Chapter 25
Processing of Dairy Products Utilizing High Pressure

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Abstract Current knowledge of the main changes induced in milk (including goat, ewe, and buffalo milks) and milk products when treated by high hydrostatic pressure (HHP) is presented. The effects of HHP on casein micelles, whey proteins, lipids, indigenous enzymes, mineral equilibrium, and microorganisms are described. The significance of these effects on the technological properties of milk, particularly in cheese- and yogurt-making applications, and functional properties is also discussed.

Keywords High pressure • Dairy product • Microbial safety • Chemical changes

25.1 Introduction

Milk was one of the first foods to be subjected to high hydrostatic pressure (HHP) in 1897, when Bert H. Hite, a researcher working at the Agricultural Experiment Station, University of West Virginia, began to study the effects of HHP on food preservation. However, at that time, it was technically impossible to work at the industrial level, so there was reduced interest in such treatment. Nowadays, a number of industrially relevant applications exist for fruit juices, meat, and fish (Rastogi et al. 2007), but there are few applications for dairy products, although patents for milk and dairy products have been published, showing the industrial relevance of this technology.

One reason for this lack of application for dairy products might be that high hydrostatic pressure (HHP) treatment affects many constituents of milk, especially protein and mineral equilibrium, and induces changes in the functional properties of such products (Trujillo et al. 1997, 2002; Huppertz et al. 2002, 2006b; Needs 2002; López-Fandiño 2006).

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Current knowledge of the main changes induced in milks (including goat, ewe, and buffalo) and milk products when treated by HHP is presented in this chapter. The effects of HHP on casein micelles, whey proteins, lipids, indigenous enzymes, mineral equilibrium, and microorganisms are described. The significance of these effects on the technological and functional properties of milk, particularly in cheese and yogurt making, is also discussed.

25.2 Effects of HHP on Milk Constituents

25.2.1 Effects of HHP on Casein Micelles

Casein micelles contain four protein species, α_{s1}-, α_{s2}-, β-, and κ-casein (CN), which are bound together by amorphous calcium phosphate and by hydrophobic interactions. These are hydrophobic, phosphorylated proteins, always occurring as large, polydisperse aggregates in aqueous solution at ambient temperature with neutral pH.

Considerable research has been carried out on the effects of HHP on casein micelles using model systems and in milk. HHP treatment induces disruption of casein micelles and dissociation of caseins from the micelle, increasing the level of non-micellar caseins; two main mechanisms are possibly responsible: the solubilization of micellar calcium phosphate and the disruption of intramicellar hydrophobic and electrostatic interactions (Schrader et al. 1997; Needs et al. 2000a; Huppertz et al. 2004a, d, f). Electron microscopy images have indicated that micellar substructures are similar for pressure-modified casein micelles and casein micelles in untreated milk (Knudsen and Skibsted 2010).

Increases in micelle size depend on treatment temperature, time, milk pH, HHP cycles, and the presence of hydrophobic solvents (Gaucheron et al. 1997; Huppertz et al. 2004d, f).

HHP treatment increases the hydration of casein micelles, due to the association of denatured beta-lactoglobulin (β-LG) with the casein micelles, which increases the net negative charge of micelles enhancing micellar solvation, and by the HHP-induced disruption of casein micelles into smaller units, which also increases micellar hydration (Gaucheron et al. 1997; Huppertz et al. 2004a).

HHP-induced increase of non-micellar caseins is affected by pH; HHP treatment of acidified milk ($pH \leq 6$) and at $pH = 7$ increases the level of soluble caseins related to that found at $pH = 6.7$, probably due to destruction of colloidal structure or enhanced electrostatic repulsion, respectively (Arias et al. 2000).

25.2.2 Effects of HHP on Whey Proteins

Unlike caseins, whey proteins are classic globular proteins with a tight tertiary structure, occurring in milk as monomers or oligomers. Two of these proteins are dominant, β-LG and α-lactalbumin (α-LA), although several other proteins are also

present in milk: bovine serum albumin (BSA), immunoglobulins, lactoferrin, and various enzymes.

β-LG is denatured (estimated by the loss of solubility) in milk by pressure over 100 MPa; however, α-LA and BSA appear to be completely resistant to pressures up to 400–500 MPa. Pressurization of milk for 30 min at 200 and 300 MPa brought about a 20 % and 80 % denaturation of β-LG, respectively, but little further denaturation occurs at 400–800 MPa (López-Fandiño et al. 1996; García-Risco et al. 2000; Scollard et al. 2000b). A pressure of 600 MPa for 15–30 min denatures between 15 and 33 % of α-LA (Needs et al. 2000a; Huppertz et al. 2004c).

The higher-pressure resistance of α-LA and BSA than β-LG has been related to the more rigid molecular structure of the former, due to the presence of a higher number of intramolecular disulfide bonds (4 and 17 compared to 2) and to the free sulfhydryl group of β-LG, which can act as an initiator in sulfhydryl-oxidation or sulfhydryl-disulfide interchange reactions (López-Fandiño et al. 1996; Gaucheron et al. 1997).

The majority of denatured β-LG in HP-treated milk is associated with casein micelles, with a small proportion remaining nonsedimentable during ultracentrifugal operation, either in the form of whey protein aggregates or associated with very small casein particles (Felipe et al. 1997; Huppertz et al. 2004d). The free sulfhydryl group of β-LG can interact with other proteins such as κ-casein, α-LA, β-LG, and α_{s2}-CN and also with proteins associated with the milk fat globule membrane through sulfhydryl-disulfide interchange reactions (Huppertz et al. 2004c; Ye et al. 2004; Considine et al. 2007).

The extent of denaturation by HHP of α-LA and β-LG is dependent on different factors; it increases with increasing treatment time (López-Fandiño et al. 1996; Scollard et al. 2000b; Huppertz et al. 2004d), temperature (Gaucheron et al. 1997; García-Risco et al. 2000; Huppertz et al. 2004d), and milk pH (Arias et al. 2000) but is reduced by diminishing the level of micellar calcium phosphate in the milk (Huppertz et al. 2004c). Furthermore, denaturation of these proteins is prevented by adding a sulfhydryl-blocking agent to milk prior to pressurization (Huppertz et al. 2004c), and denaturation of β-LG is enhanced by the addition of a sulfhydryl-oxidizing agent (KIO_3) but is reduced in the case of α-LA (Huppertz et al. 2004c; Zobrist et al. 2005).

HHP-induced denaturation of whey proteins from species other than bovine has received little attention. There are differences in whey protein denaturation by HHP from milk of different species; ewe, goat and buffalo β-Lg denaturation by HHP at 250 MPa occurs at a faster rate than that of cow β-LG in the order buffalo > ovine > caprine > cow (Felipe et al. 1997; López-Fandiño and Olano 1998; Huppertz et al. 2005b, 2006a). In addition, the level of α-La denatured in buffalo milk after treatment of 800 MPa (~90 %) is higher than that in bovine milk treated at the same pressure (~70 %; Huppertz et al. 2005b).

Few data are available on HHP-induced denaturation of other whey proteins. No differences were observed by Felipe et al. (1997) in levels of immunoglobulins (IGs) in goat milk up to 300 MPa, but some aggregation occurred between 400 and 500 MPa (~35 %). Tonello et al. (1992) and Trujillo et al. (2007) determined that

IGs in bovine and caprine colostrums treated up to 200 and 400–500 MPa showed partial damage (~12 % and 19–38 %, respectively). Viazis et al. (2007) and Permanyer et al. (2010) investigated the effects of HHP (400–600 MPa) and pasteurization (62.5 °C, 30 min) on total IG A and lysozyme activities in human milk, showing that HHP-treated human milk retained higher levels of IG A and lysozyme activities compared to heat-treated samples (100–75 % retention versus 51 % and 96–100 % versus 79, respectively). More recently, Mayayo et al. (2014) showed that HP treatment of human milk at pressures of 300–600 MPa for 30 min resulted in retention of immunoreactive lactoferrin concentration of 90–52 %. In contrast, LTLT treatment that is usually applied in human milk banks retained only 20 % of immunoreactive lactoferrin. These data suggest that HHP is a potential alternative to thermal pasteurization of human milk banking that can provide greater retention of some bioactive components.

25.2.3 Effects of HHP on Mineral Equilibria

Milk salts have been recognized as playing a major role in determining the stability of milk to heat treatments. Several authors have investigated the effects of HHP treatment on the distribution of minerals between colloidal and diffusible phases and on the level of mineral ionization. It has been reported that HHP treatment increases (López-Fandiño et al. 1998; Zobrist et al. 2005) or does not increase (Johnston et al. 1992; De la Fuente et al. 1999) the concentration of ionic calcium in milk. According to Zobrist et al. (2005) these differences could be explained by the fact that HHP-induced ionization of calcium is a reversible process on subsequent milk storage.

Desobry-Banon et al. (1994) showed that HHP-induced disintegration of the casein micelles is accompanied by an increase in the levels of diffusible calcium and phosphate from colloidal phase. HHP treatment also increases the levels of diffusible salts in milks from non-bovine species such as ewe, goat, and buffalo (Law et al. 1998; López-Fandiño et al. 1998; Huppertz et al. 2005b).

25.2.4 Effects of HHP on Milk Fat Globules

Milk fat globule size is not affected by HHP treatment (Kanno et al. 1998; Huppertz et al. 2003; Ye et al. 2004), although Kanno et al. (1998) described an increase in the mean diameter and modifications in the size distribution of milk fat globules at pressures between 400 and 800 MPa. Gervilla et al. (2001) showed that the distribution of milk fat globules in ewe milk is modified by HHP up to 500 MPa. Pressure treatment at 25 and 50 °C tended to increase the number of small globules in the range of 1–2 μm, while at 4 °C the tendency was the opposite. However, the milk fat

globule membrane (MFGM) was not damaged, as the lack of lipolysis increased during storage of milk at 4 °C.

