Père Joseph. Treatment at 50 MPa for 8 h markedly affected pH and proteolysis of Père Joseph. Treated samples had a higher concentration of a typical breakdown product resulting from the caseinolytic action of an extracellular proteinase of *B. linens*. This is possibly a result of the pH increase leading to a higher amount and/or activity of the

proteolytic enzymes of *B. linens* that are capable of breaking intact caseins (Messens *et al.*, 2000a).

Goat's milk cheese proteolysis was enhanced by exposure to 400 MPa for 5 min at 14°C. Cheeses were ripened in 14 days compared to the conventional 28 days, possibly due to the higher pH or the enhanced enzyme activity from inoculated starter culture. Sensory analysis, however, indicated bitter notes in the accelerated ripened cheese (Saldo *et al.*, 2000). Levels of proteolysis in hard caprine milk cheese treated at 50 MPa for 72 h were only slightly different from those in untreated cheese. Treatment at 400 MPa for 5 min increased secondary proteolysis, or conversion of peptides into FAA, whereas coagulant activity was decreased (Saldo *et al.*, 2002a).

15.7.4 Rheology and microstructure of pressure-treated cheese

HP treatment has been shown to overcome the texture problem of half-fat Cheddar cheese. Treatment at 200 MPa gave the most similar performance to full-fat Cheddar. However, the browning time of half-fat Cheddar was less than for full-fat Cheddar (Johnston *et al.*, 2002). HP treatment (200 MPa for 60 min) of immature Mozzarella cheese increased its meltability and resulted in cheeses not different from matured samples. Moisture redistribution was found to play a major part in the changes (Johnston and Darcy, 2000).

While the proteolysis of Gouda cheese was not affected by HP treatment (Messens *et al.*, 1999b), its rheological properties were altered. The samples treated at 400 MPa got less rigid, less solid-like, and more viscoelastic; from 50 MPa onwards, the samples had less resistance to flow. It was shown that HP weakened hydrophobic interactions in Gouda cheese. This could have led to structural changes of the paracasein network causing the rheological property changes. The effects on proteins in Gouda cheese are reversible as both hydrophobic interactions and rheological properties were restored during ripening (Messens *et al.*, 2000b). Saldo *et al.* (2001) concluded that the incidence of hydrophobic and hydrogen bonds in treated hard cheeses was reduced after HP treatment (50 MPa for 72 h). Water was bound more strongly and cheese became more fluid-like compared to the untreated cheese.

Goat's milk cheeses treated at 400 MPa for 5 min were less crumbly and more elastic than untreated cheeses (Saldo *et al.*, 2000). The texture and colour of Mato cheese, a fresh cheese made from goats' milk, changed slightly by treatment at 500 MPa. HP-treated cheese lost more whey with a higher total nitrogen content than untreated cheese (Capellas *et al.*, 2001). During ripening post-pressurisation, HP-treated (400 MPa) hard caprine cheese had lower lightness and higher chroma values than untreated cheese (Saldo *et al.*, 2002b).

Pressure-shift freezing and thawing of Cheddar was shown to prevent the paste-like body and appearance typically for conventionally frozen and thawed Cheddar. The product was, however, different from fresh cheese, e.g. in being more readily deformed. Pressure-shift freezing and thawing gave no advantages in the case of immature Mozzarella cheese (Johnston, 2000).

15.8 Future trends

The shelf-life of thermally pasteurised milk may be obtained by HP treatment of milk at 400–500 MPa. However, this treatment may not be sufficient to inactivate pathogens. To overcome this problem, more effective inactivation of bacterial contaminants can be achieved by combination of HP with heat or by applying bacteriocins or by the use of DHP. Inactivation then occurs at lower pressure levels, improving the milk quality. More efforts could be done to investigate the combined use of HP with bacteriocins, because this research is still premature. Since HP technology is more costly than traditional heat technologies, other substantial changes should be introduced to apply this technology in the dairy industry. The use of HP-treated milk for cheesemaking has several advantages. Depending on the treatment intensity, this could reduce the RCT and increase the cheese yield and/or produce cheeses that are microbiologically safer than raw milk cheeses. To overcome the texture problem of low-fat cheeses, pressurised skim milk can be used for cheesemaking. This outcome still needs to be confirmed and should be compared with other means to overcome the texture defects of low-fat cheeses. Possible applications can also be found in yoghurt production, due to the improvement of the texture of yoghurt made from HP-treated milk which leads to an increased firmness and a decreased syneresis.

