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Effects of Ultra-High Pressure Homogenization on Microbial and Physicochemical Shelf Life of Milk

J. Pereda, V. Ferragut, J. M. Quevedo, B. Guamis, and A. J. Trujillo[1]
Centre Especial de Recerca Planta de Tecnologia dels Aliments (CERPTA), CeRTA, XiT, Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

ABSTRACT

The effect of ultra-high pressure homogenization (UHPH) on microbial and physicochemical shelf life of milk during storage at 4°C was studied and compared with a conventional heat preservation technology used in industry. Milk was standardized at 3.5% fat and was processed using a Stansted high-pressure homogenizer. High-pressure treatments applied were 100, 200, and 300 MPa (single stage) with a milk inlet temperature of 40°C, and 200 and 300 MPa (single stage) with a milk inlet temperature of 30°C. The UHPH-treated milks were compared with high-pasteurized milk (PA; 90°C for 15 s). The microbiological quality was studied by enumerating total counts, psychrotropic bacteria, lactococci, lactobacilli, enterococci, coliforms, spores, and *Pseudomonas*. Physicochemical parameters assessed in milks were viscosity, color, pH, acidity, rate of creaming, particle size, and residual peroxidase and phosphatase activities. Immediately after treatment, UHPH was as efficient (99.99%) in reducing psychrotrophic, lactococci, and total bacteria as was the PA treatment, reaching reductions of 3.5 log cfu/mL. Coliforms, lactobacilli, and enterococci were eliminated. Microbial results of treated milks during storage at 4°C showed that UHPH treatment produced milk with a microbial shelf life between 14 and 18 d, similar to that achieved for PA milk. The UHPH treatments reduced the L* value of treated milks and induced a reduction in viscosity values of milks treated at 200 MPa compared with PA milks; however, these differences would not be appreciated by consumers. In spite of the fat aggregates detected in milks treated at 300 MPa, no creaming was observed in any UHPH-treated milk. Hence, alternative methods such as UHPH may give new opportunities to develop fluid milk with an equivalent shelf life to that of PA milk in terms of microbial and physicochemical characteristics.

Key words: ultra-high pressure homogenization, milk shelf life, microbial inactivation, physicochemical characteristic

INTRODUCTION

Milk is a nutritious medium that presents a favorable environment for the growth of spoilage and pathogenic microorganisms. Therefore, milk as a raw material has a short shelf life and needs to be processed. Milk produced industrially for commercial usage usually undergoes several processes including standardization of fat content according to the type of milk required, homogenization, and pasteurization. With conventional homogenization, milk is passed through 2 homogenization valves (2-stage homogenization) under moderate pressures (~18 to 20 MPa). This technology helps to prevent creaming during storage due to the reduction of fat globule size. Pasteurization is a thermal process usually applied to liquid milk in which milk can be heated rapidly to 72°C for 15 to 20 s (high-temperature short-time pasteurization) or heated to 80 to 90°C for 15 s (high-pasteurization). The main purpose of this treatment is to eliminate any potential pathogenic microorganisms that could be present and to reduce spoilage bacteria, which can create negative sensory attributes decreasing processed milk shelf life. However, thermal processes besides destroying microorganisms can cause changes in nutritional, organoleptic, or technological properties of milk (Andersson and Oste, 1995; Nursten, 1995; Singh, 1995).

Food preservation is a continuous fight against microorganisms spoiling the food or making it unsafe. In recent years, the food industry has investigated the replacement of traditional food preservation technologies such as heat treatment by new preservation techniques due to the increased consumer demand for tasty, nutritious, and natural products (Hayes and Kelly, 2003b). High-pressure processing is one of the food preservation treatments being developed and applied as a minimal process for the production of safe and nutritious foods (Kheadr et al., 2002; Hayes and Kelly, 2003a; Briñez et al., 2006a). One class of high-pressure technology based on homogenization is mi-

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[1]Corresponding author: Toni.Trujillo@uab.es

crofluidization, which is based on the principle of collisions between high-speed liquid jets. The fluid is divided into 2 channels at the valve inlet, which then collide in the reaction chamber. Another type of high-pressure processing that has been developed is high-pressure homogenization (**HPH**). The principle of the operation is similar to that of conventional homogenizers used in the dairy industry except that it works at higher pressures (up to 400 MPa). This technology is also called ultra-high pressure homogenization (**UHPH**) depending on the pressure achieved. However, the pressure limit between HPH and UHPH has never been established. In this article, we will refer to UHPH for pressures above 100 MPa. Ultra-high pressure homogenization technology was first used in the pharmaceutical, chemical, and biochemical industries (Popper and Knorr, 1990; Floury et al., 2000). Several applications of UHPH for the food industry have been reported. Possible uses of this technology for the dairy industry include reduction of fat globule size, inactivation of enzymes, and destruction of bacteria. The fat globule size reduction of emulsions lowers the creaming rate and consequently improves shelf life (Thiebaud et al., 2003). The effect of UHPH on native milk enzymes (lactoperoxidase, plasmin, and alkaline phosphatase) has been studied by Hayes and Kelly (2003b) and Hayes et al. (2005), who showed that greater inactivation is achieved by increasing pressure. Related to microbiology, HPH and UHPH have been commonly used for cell disruption of dense microbial cultures (Saboya et al., 2003), and to cause a reduction of the microbial population, improving the microbial safety of food products (Kheadr et al., 2002; Thiebaud et al., 2003; Hayes et al., 2005). In general, by increasing the pressure, inlet temperature, and number of passes through the machine, more microbial inactivation is achieved. Different authors (Vachon et al., 2002; Hayes et al., 2005; Pereda et al., 2006) have suggested the possible potential of UHPH as a combined pasteurization and homogenization step to obtain commercial milk with a shelf life similar to conventional market milk. However, literature related to the effects of UHPH on milk shelf life is scarce. To date, physicochemical and microbiological changes of UHPH-treated milks at different temperatures and pressures have been studied immediately after treatment but not during storage (Thiebaud et al., 2003; Hayes et al., 2005; Pereda et al., 2006). Only a recent work of Smiddy et al. (2007) in which microbial shelf life of milk was assessed is available. In that study, a microbial shelf life between 4 and 7 d was obtained for UHPH-treated milk at 200 or 250 MPa at the first valve and 5 MPa at the second valve.

The aim of this work was to study different UHPH conditions (pressure and inlet temperature combinations) to compare the effects of heat (90°C, 15 s) and UHPH treatments on the microbial and physicochemical shelf life of whole bovine milk during storage at 4°C.

