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Applications of high-hydrostatic pressure on milk and dairy products: a review

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Abstract

Interest in high-pressure (HP) applications on milk and dairy products has recently increased. Pressures between 300 and 600 MPa have shown to be an effective method to inactivate microorganisms including most infectious food-borne pathogens. In addition to microbial destruction, it has been reported that HP improves rennet or acid coagulation of milk without detrimental effects on important quality characteristics, such as taste, flavour, vitamins, and nutrients. These characteristics offer the dairy industry numerous practical applications to produce microbially safe, minimally processed dairy products with improved performances, and to develop novel dairy products of high nutritional and sensory quality, novel texture and increased shelf life.

Keywords: High pressure; Milk; Dairy products; Applications

Industrial relevance: Interesting, 100 years after the first report on the successful high pressure treatment of milk and despite initial hesitation to apply high pressure batch processing on dairy products, there is an increased interest in the development of new dairy products with high safety and nutritional quality and unique physico-chemical properties. This paper summarizes the potential of pressure – induced modifications of dairy products and offers a rich source of information for product and process development due to the unique pressure effects especially on lipids and proteins. His implementation should also lead to increased activities for the development of continuous or short time discontinuous high pressure processes.

1. Introduction

Among the modern technologies in the food industry, the most important are those involving non-thermal treatment of the product. High pressure (HP) processing (100–1000 MPa) is one of the most promising methods for the food treatment and preservation at room temperature (Cheftel, 1992), and of great concern because of its potential to achieve interesting functional effects.

Research into the application of HP processing for milk preservation began when Hite (1899) demonstrated that the shelf life of milk and other food products could be extended by pressure treatment. Unavailability of suitable equipment hampered early applications of HP. The advances achieved in ceramics and metallurgical industries in the use of HP techniques during the 1970s and 1980s, has led to the possibility of treating food by this method at industrial level. The first commercial HP-treated products appeared on the market in 1991 in Japan, where HP processing is now being used for products such as fruit juices, jams, sauces, rice, cakes and desserts. Now in Europe, sliced cooked ham (Espuña, Spain), and orange juice (Pampryl, France) are available in supermarket shelves.

In milk, HP produces casein micelles disintegration into casein particles of smaller diameter, with a decrease in milk turbidity and lightness, and an increase of viscosity of the milk (Johnston, Austin & Murphy, 1992). Furthermore, the pressure-induced dissociation of the colloidal calcium phosphate and denaturation of serum proteins in milk may change and/or improve its technological properties (López Fandiño, Carrascosa & Olano, 1996). In addition to microbial destruction, the effects of HP on protein structure and mineral equilibrium suggest different applications on dairy products

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including the microbiological stabilisation of milk and dairy products (i.e. cream, yoghurt and cheese), the processing of milk for cheese and yoghurt production, and the preparation of dairy products with novel textures.

This paper deals with the potential applications derived from the pressure-induced modifications in physico-chemical characteristics of milk and dairy products.

2. Milk

Liquid milk is a dairy product, which is heat-treated using a range of conditions to provide acceptable safety and shelf life. The resistance of microorganisms to pressure in food is very variable depending on HP processing conditions (pressure, time, temperature, cycles,...), food constituents and the properties and the physiological state of the microorganism (Smelt, 1998). Exponentially growing cells are more sensitive to pressure than cells in the stationary phase. The bacterial spores are always more resistant than vegetative cells and they can survive at pressure of 1000 MPa. Bacterial spores, however, can often be stimulated to germinate by pressures between 50–300 MPa. Germinated spores can then be killed by heat or mild pressure treatments. Gram-positive microorganisms tend to be more resistant to pressure than gram-negative microorganisms. However, a considerable variation in pressure resistance within strains of the same species has been demonstrated in both gram-positive and gram-negative microorganisms. Gram-positive microorganisms need the application of 500–600 MPa at 25 °C during 10 min to achieve inactivation, while gram-negative microorganisms are inactivated with treatments of 300–400 MPa at 25 °C during 10 min. Vegetative forms of yeasts and moulds are the most pressure sensitive (Smelt, 1998).

Many studies on the inactivation of pathogenic and spoilage microorganisms (naturally present or inoculated) by HP have been performed in milk during the last years and have generally demonstrated that it is possible to obtain 'raw' milk pressurised at 400–600 MPa with a microbiological quality comparable to that of pasteurised (72 °C, 15 s) milk depending on the microbiological quality of milk (Kolakowski, Reps, Dajnowiec, Szczepek & Porowski, 1997; Mussa & Ramaswamy, 1997; Buffa, Guamis, Royo & Trujillo, 2001b) but not sterilised milk due to HP resistant spores. For example, to achieve a shelf life of 10 days at a storage temperature of 10 °C, a pressure treatment of 400 MPa for 15 min or 600 MPa for 3 min at 20 °C is necessary (Rademacher & Kessler, 1997).

Several works have been done for studying the effect of HP on inoculated target microorganisms in ewe's milk, with the aim of determining the sensitivity of pathogenic and spoilage microorganisms in milk. In this respect five microorganisms have been studied in our laboratory: *Escherichia coli* CECT 405 (it is considered a good index of direct or indirect contamination of fecal origin), *Pseudomonas fluorescens* CECT 378 (indicator of *Pseudomonas* spp., major components of the spoilage microbiota of refrigerated milk), *Listeria innocua* CECT 910 (indicator of human-pathogen *L. monocytogenes*), *Staphylococcus aureus* CECT 534 (major components of the spoilage microbiota of mastitic milks), and *Lactobacillus helveticus* CECT 414 (a microorganism non-pathogen but representant of lactic microbiota) (Gervilla, Capellas, Ferragut & Guamis, 1997; Gervilla, Felipe, Ferragut & Guamis, 1997; Gervilla, Mor-Mur, Ferragut & Guamis, 1999; Gervilla, Sendra, Ferragut & Guamis, 1999). Kinetics of destruction for these microorganisms are shown in Fig. 1 and Table 1. In general, the HP inactivation was greater on *P. fluorescens* > *E. coli* ≥ *L. innocua* > *L. helveticus* > *S. aureus*. The temperature effect in addition to the HP on microorganisms was different; *P. fluorescens*, *L. innocua* and *L. helveticus* showed higher resistance to HP at room temperature (25 °C) than at low temperature (4 °C), whereas *E. coli* and *S. aureus* showed less resistance to HP at room temperature than at low temperature.