Safer cheese can be manufactured using pressurised milk, but also by HP treatment of the cheese itself. From a technological point of view, smaller volumes will need to be used in the latter case, but the process will be more complicated than for liquid processing. HP treatment of some cheese varieties increases proteolysis. Increased proteolysis was found for cheeses with a secondary flora, such as mould-ripened and smear-ripened cheeses and for goat's milk cheeses. The texture of several cheeses is affected in a positive way for some cheese varieties. The texture defect of low-fat cheeses can be overcome and Mozzarella cheese can be produced with improved meltability.

Nutritional consequences of HP processing of milk and dairy products, and food products in general, have been given little attention. More research should be done in this area because HP processing may give less nutritional damage than traditional processes. For future applications, this should be taken into account.

15.9 Sources of further information and advice

A review on the nutritional effects of HP and other new processing technologies is given in Gould (2001). The modification of the functionality of proteins has been reviewed by Messens *et al.* (1997), while Hendrickx *et al.* (1998) have reviewed the effects of HP on enzymes related to foods. The mechanism and efficacy of HP for the destruction of microorganisms has been reviewed by Smelt (1998), Knorr (1999), Linton and Patterson (2000) and Lado and Yousef

(2002). Recently, several review articles have been published concerning the HP processing of milk and dairy products. The paper by Datta and Deeth (1999) gives a general overview of HP processing of foods, followed by a description of the effects of HP on dairy products and processes. A review of the principles of HP and the equipment is also given. O'Reilly *et al.* (2001) focus on the applications of HP treatments on cheese manufacture and ripening. Both the treatment of milk prior to cheesemaking and the treatment of cheese are discussed in detail. The current state of knowledge of the effects of HP on constituents and properties of milk and possible applications of HP treatment of milk prior to the production of yoghurt and cheese are reviewed by Huppertz *et al.* (2002). The ongoing work towards the development of HP applications for the cheese industry is summarised in a paper by Trujillo *et al.* (2000).

15.10 References

- ARIAS, M, LÓPEZ-FANDINO, R and OLANO, A (2000), 'Influence of pH on the effects of high pressure on milk', *Milchwiss*, 55(4), 191–194.
- BUFFA, M, GUAMIS, B, PAVIA, M and TRUJILLO, A J (2001a), 'Lipolysis in cheese made from raw, pasteurized or high-pressure-treated goats' milk', *Int Dairy J*, 11(3), 175–179.
- BUFFA, M, GUAMIS, B, ROYO, C and TRUJILLO, A J (2001b), 'Microbiological changes throughout ripening of goat cheese made from raw, pasteurized and high-pressure-treated milk', *Food Microbiol*, 18(1), 45–51.
- BUFFA, M, TRUJILLO, A, PAVIA, M and GUAMIS, B (2001c), 'Changes in textural, micro structural, and colour characteristics during ripening of cheeses made from raw, pasteurized or high-pressure-treated goats' milk', *Int Dairy J*, 11(11–12), 927–934.
- BUFFA, M, TRUJILLO, A J and GUAMIS, B (2001d), 'Rennet coagulation properties of raw, pasteurised and high pressure-treated goat milk', *Milchwiss*, 56(5), 243–246.
- BUTZ, P, FERNANDEZ, A, KOLLER, W D, MESSENS, W and TAUSCHER, B (2000), 'Effects of high pressure treatment on fermentation processes during ripening of Gouda cheese', *High Pressure Res*, 19(1–6), 427–431.
- CAPELLAS, M, MOR-MUR, M, SENDRA, E and GUAMIS, B (2001), 'Effect of high-pressure processing on physico-chemical characteristics of fresh goats' milk cheese (Mato)', *Int Dairy J*, 11(3), 165–173.
- CAPELLAS, M, MOR-MUR, M, GERVILLA, R, YUSTE, J and GUAMIS, B (2000), 'Effect of high pressure combined with mild heat or nisin on inoculated bacteria and mesophiles of goat's milk fresh cheese', *Food Microbiol*, 17(6), 633–641.
- CAPELLAS, M, MOR-MUR, M, SENDRA, E, PLA, R and GUAMIS, B (1996), 'Populations of aerobic mesophils and inoculated *E. coli* during storage of fresh goat's milk cheese treated with high pressure', *J Food Prot*, 59(6), 582–587.
- CASAL, V and GOMEZ, R (1999), 'Effect of high pressure on the viability and enzymatic activity of mesophilic lactic acid bacteria isolated from caprine cheese', *J Dairy Sci*, 82(6), 1092–1098.
- DATTA, N and DEETH, H C (1999), 'High pressure processing of milk and dairy products', *Aust J Dairy Technol*, 54(1), 41–48.
- DE LA FUENTE, M A, OLANO, A, CASAL, V and JUAREZ, M (1999), 'Effects of high pressure and heat treatment on the mineral balance of goat's milk', *J Dairy Res*, 66, 65–72.