MATERIALS AND METHODS

Milk Supply

Fresh raw bovine milk (11.6 ± 1.0% TS and 3.29 ± 0.03% protein) was collected from a local farm (Can Badó, Barcelona, Spain) and was standardized at 3.5 ± 0.2% of fat, stored for 24 h at 4°C, and processed at the Center Especial de Recerca Planta de Tecnologia dels Aliments (CERPTA) at the Universitat Autònoma de Barcelona.

UHPH and Heat Pasteurization of Milk

Ultra-high pressure homogenization of raw milk was performed with a Stansted high-pressure homogenizer (model FPG11300, Stansted Fluid Power Ltd., Essex, UK). This device comprises a high-pressure ceramic valve able to support 350 MPa and a second pneumatic valve, located after the first one, able to support up to 50 MPa. The high-pressure system consisted of 2 intensifiers driven by a hydraulic pump. The flow rate of milk in the homogenizer was approximately 120 L/h. To minimize temperature retention after treatment, 2 spiral-type heat-exchangers (Garvía, Barcelona, Spain) located behind the second valve were used. The inlet temperature (**Ti**), the temperature before reaching the first homogenization valve (**T1**), the temperature before the second homogenization valve (**T2**), as well as the final temperature of the milk after passing through the heat exchanger (**TF**), were monitored throughout the experiment. Milk was UHPH-treated under the following conditions: 100, 200, and 300 MPa (single-stage) with inlet temperature of 40°C, and at 200 and 300 MPa (single-stage) with Ti = 30°C.

To obtain high-pasteurized milk (**PA**), raw milk was subjected to a 2-stage homogenization (18 MPa plus 2 MPa), using a Niro Soavi homogenizer (model X68P, Niro, Parma, Italy) and subsequently heat pasteurized using a Finamat heat exchanger (model 6500/010, Gea Finnah GmbH, Ahaus, Germany) at 90°C for 15 s.

Before treatments, equipment was disinfected by circulating a diluted disinfectant (20%) including peracetic acid and hydrogen peroxide (P3-Oxonia Active, Ecolab Hispanic Portuguese, Barcelona, Spain) in water (80%) for 15 min at 30°C. Milk samples were collected in sterile containers and stored at 4°C. Microbial

and physicochemical analyses of treated samples were conducted after treatment (d 0) and at 7, 14, 18, and 21 d of storage. Three replications were performed.

Microbiological Analysis

The microbiological quality of treated and untreated milk was assessed by enumerating the following microorganisms. Total bacteria were enumerated on plate count agar (Oxoid Ltd., Basingstoke, Hampshire, UK), pour-plated, and incubated for 48 h at 30°C. Psychrotrophic bacteria were enumerated on plate count agar (Oxoid), pour-plated, and incubated for 72 h at 21°C. Coliforms were enumerated on violet red bile agar medium (Oxoid), pour-plated, and incubated for 24 h at 37°C. Lactobacilli and lactococci were enumerated on Rogosa and M17 agar (Oxoid), respectively, pour-plated, and incubated for 72 h and 48 h, respectively, at 30°C. Enterococci were enumerated on kanamycin esculin azide agar (Oxoid), pour-plated, and incubated for 48 h at 37°C. *Pseudomonas* spp. were enumerated on pseudomonas agar base supplemented with cetrimide fucidin (Oxoid), spread-plated, and incubated for 96 h at 20°C. Milk samples were heated at 80°C for 5 min, quickly cooled in ice, and pour-plated on plate count agar for enumeration of total spores; plates were incubated for 48 h at 30°C. The presence of *Listeria monocytogenes* was detected using a 2-stage enrichment procedure. Twenty-five milliliters of milk was preenriched in half-Fraser broth (bioMérieux S.A., Marcy L'Etoile, France) and incubated at 37°C for 24 h. One milliliter of the preenriched sample was then incubated in Fraser broth (bioMérieux S.A.) at 37°C for 24 h. The enriched sample was then streaked onto Palcam agar medium (Oxoid) and incubated at 37°C for 24 h. The presence of *Salmonella* spp. was detected using a 2-stage enrichment procedure. Twenty-five milliliters of milk was preenriched in buffered peptone water (Oxoid) and incubated at 37°C for 24 h. One milliliter of the preenriched sample was then incubated in Muller-Kauffman broth (bioMérieux S.A.) and 0.1 mL in Rappaport-Vassiliadis broth (bioMérieux S.A.) at 37°C and 42°C for 24 h, respectively. Enrichments were then streaked onto XLD (Oxoid) and SMID2 media (bioMérieux S.A.) and incubated at 37°C for 24 h.

The detection limit was 1 cfu/mL of milk for all microorganisms except for *Pseudomonas*, which was 10 cfu/mL of milk.

Particle Size Determination

The particle size distribution in milk samples was determined using a Beckman Coulter Laser Diffraction particle size analyzer (LS 13 320 series, Beckman Coulter, Fullerton, CA). Milk samples were diluted in distilled water to reach appropriated laser obscuration. An optical model based on the Mie theory of light scattering by spherical particles was applied by using the following conditions: real refractive index = 1.471; refractive index of fluid (water) = 1.332; imaginary refractive index = 0; pump speed = 21%. The size distribution was characterized by the diameter below which 50 or 90% of the volume of particles are found (**D50** and **D90**, respectively), the Sauter diameter (surface-weighted mean diameter, $\mathbf{D_{3,2}}$), and the volume-weighted mean diameter ($\mathbf{D_{4,3}}$) value. Each sample was measured in triplicate.

Compositional Analysis of Milk

Fat content of milk was measured using the Gerber method (IDF, 1981) and TS and total N contents were analyzed in triplicate by the IDF (1987, 2002) standards, respectively. The pH of milk samples was monitored during storage using a micropH 2001 pH meter (Crison, Alella, Spain) and total acidity was determined by titration with 0.1 *N* NaOH.

Lactoperoxidase (**LP**; EC 1.11.1.7) activity was determined spectrophotometrically at 413 nm (CECIL 9000, CECIL Instruments, Cambridge, UK) and 20°C using 1 m*M* ABTS (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid, Sigma Aldrich Co., St Louis, MO) in phosphate buffer (0.1 *M* at pH 6) and 5 m*M* H_2O_2, according to the method described by Shindler et al. (1976). Alkaline phosphatase (**ALP**; EC 3.1.3.1) activity was determined spectrophotometrically at 400 nm using *p*-nitrophenyl disodiumphosphate (15.8 m*M* in 0.9 *M* 2-amino-2-methyl-1-propanol buffer at pH 10.45) and 37°C as described by Chávarri et al. (1998).