During HHP treatment (100–800 MPa), some denatured whey proteins associated with milk MFGM via disulfide bonds could be seen; β-LG was observed in the MFGM material isolated from milk treated at 100–800 MPa for 30 min, and small amounts of α-LA and κ-CN were also observed at pressures ≥700 and 500 MPa, respectively. Of the major original MFGM proteins, xanthine oxidase and butyrophilin are the major proteins involved in HHP-induced interaction with β-LG, but no change in butyrophilin content was observed during HHP treatment of whole milk, whereas xanthine oxidase was reduced to some extent beyond 400 MPa (Ye et al. 2004).

25.2.5 Effects of HHP on Lactose

Lactose in milk and in milk products may isomerize in lactulose by heating and then degrade to form acids and other sugars. No changes in these compounds are observed after pressurization, suggesting that neither Maillard reaction nor lactose isomerization occurs in milk after pressure treatment (López-Fandiño et al. 1996).

25.2.6 Effects of HHP on Milk Enzymes

Significant technological applications of some milk indigenous enzymes include flavor, texture, and stability in milk and dairy products. Because of the relative economic significance of various enzymes in milk, their stability when treated with HHP and their possible use as markers of the severity of treatment have been investigated. Most indigenous milk enzymes are quite resistant to moderate pressures (up to 400 MPa), and the resistance of different milk enzymes is correlated to their structures, as shown by Rademacher et al. (1999), who indicated the following order of enzyme resistance: phosphohexoisomerase < γ-glutamyltransferase < alkaline phosphatase < lactoperoxidase.

Some studies about the effect of HHP treatment on milk indigenous enzymes are given in Table 25.1.

According to Rademacher and Hinrichs (2006), regarding quality control, the kinetics of inactivation of γ-glutamyltransferase at 20 °C and pressures above 500 MPa are sufficiently close to the inactivation of *L. monocytogenes* and *E. coli* and may therefore provide a useful process marker for the destruction of these organisms and could be considered as a process marker in pressurized milk. On the other hand, xanthine oxidase has been also proposed as an indicator of the HHP treatment of milk. This enzyme is resistant at 400 MPa at 25 °C but is inactivated at higher pressures following a first-order kinetics (Olsen et al. 2004).

Table 25.1 Overview of the effects of high hydrostatic pressure on milk enzymes

Enzyme	Medium	HHP conditions	T^a (°C)	Inactivation (%)	Reference
Acid phosphatase	Raw and skim milks, acid and rennet wheys	200–800 MPa, 50 min	25	0–85	Balci et al. (2002)
Alkaline phosphatase	Raw milk	400 MPa, 60 min	20	0	López-Fandiño et al. (1996)
	Tris buffer (pH = 7) and milk	600 MPa, 30 min	55	90 (buffer)	Seyderhelm et al. (1996)
				10 (milk)	
	Goat milk	500 MPa, 10 min	20	0	Felipe et al. (1997)
	Raw milk	500 MPa, 90 min	25 or 50	50	Rademacher et al. (1998)
		600 MPa, 10 min		100	
	Raw milk	0.1–725 MPa	25–63	14–300 min	Ludikhuyze et al. (2000)
Cathepsin D	Ewe milk	200, 450, 650 MPa, 10 min	20, 40, 55	0–58	Moatsou et al. (2008)
γ-Glutamyltransferase	Raw milk	400 MPa	20–25	0	Rademacher et al. (1999)
	Raw milk	300–400 MPa, 0–180 min	3	15	Pandey and Ramaswamy (2004)
				35 activation	
Lactoperoxidase	Raw milk	400 MPa, 60 min	20	0	López-Fandiño et al. (1996)
	Tris buffer (pH = 7) and raw milk	600 MPa, 2 min	25	70	Seyderhelm et al. (1996)
		600 MPa, 5–30 min	25 or 50	30	
	Raw milk and acid whey	150–750 MPa, 0–140 min	15–73	0–25	Ludikhuyze et al. (2001)
				Antagonistic effect of T^a	
Lipase	Raw milk	Up to 600 MPa, 15 min	20	0	Kolakowski et al. (1997a)
		1000 MPa, 15 min	20	6–23	
	Raw milk	300–400 MPa, 0–100 min	3	133 activation	Pandey and Ramaswamy (2004)
Phosphohexose isomerase	Raw milk	400 MPa	20–25	0	Rademacher et al. (1999)

Plasmin	Goat milk	400 MPa, 10 min	2	0	Trujillo et al. (1997)
	Raw milk	400 MPa, 30 min	25	0	García-Risco et al. (1998)
	Raw milk	400 MPa, 15 min	60	86.5	García-Risco et al. (2000)
	Phosphate buffer (pH = 6.7) + sodium caseinate	Up to 600 MPa, 20 min	20	0	Scollard et al. (2000a)
	Phosphate buffer (pH = 6.7) + sodium caseinate + β-lactoglobulin	50–800 MPa, 1–30 min	20	0–85	Scollard et al. (2000b)
	Raw milk	400, 600 MPa, 30 min	20	30, 75	Huppertz et al. (2004f)
	Phosphate buffer (pH = 6.6)	300–800 MPa, 0–60 min	25–65	Stable at room T^a Inactivation 300–600	Borda et al. (2004)
				Antagonistic effect of T^a at >600 MPa	
	Ewe milk	200, 450, 650 MPa, 10 min	20, 40, 55	0–77	Moatsou et al. (2008)
Protease from *Bacillus subtilis*	Raw, pasteurized, and homogenized milk	300–450, 600 MPa, 0–15 min	40, 50, 60	0–35	Bilbao-Sáinz et al. (2009)
Xanthine oxidase	Raw milk	0–600 MPa	20		Olsen et al. (2004)

25.2.7 Effects of HHP on Minor Components of Milk

The effect of HHP on hydrosoluble vitamins (B_1, B_6, and C) in a multivitamin model system and in raw milk has been studied. Treatment of raw milk at 400 MPa for 30 min at 25 °C resulted in no significant losses in vitamins B_1 and B_6 (Sierra et al. 2000). Minor variations have also been found in vitamins after pressurization (200–600 MPa for 30 min at room temperature) of a multivitamin model system. Vitamins B_1 and B_6 undergo no significant losses after treatment, but vitamin C levels, although significant, are not dependent on the intensity of the HHP process (Sancho et al. 1999). More recently, Moltó-Puigmartí et al. (2010) studied the ability of HPP to maintain fatty acid, vitamin C, and vitamin E contents of human milk. Fatty acid proportions in milk, as well as levels of δ-, γ-, and α-tocopherols, did not vary with any of the treatments. Total vitamin C and ascorbic acid levels were maintained after HPP.

The effect of HHP at moderate-temperature processing on the volatile profile of milk, the kinetics of volatile formation in milk subjected to pressure-assisted thermal treatments, and the antioxidant impacts on volatile formation in HHP-treated milk have been investigated under different pressures (482, 586, 620, and 655 MPa), temperatures (25–75 °C), and holding times (1–10 min) and compared to pasteurization treatment (Vazquez-Landaverde et al. 2006, 2007; Vazquez-Landaverde and Qian 2007). These authors showed that heat treatment tends to promote the formation of methanethiol, hydrogen sulfide, methyl ketones, and aldehydes, whereas HHP treatment favors the formation of hydrogen sulfide and aldehydes. BHA and epicatechin, and ascorbic acid and β-carotene, to a lesser extent, are able to effectively inhibit aldehyde formation. On the other hand, pressure-assisted thermal treatments inhibit the formation of volatile sulfur compounds reported to be factors in consumer rejection of cooked milk flavor.

25.2.8 Changes in Characteristics of Milk Due to HHP-Induced Modifications in Milk Constituents

One effect of HHP on milk, caused by the destruction of the colloidal structure into smaller structures, is change in the visual appearance of milk, which becomes translucent and green yellow (Johnston et al. 1992; García-Risco et al. 2000; Needs et al. 2000a). Reduction in micellar size causes milk to lose its ability to scatter light, becoming translucent; this effect is particularly strong in the case of skim milk, since fat globules have light scattering properties themselves, although the effect is dependent on pressure and temperature of the treatment. In this case, skim milk HHP treated at 400 MPa for 15 min at 50 °C is not visually distinguished by sensory analysis compared to untreated milk, and these samples are preferred by taste-test panelists because of their smoother and creamier taste (García-Risco et al. 2000). This is consistent with the increase in micellar size occurring in these conditions, which increases light scattering of milk.

Creaming of raw whole bovine milk at refrigeration temperatures is generally regarded as an undesirable phenomenon; traditionally, creaming is prevented by homogenizing the milk. HHP processing at pressures ≤250 MPa increases the rate and level of creaming, an aspect that could be used to improve cream separation in the production of butter, whereas treatment at >400 MPa reduces both of these parameters, resulting in more stable milks during storage. The amount of milk protein associated with MFGM during HHP treatment and HP-induced aggregation and denaturation of agglutinins and lipoproteins may explain the changes in creaming characteristics of milk reducing rate and level of creaming (Gervilla et al. 2001; Huppertz et al. 2003).

HHP processing increases the viscosity of skim milk, and it could be related to the effect of HHP on casein micelles, e.g., disruption of casein micelles and reduction in particle size, and HP-induced increases in hydration of casein micelles, increasing voluminosity of the micelles, which should also increase the viscosity of milk (Huppertz et al. 2003).