- DESOBRY-BANON, S, RICHARD, F and HARDY, J (1994), 'Study of acid and rennet coagulation of high pressurized milk', *J Dairy Sci*, 77, 3267–3274.
- DRAKE, M A, HARRISON, S L, ASPLUND, M, BARBOSA-CANOVAS, G and SWANSON, B G (1997), 'High pressure treatment of milk and effects on microbiological and sensory quality of cheddar cheese', *J Food Sci*, 62(4), 843.
- EARNSHAW, R (1996), 'High pressure food processing', *Nutr Food Sci*, (2), 8–11.
- ERKMEN, O AND KARATAS, S (1997), 'Effect of high hydrostatic pressure on *Staphylococcus aureus* in milk', *J Food Eng*, 33, 257–262.
- FAMELART, M H, GAUCHERON, F, MARIETTE, F, LE GRAËT, Y, RAULOT, K and BOYAVAL, E (1997), 'Acidification of pressure-treated milk', *Int Dairy J*, 7, 325–330.
- FELIPE, X, CAPELLAS, M and LAW, A J R (1997), 'Comparison of the effects of high-pressure-treatments and heat pasteurization on the whey proteins in goat's milk', *J Agric Food Chem*, 45, 627–631.
- FERRAGUT, V, MARTINEZ, V M, TRUJILLO, A J and GUAMIS, B (2000), 'Properties of yoghurts made from whole ewe's milk treated by high hydrostatic pressure', *Milchwiss*, 55, 267–269.
- GALLOT-LAVALLEE, T (1998), 'Efficiency of high pressure treatment for destruction of *Listeria monocytogenes* in goat cheese from raw milk', *Sci Aliments*, 18(6), 647–655.
- GARCIA-GRAELLS, C, MASSCHALCK, B and MICHEL, C W (1999), 'Inactivation of *Escherichia coli* in milk by high-hydrostatic-pressure treatment in combination with antimicrobial peptides', *J Food Prot*, 62(11), 1248–1254.
- GARCIA-RISCO, M R, CORTES, E, CARRASCOSA, A V and LÓPEZ-FANDINO, R (1998), 'Microbiological and chemical changes in high-pressure-treated milk during refrigerated storage', *J Food Prot*, 61(6), 735–737.
- GARCIA-RISCO, M R, OLANO, A, RAMOS, M and LÓPEZ-FANDINO, R (2000), 'Micellar changes induced by high pressure. Influence in the proteolytic activity and organoleptic properties of milk', *J Dairy Sci*, 83(10), 2184–2189.
- GAUCHERON, F, FAMELART, M H, MARIETTE, F, RAULOT, K, MICHEL, F and LE GRAËT, Y (1997), 'Combined effects of temperature and high-pressure treatments on physicochemical characteristics of skim milk', *Food Chem*, 59, 439–447.
- GERVILLA, R, CAPELLAS, M, FERRAGUT, V and GUAMIS, B (1997), 'Effect of high hydrostatic pressure on *Listeria innocua* 910 CECT inoculated into ewe's milk', *J Food Prot*, 60(1), 33–37.
- GERVILLA, R, FERRAGUT, V and GUAMIS, B (2000), 'High pressure inactivation of microorganisms inoculated into ovine milk of different fat contents', *J Dairy Sci*, 83(4), 674–682.
- GOULD, G W (2001), 'Symposium on "nutritional effects of new processing technologies". New processing technologies: an overview', *Proc Nutr Soc*, 60(4), 463–474.
- HAYASHI, R, KAWAMURA, Y and KUNUGI, S (1987), 'Introduction of high pressure to food processing: preferential proteolysis of b-lactoglobulin in milk whey', *J Food Sci*, 52(4), 1107–1108.
- HENDRICKX, M E, LUDIKHUYZE, L, VAN DEN BROECK, I and WEEMAES, C (1998), 'Effects of high pressure on enzymes related to food quality', *Trends Food Sci Technol*, 9, 197–203.
- HEREMANS, K (1982), 'High pressure effects on proteins and other biomolecules', *Ann Rev Biophys Bioeng*, 11, 1–21.
- HILL, V M, LEDWARD, D A and AMES, J M (1996), 'Influence of high hydrostatic pressure and pH on the rate of Maillard browning in a glucose–lysine system', *J Agric Food*