In addition, a number of researchers have investigated the combined efficacy of HP in combination with mild temperatures (30–50 °C) and/or with bacteriocins (nisin, pediocin, lacticin,...) for the inhibition of foodborne bacteria and spores, demonstrating that some of these combination substantially enhance the efficiency of HP treatment, even sometimes showing phenomenons of synergistic inactivation between HP treatments and natural antimicrobial substances (García Graells, Masschalck & Michiels, 1999; Alpas & Bozoglu, 2000; Morgan, Ross, Beresford & Hill, 2000).

In addition to the inhibition and destruction of microorganisms, HP influences the physico-chemical and technological properties of milk. When milk is subjected to HP, the casein micelles are disintegrated into smaller particles (Schmidt & Koops, 1977). This disintegration is accompanied by an increase of caseins and calcium phosphate levels in the diffusible or serum phase of milk and by a decrease in the both non-casein nitrogen and serum nitrogen fractions, suggesting that the whey proteins become sedimentable by centrifugation and precipitable at pH 4.6 (Johnston et al., 1992; Law et al., 1998). Felipe, Capellas and Law (1997) reported that on pressure treatment up to 500 MPa at 25 °C, β-lactoglobulin is the most easily denatured serum protein, and denaturation of the immunoglobulins and α-lactalbumin only occurs at the highest pressures and particularly at 50 °C. An application derived from this observation is the preservation of colostrum immunoglobulins as alternative to heat treatment which induces immunoglobulins damage.

On the other hand, it appears essential to note that

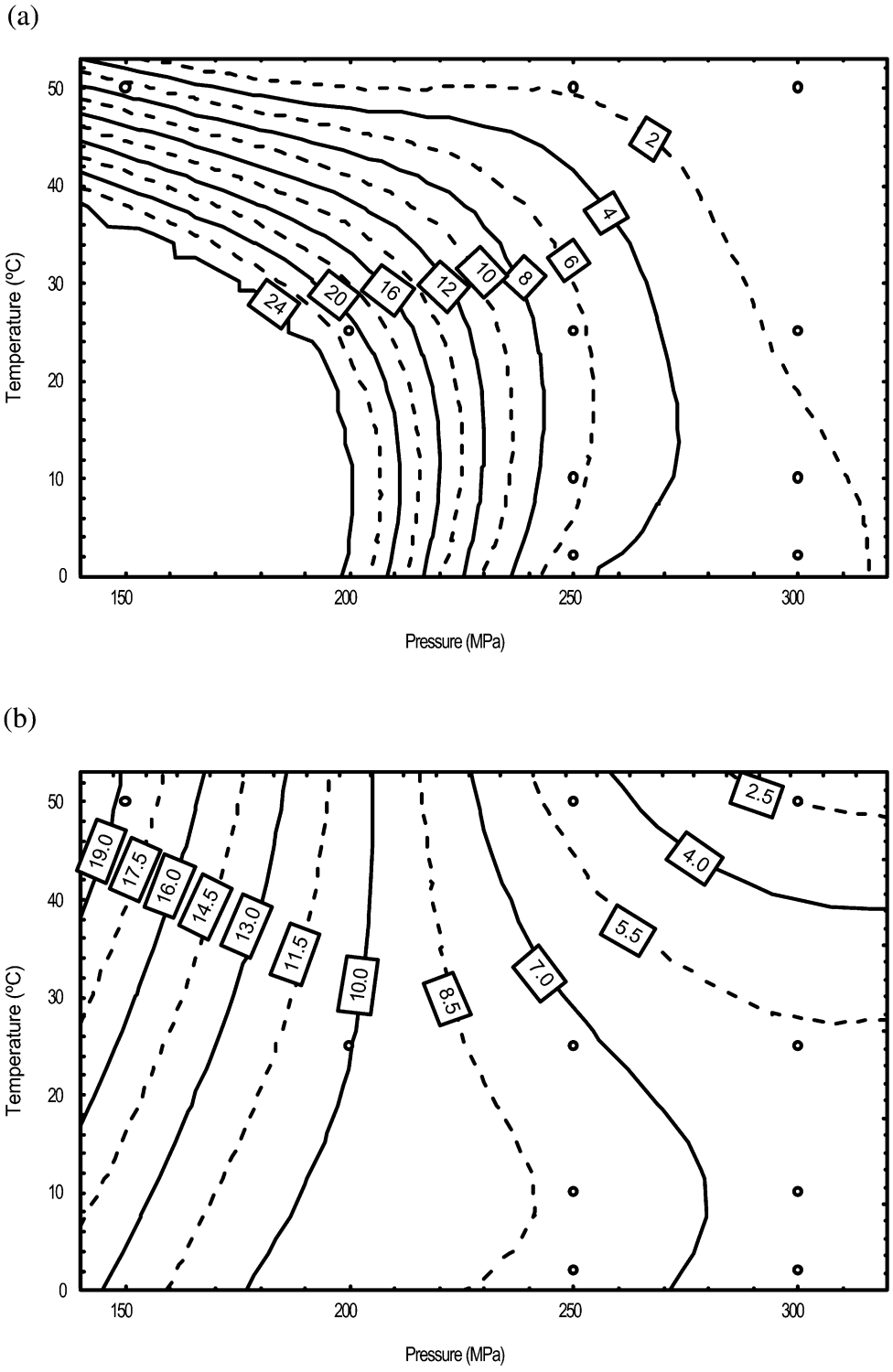

Fig. 1. Contour plot of *D*-values (min). Kinetics of microbial inactivation by high-pressure treatments on ewe's milk. Points actually measured are represented as open circles. (a) *Pseudomonas fluorescens*. (b) *Escherichia coli*.