HHP had little overall effect on heat stability of raw, preheated, or serum protein-free skim milk at pH values in the range 6.1–7.0. This result may possibly be related to reversibility of HP-induced changes in milk, either on subsequent storage or on severe heating of milk during determination of heat stability. However, HHP treatment at 600 MPa considerably increases heat stability of concentrated skim milk at pH values >6.7, which may be related to the formation of stabilizing κ-CN/β-LG complexes on the micelle surface (Huppertz et al. 2004c, d).

Ethanol stability of raw skim milk is reduced by HHP treatment, although it is partially reversible on subsequent storage for up to 24 h at 5 °C (Johnston et al. 2002b; Huppertz et al. 2004g). Increases in the level of soluble Ca and the dissociation of κ-CN from the micelle, which reduces steric stability of casein micelles, could facilitate the ethanol-mediated coagulation in HHP-treated milk (Huppertz et al. 2004g).

HHP-treated milk undergoes proteolysis after treatment during storage depending on the pressure and temperature of the treatment applied and the storing temperature. The level of proteolysis in HHP-treated milk at 100–400 MPa at room temperature and in untreated milk was similar during refrigerated storage (García-Risco et al. 2003), although some proteolysis of milk treated at 400 MPa for 30 min and then held at 5 °C was observed by Huppertz et al. (2004e) in comparison to untreated milk. According to García-Risco et al. (2003), although pressure conditions assayed does not lead to great plasmin inactivation (~20 %), it is likely that serum-liberated enzymes become more vulnerable to the action of proteinase inhibitors normally found in the soluble fraction, thus counteracting the enhanced susceptibility of caseins to the enzyme due to micellar disruption and protein solubilization, resulting in proteolysis levels on refrigerated storage similar to those of untreated milk.

However, when HHP-treated milk (300–400 MPa for 30 min) is stored at 37 °C, proteolysis of milk increases as a result of combination of disruption of casein micelles, increased availability of substrate to plasmin, and low inactivation of plasmin (Scollard et al. 2000b; Huppertz et al. 2004e). In contrast, proteolysis is considerably reduced if milk is HHP treated at 600 MPa for 30 min at room temperature, probably due to reduced plasmin activity after treatment (Scollard et al. 2000b).

25.3 Effects of HHP on Milk Microorganisms

25.3.1 Effect of HHP on Milk Shelf Life

Interest in high hydrostatic pressure (HHP) for treatment of milk has increased, mainly due to the possibility of reducing the number of spoiling and pathogenic microorganisms without causing significant effects on flavor and nutritional value. Several studies demonstrated that milk pressurized at 400–600 MPa by 10–60 min presents a microbiological quality comparable to that submitted to standard thermal pasteurization (72 °C during 15 s), but not to sterilized milk (UHT or similar) due to high resistance of bacterial spores to high pressures (Kolakowski et al. 1997b; Mussa and Ramaswamy 1997; Buffa et al. 2001a). Attempts to increase the lethal effect over bacterial spores by combining HHP with mild temperatures were not completely satisfactory on milk (Scurrah et al. 2006).

Molina-Hoppner et al. (2004) reported that HHP treatments at 300 MPa for 5 min on *L. lactis* ssp. *cremoris* MG1363 reduced metabolic activity to 10–12 % with respect to untreated microorganisms; after 12 min of treatment, cells did not show any metabolic activity. Gervilla et al. (1999) reported for *L. helveticus* that pressure treatments were more effective at low (2 and 10 °C) and moderately high (50 °C) temperatures than at room temperature (25 °C).

Bacteria are expected to be injured or inactivated by HHP, depending on the pressure level, species, and strain of the microorganism and subsequent storage conditions. Sublethal injured bacteria may be able to repair in a medium containing necessary nutrients under conditions of optimum pH and temperature, affecting quality and safety, especially in nutritive and low-acid food products such as milk. Two types of injury were described after HHP treatments by Bozoglu et al. (2004): I1 and I2. I2-type injury is a major injury, and after its repair (I2 to I1), cells can form colonies on nonselective but not on selective agar. Therefore, it is imperative that shelf life studies must be conducted over a period of time for potential repair of I2-type injury either to detectable injury (I1) or to active cells (AC) to ascertain the microbiological safety of low-acid food products, such as milk. During storage at low temperatures, milk retains optimum conditions to repair psychrotrophic microorganisms, such as *L. monocytogenes*, *Yersinia enterocolitica*, and *Pseudomonas* spp. (Bozoglu et al. 2004; De Lamo-Castellví et al. 2005).

25.3.2 Effect of HHP on Main Food-Borne Pathogens Present in Milk

25.3.2.1 Bacillus cereus

Bacillus cereus spores most often enter milk from water, soil, feces, bedding, cattle feed, milkstone deposits on farm bulk tanks, pumps, pipelines, gaskets, processing equipment, and packing material of the dairy industry, as well as from udders during

milking or as a result of mastitis (Meer et al. 1991; Andersson et al. 1995), but values of *B. cereus* usually reported in pasteurized milk are less than 3 log cfu/ml (Van Netten et al. 1990; Lin et al. 1998). The amount of viable cells or spores to form enterotoxins seems to vary between about 5 and 7 logs, partly due to large differences in the amount of enterotoxins produced by different strains (Granum 1994; Granum and Lund 1997). López-Pedemonte et al. (2003a) studied the effect of HHP (300, 400, or 500 MPa at 30 °C during 15 min) on inactivation of spores of *B. cereus* ATCC 9139 inoculated into cheese made of raw cow's milk with and without a previous germination cycle of 60 MPa at 30 °C for 210 min, observing that adding the germinative cycle resulted in higher efficiency when applied with a 500 MPa HHP treatment; however, maximum reduction achieved was only about 2.0 log cfu/ml. Van Opstal et al. (2004) identified two possible approaches to inactivate spores of *B. cereus* in milk: a single-step treatment at 500 MPa and 60 °C for 30 min and a two-step treatment consisting of 30 min at 200 MPa and 45 °C to induce spore germination, followed by mild-heat treatment at 60 °C for 10 min to inactivate germinated spores. Both treatments achieved a ≥6 log inactivation, but a small fraction of spores always remained ungerminated. Further, not all germinated spores were inactivated by pressure treatment, even under the most severe conditions, probably due to the existence of a fraction of superdormant spores that resist germination under high pressure.

This combined effect with lysozyme and/or nisin has also been suggested for increasing the lethal effect of HHP. López-Pedemonte et al. (2003b) studied the combined effect of HHP and nisin or lysozyme on the inactivation of spores of *B. cereus* (ATCC 9139) in model cheeses made of raw milk submitted to a germination cycle of 60 MPa at 30 °C for 210 min, to a vegetative cell destruction cycle of 300 or 400 MPa at 30 °C for 15 min, or to both treatments. The combination of both cycles improved the efficiency of the whole treatment, obtaining the highest inactivation (2.4 ± 0.1 log cfu/g) when the second pressure cycle of 400 MPa was applied with the presence of nisin (1.56 mg/l of milk), whereas lysozyme (22.4 mg/l of milk) did not increase sensitivity of the spores to HHP. Black et al. (2008) investigated the germination and inactivation of spores of *B. cereus* suspended in milk. Treating four strains of *B. cereus* at 500 MPa for 5 min twice at 40 °C in the presence of 500 IU/ml nisin proved to be less effective at inactivating the spores compared with *B. subtilis*, where a log reduction of 5.9 was obtained when nisin was added to milk prior to HHP treatment.

25.3.2.2 Escherichia coli

Different types of *E. coli* have been reported as a cause of food-borne diseases. *E. coli* O157:H7 has emerged as a food-borne pathogen of major concern for the food industry due to its ability to cause severe illness, particularly in children. Dairy cattle are considered to be the main reservoir of *E. coli* O157:H7 for human infection (Weeratna and Doyle 1991), with fecal contamination of milk being an important vehicle for its transmission (Borczyk et al. 1987; Gonzalez 2002). Patterson and Kilpatrick (1998) used different combinations of pressures, temperatures, and

times to eliminate the population of *E. coli* O157:H7 inoculated in UHT milk. Population was reduced by 5 log cfu/ml when milk was treated at 400 MPa for 15 min at 50 °C. Linton et al. (2001) reported only a 4 log (cfu/ml) reduction of the two most resistant strains (NCTC 11601 and NCTC 9706) after applying 500 MPa for 40 min. Nevertheless, no survivors of either strain could be detected after an HHP treatment of 600 MPa for 30 min, which would mean a reduction above 7 log cfu/ml.

Dogan and Erkmen (2003) achieved complete inactivation of *E. coli* inoculated in raw milk (ranging from 6.14 to 6.98 log cfu/ml) with HHP treatment of 600 MPa for 30 min. The required time to achieve this goal in milk was significantly higher than in other matrices, like peach and orange juices, where 12 and 10 min were needed, respectively. A protective effect has been described for skim milk on HHP-mediated inactivation and injury of *E. coli.* However, protein fractions derived from skim milk (casein, whey, globulin, and albumin) did not exhibit this protective effect. Microscopy analysis by DAPI/PI staining indicated that some cells were localized in the solid portion of skim milk and that this would protect those cells from the effect of HHP (Narisawa et al. 2008).