Enzyme units were expressed in units/mL, where one unit (U) is defined as the amount of enzyme that catalyzes the production of 1 micromole of product per minute. All enzyme determinations were done in triplicate.

Color Determination

Color values of milk samples were determined using a Hunter Lab colorimeter (MiniScan XETM, Hunter Associates Laboratory Inc., Reston, VA). Color coordinates were measured with an illuminant of D65 and a standard observer of 10° and the colorimeter was calibrated against white and black tile standards. Fifty milliliters of each milk sample was warmed to 20°C before analysis. The Commission Internationale de l'Eclairage (CIE) L*, a*, and b* values were measured in triplicate. The L* value represents the

lightness with values from 0 (black) to 100 (white), which indicates a perfect reflecting diffuser; the a* and b* axes have no specific numerical limits and represent chromatic components. Positive values of a* are red and negative values are green, whereas positive values of b* are yellow and negative ones are blue.

Viscosity Determination

Viscosity of milk samples was measured in duplicate at 20°C with a rotational rheometer (Haake Rheo Stress 1, Thermo Electron Corporation, Karlsruhe, Germany) using an internal cylinder Z34 (DIN53019) and an external cylinder Z34 (DIN53018). Samples were subjected to an increasing shear rate from 0 to 140 s^{-1} in 3 min and the experimental flow curves were fitted to the Newton model, $\tau = \eta\gamma$, where τ is the shear stress (Pa), η is the viscosity (Pa × s), and γ is the shear rate (s^{-1}).

Rate of Creaming

Immediately after UHPH or heat treatment, milk samples (100 mL) were placed in graduated cylinder tubes (100 ± 0.30 mL), which were sealed and stored in a cool room at 4°C. The volume of the cream separated from the milk was measured at 0, 4, 24, 48, 72, 96, 120, 144, and 168 h and expressed as milliliters of cream per 100 mL of milk.

Statistical Analysis

Results were analyzed by an ANOVA using the GLM procedure of SAS (SAS Institute, 2004). Data were presented as least squares means. The Tukey test was used for comparison of sample data. Evaluations were based on a significant level of $P < 0.05$.

RESULTS AND DISCUSSION

Temperature Increase During UHPH Treatment

The milk temperatures measured for the conditions used in this study are shown in Table 1. A linear temperature increase of 19.15°C per 100 MPa in the pressure range from 100 to 300 MPa at Ti = 40°C was calculated by plotting T2 vs. pressure. This linear rise of the temperature with the homogenization pressure is in agreement with the values obtained by Thiebaud et al. (2003) of 18.5°C per 100 MPa working from 100 to 300 MPa with inlet temperatures of 4, 14, and 24°C. Increases of 16.6°C per 100 MPa at 150 to 250 MPa and Ti = 45°C, and 17.6°C per 100 MPa in the pressure range of 50 to 200 MPa at Ti from 6 to 10°C, were observed by Hayes et al. (2005) and Hayes and Kelly (2003a), respectively. The increase of temperature during UHPH treatments is a consequence of the adiabatic heating generated in the machine in addition to the high turbulence, shear, and cavitation forces that the fluid suffers in the homogenization valve (Hayes and Kelly, 2003a; Thiebaud et al., 2003).

Table 1. Temperature changes[1] of milk during ultra-high pressure homogenization treatment[2]

Ti (°C)	Homogenization pressure (MPa)	T1 (°C)	T2 (°C)
40	100	43.0 ± 1.0	64.7 ± 1.5
	200	46.7 ± 0.6	84.0 ± 1.0
	300	50.0 ± 1.0	103.0 ± 1.0
30	200	39.0 ± 0.0	78.7 ± 1.2
	300	42.7 ± 0.6	97.3 ± 0.6

[1]Ti = inlet temperature; T1 = temperature before the first homogenization valve; T2 = temperature before the second homogenization valve.

[2]All temperature values are means (± standard deviations) of data from 3 independent experiments.

After reaching T2, milk was quickly cooled by passing through a heat exchanger, therefore the holding time at the higher temperature T2 was very short (≤0.7 s). This reduction of heating time during the UHPH treatment in comparison with heating time during pasteurization is very important to obtain products with less thermal damage, thus avoiding adverse effects on milk flavor and nutrients.

Microbial Inactivation

Microbial populations found in raw, PA-, and UHPH-treated milks immediately after treatments and during storage are shown in Table 2.

In this study, psychrotrophic counts of raw refrigerated milks ranged between 5.3×10^4 to 9.5×10^4 cfu/mL. Significant decreases in psychrotrophic counts at d 0 were observed for milk samples treated at 200 and 300 MPa at both inlet temperatures and for PA milk. Results showed that UHPH treatments were as efficient (99.99%) in reducing the psychrotrophic bacterial population as PA treatment, reaching reductions of approximately 3.5 log cfu/mL. Nevertheless, treatment at 100 MPa at 40°C was not able to reduce microbial counts to significant levels in comparison with raw milk, and the reduction achieved was close to the limit established by the European normative (n = 5, c = 1, m = 5×10^4, M = 5×10^5) for pasteurized milks where n = number of sample units comprising the sample; m = threshold value for the number of bacteria, and the result is considered satisfactory if the number of bacteria in all sample units does not exceed "m"; M = maximum value for the number of bacteria, and the result is considered unsatisfactory if the number

Table 2. Microbial populations (log cfu/mL) of raw and treated milks during storage at 4°C[1]