HP no only solubilises colloidal calcium phosphate but also resolubilises insoluble heat-induced crystalline calcium phosphate (i.e. in UHT milk). Milk serum is, after certain time at defined environmental conditions, always saturated with respect to calcium phosphate. However, its solubility decreases with increasing temperature but increases with pressure (Schrader, Buchheim & Mor, 1997).

Table 1
Decimal reduction times (*D*-values) at different pressures and temperatures on some studied microorganisms inoculated into ewe's milk

Microorganisms	Treatments	*D*-values (min)
Escherichia coli	300 MPa/2 °C	5.35
	300 MPa/25 °C	5.19
Pseudomonas fluorescens	250 MPa/2 °C	3.87
	250 MPa/25 °C	4.58
Listeria innocua	400 MPa/2 °C	3.12
	400 MPa/25 °C	4.00
Staphylococcus aureus	450 MPa/2 °C	20.00
	450 MPa/25 °C	16.70
Lactobacillus helveticus	450 MPa/2 °C	7.10
	450 MPa/25 °C	9.10

Studies carried out by Gervilla, Ferragut and Guamis (2001) on free fatty acids (FFA) content (lipolysis of milk fat) in ewe's milk have showed that HP treatments between 100–500 MPa at 4, 25 and 50 °C did not increase FFA content, even some treatments at 50 °C showed lower FFA content than fresh raw milk. This phenomenon of great interest to avoid off flavours derived from lipolytic rancidity in milk.

Hydrostatic pressure up to 500 MPa produces some modifications on size and distribution of milk fat globules of ewe's milk. HP treatments at 25 and 50 °C showed a tendency to increase the number of small globules in the range 1–2 μm, whereas at 4 °C the tendency was the reverse (Gervilla et al., 2001). However, no damage on the milk fat globule membrane occurred, being proved by the lack of lipolysis increase as it was mentioned above. These modifications on distribution of milk fat globules could be due to phenomenons of aggregation and disaggregation/disintegration. This provides some advantages for HP-treated milk, because HP treatment increases the stability of milk treated at 25 and 50 °C, whereas at 4 °C (low temperatures) increases the creaming-off (Gervilla et al., 2001). This last aspect could improve cream separation in the elaboration of butter.

Lactose in milk and milk products may isomerise in lactulose by heating and then degrade to form acids and other sugars. No changes in these compounds are observed after pressurisation (100–400 MPa for 10–60 min at 25 °C), suggesting that no Maillard reaction or lactose isomerisation occur in milk after pressure treatment (López Fandiño et al., 1996).

In general, rennet coagulation properties of milk subjected to pressures of 100–500 MPa for 30 min are enhanced, although pressures above 300 MPa increase the rennet coagulation time of milk (López Fandiño et al., 1996; Buffa, Trujillo & Guamis, 2001d). It is known that milk with casein micelles of reduced diameter enhance milk coagulation properties. The drop in particle size produced by HP treatment is accompanied by conformational changes of spherical particles into chains or cluster of submicelles and could be responsible for the enhanced coagulation properties.

Micelle desintegration induced by HP treatment also affects milk colour. A decrease of L^* (lightness) and an increase of greenness ($-a^*$) and yellowness ($+b^*$) was observed when ewe's milk was HP treated (Gervilla et al., 2001). The decrease in L^* value could have been mainly due to disintegration of casein micelles by pressure into small fragments that increase the translucence of the milk (Johnston, 1995). These changes were visually negligible in whole ewe's milk, which is an interesting aspect to avoid reject by the potential consumers. Similar results were found by Mussa and Ramaswamy (1997) in cow's milk, showing the low sensitivity of milk colour to pressure. However, in skim milk treated at 600 MPa for 15 min significant changes in L^*, b^* and a^* values, that could be also perceived visually, were observed. Warming of samples from 4° to 43 °C derived back colour values of HP milk towards the values of untreated milk, although not to the same initial point (Needs et al., 2000).

Contrary to thermal treatments, where covalent as well as non-covalents bonds are affected, HP treatment at room and mild temperatures only disrupts relatively weak chemical bonds (hydrogen bonds, hydrophobic bonds, ionic bonds). Thus, small molecules such as vitamins, amino acids, simple sugars and flavour compounds remain unaffected by the HP treatment (Cheftel, 1992). HP treatment of milk at 400 MPa (at a rate of 2.5 MPa/s for 30 min at 25 °C) results in no significant loss of vitamins B_1 and B_6 (Sierra, Vidal Valverde & López Fandiño, 2000). García Risco, Olano, Ramos and López Fandiño (2000) found that HP treatments at 400 MPa for 15 min at 40–60 °C reduce the proteolytic activity, and at 25–60 °C maintain or improve the organoleptical properties of milk, suggesting that these combined treatments could be used to produce milk of good sensory properties with an increased shelf-life.

3. Cheese

There are numerous areas of interest regarding the HP processing of cheese (Table 2), the more important including cheese making from HP-treated milk, acceleration of cheese ripening and inactivation or reduction of pathogenic or spoilage microorganisms in cheese to increase cheese safety and shelf life.