25.3.2.3 Listeria monocytogenes

Outbreaks of listeriosis have often been related to the consumption of raw and pasteurized milk, sour milk, chocolate milk, butter, and ice cream. Its presence in milk-based products can be a result of either raw milk direct contamination from dairy cattle, reaching concentrations above 3 log cfu/ml (Sanaa et al. 2004), or post-processing contamination in the case of dairy products like cheese (Borucki et al. 2004; Carminati et al. 2004).

Styles et al. (1991) reported inactivation above 6 log cfu/ml of *L. monocytogenes* Scott A after treating inoculated UHT milk samples at 340 MPa for 80 min. Mussa et al. (1999) observed that *L. monocytogenes* Scott A was more pressure resistant than the natural microbiota of raw milk after applying HHP treatments from 150 to 350 MPa during 0 to 120 min. Erkmen and Dogan (2004) described reductions of about 2.09 and 2.76 log cfu/ml in aerobic bacteria and *L. monocytogenes*, respectively, after 10-min pressure treatment at 400 MPa in raw milk, increasing to 5.09 and 6.47 log cfu/ml, respectively, at 600 MPa.

Chen and Hoover (2003) studied HHP inactivation of *L. monocytogenes* Scott A in whole milk combining higher pressures and temperatures (400 and 500 MPa; at 22, 40, 45, and 50 °C; holding time, 0–120 min) and observed a tailing phenomenon in all survival curves, indicating that linear models were not adequate for describing the effect of HHP. The log-logistic model produced the best fits to all survival curves, but the Weibull model provided good fit at the range 40–50 °C and reasonable predictions of inactivation.

Growth temperatures and growth phases have a significant effect on the inactivation of *Listeria monocytogenes* by HPP into milk. Cell growths at 15 °C were more

sensitive than cell growths at 4, 25 or 35, or 43 °C, which were the most resistant. Inactivation of cell growths at 4, 15, or 25 °C followed first-order kinetics, whereas cells grown at 35 or 43 °C displayed nonlinear inactivation kinetics due to tailing. Growth phase also significantly influenced inactivation of *L. monocytogenes* by HPP. Cells at the mid-stationary phase were significantly more resistant than cells grown at the mid- and late-exponential phase. This was probably due to changes in membrane composition and synthesis of stationary phase proteins and/or stress proteins (Hayman et al. 2007).

Post-processingtemperatures influence in recovering ability of injured *L. monocytogenes*. Koseki et al. (2008) observed that immediately after HPP treatment (550 MPa at 25 °C for 5 min), no *L. monocytogenes* cells were detected in milk regardless of the inoculum level (up to 7 log cfu/ml). However, the number of *L. monocytogenes* cells increased by >8 log cfu/ml after 3–28 days of storage at 4 °C. This recovery was not observed when storage was at 37 °C for 28 days. Mild-heat treatments (37 °C for 240 min or 50 °C for 10 min) following HPP (550 MPa at 25 °C for 5 min) inhibited the recovery of *L. monocytogenes* in milk after HPP during 70-day storage at 25 °C. Low pH also resulted in a noticeable synergistic effect on inactivation of *L. monocytogenes*. Xu et al. (2009) evaluated the effects of pressure come-up and holding times on the inactivation of *Listeria monocytogenes* in milk subjected to high-pressure treatments at 300, 400, and 500 MPa for less than 10 min at 30 °C. Milk showed a considerable baroprotective effect against *L. monocytogenes*. At 300 MPa, the *D* values for *L. monocytogenes* were 9.56, 1.11, and 0.94 min in milk, orange juice, and tomato juice, respectively.

25.3.2.4 Mycobacterium avium ssp. paratuberculosis

Mycobacterium avium ssp. *paratuberculosis* (MAP) causes Johne's disease, a chronic granulomatous enteritis that affects cattle and other ruminants. The isolation of MAP from breast milk, intestinal and lymph nodes, and blood from patients with Crohn's disease suggests a link between MAP and Crohn's disease, although this connection may not definitively be proved to date (Naser et al. 2000, 2004; Schwartz et al. 2000; Greenstein 2003; Selby 2004). MAP has been of concern to the dairy industry due to some reports supporting that it may not be effectively inactivated by conventional HTST pasteurization (72 °C, 15 s) (Lund et al. 2002). Few data have been published concerning the effect of HHP treatments on MAP. López-Pedemonte et al. (2006) inoculated two strains of MAP into sterilized milk to evaluate inactivation by HHP. Significant differences were also found between MAP strains as reported for other microorganisms, obtaining average reductions of 4 log cfu/ml after treatment with 500 MPa, which is comparable to results reported for thermal treatments. Donaghy et al. (2007) determined the effect of HHP alone and in conjunction with pasteurization (72 °C for 15 s) on the viability of two strains of MAP. A significantly greater ($P < 0.001$) reduction in viable numbers (mean log reduction of 6.52 cells/ml) was observed when using 500 MPa compared with 400 MPa (mean

log reduction of 2.56 cells/ml) for 10-min treatments, and the number of survivors was significantly lower ($P<0.001$) after a 10-min treatment with respect to a 5-min treatment. The use of high pressure was even more effective when combined with pasteurization, although there were still survivors when high inoculum levels of MAP were used.

25.3.2.5 Salmonella spp.

Raw and pasteurized milks have been involved in several outbreaks of salmonellosis. *Salmonella enterica* sv. *typhimurium* is most often associated with milk and dairy products; of great concern nowadays is *Salmonella typhimurium* DT 104, which is resistant to multiple antibiotics and has been documented in the UK in several outbreaks, some of which were determined to be caused by unpasteurized milk. In the USA, this serotype of *Salmonella* is the second most commonly reported cause of food-borne salmonellosis (Guan et al. 2005).

Tholozan et al. (2000) obtained complete inactivation of *S. typhimurium* strain Mutton (ATCC 13311) (>8 log reduction) in sodium citrate (pH 5.6) and sodium phosphate (pH 7.0) buffers after applying a 400 MPa HHP treatment for 10 min at 20 °C. However, Guan et al. (2005) reported that 350 MPa applied to UHT whole milk inoculated with *Salmonella typhimurium* DT 104 at ambient temperature (21 °C) during 60 min had little effect on this bacterium and only 3 log reductions were observed after 120 min. Cell count reductions reported at 350, 400, and 450 MPa for 30-min treatment were approximately 0.6, 1.8, and 5.0 log cfu/ml, respectively. Pressures of 500, 550, and 600 MPa reduced counts 4.5–5.1 log cycles within 10 min. A tailing was observed in all survival curves. The log-logistic model produced the best fit to data. Consequently, a 5.0 log reduction of *S. typhimurium* DT 104 in UHT milk required pressurization at 550 MPa for 50 min or 600 MPa for 30 min.

25.3.2.6 Staphylococcus aureus

Staphylococcus aureus are frequent contaminants of raw milk, being widely recognized as a common cause of clinical and subclinical mastitis in dairy cattle, sheep, and goats.

As is the case for most of vegetative bacteria, viability loss of *S. aureus* is enhanced as level of pressure, time, and temperature increases but shows greater resistance to HHP than other non-spore-forming bacteria (Patterson et al. 1995; Alpas et al. 2000; Trujillo et al. 2002). Patterson and Kilpatrick (1998) achieved a maximum reduction of approximately 6 log cfu/ml of *S. aureus* only when milk was treated at 500 MPa for 15 min at 50 °C. Gervilla et al. (1999) also found that in ovine milk *S. aureus* was extremely resistant to pressure and cell reductions above 7 log (cfu/ml) were only achieved after applying treatments of 500 MPa at 50 °C for 15 min.

García-Graells et al. (2003) observed that the lactoperoxidase system increased HHP inactivation of *S. aureus* in skim milk at pressures above 500 MPa at 20 °C

when it was present in loads under 10^6 cfu/ml. Under HHP it is sensitive to lysozyme, although it is not lysed, which suggested the existence of a non-lytic mechanism of bactericidal action of lysozyme against *S. aureus* (Masschalck et al. 2002).

25.3.2.7 Yersinia enterocolitica

Yersinia enterocolitica has been frequently isolated from raw milk and even from pasteurized milk (Larkin et al. 1991), and milk has been implicated in several outbreaks of yersiniosis (Tacket et al. 1984). In pasteurized milk, contamination has been mainly attributed to inadequate pasteurization or post-processing contamination (Klausner and Donnelly 1991; Kushal and Anand 1999). The psychrotrophic nature of this organism is of particular significance in milk and milk products that are normally stored at low temperatures.

Chen and Hoover (2003) studied the survival curves of *Yersinia enterocolitica* ATCC 35669 inactivated by high hydrostatic pressure in UHT whole milk at pressures ranging from 350 to 500 MPa. Tailing was observed in all survival curves and there was strong curvature in the plotted data so that the log-logistic Weibull models showed better R^2 and MSE values than the linear regression model. De Lamo-Castellví et al. (2005) also observed a tailing after 35 min of HHP treatment when the kinetics of population reduction were determined in one of the most baroresistant strains of *Yersinia enterocolitica* (serotype O:8). In that case, quadratic adjustment was the mathematical model that better fitted the results ($R^2 = 0.992$).

25.4 Effects of HHP on Dairy Products

25.4.1 Cheese

HHP treatment is a potential technology in the dairy industry for the manufacture of cheese, due to its effects on rennet coagulation time, cheese yield, ripening characteristics, extent of cheese shelf life, cheese functionality, and development of new textures.