Microbial group	Day	Treatment						
		Raw	Pasteurized: 90°C for 15 s	200 MPa at 30°C	300 MPa at 30°C	100 MPa at 40°C	200 MPa at 40°C	300 MPa at 40°C
Psychrotrophs	0	4.9[a] ± 0.1	1.4[b] ± 0.2	1.5[b] ± 0.6	1.6[b] ± 0.2	4.2[a] ± 0.5	1.5[b] ± 0.6	1.2[b] ± 0.6
	7		1.4[a] ± 0.4	1.2[a] ± 0.5	1.7[a] ± 0.2		1.5[a] ± 0.4	1.2[a] ± 0.4
	14		2.7[b] ± 0.9	2.7[b] ± 0.9	3.7[a] ± 0.4		3.7[a] ± 0.6	4.1[a] ± 0.2
	18		5.3[a] ± 0.8	3.5[b] ± 1.1	6.4[a] ± 0.1		5.4[a] ± 0.5	6.0[a] ± 0.5
	21		5.8[b] ± 1.2	3.8[c] ± 0.9	6.9[a] ± 0.3		6.7[a] ± 0.7	7.1[a] ± 0.4
Total bacteria	0	4.9[a] ± 0.1	1.4[b] ± 0.2	1.6[b] ± 0.2	1.7[b] ± 0.3	4.4[a] ± 0.4	1.7[b] ± 0.2	1.4[b] ± 0.5
	7		1.4[a] ± 0.4	1.4[a] ± 0.2	1.8[a] ± 0.2		1.5[a] ± 0.2	1.4[a] ± 0.4
	14		2.8[b] ± 0.8	2.5[b] ± 0.6	3.6[a] ± 0.3		3.8[a] ± 0.6	3.7[a] ± 0.3
	18		5.2[a] ± 0.8	3.6[b] ± 1.1	6.3[a] ± 0.2		5.7[a] ± 0.2	6.2[a] ± 0.4
	21		6.2[b] ± 0.7	3.9[c] ± 0.9	6.9[a] ± 0.3		7.4[a] ± 0.4	7.1[a] ± 0.4
Lactococci	0	4.9[a] ± 0.3	1.5[b] ± 0.1	1.5[b] ± 0.4	1.7[b] ± 0.3		1.4[b] ± 0.6	1.3[b] ± 0.8
	7		1.6[a] ± 0.2	1.4[a] ± 0.5	1.6[a] ± 0.2		1.4[a] ± 0.4	1.2[a] ± 0.5
	14		2.9[b] ± 0.9	2.5[b] ± 0.9	3.8[a] ± 0.3		3.8[a] ± 0.4	4.1[a] ± 0.3
	18		5.1[b] ± 0.6	3.6[c] ± 1.0	6.2[a] ± 0.3		5.3[b] ± 0.7	5.9[ab] ± 0.2
	21		5.6[b] ± 1.0	3.9[c] ± 0.7	6.6[a] ± 0.5		7.2[a] ± 0.5	6.9[a] ± 0.5
Coliforms	0	3.5[a] ± 0.3	ND[c,2]	ND[c]	ND[c]	0.5[b] ± 0.6	ND[c]	ND[c]
	7		ND	ND	ND		ND	ND
	14		ND	ND	ND		ND	ND
	18		ND	ND	ND		ND	ND
	21		ND	ND	ND		ND	ND
Lactobacilli	0	2.9[a] ± 0.2	ND[c]	ND[c]	ND[c]	1.8[b] ± 0.2	ND[c]	ND[c]
	7		ND	ND	ND		ND	ND
	14		ND	ND	ND		ND	ND
	18		ND	ND	ND		ND	ND
	21		ND	ND	ND		ND	ND
Enterococci	0	3.9[a] ± 0.2	ND[c]	ND[c]	ND[c]	2.4[b] ± 0.1	ND[c]	ND[c]
	7		ND	ND	ND		ND	ND
	14		ND	ND	ND		ND	ND
	18		ND	ND	ND		ND	ND
	21		ND	ND	ND		ND	ND
Spores	0	1.7[a] ± 0.1	0.9[c] ± 0.2	0.6[c] ± 0.3	0.7[c] ± 0.3	1.3[b] ± 0.3	0.8[c] ± 0.31	0.6[c] ± 0.1
	7		0.8[a] ± 0.3	0.6[a] ± 0.2	0.8[a] ± 0.1		0.7[a] ± 0.19	0.5[a] ± 0.3
	14		0.9[a] ± 0.2	0.4[b] ± 0.1	0.7[a] ± 0.2		0.7[a] ± 0.27	0.7[a] ± 0.3
	18		0.9[c] ± 0.2	1.0[c] ± 0.3	1.7[b] ± 0.2		1.5[b] ± 1.04	2.8[a] ± 0.6
	21		1.1[b] ± 0.2	1.1[b] ± 0.7	2.8[a] ± 0.3		2.7[a] ± 0.12	2.6[a] ± 0.3
Pseudomonas	0	3.7[a] ± 0.3	ND[c]	ND[c]	ND[c]	2.6[b] ± 0.0	ND[c]	ND[c]
	7		ND	ND	ND		ND	ND
	14		ND	ND	ND		ND	ND
	18		ND[c]	ND[c]	ND[c]		2.1[b] ± 2.5	4.5[a] ± 0.2
	21		ND[b]	ND[b]	ND[b]		4.8[a] ± 0.4	5.1[a] ± 0.6

[a–c]Values in the same row with different superscripts differ significant ($P < 0.05$).

[1]Values are means ± standard deviations of duplicate analysis from 3 different productions.

[2]ND = not detected.

of bacteria in one or more sample units is "M" or more; c = number of sample units where the bacteria count may be between m and M, the sample being considered acceptable if the bacteria count of the other sample unit is m or less (European Union, 1992). Therefore, neither microbial changes nor physicochemical changes of milks treated at 100 MPa were studied during storage at 4°C.

No detectable levels of psychrotrophic bacteria at 200 and 250 MPa with inlet temperatures of 45°C and 55 to 70°C were reported by Hayes et al. (2005) and Smiddy et al. (2007), respectively by using a Stansted HP homogenizer (model nm-GEN 7400H). Lower reductions for this microbial group have been reported by Thiebaud et al. (2003), who obtained 1.3 to 1.6 log cycle reductions at 200 MPa and Ti = 24°C and 2.7 to 3.1 log cycle reductions at 300 MPa with the same Ti (Stansted HP homogenizer, model FPG7400 H).

By comparing psychrotrophic counts of each treatment during storage, no differences ($P > 0.05$) were detected until d 14. This indicated that the lag phase ended approximately on the seventh day of storage, after which counts started to increase progressively. Psychrotrophic counts increased during storage in all milks, but rates between samples were different. At d 14 UHPH-treated milks at both pressures and Ti = 40°C, and milks treated at 300 MPa at 30°C had higher counts than PA and 200 MPa at 30°C milks. At d 18,

all milks except those treated at 200 MPa at 30°C had psychrotrophic counts above the legal limit established for heat-treated milks, whereas milk treated at 200 MPa at 30°C remained below this limit even at 21 d of storage. This unexpected result (longer microbial shelf life with the lower pressure and Ti applied) could be related to the partial inactivation of LP that occurs in this treatment, whereas in the other treatments a complete inactivation of the enzyme was achieved (see results in LP and ALP determination). The LP system is a natural antimicrobial system of milk that is effective against some microorganisms. The central component of the system is the LP enzyme, which catalyzes the oxidation by hydrogen peroxide of thiocyanate (SCN^-) into hypothiocyanite ($OSCN^-$). This last component acts at the bacterial cell membrane by oxidizing the sulfydryl groups of proteins to disulfides. Barrett et al. (1999) found that milk pasteurized at 80°C for 15 s deteriorates faster than milk pasteurized at 72°C for 15 s. This phenomenon has been attributed to heat shocking of spores but it can also be related to the complete inactivation of LP that occurs at 80°C, whereas at 72°C, the residual LP activity was 70%. Vannini et al. (2004) reported that HPH and antimicrobial enzymes such as LP demonstrated an enhanced activity against several spoilage and pathogenic microorganisms. The authors attributed this phenomenon to an increased exposure of hydrophobic regions of proteins.