3.1. Cheese production from HP-treated milk

Milk pasteurisation (heating at 72–74 °C for 15 s or equivalent treatments) destroys pathogenic and most, but not all, spoilage microorganisms, and it is the most important heat treatment applied to cheese milk to provide acceptable safety and quality. However, milk pasteurisation is known to adversely affect the devel-

Table 2
Practical applications related to high-pressure processing of cheese

Applications	Cheese variety	Pressure conditions	References
Reduction of great variability of moisture content existing within a block of cheese or between different blocks and generation of desirable new cheese textures	Reduced fat Cheddar	Not specified	(Torres Mora et al., 1996)
High-pressure cheese brining	Semi hard ewe milk cheese	50 and 200 MPa, 45 min, 14 °C, 24% brine	(Pavia, Trujillo, Guamis & Ferragut, 2000)
	Gouda	100–300 MPa, 15–130 min, 14 °C, 19.5% brine	(Messens, Dewettinck, Van Camp & Huyghebaert, 1998 Messens, Dewettinck & Huyghebaert, 1999)
Use of high pressure-treated milk to made 'raw' milk cheese and increase of cheese yield	Semi-hard goat milk cheese	500 MPa, 15 min, 20 °C	(Trujillo et al., 1999a,b)
	Cheddar	586 MPa, three 1-min cycles, 5 °C	(Drake et al., 1997)
Cheese ripening acceleration	Cheddar	50 MPa, 72 h, 25 °C	(Yokoyama et al., 1992)
Production of low fat cheese with improved texture	Semi-hard bovine milk cheese	400 MPa, 15 min, 22 °C	(Molina, Álvarez, Ramos, Olano & López Fandiño, 2000)
Inactivation or reduction of pathogenic and spoilage microorganisms by high pressure or by combining HP with other processes such as bacteriocins in cheese	Gouda, Kurpiowski and Cammembert	200–1000 MPa at 200 MPa 20 °C intervals, three 5-min cycles, 20 °C	(Kolakowski et al., 1998)
	Cheddar	100–500, 20 min, 20 °C	(O'Reilly et al., 2000a)
Increase of fresh cheese shelf life	Mató	450–500 MPa, 5–30 min, 2–25 °C	(Capellas et al., 1996; Trujillo et al., 2000a)
Pressure-shift freezing and thawing of cheese	Cheddar and Mozzarella	200 MPa, 70 min, −20 °C	(Johnston, 2000)
Preservation of cheese at the optimum point of ripening	Suggested application	700–800 MPa, 15 min	(Reps, Wisniewska, Dajnowiec & Iwanczak, 2000)
Pressurisation of lactic bacteria to create an extra supply of enzymes with debittering properties	Suggested application	300–350 MPa, 20 min, 20 °C	(Casal & Gómez, 1999)

opment of many sensory characteristics of cheese, leading to alterations in texture and often delayed maturation (Grappin & Beuvier, 1997). HP technology can be used to increase the microbiological safety and quality of milk to produce high quality cheeses. As it was mentioned above, HP processing of milk at room temperature causes several protein modifications, such as whey protein denaturation and micelle fragmentation, and alters mineral equilibrium. These changes modify the technological aptitude of milk to make cheese, improving the rennet coagulation and yield properties of cheese milk (Trujillo, Royo, Guamis & Ferragut, 1999b; Buffa et al., 2001d).

Drake, Harrison, Asplund, Barbosa Canovas and Swanson (1997) in Cheddar and Trujillo, Royo, Ferragut and Guamis (1999a) and Trujillo et al. (1999b) in semi-hard goat milk cheeses reported higher yields and no detrimental effects on cheese flavour in cheese made from HP-treated milk related to cheeses made from raw or pasteurised milks. In addition, microbiological quality of cheeses from HP-treated milk (500 MPa for 15 min at 20 °C) was comparable to pasteurised milk (72 °C for 15 s) cheeses (Buffa et al., 2001b). However, the application of HP technology to cheese milk causes differences in cheese composition and ripening in comparison to pasteurised milk cheese. The HP-treated milk cheeses have higher moisture, salt and total free amino acids contents than raw or pasteurised milk cheeses (Trujillo et al., 1999a,b). On the other hand, cheeses made from HP-treated 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 (Buffa, Guamis, Pavia & Trujillo, 2001a). This behaviour was explained by heat-sensitive but partial pressure-resistant characteristics of the indogenous milk lipase.

In relation to cheese texture and microstructure, Buffa, Trujillo and Guamis (2001c) using uniaxial compression and stress relaxation tests, and confocal laser scanning microscopy showed that cheeses made from raw or HP-treated milk were firmer and less fracturable than cheeses made from pasteurised milk, but differences became less notable toward the end of ripening. Cheeses from pasteurised and HP-treated milk were less cohesive than from raw milk. Although cheese exhibited a loss of elastic characteristics with ageing, cheeses from HP-treated milk were the most elastic initially. Confocal laser scanning micrographs displayed cheeses from HP-treated milk with a regular and compact protein matrix, with small and uniform fat globules resembling the structure of cheeses made from raw milk.

These reports show that HP processing of milk could be an alternative to heat treatment for the production of fresh or ripened cheese with improved performances.

3.2. Inactivation or reduction of pathogenic and spoilage microorganisms in cheese

Most research to date has concentrated on the application of HP to inactivate microorganisms in cheese to increase cheese safety and shelf life. Szczawinski, Szczawinska, Stanczack, Fonberg-Broczek and Arabas (1997) achieved a 6 log reduction of inoculated *Listeria monocytogenes* in ripened sliced cheese with a treatment of 500 MPa for 15 min and a significant decrease of cheese microbiota. Gallot-Lavallée (1998) studied the efficiency of HP treatment for destruction of *L. monocytogenes* in goat cheese from raw milk finding that 450 MPa/10 min or 500 MPa/5 min treatments achieve more than 5.6 log units of reduction of this microorganism without significantly affecting sensory characteristics of cheese. Reps, Kolakowski and Dajnowiec (1998) achieved a significant decrease of total microbial counts at pressure above 400 MPa in Gouda and Camembert cheeses, whereas spore count was unaffected even at 1000 MPa. They recorded inactivation of proteolytic enzymes at pressures over 800 MPa and suggest that HP has a beneficial effect on organoleptic properties of cheese, mainly on its consistency.