25.4.1.1 Effects of HHP Processing on Cheese-Making Properties of Milk

As reported previously, HHP treatment reduces the number of microorganisms in milk, so it can be used to increase the microbiological safety and quality of milk to produce high-quality cheeses. Drake et al. (1997) reported a comparable microbiological quality of cheeses made with pasteurized milk and HHP-treated milk (3 cycles of 1 min at 586 MPa) with no detrimental effects on cheese flavor. In accordance with this finding, microbiological quality of cheeses made with HHP-treated goat

milk (500 MPa for 15 min at 20 °C) was comparable to pasteurized milk (72 °C for 15 s) cheeses (Trujillo et al. 1999; Buffa et al. 2001b). Cheeses elaborated with HHP-treated milk showed a similar level of lipolysis to cheeses made from raw and pasteurized milk and received the highest scores for overall aroma and taste (Buffa et al. 2001a), suggesting that it is possible to apply HHP processing to milk for making cheese of satisfactory hygienic quality.

In addition to improving the safety of cheeses, the application of HHP treatment of milk causes several protein modifications, such as whey protein denaturation and micelle fragmentation, and alters mineral equilibrium. Consequently, these changes modify the technological capacity of milk for making cheese.

Coagulation Properties

Many authors have found an improvement in the coagulation characteristics of cheese milk by HHP treatments. Desobry-Banon et al. (1994) showed that pressurization from 230 MPa enhanced acid and rennet milk coagulation in reconstituted milk prepared from low-heat skim milk powder. In other skim milk samples, rennet clotting time decreased as a result of pressure treatments up to 200 MPa but increased with higher pressures (Needs et al. 2000b). In agreement, coagulation time in raw bovine whole milk decreased as pressure increased $\leq$200 MPa and then increased again until at 400 MPa, reaching values comparable to that of raw milk (López-Fandiño et al. 1996, 1997). In goat's milk coagulation time did not change significantly with pressures up to 200 MPa, but treatments at 300, 400, and 500 MPa increased the coagulation time of milks (López-Fandiño and Olano 1998; Buffa et al. 2001c). In the case of ewe's milk, coagulation time decreased slightly with pressurization at 100 MPa and then increased significantly at 200 and 300 MPa, decreasing again at 400 MPa, with values similar to untreated milk (López-Fandiño and Olano 1998). Pandey et al. (2003) showed that coagulation rate is a function of pressure and temperature. At lower pressures (200 MPa), a change in treatment temperature from 3 °C to 21 °C increased the coagulation rate; however, at higher-pressure level (400 MPa), the coagulation rate was lower at higher temperatures (21 °C). Coagulation of milk is the result of two processes, i.e., enzymatic hydrolysis of κ-CN, which destabilizes casein micelles, and the aggregation of micelles leading to formation of a gel. These processes are governed by the stability of casein and mineral balances in milk, especially calcium and pH. HHP treatment of milk has been shown to cause a reduction in colloidal calcium phosphate concentration and reduction of enzymatic coagulation time, due to an increase in Ca^{2+} activity (Schrader et al. 1997). Furthermore, pressure treatment of milk affects milk proteins, including reduction in the size of casein micelles and denaturation of β-LG, probably followed by interaction with micellar κ-CN (O'Reilly et al. 2001).

López-Fandiño et al. (1997) found that the initial stage and extent of the enzymatic phase of coagulation were inhibited by pressures over 200 MPa and explained

by the attachment of β-LG to κ-CN under pressure, which restricts the hydrolysis of κ-CN by chymosin. However, Ohmiya et al. (1987) did not observe differences in the primary phase reaction of milk curdling by rennet with pressures up to 130 MPa. Similarly, Needs et al. (2000b) showed that the first stage of coagulation was unaffected by pressure, but HHP processing affected the second phase of rennet coagulation, which was explained with two opposing mechanisms: the effect of pressure on the properties of the micelles resulted in their rapid aggregation, while increasing β-LG denaturation progressively reduced the aggregation rate. In conclusion, it is clear that the effects of HHP on cheese coagulation properties depend on the substrate and the HHP conditions (pressure, time, and temperature).

Cheese Yield

An interesting application of high-pressure treatment on milk in cheese manufacture is the possibility to increase cheese yield.

Cheese yield increases with pressurization at 300 and 400 MPa in raw and heated milks (López-Fandiño et al. 1996; Huppertz et al. 2005a). This increase in cheese yield could be explained by the denaturation and incorporation of additional β-LG into the curd and greater degree of moisture content. Besides the economic interest in increasing cheese yield, gel characteristics of rennet curd is an important parameter in the cheese-making process, because it can affect textural attributes of cheese. A treatment of 300 MPa for 30 min in cow's milk significantly increased the firmness of gels (López-Fandiño et al. 1996). Pandey et al. (2000) studied the effect of HHP treatment and temperature on the water holding capacity and gel strength of rennet curds and found that lower pressures (200 MPa) and lower temperatures (3 °C) decreased water holding capacity but increased gel strength. Queso fresco made from pressure-treated milk (400 MPa, 20 min, 20 °C) contained more moisture and was less firm, less crumbly, and more sticky than cheeses made from raw milk and decreased in firmness during storage (Sandra et al. 2004). Nevertheless, pressurization (400 MPa, 22 °C, 15 min) of reduced-fat cow's milk prior to cheese making increased yield and improved cheese texture and accounted for higher overall acceptability (Molina et al. 2000). In a study of cheddar cheese made with high-pressure-treated milk, HHP treatment of raw milk (483 and 676 MPa) augmented cheddar cheese yield with better curd formation properties. HHP-treated cheeses at 10 °C showed higher cheese yield and protein retention compared to cheeses made from raw or pasteurized milk, which can be attributed to a combined action of protein and moisture retention (San Martín-González et al. 2007). Similarly, the yield of cheddar cheese made from HHP-treated milk (three cycles of 1 min at 586 MPa) was 7 % higher than that from raw or pasteurized milk (Drake et al. 1997); however, texture defects were present in pressurized milk cheeses, which were attributed to an excess of moisture. Arias et al. (2000) reported that curd yield and moisture retention in the curd increased with an increase in pH of milk, in the range 5.5–7.0, for milk treated at 400 MPa. In agreement, yield of curd from cow's milk was

slightly influenced by treatment at pressures ≤250 MPa, but treatment at 400, 600, or 800 MPa for 30 min significantly increased curd yield by 10 %, 24 %, or 25 %, respectively (Huppertz et al. 2004b). Needs et al. (2000b) did not observe significant differences in syneresis from curds prepared from untreated milk and milks pressure treated at 200 or 400 MPa; however, a treatment of 600 MPa significantly decreased the syneresis of the curd, possibly due to the effects of a finer gel network and increased inclusion of whey protein. The microstructure of curds formed from pressure-treated (600 MPa) milks showed a gel formed with a dense network of fine strands, which appeared to be continuous over long distances, as a result of an increase in the number of protein particles and modification of the properties of these particles that occurred during pressure treatment, for example, through disruption of casein micelles and denaturation of β-LG (Needs et al. 2000b).

Goat's milk behaves very similarly to cow's milk, and treatments at 300 and 400 MPa applied for 30 min in milk significantly improve cheese yield (López-Fandiño and Olano 1998). However, in goat's milk, firmness of curd is also improved with pressurization. Trujillo et al. (1999) found that the yield of cheese made from goat's milk treated at 500 MPa for 15 min was 5 % higher than that from pasteurized milk (Trujillo et al. 1999). Texture characteristics of cheeses made with HHP-treated milk were firmer and less fracturable than pasteurized milk cheeses but less cohesive than raw milk cheeses. HHP-treated milk cheeses have the most regular and close protein matrix, with small and uniform fat globules, resembling the structure of raw milk cheeses (Buffa et al. 2001d).

In the case of ewe milk, cheese yield also increases with pressurization from 200 to 400 MPa (López-Fandiño and Olano 1998). Higher increase in cheese yield of ewe milk at lower pressures is in accordance with the higher level and higher denaturation of β-LG in ewe milks (López-Fandiño and Olano 1998). However, firmness of curds was not affected by pressure treatment.

Rennet coagulation time of buffalo milk increases with increasing pressure, whereas the strength of the coagulum formed decreases after treatment at 250–800 MPa (Huppertz et al. 2005b).

25.4.1.2 Use of HHP Processing to Reduce or Inactivate Microorganisms in Cheese

HHP application in cheese can reduce total numbers of microorganisms or inactivate pathogenic microorganisms, thus increasing the shelf life and safety of the product. However, the application of pressure treatment could affect the sensorial characteristics of cheeses, which will be dependent on the type of cheese, HHP conditions, and cheese age at the time of treatment.

Ewe's milk cheeses pressurized at 15 days of ripening exhibit lower counts than those treated at day 1, demonstrating that the degree of maturity of cheeses affects the degree of microbial inactivation. However, pressurization at day 1 significantly changed the sensorial characteristics of cheeses, with a more elastic, softer, and less

crumbly texture (Juan et al. 2007d, 2008). In agreement, HHP treatments at 300 or 400 MPa on 2 or 50 days of ripening significantly reduced counts of undesirable microorganisms, improving the microbiological quality and safety of La Serena cheese immediately after treatment and at the end of the ripening period (Arqués et al. 2006). Cheeses treated by HHP on day 2 presented higher fracturability, hardness, and elasticity values than untreated cheeses or cheeses treated on day 50 (Garde et al. 2007). The aroma also was affected by HHP treatments on day 2, showing lower quality and intensity scores in comparison with untreated cheeses. On the other hand, HHP treatment on day 50 did not influence the sensory characteristics of 60-day-old cheeses (Arqués et al. 2007).

Gallot-Lavallée (1998) studied the efficiency of HHP treatment for destruction of *L. monocytogenes* in goat cheese from raw milk, finding that 450 MPa for 10-min or 500 MPa for 5-min treatments achieve more than 5.6 log units of reduction of this microorganism without significantly affecting sensory characteristics of cheeses.