Total plate counts of raw milk were between 5.9×10^4 and 1×10^5 cfu/mL. Both total plate count and lactococci bacteria showed a similar behavior as psychrotrophic bacteria, being initially reduced by 3.5 log. Saboya et al. (2003), working on cell suspensions, reported that to break 80% of lactococci cells, one pass at 200 MPa was required. In relation to total plate count, Thiebaud et al. (2003) obtained lower microbial reductions (1 to 1.15 log cfu/mL) working at 200 MPa and Ti = 24°C. Kheadr et al. (2002) obtained 2 log cycles of inactivation for total counts in whole bovine milk (3.4% fat) after homogenization at 200 MPa (5 passes) with Ti = 28°C. The reduction achieved in the present study was also higher than that obtained by Hayes et al. (2005) of 1.83 log cfu/mL working at 200 MPa and Ti = 45°C and 3 log cfu/mL at 250 MPa, and it was similar to the reduction reached by Hayes and Kelly (2003a) of 75% of total bacterial count at 200 MPa. Smiddy et al. (2007) found a reduction of ~5 log units for UHPH-treated milk at 250 MPa but at inlet temperatures of 55 and 70°C.

Differences observed in total plate counts between this study and others could be explained by a combination of differences in the valve construction and machine design, as well as in the inlet temperature and the time at which milk is maintained at the higher temperature reached during the treatment. In this study, the higher temperature reached was approximately 100°C especially in treatments at 300 MPa with Ti = 40°C. However, as mentioned above, the temperature retention during treatment was very short (~0.7 s). In comparison, in the studies followed by Hayes and Kelly (2003a) and Hayes et al. (2005), the holding time at the outlet temperature was calculated to be 20 s; however, they reached lower microbial reductions compared with our results. Possible mechanisms of UHPH responsible for destruction of bacteria include sudden pressure drops, torsion and shear stresses, cavitation shock waves, turbulence, viscous shear, and high velocity collisions (Popper and Knorr, 1990; Wuytack et al., 2002; Hayes and Kelly, 2003a). Therefore, we can presume that homogenization valves and machine design could be one of the most important factors, although the temperature increase produced in the UHPH valve may also play an important role in microbial inactivation.

Some authors (Thiebaud et al., 2003; Hayes et al., 2005) found that reduction in total plate counts was pressure dependent, with better inactivation as homogenization pressure increased. However, in the present study, no significant differences were detected between UHPH samples (200 and 300 MPa) after treatment. Moreover, Hayes and Kelly (2003a) found that a 2-stage treatment had a greater destructive effect on milk microflora. In preliminary experiments, 2-stage homogenization treatments were also performed in our laboratories, but microbial counts were not improved compared with a single-stage treatment (results not shown); therefore, it was decided not to include these treatments in the study.

Coliform counts are widely used as a contamination index. An elevated number in milk indicates deficient handling during milking, collection, or manipulation. In the present study, coliform counts in raw milk were on average 3.1×10^4 cfu/mL. Coliforms were completely inactivated by both heat and UHPH treatments, except at 100 MPa. Similar results were obtained by Hayes et al. (2005).

Enterococci were only detected in milk treated at 100 MPa. In spite of the fact that enterococci are highly resistant to freezing, drying, and heat treatments such as conventional pasteurization, it could be observed that UHPH treatments were very active against this microbial group. However, results published by Wuytack et al. (2002) have shown that enterococci were one of the most resistant microorganisms to UHPH obtaining a microbial inactivation of less than 1 log at a working pressure of 200 MPa.

Average lactobacilli counts of raw milk were 7.94×10^2 cfu/mL. However, lactobacilli were absent in heat- and UHPH-treated milks, except for those undergoing the 100 MPa treatment, where 1.8 log units were detected. These results do not agree with those of Thiebaud et al. (2003), who obtained inactivation ratios of 1.0 to 1.6 and 1.2 to 2.3 at 200 and 300 MPa respectively, with Ti = 24°C.

Undetectable levels of coliforms, enterococci, and lactobacilli were obtained in different milks during storage at 4°C. However, in the study of Smiddy et al. (2007), coliforms were detected at d 4 of storage in milk treated at 200 MPa and 55°C, and a slight increase of coliforms was observed at d 14 in milk treated at 200 MPa at 70°C.

Spore counts in raw milk ranged between 3.99×10 and 6.31×10 cfu/mL. Spores were highly resistant to heat and UHPH treatments, but they were reduced significantly for all UHPH and heat treatments, although not completely eliminated (0.8 to 1.1 logs of inactivation depending on the treatment), and the lower spore reduction achieved was for milk treated at 100 MPa (0.4 log of inactivation). As in the case of the other microbial groups, no significant differences were observed between samples up to 14 d of storage. At d 18 spore counts increased for all milks, but this increase was faster for milk treated at 300 MPa and 40°C, followed by treatments at 200 MPa at 40°C and 300 MPa at 30°C. Pasteurized and UHPH-treated milk at 200 MPa at 30°C had the lowest spore counts. Feijoo et al. (1997), studying the effect of HPH (microfluidization) on spores of ice cream at pressures from 50 to 200 MPa, obtained percentages of spore reduction from 6 to 68%. Their results showed that the number of spores was reduced by forces generated in the machine but that spores were not completely destroyed. In the same way, combinations of pressure and temperature used in this study were not sufficient to eliminate spores. Heat-resistant spores are usually present in raw milk and the literature suggests that there are some spore-forming microorganisms able to grow at refrigeration temperatures (Meer et al., 1991), which is in accordance with our results. These microorganisms with heat-resistant and psychrotrophic properties could present a problem for the milk shelf life.

Pseudomonads are one of the major microorganisms responsible for milk spoilage. At d 0, they were detected only in milk treated at 100 MPa. Levels of this group of microorganisms remained undetectable during storage in PA milk and milks treated at both pressures with Ti = 30°C. However, bacterial growth in pseudomonas agar base medium was observed in UHPH- treated milks at 40°C during the last days of storage. The increase in microbial counts was higher for milk treated at 300 MPa (4.5 log cycles) than for that treated at 200 MPa (2.1 log cycles). No detectable levels at d 0 in milks treated at pressures ≥200 MPa are in accordance with the results of Wuytack et al. (2002) and Smiddy et al. (2007). In the latter study, an increase of pseudomonads was also observed during storage for all UHPH treatments.