Chem, 44, 594–598.

- HILL, V M, ISAACS, N S, LEDWARD, D A and AMES, J M (1999), 'Effect of high hydrostatic pressure on the volatile components of a glucose–lysine model system', *J Agric Food Chem*, 47, 3675–3681.
- HITE, B H (1899), 'The effect of pressure in the preservation of milk', *Bull. W. Virginia Univ. Agr. Exp. Stat*, 58, 15–35.
- HUPPERTZ, T, KELLY, A L and FOX, P F (2002), 'Effects of high pressure on constituents and properties of milk', *Int Dairy J*, 12(7), 561–572.
- JIN, Z T, HARPER, W J and FARKAS, D (1996), 'Effects of high pressure treatment on changes of microflora and aroma profile in accelerated ripening of cheese slurry', *J Dairy Sci*, 79, suppl. 1, 114.
- JOHNSTON, D E (1995) 'High pressure effects on milk and meat', in Ledward, D A, Johnston, D E, Earnshaw, R G and Hasting, A P M, *High Pressure Processing of Foods*, Nottingham, University Press, 99–121.
- JOHNSTON, D E (2000), 'The effects of freezing at high pressure on the rheology of Cheddar and Mozzarella cheeses', *Milchwiss*, 55(10), 559–562.
- JOHNSTON, D E and DARCY, P C (2000), 'The effects of high pressure treatment on immature Mozzarella cheese', *Milchwiss*, 55(11), 617–620.
- JOHNSTON, D E, AUSTIN, B A and MURPHY, P M (1992), 'Effects of high hydrostatic pressure on milk', *Milchwiss*, 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', *Milchwiss*, 48, 206–209.
- JOHNSTON, D E, MURPHY, R J and BIRKS, A W (1994), 'Stirred-style yoghurt type product prepared from pressure treated skim-milk', *High Pressure Res*, 12, 215–219.
- JOHNSTON, D E, O'HAGAN, M and BALMER, D W (2002), 'Effects of high pressure treatment on the texture and cooking performance of half-fat Cheddar cheese', *Milchwiss*, 57(4), 198–201.
- KHEADR, E E, VACHON, J F, PAQUIN, P and FLISS, I (2002), 'Effect of dynamic high pressure on microbiological, rheological and microstructural quality of Cheddar cheese', *Int Dairy J*, 12(5), 435–446.
- KNORR, D (1999), 'Novel approaches in food-processing technology: new technologies for preserving foods and modifying function', *Curr Opin Biotechnol*, 10(5), 485–491.
- KUMENO, K, NAKAHAMA, N, HONMA, K, MAKINO, T and WATANABE, M (1993), 'Production and characterization of a pressure-induced gel from freeze-concentrated milk', *Biosci Biotechnol Biochem*, 57, 750–752.
- LADO, B H and YOUSEF, A E (2002), 'Alternative food-preservation technologies: efficacy and mechanisms', *Microbes Infect*, 4(4), 433–440.
- LINTON, M and PATTERSON, M F (2000), 'High pressure processing of foods for microbiological safety and quality (a short review)', *Acta Microbiol Immunol Hung*, 47(2–3), 175–82.
- LÓPEZ-FANDINO, R and OLANO, A (1998a), 'Cheese-making properties of ovine and caprine milks submitted to high pressures', *Lait*, 78(3), 341–350.
- LÓPEZ-FANDINO, R and OLANO, A (1998b), 'Effects of high pressures combined with moderate temperatures on the rennet coagulation properties of milk', *Int Dairy J*, 8(7), 623–627.
- LÓPEZ-FANDINO, R, CARRASCOSA, A V and OLANO, A (1996), 'The effects of high pressure on whey protein denaturation and cheese-making properties of raw milk', *J Dairy Sci*, 79(6), 923–936.
- LÓPEZ-FANDINO, R, DE LA FUENTE, M A, RAMOS, M and OLANO, A (1998), 'Distribution of