Calzada et al. (2013) pressurized blue-veined cheeses made from pasteurized ovine milk at 400 or 600 MPa after 3, 6, or 9 weeks of ripening. On day 90, treatments at 400 MPa had lowered counts of lactic acid bacteria and *P. roqueforti* by less than 2 log units, whereas treatments at 600 MPa had reduced lactic acid bacteria counts by more than 4 log units and *P. roqueforti* counts by more than 6 log units. Differences in sensory characteristics between pressurized and untreated cheeses were generally negligible, with the only exception being treatment at 600 MPa at 3 weeks, which affected sensory characteristics of cheese.

HHP processing can be applied to increase fresh cheese shelf life to maintain its organoleptic acceptance. Capellas et al. (1996) showed that treatment at 500 MPa for 5 min at 25 °C delayed cheese spoilage during 2 and 3 months when applied during 5 or 30 min, respectively. When HHP treatment was combined with nisin, the extended cheese shelf life was more effective. In agreement, HHP treatment of fresh lactic curd cheese between 300 and 600 MPa for 5 min at ambient temperature effectively controlled the occurrence of spoilage yeasts and extended product shelf life for up to 8 weeks (Daryaei et al. 2008) without adverse effects on sensory and textural attributes of the product (Daryaei et al. 2006). In contrast, a significant effect of HHP on textural properties of rennet-coagulated fresh Scottish cheese was observed by Okpala et al. (2010). Fresh cheeses treated at 300 and 400 MPa for 5 min, stored at 4 °C, presented a shelf life of 14 and 21 days, respectively, compared to untreated control cheese, which presented a shelf life of 7 days. On the other hand, HHP treatments produced firmer texture and more yellow color (Evert-Arriagada et al. 2012). On the other hand, the application of 500 MPa (5 min, 16 °C) on fresh cheeses produced a considerable increase in shelf life, achieving 19–21 days when stored at 4 °C, whereas untreated cheese became unsuitable for consumption on days 7–8. Cheeses that were HHP treated at 500 MPa were firmer and more yellow than untreated ones. However, these changes, which were detected by instrumental and sensory analysis, did not affect the preference for pressurized cheese (Evert-Arriagada et al. 2014).

25.4.1.3 Use of HHP Processing for Cheese Ripening Acceleration

Ripening of cheese is a long and costly process; consequently, many methods to shorten ripening have been studied, including the use of elevated ripening temperatures, addition of enzymes, use of cheese slurries and selection, and attenuation or genetic modification of the starter. Ripening of cheeses is determined mainly by proteolysis; however, glycolysis and lipolysis are also necessary for the development of flavor and texture characteristics. Many authors have reported the possibility to ripening acceleration by HHP processing. Yokohama et al. (1992) described in a patent proposal the potential use of HHP technology for accelerating the ripening of cheddar cheese. Cheese samples were exposed to pressure from 0.1 to 300 MPa at 25 °C for 3 days. O'Reilly et al. (2000) tested the same conditions and showed an increase in proteolysis rates. Furthermore, studying different ranges of pressures and times to determine optimal conditions for acceleration the ripening of cheddar cheeses, O'Reilly et al. (2003) found that use of pressures below 150 MPa gave the largest increases in proteolysis parameters, in terms of %pH 4.6 SN/TN. However, levels of free amino acids (FAA) progressively decreased as the pressure was increased above 50 MPa. The increase in primary proteolysis in cheese by HHP may be explained by conformational changes in casein structure post-pressurization, making the protein more susceptible to the action of proteases. In Camembert cheese, the highest degree of proteolysis was observed with 50 MPa for 4 h and was dependent on the maturity of the cheese. Application of 50 MPa when treatment was applied on 4-day-old cheeses enhanced the primary proteolysis of cheeses, while the subsequent peptidolysis remained unaffected (Saldo et al. 2001). Increased levels of proteolysis have been found for surface-mold ripened cheese (Paillardin cheese, containing a secondary inoculum *Penicillium camemberti*) which was HHP treated at 50 MPa for 8 h at 20 ° C (Messens et al. 2001).

Other HHP conditions have been tested for accelerating cheese ripening that involves high HHP treatments (400–600 MPa) at short times (5–15 min) or an initial high HHP treatment at short times followed by low HHP treatment (50 MPa) for long times (72 h) in different cheese varieties. Saldo et al. (2002) subjected goat milk cheeses to pressures of 400 MPa for 5 min and lowered the breakdown of α_s-CNs, explained by the reduction of residual rennet and activity of some proteases. However, the HHP treatment increased FAA release, reaching twice the value found in untreated cheeses after 28 days, possibly as a result of higher peptidase activity. These authors observed that pressurization of cheese increased pH levels, which could enhance enzymatic activity. Levels of proteolysis of goat milk cheeses increased when the previous treatment (400 MPa for 5 min) was followed by 50 MPa for 72 h (Saldo et al. 2000). These results suggest that a combination of shock high-pressure treatment, which causes enzyme release, followed by a long and moderate-pressure treatment, which enhances enzymatic activity, would be best for accelerating the ripening of goat milk cheeses (Saldo et al. 2000). In Spanish cheese manufactured with a mixture of cow and ewe milks, HHP treatment of 400 MPa for 5 min accelerated casein degradation and increased FAA; however, this treatment did not influence the taste quality of cheeses (Ávila et al. 2006).

On the other hand, treatment of 400 MPa for 10 min at room temperature applied on 1-day post-manufacture on full-fat cheddar cheese did not influence the primary or secondary proteolysis of cheeses but could be discriminated from untreated cheeses by higher cooked animal flavor and butter odor at 90 days of ripening. When cheeses were ripened until 180 days, HHP-treated cheeses presented lower intensity of flavor attributes (Rynne et al. 2008).

Juan et al. (2004, 2007a) studied the possibility of accelerating the ripening of ewe milk cheeses by HHP treatments from 200 to 500 MPa for 10 min applied on 1- and 15-day-old cheeses. They found that primary proteolysis was enhanced by pressures of 300 and 400 MPa applied on the first day of ripening with 200–500 MPa applied at 15 days of manufacturing and attributed this to conformational changes in casein structure produced by pressure, which makes protein more susceptible to the action of proteases. Secondary proteolysis was enhanced by treatment at 300 MPa. Cheeses HHP treated on 1 day of ripening presented the highest FAA levels, whereas those treated at the same pressure on day 15 of manufacturing had the highest levels of water-soluble nitrogen (Juan et al. 2004). The lipolytic process of ewe milk cheeses was also improved by pressurization at 300 MPa on 1 day of ripening, showing twice the level of FFA after pressurization, which could be caused by the early lysis of cells and better interaction of microbial lipase with fat (Juan et al. 2007b).

On the other hand, pressurization at 500 MPa drastically reduced proteolysis and lipolysis of ewe milk cheeses, probably due to the reduction of starter bacteria and enzyme inactivation by pressure, suggesting that it may be useful for slowing or arresting cheese ripening at the optimum stage of ripening (Juan et al. 2004, 2007c).

The potential application of HHP processing for cheese ripening is evident from the described results; however, it depends on the stage of ripening and the variety of cheese. HHP treatment increases cell membrane permeability (Cheftel 1992), which favors the release of intracellular material to the medium, and consequently improves the access of enzymes to their substrates. Furthermore, HHP causes destabilization of micelles, which might render caseins more susceptible to the action of proteolytic enzymes. It seems that the application of HHP during the early stages of ripening tends to have a greater effect the on ripening process than when HHP is applied at a later stage of ripening. Furthermore, the application of higher pressures ($\geq$500 MPa) drastically reduces microbial counts and inactivates enzymes, so it may be useful to arrest the ripening of cheeses and maintain the optimum characteristics at a given time (Juan et al. 2004, 2007c; Calzada et al. 2014a, b).

25.4.1.4 Effects of HHP Processing on Sensorial Characteristics of Cheese

Despite the acceleration of the ripening of cheeses, HHP treatment can alter their rheological characteristics. Torres-Mora et al. (1996) suggested the possibility of the generation of desirable new cheese textures and reduced variability of moisture contents within blocks of reduced-fat cheddar cheese by the use of HHP, which produced a more continuous microstructure in cheddar cheese curd post-pressurization.

HHP treatment of 50 MPa for 72 h resulted in a softer texture of goat milk cheeses, with a more uniform structure (Saldo et al. 2001). When these cheeses were pressurized at 400 MPa or 400 MPa followed by 50 MPa, their elasticity and mouthfeel increased, and crumbliness decreased, but did not affect the acceptability of cheeses. Textural changes seem to be related to changes in calcium equilibrium, which is reestablished after HHP treatment, but with different associations between caseins (Saldo et al. 2000). Texture of cheddar cheese HHP treated at 50 MPa for 72 h became softer (Saldo et al. 2001) but did not show gross structural changes in the cheese matrix (O'Reilly et al. 2000). However, cheddar cheeses pressurized at 350 MPa for 70 h at 25 °C showed more fat emulsification, and the fat appeared to be encapsulated by protein (O'Reilly et al. 2003). Serrano et al. (2004, 2005) described that the application of moderate (345 MPa) and higher (483 MPa) pressures for 3 and 7 min accelerated the shredability of cheddar cheeses, showing similar attributes to those produced after 27 days of ripening. Pressure treatments reduced the presence of crumbles, increased mean shred particle length, improved length uniformity, and enhanced surface smoothness, so this treatment can be used to shred cheddar cheese immediately after block cooling, thus reducing refrigerated storage costs and simplifying the handling of cheese for shredding. In a half-fat cheddar cheese, pressurization from 100 to 800 MPa for 2 h induced softening of cheeses and increased meltability, cohesiveness, and chewiness (Johnston et al. 2002a). Increase in cohesiveness with pressure also was observed by Nienaber et al. (2000) in cheddar cheese with the same pressure treatments, explained by the physical effect of HHP that fuses particles together and strengthens internal binding forces. Full-fat cheddar cheese HHP treated at 400 MPa for 10 min at room temperature presented lower fracturability and flowability and higher deformability, explained by increases in cheese pH due to HHP that would be conductive to decreases in the ratio of soluble-to-colloidal calcium and degree of paracasein hydration and an increase of paracasein aggregation (Rynne et al. 2008).