Capellas, Mor-Mur, Sendra, Pla and Guamis (1996) observed reductions of 7 log units of *Escherichia coli* populations working on inoculated Mató cheese (fresh goat's milk cheese) and HP-treated from 400 to 500 MPa for 5–15 min at refrigeration and room temperature and the extension of refrigerated storage life of the cheese. These authors also studied the resistance of cocci (*Staphylococcus carnosus*) and spores (*Bacillus subtilis*) in fresh cheese as these groups of microorganisms are acknowledged as pressure resistant. The treatments that provided a total inactivation of *E. coli* only reduced *S. carnosus* population in 2 log units. To achieve a higher inactivation, it was necessary an increase in the temperature of the treatment, the application of cycle treatments or the use of bacteriocins together with pressure. HP treatment at 50 °C for 5 min caused a reduction of 7 log units. Multiple-cycle treatments of 500 MPa at 10 °C and times between 15 and 30 min also improved the inactivation rate and the combination of 500 MPa and nisin was the most effective treatment to inactivate cheese indigenous microbiota. Germination treatments on *B. subtilis* of 60 MPa at 40 °C for 210 min followed by vegetative cells inactivation treatments of 500 MPa at 40 °C for 15 min caused a lethality of 4.9 log units (Capellas, Mor-Mur, Gervilla, Yuste & Guamis, 2000). Application of 500 MPa at 25 °C during 5 or 30 min increased shelf-life of refrigerated (4 °C) vaccuum-packaged fresh cheese to 2 or 3 months, respectively (Fig. 2). When these treatments were applied to cheese with 7.1 mg nisin/kg, no development of initial counts was observed during 4 months of refrigerated storage (Trujillo et al., 2000a). These treat-

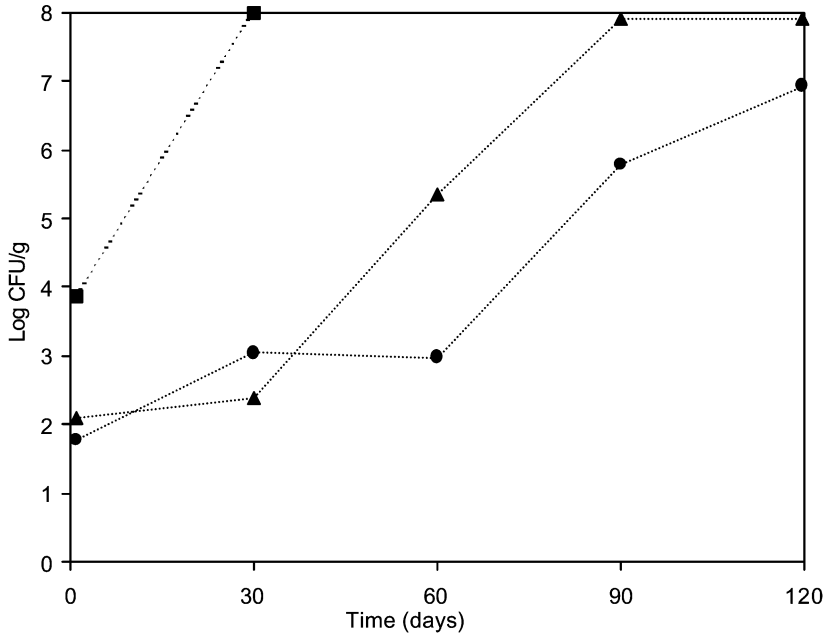

Fig. 2. Counts of aerobic mesophilic population of *Mató* cheese during vaccuum-packaged storage at 4 °C. ■: non pressure-treated cheese; ▲: 5 min of pressure treatment at 500 MPa/25 °C; ●: 30 min of pressure treatment at 500 MPa/25 °C. Reprinted from Food Research International 33, (Trujillo et al., Application of high pressure treatment for cheese production, pp. 311–316, 2000), with permission from Elsevier Science.

ments caused a higher loss of whey in HP-processed cheeses and texture and cheese colour were slightly changed but composition was not affected, (Capellas, Mor-Mur, Sendra & Guamis 2001a). Although these changes were detected by panellists during sensory analysis, pressure-treated cheeses received the same score in ranking tests when compared with untreated cheeses (Capellas, Mor-Mur, Sendra & Guamis, 2001b). These results confirm the practical application of HP in shelf-life extension of highly perishable products, with high pH and a_w values, as this type of fresh cheese.

A procedure for the manufacture of cheese with controlled microbiota by means of HP has been described by Trujillo, Guamis and Carretero (2000b). With this treatment, it is possible to obtain model cheeses, which would permit the quantitation of the contribution of individual proteolytic agents to cheese ripening.

O'Reilly, O'Connor, Kelly, Beresford and Murphy (2000a) have determined the effect of HP (50–800 MPa for 20 min at 10–30 °C) on the inactivacion of microbial contaminants (*Staphylococcus aureus*, *E. coli* and *Penicillium roqueforti* spores) in model cheese systems (phosphate buffer at pH 5.3 and cheese slurries) and in Cheddar cheese. Relative sensitivity of the microbial species to HP in Cheddar cheese was, as it was demonstrated previously in model cheese slurry system, *P. roqueforti* > *E. coli* > *S. aureus*. However, pressure inactivation of *S. aureus* and *P. roqueforti* was most extensive in buffer while, a greatest sensitivity was exhibited by *E. coli* in Cheddar cheese at pressures <200 MPa, possibly due to acid injury during the cheese fermentation. These results underline the importance of taking product and processing conditions into account when assessing the impact of HP on the viability of food microorganisms.