- minerals and proteins between the soluble and colloidal phases of pressurized milks from different species', *J Dairy Res*, 65, 69–78.
- LUDIKHUYZE, L R, CLAEYS, W L and HENDRICKX, M E (2001), 'Effect of temperature and/or pressure on lactoperoxidase activity in bovine milk and acid whey', *J Dairy Res*, 68(4), 625–637.
- MCCLEMENTS, J M, PATTERSON, M F and LINTON, M (2001), 'The effect of growth stage and growth temperature on high hydrostatic pressure inactivation of some psychrotrophic bacteria in milk', *J Food Prot*, 64(4), 514–522.
- MERTENS, B and DEPLACE, G (1993), 'Engineering aspects of high-pressure technology in the food industry', *Food Technol*, 47(6), 164–169.
- MESSENS, W (2000), 'High-pressure-brining and ripening of hard and semi-hard cheeses', Faculty of Agricultural and Applied Biological Sciences, Ghent, Ghent University, 154.
- MESSENS, W, VAN CAMP, J and HUYGHEBAERT, A (1997), 'Review article: the use of high pressure to modify the functionality of food proteins', *Trends Food Sci Technol*, 4(8), 107–112.
- MESSENS, W, DEWETTINCK, K, VAN CAMP, J and HUYGHEBAERT, A (1998), 'High pressure brining of Gouda cheese and its effect on the cheese serum', *Food Sci Technol*, 31(6), 552–558.
- MESSENS, W, DEWETTINCK, K and HUYGHEBAERT, A (1999a), 'Transport of sodium chloride and water in Gouda cheese as affected by high-pressure brining', *Int Dairy J*, 9(8), 569–576.
- MESSENS, W, ESTEPAR-GARCIA, J, DEWETTINCK, K and HUYGHEBAERT, A (1999b), 'Proteolysis of high-pressure-treated Gouda cheese', *Int Dairy J*, 9(11), 775–782.
- MESSENS, W, FOUBERT, I, DEWETTINCK, K and HUYGHEBAERT, A (2000a), 'Proteolysis of a high-pressure-treated smear-ripened cheese', *Milchwiss*, 55(6), 328–332.
- MESSENS, W, VAN DE WALLE, D, AREVALO, J, DEWETTINCK, K and HUYGHEBAERT, A (2000b), 'Rheological properties of high-pressure-treated Goat cheese', *Int Dairy J*, 10(5–6), 359–367.
- MESSENS, W, FOUBERT, I, DEWETTINCK, K and HUYGHEBAERT, A (2001), 'Proteolysis of a high-pressure-treated mould-ripen cheese', *Milchwiss*, 56(4), 201–204.
- MOLINA, E, ALVAREZ, M D, RAMOS, M, OLANO, A and LÓPEZ-FANDINO, R (2000), 'Use of high-pressure-treated milk for the production of reduced-fat cheese', *Int Dairy J*, 10(7), 467–475.
- MORGAN, S M, ROSS, R P, BERESFORD, T and HILL, C (2000), 'Combination of hydrostatic pressure and lacticin 3147 causes increased killing of *Staphylococcus* and *Listeria*', *J Appl Microbiol*, 88(3), 414–420.
- MORRIS, H A, GUINEE, T P and FOX, P F (1985), 'Salt diffusion in Cheddar cheese', *J Dairy Sci*, 68, 1851–1858.
- MUSSA, D M and RAMASWAMY, H S (1997), 'Ultra high pressure pasteurization of milk: kinetics of microbial destruction and changes in physico-chemical characteristics', *Food Sci Technol*, 30, 551–557.
- MYLLYMÄKI, O (1996) 'High pressure food processors', in Ohlsson, T, *High Pressure Processing of Food and Food Components – a Literature Survey and Bibliography*, Göteborg, Kompendiet, 29–46.
- NAKAMURA, T, SADO, H and SYUKUNOBE, Y (1993), 'Production of low antigenic whey protein hydrolysates by enzymatic hydrolysis and denaturation with high pressure', *Milchwiss*, 48(3), 141–145.
- NEEDS, E C, CAPELLAS, M, BLAND, A P, MANOJ, P, MACDOUGAL, D and PAUL, G (2000a),