O'Reilly et al. (2002) described that it is possible to accelerate the ripening process of low-moisture mozzarella cheese by pressurization of 400 MPa for 20 min at 25 °C. HHP treatment enhanced the development of cooking-related functional characteristics, which would normally happen gradually during storage time, resulting in an increase in water holding capacity of the matrix and an increase in the flowability and fluidity of heated cheeses. Hence, HHP treatment reduced the time required to attain satisfactory cooking performance (O'Reilly et al. 2002). Furthermore, Johnston and Darcy (2000) found that ripening acceleration of mozzarella cheese was possible with 200 MPa during 60 min, resulting in a decrease in hardness and an increase in flow of the heated cheese. On the other hand, pressurization (400 MPa for 5 min) of reduced-fat mozzarella cheese did not affect its rheological properties (Sheehan et al. 2005).

For ewe milk cheeses, Juan et al. (2007d) observed that pressurization of cheeses (from 200 to 500 MPa) on the first day of ripening decreased fracturability and increased deformability of cheeses. This phenomenon could be explained by the increase in water retention capacity and the more homogeneous microstructure produced by HHP, thus reducing possible areas of fracture. The highest treatment (500 MPa) significantly changed the texture of cheese, producing the least crumbly,

most elastic, and softest cheese. When ewe raw milk cheeses were HHP treated at 300 or 400 MPa for 10 min on day 2 of ripening, fracturability, hardness, and elasticity values increased (Garde et al. 2007). In this case, pressurized cheeses presented lower breakdown of α_{s1}-CN, which could produce firmer cheeses. According to Juan et al. (2007d), when pressure treatment was applied at 50 days of ripening, cheese texture was not affected.

25.4.1.5 Other HHP Processing Applications in Cheese

Besides the reduction of microbial counts, increase in cheese yield, modification of cheese ripening, and development of products with new sensory characteristics, other applications of high pressure in cheese have been proposed.

The possibility of accelerating cheese brining by HHP treatment has been suggested in Gouda (Messens et al. 1998, 1999) and Manchego (Pavia et al. 2000) cheeses, but salt uptake and salt diffusion were not accelerated by the pressure conditions tested (100–500 MPa, 15–130 min in Gouda and 50–200 MPa in Manchego, respectively).

Another proposed application was use of HHP processing to attenuate starter bacteria to be used as adjuncts in cheese manufacture. Attenuated starter bacteria cannot produce acid during cheese manufacture, but they do contain enzymes that contribute to cheese ripening. Casal and Gómez (1999) suggested that *Lactococcus lactis* ssp. *lactis* treated at 300 MPa and *Lactobacillus casei* ssp. *casei* treated at 350 MPa may be added during cheese making to provide an extra supply of enzymes with potential debittering properties, which may be used to accelerate cheese ripening. Upadhyay et al. (2007) observed that pressurization at 200 MPa for 20 min at 20 °C was successfully used to attenuate *L. lactis* ssp. *cremoris*, which may be used in combination with primary strains in cheddar cheese making, producing higher levels of FAA and acceleration of secondary proteolysis in cheese.

25.4.2 Yogurt and Acid Gels

Commercial yogurt is available in different presentations such as set gel, stirred gel, or liquid. Varieties of presentations are also related to milk composition, especially solids and fat content, which depend on milk origin and the preliminary step of skimming. The composition of yogurt cultures is typically a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, but increasingly, a probiotic strain is being added for health purposes. The effect of HHP on yogurt characteristics has been studied by several authors, and some of the abovementioned types of raw material commercial presentation and approaches have been raised (Table 25.2).

Yogurt quality must meet two relevant aspects. One is that the milk must be of very good hygienic quality. Also, from a physical point of view, yogurt should be stable during its commercial life (approximately 1 month), which in most cases

Table 25.2 Overview of the effects of high hydrostatic pressure on yogurt and acid gels

Type of yogurt	HHP (MPa) conditions Time (min)	*T* (°C)	Parameter evaluation	Authors
Stirred skim milk acid gels (GDL)	200, 400, 600	NS	Viscosity	Johnston et al. (1994)
Set yogurts treated	200–1000 (15)	Ambient	Activity of starters	Reps et al. (1998a)
Set yogurts from ewe's milk	200, 350, 500 (15)	10, 25, 55	Firmness Coagulation properties Syneresis	Ferragut et al. (2000)
Stirred low-fat yogurts	100–400 (15)	20	Culture counts Organic acids Free amino acids Viscosity Sensory evaluation	de Ancos et al. (2000)
Set skim fortified milk yogurts	600 (15)	Ambient	Color Texture Viscoelasticity Whey protein denaturation Microstructure	Needs et al. (2000a)
Acid-set gels (GDL): WP treated (H, HHP, native) added to simulated milk treated (H, HHP, native)	250, 400, 600, 700 (20)	25	Gel formation Whey protein denaturation Acidification curves	Walsh-O'Grady et al. (2001)
Set skim milk with WPC	600 (15)	Ambient	Consumer test Texture	Capellas et al. (2002)
Set whole milk fortified with WPC	676, (5, 30) 193, (5, 30)	Ambient	Yield stress Water holding capacity Microstructure	Harte et al. (2002)
Low-fat set yogurt	300, 400, 500, 676 (5) and combined with HT(HHP + H and H + HHP)	Ambient	Luminosity Yield stress, G' Penetration Water holding capacity Microstructure (TEM)	Harte et al. (2003)
Set yogurt of fortified (WPC) or concentrated skim milk	600, 15 min	Ambient	Syneresis Texture	Capellas and Needs (2003)
Stirred low-fat fortified with different cultures including *Bifidobacterium longum*	676, 5 min HT HHP HHP + H	Ambient	Yield stress, K, n TPA	Penna et al. (2006)

(continued)

Table 25.2 (continued)

Type of yogurt	HHP (MPa) conditions Time (min)	*T* (°C)	Parameter evaluation	Authors
Stirred low-fat fortified with different cultures including *Bifidobacterium longum*	676 (5) H HHP HHP + H	Ambient	Yield stress, K, n Water holding capacity Microstructure	Penna et al. (2007)
Low-fat stirred yogurt	100, 250, 400 (10)	25, 70, 90	Viscosity Whey protein denaturation Water holding capacity Particle size Microstructure	Udabage et al. (2010)
Acid gels (GDL) of pressurized skim milk between pH 6.4 and 7.3	200–600 (30)	Ambient	Particle size Whey protein denaturation Gel formation	Anema (2010)

means accomplishing good water retention capacity of the gel, minimizing the expulsion of whey (minimum syneresis) and good texture characteristics (firmness and creamy mouthfeel of gels).

To discover the best conditions of HHP treatment for yogurt making and mechanisms involved in acid gel formation, studies performed in this field have used different degrees of raw material complexity and treatment combinations and sequences (heat and HHP treatments).

Needs et al. (2000a) compared set yogurts made from skim fortified milk HHP treated (600 MPa, 15 min, ambient temperature) and heat treated (85 °C, 20 min). Viscoelastic characteristics of yogurts when strain sweeps were performed showed lower resistance to strain (loss of linear behavior) of HHP than those produced from heated milk. However, G' (elastic modules related to the interacting forces in the solid structure) values in the linear region were higher in HHP than in heat-treated yogurts. This behavior could be interpreted as HHP yogurt having more interacting micelles, probably with less intense forces, than yogurts produced from heat-treated milk, in which interactions were produced through more resistant strands, giving a deformable and resistant character to breakdown of the latter. In a study performed by Harte et al. (2002), the application of 676 MPa and long holding times (30 min) to milk (full fat) resulted in yogurts with equivalent rheological properties and WHC yogurts made from heat-treated (85 °C, 30 min) milk. In a further work, Harte et al. (2003) studied the effect of combining HHP (300, 400, 500, and 676 MPa for 5 min at ambient temperature) and the same heat treatment in low-fat fortified milk. Results showed improvement in the most important quality characteristics of yogurts when HHP and heat treatment were combined; in most conditions the combination was better than when each treatment was applied alone, although the order of treatment application gave different results. Generally, better WHC and mechanical

properties were observed in milk heat treated and then HHP treated, particularly in the intermediate range of 400–500 MPa. However, microstructure of yogurts was similar independently of whether thermal treatment was applied after or before HHP. Penna et al. (2006, 2007) studied mechanical properties, microstructure, and water retention capacity of stirred low-fat fortified yogurts made with two different probiotic starter cultures. HHP conditions applied were 676 MPa, 5 min at ambient temperature. The effect of HHP was compared to heat treatment (85 °C, 30 min) alone and with yogurts obtained from milk processed by combining HHP and heat treatment. Milk treatment was applied before yogurt fermentation, type of starter culture, and inoculation rate modified the gel properties. Combined HHP and heat treatment of milk with 0.1 % inoculation rate (for both cultures) led to attractive rheological and textural properties, i.e., creamy and thick consistency with good water retention properties, although WHC was not always the best compared to heat and HHP treatments applied alone. The authors explained these results by focusing mainly on yogurt microstructure characteristics, and they proposed a scheme of microstructure gel formation process as pH dropped during fermentation (Penna et al. 2007), in which the microstructure of heat-treated milk yogurt was composed of fewer interconnected chains of irregularly shaped casein micelle structures, forming a network that enclosed the void spaces, while the microstructure of HHP yogurt presented more interconnected clusters of densely aggregated protein with reduced and uniform particle size. The combined HHP and heat milk treatments led to compact yogurt gels with larger casein micelle clusters interspaced by void spaces and exhibited a higher degree of cross-linking. These aggregates in association with clumps of dense amorphous material resulted in improved gel texture and viscosity.