3.3. Cheese ripening acceleration

Being cheese ripening a quite expensive process, acceleration of ripening is highly desirable. Most of the work in this field has been done by elevation of ripening temperature, addition of cheese slurries or exogenous enzymes or by the use of adjunct starters, either as such or in modified form. The potential use of HP to accelerate cheese ripening was first shown in a patent by Yokoyama, Sawamura and Motobayashi, (1992). Experimental Cheddar cheese samples were exposed to pressure from 0.1 to 300 MPa for 3 days at 25 °C after cheese making. Best results were obtained at 50 MPa, at which pressure a cheese with free amino acid amount and taste comparable to that of a 6-month-old commercial cheese was obtained. However, similar studies in Cheddar cheese (O'Reilly, O'Connor, Murphy, Kelly & Beresford, 2000b) and in other cheese varieties (Kolakowski, Reps & Babuchowski, 1998; Saldo, Sendra & Guamis, 1999) have shown notable differences respect to the level of proteolysis claimed by the Yokoyama's patent. It should be noted that the method of Cheddar cheese making reported by these authors was substan-

Table 3
High-pressure conditions tested in different cheese varieties for accelerating ripening

Cheese variety	High Pressure Conditions	References
Cheddar and Parmensan	50 MPa, 72 h, 25 °C, after salting	(Yokoyama et al., 1992)
Camembert and Gouda	0.1–500 MPa, 4 h, 5 °C, 5 and 10 days-old cheese. Cyclic pressure 3×5 min in the range 200–1000 MPa at 200 MPa intervals, room temperature, 5 and 10 days-old Camembert and 2 and 6 weeks-old Gouda	(Kolakowski et al., 1998; Reps et al., 1998)
Kurpiowski (Emmental type)	Cyclic pressure 3×5 min in the range 200–1000 MPa at 200 MPa intervals, room temperature, 2 and 5 weeks-old cheese	(Reps et al., 1998)
Goat's milk cheese	50 MPa, 72 h, 14 °C, after salting 400 MPa, 5 min, 14 °C, after salting 400 MPa, 5 min plus 50 MPa, 72 h, 14 °C, after salting	(Saldo, Sendra & Guamis, 2000)
Mozzarella	100–800 MPa at 200 MPa intervals, 5–120 min, 20 °C, immature	(Johnston & Darcy, 2000)
Cheddar	50 MPa, 72 h, 25 °C, after salting	(O'Reilly et al., 2000b)
Père Joseph	50 MPa, 8 h, 20 °C, after salting	(Messens et al., 2000)

tially different from conventional procedure. In particular, the kind of starter bacteria added to the cheese milk was highly proteolytic and at least 10-fold higher than conventional inoculation rates.

The effect of an early pressure treatment on the proteolysis increase can be related with a pH increase and the selection of non-starter bacteria (Messens, Foubert, Dewettinck & Huyghebaert, 2000) on smear-surface ripened cheese.

Other HP conditions have been tested for accelerating cheese ripening that involved 'high' HP treatments (400–600 MPa) short times (5–15 min) or an initial 'high' HP treatment (400–600) short times (5–15 min) followed by a 'low' HP treatment (50 MPa) long times (72 h) and different cheese varieties (Table 3). While long treatments at moderate pressure produce an increase in proteolysis while the treatment is applied, short and intense treatments produce a permanent effect on proteolysis rates (Fig. 3). The enhancement effect is presumed to be caused by the release of starter enzymes. An increase in free amino acid amount was found on cheese two weeks after pressure treatment at 400 MPa for 5 min (Saldo, McSweeney, Sendra, Kelly & Guamis, 2002).

The application of HP processing to cheese ripening is evident from the results obtained by different authors in some cheese varieties. However, further research (i.e. rheology and sensory characteristics) is required to evaluate the full applicability of HP in accelerating the ripening.

4. Yoghurt and fermented milks

Yoghurt can be found in three states: set yoghurt, stirred yoghurt and fluid or drinking yoghurt. Most common defects of yoghurt are low rigidity or viscosity in set or stirred yoghurts, respectively and syneresis (separation of an aqueous whey phase). Two strategies have been used to improve yoghurt quality and preservation by means of HP: yoghurt making from HP-treated milk and pressurisation of yoghurt to inactivate microbiota.

The properties of acid-set gels prepared from HP-treated milk have been reported by Johnston, Murphy and Birks (1994). Results indicate improved texture (rigidity and resistance to breaking) and syneresis resistance of the gels, measured by drainage or by centrifugation. Furthermore, authors reported viscosity improvement of stirred-style yoghurt-type product prepared from HP-treated skim milk (100–600 MPa, up to 1 h). Most of the viscosity improvement was achieved after 15-min pressurisation at 400 MPa and after 5 min at 600 MPa with slight further increases up to 60 min.

Ferragut, Martínez, Trujillo and Guamis (2000) elaborated ewe's milk yoghurt from HP-treated milk using different combinations of temperature and pressure (10, 25 and 55 °C; 200, 350 and 500 MPa for 15 min) and from pasteurised (70 °C, 10 min) milk. Yoghurt firmness increased as pressure increased, and treatments of 350 MPa at 25°C and 500 MPa at 55 °C showed no differences in whey syneresis compared with pasteurised milk. Yoghurt evolution in storage at 4 °C for 20 days showed a good stability in terms of firmness in all treatments but water retention was only maintained in yoghurts made from HP-treated milk.