- 'Comparison of heat and pressure treatments of skim milk, fortified with whey protein concentrate, for set yogurt preparation: effects on milk proteins and gel structure', *J Dairy Res*, 67(3), 329–348.
- NEEDS, E C, STENNING, R A, GILL, A L, FERRAGUT, V and RICH, G T (2000b), 'High-pressure treatment of milk: effects on casein micelle structure and on enzymic coagulation', *J Dairy Res*, 67(1), 31–42.
- OHMIYA, K, FUKAMI, K, SHIMIZU, S and GEKKO, K (1987), 'Milk curdling by rennet under high pressure', *J Food Sci*, 52(1), 84–87.
- OKAMOTO, M, HAYASHI, R, ENOMOTO, A, KAMINOGAWA, S and YAMAUCHI, K (1991), 'High-pressure proteolytic digestion of food proteins: Selective elimination of β -lactoglobulin in bovine milk whey concentrate', *Agr Biol Chem*, 55(5), 1253–1257.
- 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', *Appl Environ Microbiol*, 66(11), 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', *Inn Food Sci Emerg Technol*, 1, 109–117.
- O'REILLY, C E, KELLY, A L, MURPHY, P M and BERESFORD, T P (2001), 'High pressure treatment: applications in cheese manufacture and ripening', *Trends Food Sci Technol*, 12(2), 51–59.
- PANDEY, P K, RAMASWAMY, H S and ST-GELAIS, D (2000), 'Water-holding capacity and gel strength of rennet curd as affected by high-pressure treatment of milk', *Food Sci Technol*, 33(8), 655–663.
- PATTERSON, M F and KILPATRICK, D J (1998), 'The combined effect of high hydrostatic pressure and mild heat on inactivation of pathogens in milk and poultry', *J Food Prot*, 61(4), 432–436.
- PAVIA, M, TRUJILLO, A J, GUAMIS, B and FERRAGUT, V (2000), 'Effectiveness of high-pressure brining of Manchego-type cheese', *Food Sci Technol*, 33(5), 401–403.
- REPS, A, KOLAKOWSKI, P and DAJNOWIEC, F (1998), 'The effect of high pressure on microorganisms and enzymes of ripening cheeses', in Isaacs, N S, *High Pressure Food Science, Bioscience and Chemistry*, Reading, Royal Society of Chemistry, Cambridge, UK, 265–270.
- SALDO, J, SENDRA, E and GUAMIS, B (2000), 'High hydrostatic pressure for accelerating ripening of goat's milk cheese: proteolysis and texture', *J Food Sci*, 65(4), 636–640.
- SALDO, J, SENDRA, E and GUAMIS, B (2001), 'Hard cheese structure after a high hydrostatic pressure treatment at 50 MPa for 72 h applied to cheese after brining', *Lait*, 81(5), 625–635.
- SALDO, J, MCSWEENEY, P L H, SENDRA, E, KELLY, A L and GUAMIS, B (2002a), 'Proteolysis in caprine milk cheese treated by high pressure to accelerate cheese ripening', *Int Dairy J*, 12(1), 35–44.
- SALDO, J, SENDRA, E and GUAMIS, B (2002b), 'Colour changes during ripening of high pressure treated hard caprine cheese', *High Pressure Res*, 22(3–4), 659–663.
- 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 Milchwirtschaft Forsch*, 50, 79–88.
- SCHRADER, K, BUCHHEIM, W and MORR, C V (1997), 'High pressure effects on the colloidal calcium phosphate and the structural integrity of micellar casein in milk. I. High