No *Salmonella* spp. or *L. monocytogenes* were detected in any raw milk sample using the protocols described above. Therefore, we can draw no conclusion about the efficiency of UHPH treatments on the destruction of these pathogenic microorganisms. However, some studies on the destruction of foodborne pathogens in milk have been carried out by different researchers. Briñez et al. (2006a,b) inoculated 2 different strains of *Escherichia coli* (*E. coli* ATCC 10536 and *E. coli* O157:H7 CCUG 44857) and *Listeria innocua* in milk and, using Ti = 20°C and 300 MPa in the first homogenizing valve and 30 MPa in the second valve, obtained lethality values of 4.3, 3.94, and 4.31 log, respectively. Vachon et al. (2002) found that *L. monocytogenes* was more resistant to the pressure treatments than *Salmonella*. For *Salmonella enteritidis*, they obtained a reduction of 4 log and complete inactivation at 200 and 300 MPa, respectively (Ti = 25°C), whereas complete inactivation of *L. monocytogenes* was only achieved by applying 300 MPa for 3 passes.

Particle Size Determination

The particle size distribution curve of raw milk was characterized by a main peak at 3.7 μm that corresponds to fat globules and a small peak at 0.2 μm that corresponds to casein micelles. Curves of size distribution for PA- and UHPH-treated milks at 200 MPa were characterized by one peak, whereas milks treated at 300 MPa showed the presence of a second small peak at higher diameters due to the formation of large particles or fat aggregates. Fat globules <0.6 μm represented approximately 90 and 95% of the total fat volume at 300 MPa at 40 and 30°C, respectively, whereas fat aggregates >1 μm were about 10 and 5% of the total fat volume at 300 MPa at 40 and 30°C, respectively. The polydisperse distributions obtained for 300 MPa are in accordance with results of Thiebaud et al. (2003). These clusters can be formed through shared protein constituents adsorbed onto the surface (Thiebaud et al., 2003) or by coalescence of fat globules. The formation of clusters of fat globules can originate from different causes. One explanation was given by Hayes et al. (2005) who stated that at higher pressures there would be more exposed fat interface. The amount of casein may become limiting, resulting in insufficiently covered fat globules that can agglomerate. Floury et

Table 3. Fat globule size parameters for raw and treated milks[1]

Treatment	Fat globule size parameter[2]			
	D50	D90	$D_{3,2}$	$D_{4,3}$
Raw	3.05[a] ± 0.03	5.08[a] ± 0.08	0.55[a] ± 0.02	2.80[a] ± 0.04
Pasteurized[3]	0.38[b] ± 0.01	0.92[bc] ± 0.03	0.30[b] ± 0.00	0.47[c] ± 0.01
200 MPa at 30°C	0.14[d] ± 0.01	0.32[c] ± 0.06	0.20[c] ± 0.11	0.17[c] ± 0.02
200 MPa at 40°C	0.12[e] ± 0.01	0.23[c] ± 0.00	0.11[d] ± 0.00	0.14[c] ± 1.07
300 MPa at 30°C	0.15[cd] ± 0.00	0.36[c] ± 0.07	0.14[d] ± 0.00	1.97[b] ± 1.07
300 MPa at 40°C	0.16[c] ± 0.01	1.38[b] ± 1.59	0.15[cd] ± 0.01	2.32[ab] ± 0.56

[a–e]Different superscripts in the same column indicate significant differences ($P < 0.05$).

[1]Values are means ± standard deviations of triplicate analysis from 3 different productions.

[2]D50 = diameter below which 50% of the volume of particles are found; D90 = diameter below which 90% of the volume of particles are found; $D_{3,2}$ = Sauter diameter (surface-weighted mean diameter); $D_{4,3}$ = volume-weighted mean diameter.

[3]Pasteurized = high-pasteurized milk, 90°C for 15 s.

al. (2000) suggested that at pressure of 300 MPa, whey proteins are denatured and would not be able to stabilize fat globules.

Fat globule size parameters (D90, D50, $D_{3,2}$, and $D_{4,3}$) for the different milks are shown in Table 3. As expected, PA milk obtained by traditional homogenization underwent a reduction in milk fat globule size ($P < 0.05$) in terms of all parameters studied compared with raw milk. The reduction in fat globule size achieved in UHPH-treated milks at 200 MPa (Ti = 30 and 40°C) was higher than that obtained by traditional homogenization. Although milks treated at 200 MPa were numerically smaller than pasteurized milk for all fat globule size parameters, significant differences were only detected for the D50 and $D_{3,2}$ values. The UHPH treatment achieved a great reduction of particle size at 200 MPa; however, no further reduction could be obtained at 300 MPa. Milks treated at 300 MPa showed a significantly lower D50 value compared with PA milk but slightly higher compared with milks treated at 200 MPa. The $D_{4,3}$ parameter is very sensitive to the presence of small amounts of large particles. At 300 MPa, an increase in $D_{4,3}$ was observed, which is in relation to the formation of aggregates previously mentioned.

pH and Total Acidity

Immediately after treatment, small but significant differences were detected in the pH value of milk treated at 200 MPa at 30°C (~6.72) compared with raw milk and to the other treated milks (~6.74). For each day of sampling, the pH of milks treated at 200 MPa at 30°C was always below the pH values of the other milks as can be seen in Figure 1.

After 18 d of storage, a decrease in pH was observed except for pasteurized milks and milks treated at 300 MPa at 30°C, which had a constant pH until the end of storage. The reduction in pH was accompanied by an increase in acidity.