More recently, Udabage et al. (2010) also studied physical properties of low-fat stirred yogurts prepared from reconstituted skim milk (14 % solids) treated in some combinations of HHP (100, 250, or 400 MPa at 25, 70, or 90 °C for 10 min) before (HHP + H) and after (H + HHP) heat treatment (90 °C, 10 min). HHP treatment of skim milk at 25 °C with prior or later heat treatment resulted in stirred yogurts of similar consistency to those produced by heat treatment alone. The application of only HHP at 70 and 90 °C did not produce any improvement in yogurt characteristics. However, as HHP promotes whey protein denaturation, there is a possibility to reduce the severity of the standard heat treatment traditionally applied to yogurt milk by incorporating an HHP processing step in yogurt production. This treatment combination may be a strategy to incorporate heat-sensitive ingredients in yogurt formulation.

The observation that heating was more efficient in producing casein/whey protein interaction products as acid gels was also observed by Walsh-O'Grady et al. (2001) in a study of acid-set simulated yogurt milk gels in which HHP was applied and compared to heat treatment. In this study it was confirmed that to obtain acid gel of certain consistency ($G' > 500$ Pa), denaturation of whey protein is a prerequisite.

A more recent contribution to understanding the HHP acid gel formation by GLD in skim milk has been made by Anema (2010), who studied the effect of initial pH of milk (from 6.4 to 7.3). Anema begins with the idea that denaturation of whey

proteins and the disruption of casein micelles could not entirely account for changes in the rheological properties of acid gels, as denaturation of up 50 % of the whey proteins produced acid gels with very low G' and yield stresses. It is proposed that the pH and magnitude of pressure treatment affect the interactions of denaturated β-LG with casein in the acid gel structures. At low pressures and/or initial pHs, denatured β-LG acts predominantly as an inert filler in the acid gel structures, whereas, at higher pressures and/or initial pHs, denatured β-LG actively participates in the formation of the gel network during acidification. However, to confirm this hypothesis, further studies about the kinetics of β-LG denaturation and localization of the denatured whey protein are needed.

Apart from yogurt characteristics produced from HHP-treated milk, few studies have been focused on the application of HHP to the gelled final product. Reps et al. (1998b) applied pressures in the range of 200–1000 MPa in 200 MPa intervals for 15 min to yogurts to study the effect on microflora. It was found that pressure of 400 MPa was the minimum pressure necessary to inactivate *Lactobacillus delbrueckii* sp. *bulgaricus*. However, *Streptococcus thermophilus* was resistant to pressurization. During storage of yogurt after pressurization, a decrease in *Streptococcus thermophilus*counts was observed. During cold storage of yogurt pressurized under 400 MPa and higher, no further acidification was observed.

De Ancos et al. (2000) studied the effects of HHP (100–400 MPa for 15 min) on physicochemical, microbiological, and sensory characteristics of packed stirred low-fat yogurt. Pressures over 200 MPa prevented post-acidification of yogurt during cold storage. HHP-treated yogurts presented higher viscosity than did untreated controls, and these differences were maintained during storage. HHP treatments of 300 and 400 MPa reduced the number of viable cells of lactobacilli to below the legal minimum permitted in many countries.

25.4.3 Other Dairy Products

The effect of HHP on other dairy products such as cream, ice cream, and whey protein concentrate used as an ingredient have been conducted in different studies.

HHP modification of whey protein concentrates (WPC) added in low-fat whipping cream has been studied by Padiernos et al. (2009). Whipping cream containing HHP-treated WPC (300 MPa at 25 °C for 15 min) was compared with samples not containing WPC or with untreated WPC. Overall, the most relevant quality characteristics of whipping cream, i.e., viscosity, water retention capacity and overrun, and formulations containing HHP-treated WPC, were the best, indicating improvement of foaming properties by using this technology in WPC as an ingredient. A similar study was performed by the same research group with the same HHP conditions, using HHP-treated WPC added to low-fat ice cream mixes (Lim et al. 2008). Ice cream containing HHP-treated WPC exhibited the greatest overrun and foam stability, confirming the effect of HHP on foaming properties of whey proteins in a complex system. Improvements of overrun and foam stability were

observed when HHP-treated whey protein was used at a concentration as low as 10 % (wt/wt) in ice cream mix.

Other studies in dairy products have been related to physical changes in milk fat. HHP-induced crystallization of milk fat is one of the main effects observed in cream and butter. Buchheim and Abou El Nour (1992) applied HHP (100–500 MPa at 23 °C for 1–15 min) to dairy cream. Crystallization was observed in fat droplets, mainly in the globule periphery, and increased with duration and magnitude of pressure application (maximum at 300–500 MPa). It was also observed that after HHP treatment at 23 °C and subsequent storage at the same temperature, crystallization was maintained. The authors mentioned two potential applications of this observed phenomenon: fast aging of ice cream mix and physical ripening of dairy cream for butter making. Another possible consequence of HHP-induced crystallization of milk fat was the improvement in whipping properties observed by Eberhard et al. (1999). The best conditions were between 400 and 600 MPa for up to 2 min. However, at higher pressures, denaturation of whey protein occurs, causing destabilization of whipping cream.

For water and nonfatty products, adiabatic heat is approximately 3 °C per 100 MPa. Fats have larger adiabatic heat (up to 10 °C per 100 MPa) due to higher compressibility of fat compared to water (Ting et al. 2002). Depending on the fat studied and HHP conditions applied, adiabatic temperature is variable and also different for compression and decompression processes (Buchheim et al. 1999). These observed differences are mainly due to transitions between polymorphic states of the fats. In the case of milk fat, the increase of pressure magnitude leads to an increase of crystallization and melting temperatures, approximately 16 °C per 100 MPa (Frede and Buchheim 2000).

Another aspect studied on cream is the physical stability of emulsion when submitted to HHP. Dumay et al. (1996) compared the effect of HHP (450 MPa at 25 °C for 15 or 30 min or at 10 or 40 °C for 30 min) on pasteurized and UHT dairy creams (35 % fat). Pressure applied at 10 or 25 °C did not affect fat globule size distribution or rheological behavior, and samples were stable during cold storage for 8 days. However, the application of 450 MPa at 40 °C induced surface changes in fat globules, which were reversible in part during cold storage. UHT-treated creams were more sensitive to globule aggregation than pasteurized creams. This observation suggests a possible application to cream churning or whipping.

HHP may have interesting applications related to processes in which phase transitions are implied. HHP reduces the freezing and melting points of water to a minimum of −22 °C at 201.5 MPa. The primary applications of pressure in relation to water phase diagram are increased freezing rates obtained using pressure-assisted freezing (resulting in rapid and uniform nucleation and growth of ice crystals on releasing the pressure), increased thawing rates, and also the possibility of nonfrozen storage at subzero temperatures (Kalichevsky et al. 1995). Pressure-assisted freezing may be of special interest to avoid coarse ice crystallization and obtaining a smooth texture in various types of ice creams (including low fat) or sherbets. The Unilever company has patented combinations of HHP processing and freezing for improving consistency and smoothness and slower melting of ice creams (Keenan et al. 1998).

25.5 Conclusions

High hydrostatic pressure induces inhibition and destruction of microorganisms, influences the physicochemical and technological properties of milk, and is able to produce high-quality dairy products with improved characteristics. However, and despite these possibilities, the application of this technology on milk and dairy products at industrial-level HHP is scarce, given the limited number of dairy companies applying this technology. However, a series of dairy products are already being treated using this technology, such as: (1) cold pasteurized milk (Villa de Patos, Mexico); (2) free-starter fresh cheese with a shelf life of 45 days in cold storage (Pastoret La Segarra, Spain); (3) cheese-based sandwich fillings (Rodilla, Spain); (4) different yogurt-based products (drinkable yogurt, yogurt dressing, "clouds of yogurts") by Pulmuone (Korea), Bolthouse (USA), and Romantics (Spain), respectively; (5) colostrum-based beverages that preserve the functionality of heat-sensitive bioactive components present in colostrum such as immunoglobulins, lactoferrin, and growth factors (New Image Group, New Zealand); and (6) different snacks made from mozzarella and beef jerky (Snack Patrol, USA) or ham pork and cheddar cheese (Deli24, UK). In addition, there are other very interesting research dairy applications, including cheese ripening acceleration, the arresting of cheese ripening at the optimal point, and the improvement of set yogurt characteristics (water retention and texture) which have not been transferred to the market, although they possess numerous potential advantages. Although the industrial HHP equipment used at present is discontinuous, from 200 to 600 L of capacity, current HHP machines are able to process from 1000 kg to 2.2 tn/h (www.hyperbaric.com), so the implementation of this technology in the dairy industry today is possible, with a reasonable price.

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