Needs et al. (2000) recorded lower values of fracture stress in set yoghurts made from milk pressure-treated at 600 MPa for 15 min compared to heat treated milk. When HP milk yoghurt was compared with conventional heat-treated milk yoghurt in a consumer's test, it was observed that lower fracture stress values in HP milk yoghurt were correlated with a higher score in creaminess. When ranking both products, consumers preferred the taste of conventional yoghurt. The use of a mixture

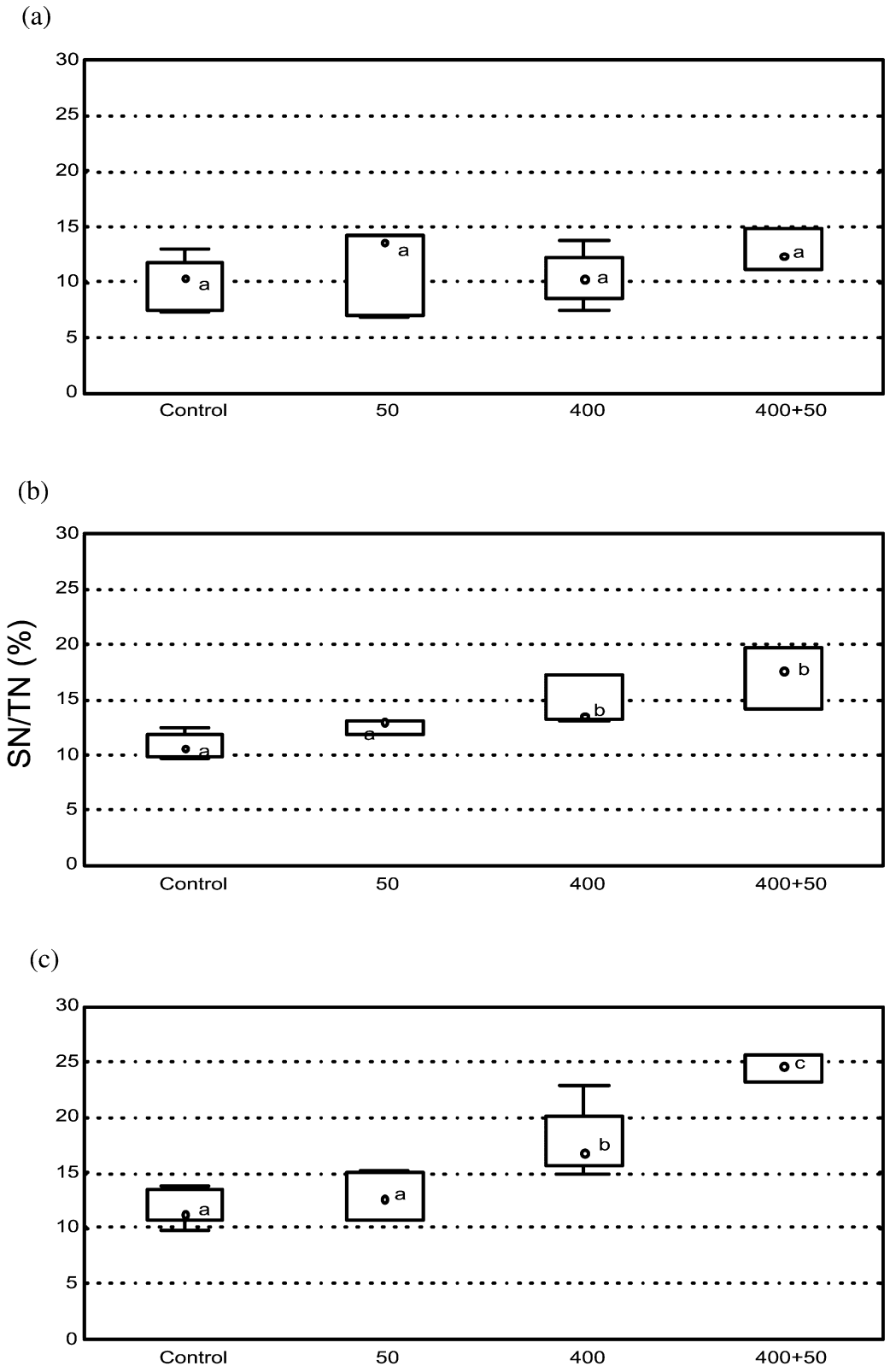

Fig. 3. Evolution of the amount of nitrogen products soluble at pH 4.6 (SN) referred to total nitrogen content (TN) in goat's cheese exposed to several different high pressure treatments after salting in brine. 50 treatment at 50 MPa for 72 h, 400 treatment at 400 MPa for 5 min, 400+50 treatment at 400 MPa for 5 min followed by 50 MPa for 72 h. The three plots correspond with three ripening times studied [(a) 4 days; (b) 14 days; and (c) 28 days]. In each plot the central point represent the median value, the box contains the two central quartiles and the whiskers the amplitude of the variable. Different letters in the same graph correspond to cheeses that are not different ($P<0.05$) on primary proteolysis.

containing only 10% of pressure-treated milk resulted in a creamy product that maintained the taste of the conventional yoghurt, which would be advantageous since only a small proportion of the milk has to be pressurised and the improvement in texture is clearly perceived (Capellas, Noronha, Mor-Mur & Needs, 2001). In a further study that compared properties of yoghurts made from pressure or heat-treated milk that had previously been concentrated or fortified with skim milk powder, Capellas, Mor-Mur and Needs (2001) recorded that yoghurts from HP milk did not show the spontaneous syneresis observed in yoghurts from heat-treated milk.

Tanaka and Hatanaka (1992) studied the effect of HP (200–300 MPa at 10–20 °C for 10 min) on packaged yoghurt. This treatment did not modify the yoghurt texture nor reduced the number of viable lactic acid bacteria and prevented the continued development of acidity, which can lead to syneresis. Furthermore, pressures above 300 MPa prevented over-acidification, but the number of viable lactic acid bacteria was reduced. In this concern, Krompkamp, Moreira, Langeveld and Van Mil (1995) also detected a reduction of yoghurt bacteria at 300 MPa, and they also found that a more solid gel structure was formed above 200 MPa. Reps, Warminska Radyko and Dajnowiec (1999) and Reps, Warminska Radyko, Krzyzewska and Tomasik (2001) investigated the inactivation of yoghurt microbiota by HP (400 MPa, 15 min) to state whether the complete inactivation of bacteria was necessary to preserve yoghurt. HP treatment completely inactivated *Lactobacillus delbruecki* sp. *bulgaricus*. However, *Streptococcus salivarius* sp. *thermophilus* was more resistant to the pressurisation, and it was found that the degree of inactivation was dependent on the strain and varied from 35.3 to 99.9%. These results show that prolongation of the shelf life of yoghurt by HP processing can be obtained by complete inactivation of lactic acid bacteria.