Hayes and Kelly (2003a) explained that the decrease in pH of homogenized milks observed after 24 h of storage at 4°C could be related to the partial inactivation of milk lipoprotein lipase (**LPL**). This fact could explain the marked pH reduction that occurred at 200 MPa at 30°C. Lipoprotein lipase is an enzyme associated with the casein micelle. Due to destruction of milk fat globule membrane and reduction of fat globules after treatment, LPL can easily access the fat and can find greater interfacial fat surface area on which to act. In this study the constant pH value for PA milks and milks treated at 300 MPa and 30°C could be related to the temperature achieved, at which complete inactivation of LPL could be obtained. A preliminary experiment performed in our laboratories (unpub-

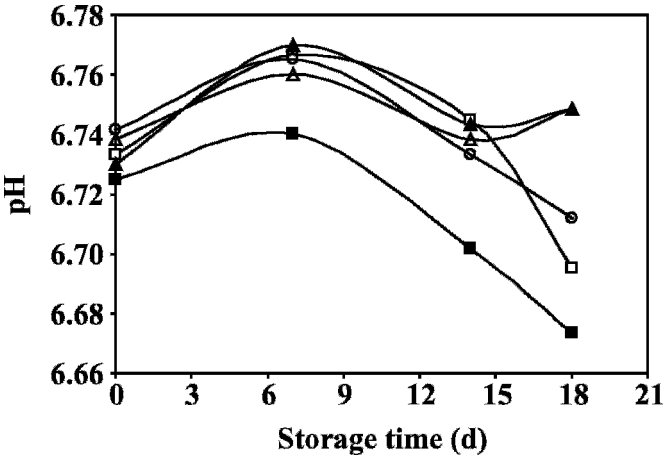

Figure 1. Evolution of pH during refrigerated storage at 4°C of treated milks: 200 MPa at 30°C (■), 200 MPa at 40°C (□), 300 MPa at 30°C (▲), 300 MPa at 40°C (○), and high-pasteurized at 90°C for 15 s (△).

Table 4. Peroxidase and phosphatase activities for raw and treated milks[1]

Treatment	Peroxidase (U/mL)	Phosphatase (U/mL)
Raw milk	1.34[a] ± 0.03	0.88[a] ± 0.01
Pasteurized[2]	ND[c,3]	ND[b]
200 MPa at 30°C	0.46[b] ± 0.01	ND[b]
200 MPa at 40°C	0.01[c] ± 0.00	ND[b]
300 MPa at 30°C	ND[c]	ND[b]
300 MPa at 40°C	ND[c]	ND[b]

[a–c]Different superscripts indicate significant differences ($P < 0.05$).

[1]Data are means ± standard deviations of triplicate analysis from 3 different productions.

[2]Pasteurized = high-pasteurized milk (90°C for 15 s).

[3]ND = not detected.

lished results) on whole milk at the inlet temperatures mentioned above, with the aim to verify the effect of UHPH treatments (100 to 300 MPa) on milk lipase (assessed by total free fatty acids determination after treatment), showed that there is a positive effect of pressure on the inactivation of lipase. However, total inactivation of milk lipase was not achieved at 100 to 200 MPa at 30°C.

Surprisingly, at 300 MPa at 40°C (the most intense treatment), a reduction of pH during storage at 4°C was observed. At this treatment, a T2 around 100°C (able to inactivate LPL) was achieved; therefore, this behavior could be explained by the production of microbial lipases from psychrotrophic bacteria, mostly pseudomonads, which are stable at high temperatures and survive pasteurization and UHT treatments (Stepaniak and Sorhaug, 1995). Pseudomonads were present at the end of storage in milks treated at 300 and 200 MPa at Ti = 40°C. Production of enzymes at low temperatures is limited but possible for the pseudomonads. Hayes et al. (2005) reported a pH decrease at 150 MPa, but no decrease at 200 or 250 MPa after 14 d of storage at 4°C. This finding strengthens the results of the present study because the pH decrease was observed from d 18. However, the reduction in milk pH could also be related to the production of lactic acid by microorganisms. A good correlation between acidity and concentration of microorganisms was found by Odriozola-Serrano et al. (2006). Additional work is being carried out in our laboratories to prove these hypotheses about the reduction of pH in UHPH-treated milks at 300 MPa at 40°C.

LP and ALP Determinations

The effect of heat and UHPH treatments on LP and ALP was evaluated by determining the residual enzymatic activities (Table 4). Lactoperoxidase is an enzyme that is relatively sensitive to heat. As can be seen, heat treatment at 90°C for 15 s completely inactivated LP, as occurs with high-pasteurized milks. Temperature achieved during UHPH treatments at 300 MPa also produced total enzyme inactivation. Milk treated at 200 MPa at 40°C had a residual activity less than 1%; however, only milk treated at 200 MPa at 30°C showed significant differences compared with the other UHPH-treated milks. This treatment was able to reduce LP, but a residual activity of 35% was maintained. Residual LP activities in milk of 70, 40, and 0% were observed by Barrett et al. (1999) for treatments at 72°C for 15 s, 72°C for 80 s, and 80°C for 15 s, respectively. It seems that LP can remain active depending on the temperature–time combination applied, being very sensitive to temperatures around 80°C. Working with UHPH at Ti = 45°C, Hayes et al. (2005) reported 91, 34, and 0% of residual LP activity at 150, 200, and 250 MPa, respectively. These results are similar to those observed in this study. Treatment at 200 MPa and Ti = 40°C inactivated almost 100% of the enzyme. Differences in enzyme inactivation between various studies could be due to differences in T2 temperature and holding time: 77°C for 20 s in the study of Hayes et al. (2005) and 84°C for 0.7 s in this study. These results agree with the study of Barrett et al. (1999), in which the holding time had a limited effect on heat inactivation in the range of 68 to 80°C. The LP inactivation achieved by Hayes et al. (2005) at 200 MPa was similar to that obtained in this study at 200 MPa with Ti = 30°C where milk reached a T2 of ~78°C. Therefore, the relevant factor in relation to LP inactivation by UHPH could be ascribed to the temperature achieved by samples (T2) in the different treatments, which depends on the inlet temperature and pressure applied.

Both heat and UHPH treatments achieved complete inactivation of ALP. Hayes et al. (2005) had no measurable ALP activity in pasteurized, homogenized commercial milk and in UHPH-treated milk at 250 MPa. However, residual activity of 2 and 29% was observed at 200 MPa and 150 MPa, respectively (Ti = 45°C). Hayes and Kelly (2003b), using a qualitative assay, obtained positive results for milks treated from 50 to 200 MPa with inlet temperatures of 6 to 9°C. However, temperatures reached during treatments never exceeded 53°C. Alkaline phosphatase is an enzyme that is associated with the MFGM and usually a treatment of 72°C for 15 s is enough to inactivate it. The total inactivation of ALP achieved in this study could be due to different factors: the milk fat globule size reduction (which implies the destruction of milk fat globule membrane), the temperatures achieved before the second homogenization valve, and the high shear forces produced during treatment.