- pressure dissolution of colloidal calcium phosphate in heated milk systems', *Nahrung*, 41, 133–138.
- SCHWARZENBOLZ, U, KLOSTERMEYER, H and HENLE, T (2000), 'Maillard-type reactions under high hydrostatic pressure: formation of pentosidine', *Eur Food Res Technol*, 211, 208–210.
- SCHWERTFEGER, M and BUCHHEIM, W (1999), 'Coagulation of skim milk under high hydrostatic pressure with acidification by glucono- δ -lactone', *Int Dairy J*, 9, 487–492.
- SCOLLARD, P G, BERESFORD, T P, NEEDS, E C, MURPHY, P M and KELLY, A L (2000), 'Plasmin activity, beta-lactoglobulin denaturation and proteolysis in high pressure treated milk', *Int Dairy J*, 10(12), 835–841.
- SENDRA, E, SALDO, J and GUAMIS, B (1999), 'Goat's milk cheese accelerated ripening. Compositional indexes', in Ludwig, H, *Advances in High Pressure Bioscience and Biotechnology*, Heidelberg, Springer, 465–468.
- SHIBAUCHI, Y, YAMAMOTO, H and SAGARA, Y (1992), 'Conformational change of casein micelles by high pressure treatment', in Balny, C, Hayashi, R, Heremans, K and Masson, P, *High Pressure and Biotechnology, Colloque INSERM*, London, John Libbey, 239–242.
- SIERRA, I, VIDAL-VALVERDE, C and LÓPEZ-FANDINO, R (2000), 'Effect of high pressure on the vitamin B1 and B6 content of milk', *Milchwiss*, 55(7), 365–367.
- SMELT, J P P M (1998), 'Recent advances in the microbiology of high pressure processing', *Trends Food Sci Technol*, 9, 152–158.
- STAPELFELDT, H, PETERSEN, P H, KRISTIANSEN, K R, QVIST, K B and SKIBSTED, L H (1996), 'Effect of high hydrostatic pressure on the enzymatic hydrolysis of β -lactoglobulin B by trypsin, thermolysin and pepsin', *J Dairy Res*, 63, 111–118.
- STYLES, M F, HOOVER, D G and FARKAS, D F (1991), 'Response of *Listeria monocytogenes* and *Vibrio parahaemolyticus* to high hydrostatic pressure', *J Food Sci*, 56, 1404–1407.
- SZCZAWINSKI, J, SZCZAWINSKA, M, STANCZAK, B, FONBERG-BROCZEK, M, ARABAS, J and SZCZEPEK, J (1997), 'Effect of high pressure on survival of *Listeria monocytogenes* in ripened, sliced cheeses at ambient temperature', in Heremans, K, *High Pressure Research in the Biosciences and Biotechnology*, Leuven, Belgium, Leuven University Press, 295–298.
- TAUSCHER, B (1995), 'Pasteurization of food by hydrostatic high pressure: chemical aspects', *Z Lebensm Unters Forsch*, 200, 3–13.
- TRUJILLO, A J, FERRAGUT, V, GERVILLA, R, CAPELLAS, M and GUAMIS, B (1997), 'High pressure effects on milk and milk products', *Rec Res Dev in Agric Food Chem*, 1, 137–159.
- TRUJILLO, A J, ROYO, C, FERRAGUT, V and GUAMIS, B (1999a), 'Ripening profiles of goat cheese produced from milk treated with high pressure', *J Food Sci*, 64(5), 833–837.
- TRUJILLO, A J, ROYO, C, GUAMIS, B and FERRAGUT, V (1999b), 'Influence of pressurization on goat milk and cheese composition and yield', *Milchwiss*, 54(4), 197–199.
- TRUJILLO, A J, CAPELLAS, M, BUFFA, M, ROYO, C, GERVILLA, R, FELIPE, X, SENDRA, E, SALDO, J, FERRAGUT, V and GUAMIS, B (2000), 'Application of high pressure treatment for cheese production', *Food Sci Technol*, 33(3–4), 311–316.
- VACHON, J F, KHEADR, E E, GIASSON, J, PAQUIN, P and FLISS, I (2002), 'Inactivation of foodborne pathogens in milk using dynamic high pressure', *J Food Prot*, 65(2), 345–352.

- VAN WILLIGE, R W G and FITZGERALD, R J (1995), 'Tryptic and chymotryptic hydrolysis of β -lactoglobulin A, B and AB at ambient and high pressure', *Milchwiss*, 50(4), 183–186.
- VARDAG, T, DIEKES, H and KÖRNER, P (1995), 'High pressure food processing', *Food Technol Europe* (Sept/Oct), 106–110.
- VÉLEZ-RUIZ, J F, SWANSON, B G and BARBOSA-CANOVAS, G V (1998), 'Flow and viscoelastic properties of concentrated milk treated by high hydrostatic pressure', *Food Sci Technol*, 31, 182–195.
- YOKOYAMA, H, SAWAMURA, N and MOTOBAYASHI, N (1992), 'Method for accelerating cheese ripening', *European Patent Application EP 0469857 A1*.