The application of HP for kefir preservation has also been studied. Reps, Krzyzewska, Lanievska-Moroz and Iwanczak (2000) studied the microbial populations and acidifying activity of kefir treated at 200–800 MPa during 15 min and stored for 3 weeks. Reduction of bacterial counts increased with increasing pressures and yeasts were completely inactivated at 400 MPa. Acidification of kefir pressurised at 600 and 800 MPa only increased slightly during the storage. Mainville, Montpetit, Durand and Farnworth (2001) have also studied the deactivation of bacteria and yeast in kefir using heat treatment, irradiation and HP. Heat treatments (autoclaving at 110 °C for 3 min and ohmic heating at 72 °C internal temperature) deactivated the bacteria and yeast in kefir (8.58 log cfu/g lactobacilli and 5.09 log cfu/g total yeasts) but changes in structure of the kefir protein and lipids were seen in transmission electron micrographs. Irradiation of kefir at 5 kGy, and HP treatment at 400 MPa for 5 or 30 min deactivated the bacteria and yeast in kefir and left the protein and lipid structure of the product unchanged.

5. Cream, butter and ice cream

Only a few studies have dealt with the effects of HP on cream, butter and ice cream. Buchheim and Abou El Nour (1992) subjected pasteurised dairy creams (35 and 43% fat) from 100 to 500 MPa at 23 °C for 1–15 min. Using the freeze fracture technique and transmission electron microscopy, they found that pressurisation induced fat crystallisation within the small emulsion droplets, mainly at the globule periphery. Fat crystallisation increased with the length of pressure treatment and was maximal after processing at 300–500 MPa. Moreover, the crystallisation proceeded during further storage at 23 °C after pressure release. Two potential applications of this phenomenon were mentioned by the authors: fast ageing of ice cream mix and physical ripening of dairy cream for butter making. In a subsequent study an equivalence of pressure and temperature was established (Buchheim & Frede, 1996) on the crystallisation process of emulsified fats. Whipping properties improved when cream was treated at pressures up to 600 MPa for up to 2 min (Eberhard, Strahm & Eyer, 1999) probably due to better crystallisation of milk fat. When treatment conditions exceed the optimum an excessive denaturation of whey protein occurs and results in longer whipping time and destabilisation of whipped cream. Below 400 MPa no noticeable effects on whipping properties of cream were found.

For water and non-fatty 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, Balasubramaniam & Raghubeer, 2002). The adiabatic temperature change is not the same for compression and decompression processes, and it depends on the fat studied, pressure applied and testing temperature (Buchheim, Frede, Wolf & Baldenegger, 1999). These differences are due to crystallisation/melting processes or transitions between polymorphic states of fat. Milk fat is a highly complex triglyceride system, and the higher melting point fraction of milk fat shows crystallisation properties affected by pressure. Pressure increase elevates its crystallisation and melting temperatures, approximately 16 °C per 100 MPa (Frede & Buchheim, 2000).

Studies carried out by Raffalli et al. (1994) have shown that it is possible to reduce significantly the microbial load of a dairy cream (35% fat) by HP at 450 MPa and 25 °C for 10 to 30 min. Inactivation followed apparent first order kinetics, with a decimal reduction time of 7.4 min under the pressure treatment conditions used. Pressure could thus be used to extend the refrig-

erated shelf-life of dairy creams. Nevertheless, the authors found resuscitation of pressure-stressed bacteria after incubation under optimal conditions. Other potential application cited by the authors concerns on the preparation of 'raw' milk cheeses by processing separately dairy cream by HP, and skimmed milk through microfiltration. However, this area has been largely unexplored and should make an interesting area of study.

Dumay, Lambert, Funtenberger and Cheftel (1996) studied the effect of HP processing on pasteurised and UHT sterilised dairy creams (35% fat) at 450 MPa at 25 °C for 15 or 30 min, or at 10 or 40 °C for 30 min. In the case of pasteurised creams, pressurisation at 450 MPa at 10 or 25 °C did not modify its fat globule size distribution, or its flow behaviour. Furthermore, the pH of the cream remained unchanged immediately after treatment and no further acidification was observed on storage for 8 days at 4 °C. In contrast, HP treatment carried out at 40 °C induced surface changes in fat globules, these changes being partly reversible with storage time. Sterilised cream was more sensitive than pasteurised cream to aggregation phenomena. This observation suggests a possible application to cream churning or whipping.

A limiting effect of high-pressure application on fat rich products could be the induction of autoxidation caused by the treatment. Butz, Zielinski, Ludwig and Tauscher (1999) studied the influence of pressures up to 600 MPa on a model system close to milk fatty acid composition, concluding that oleic acid was not affected but autoxidation of linoleic acid was increased by pressures from 350 MPa though the effects were small and no new oxidation products, compared with thermal treatment, were formed. The authors advise the need for complementary studies in dairy product matrices.

High pressure has interesting effects on the solid–liquid phase diagram of water. The application of HP 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 the increased freezing rates obtained using pressure-assisted freezing (resulting in rapid and uniform nucleation and growth of ice crystals on releasing the pressure), the increased thawing rates and also the possibility of non-frozen storage at subzero temperatures (Kalichevsky, Knorr & Lillford, 1995). Pressure-assisted freezing may be of special interest to avoid coarse ice crystallisation and obtain a smooth texture in various types of ice creams (including low fat) or sherbets. The Unilever Company has patented combinations of HP processing and freezing for improved consistency and smoothness, and slower melting of ice creams (Keenan, Wix & Young, 1998).

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