Table 5. Changes in color of different milks during storage at 4°C[1]

Color parameter	Treatment					
	Raw	Pasteurized: 90°C for 15 s	200 at 30°C	300 at 30°C	200 at 40°C	300 at 40°C
Day 0						
L	90.28^{c} ± 0.18	93.24^{a} ± 0.03	92.68^{b} ± 0.16	92.77^{b} ± 0.11	92.73^{b} ± 0.06	92.72^{b} ± 0.14
a	–1.58^{a} ± 0.03	–1.64^{a} ± 0.08	–1.64^{a} ± 0.06	–1.49^{b} ± 0.07	–1.88^{b} ± 0.16	–2.01^{c} ± 0.09
b	9.6^{a} ± 0.16	8.68^{b} ± 0.14	8.09^{c} ± 0.17	7.79^{d} ± 0.14	7.84^{d} ± 0.17	7.80^{d} ± 0.17
Day 7						
L		93.40^{a} ± 0.05	92.94^{d} ± 0.17	93.15^{b} ± 0.21	93.08^{bc} ± 0.24	93.01^{dc} ± 0.19
a		–1.68^{c} ± 0.07	–1.58^{a} ± 0.05	–1.99^{d} ± 0.08	–1.91^{c} ± 0.06	–2.07^{e} ± 0.07
b		8.83^{a} ± 0.21	8.24^{b} ± 0.20	7.95^{c} ± 0.19	7.93^{c} ± 0.17	7.93^{c} ± 0.17
Day 14						
L		93.37^{a} ± 0.11	92.94^{b} ± 0.06	92.92^{b} ± 0.32	92.88^{b} ± 0.17	92.99^{b} ± 0.15
a		–1.69^{b} ± 0.05	–1.54^{a} ± 0.11	–1.99^{d} ± 0.06	–1.87^{c} ± 0.06	–2.04^{e} ± 0.06
b		8.92^{a} ± 0.20	8.29^{b} ± 0.22	8.08^{c} ± 0.22	8.08^{c} ± 0.29	8.11^{c} ± 0.15
Day 18						
L		93.42^{a} ± 0.17	92.92^{c} ± 0.09	93.12^{b} ± 0.14	93.06^{bc} ± 0.16	92.97^{bc} ± 0.13
a		–1.72^{b} ± 0.03	–1.59^{a} ± 0.07	–2.01^{d} ± 0.06	–1.90^{c} ± 0.05	–2.08^{e} ± 0.04
b		8.98^{a} ± 0.18	8.35^{b} ± 0.19	8.12^{c} ± 0.23	8.13^{c} ± 0.35	8.14^{c} ± 0.17
Day 21						
L		93.35^{a} ± 0.19	93.09^{ab} ± 0.19	93.32^{b} ± 0.15	93.14^{b} ± 0.04	93.09^{b} ± 0.38
a		–1.80^{b} ± 0.10	–1.63^{a} ± 0.17	–2.11^{d} ± 0.17	–1.99^{c} ± 0.09	–2.05^{cd} ± 0.18
b		8.99^{a} ± 0.22	8.41^{b} ± 0.23	8.15^{c} ± 0.18	8.18^{c} ± 0.32	7.88^{d} ± 0.21

$^{a–e}$Different superscripts in the same row indicate significant differences ($P < 0.05$).

[1]Data are means ± standard deviations of triplicate analysis from 3 different productions.

Color

Significant differences ($P < 0.05$) in L* values were observed between raw, PA-, and UHPH-treated milks at d 0 (Table 5). Pasteurized milk was whiter than UHPH-treated milks and raw milk; nevertheless, differences in instrumental color measurements were not visually obvious.

No significant differences among raw milk, PA milk, and milk treated at 200 MPa at 30°C were detected in relation to a* value. All of these treatments exhibited more negative a* values (which indicates more green) compared with other treatments. In relation to b* values, raw milk had the highest value, which was significantly different from PA and UHPH-treated milks. No significant differences were detected between milks treated at 300 MPa at both inlet temperatures and 200 MPa at 40°C. The decrease in milk b* value was in the order of raw > pasteurized > 200 MPa at 30°C > 300 MPa at 30°C = 200 MPa at 40°C = 300 MPa at 40°C.

Increased lightness of PA- and UHPH-treated milks and small changes in a* and b* values after treatment compared with raw milk were also observed by Hayes and Kelly (2003a) and Hayes et al. (2005). Treated milks were whiter than raw milk due to the increase of fat globules, which diffract light more efficiently. On the other hand, differences between UHPH-treated milks and conventional homogenized milk could be related to the state of the casein micelles. In general, lower L* values indicate disintegration of the casein micelles (O'Sullivan et al., 2002). In this study, the average casein micelle size was not studied; however, no change in micelle size at pressures below 150 MPa but a 5% decrease in micelle size in samples homogenized at 200 MPa was observed by Hayes and Kelly (2003a) in skim milk. Therefore, we could suppose that conventional homogenized milk was whiter than UHPH-treated milks due to a lesser reduction in micelle casein size.

During storage, PA-treated milk always had a higher L* value compared with UHPH-treated milks. Over time, the lightness (L*) of all milks did not show a defined tendency, but in general, an increase ($P < 0.05$) in this parameter was observed between d 0 and 21. The a* value remained largely unchanged during storage, whereas b* showed a tendency to a linear increase.

Viscosity

Viscosity, besides affecting the flow conditions in dairy processes, is an important physical property related to milk shelf life because it is associated with the rate of creaming. Viscosity values measured in milks treated at 200 MPa were significantly different from those of raw, PA, and UHPH-treated milks at 300 MPa (Table 6). At each sampling time during the storage study, milks treated at 200 MPa always had the lowest viscosity value ($P < 0.05$). During milk storage an increase ($P < 0.05$) of viscosity was observed, but increases after d 7 were not significantly different,

Table 6. Evolution of viscosity (mPa × s) of raw and treated milks during storage at 4°C[1]

Milk	Storage day				
	Day 1	Day 7	Day 14	Day 18	Day 21
Raw	2.405[a] ± 0.118				
Pasteurized[2]	2.409[a] ± 0.043	2.552[a] ± 0.029	2.592[b] ± 0.039	2.553[a] ± 0.150	2.647[a] ± 0.077
200 MPa at 30°C	2.274[b] ± 0.038	2.346[c] ± 0.027	2.368[c] ± 0.035	2.375[b] ± 0.023	2.433[c] ± 0.022
300 MPa at 30°C	2.425[a] ± 0.050	2.524[ab] ± 0.056	2.543[b] ± 0.032	2.541[a] ± 0.097	2.554[b] ± 0.040
200 MPa at 40°C	2.240[b] ± 0.035	2.314[c] ± 0.031	2.348[c] ± 0.048	2.348[b] ± 0.032	2.363[c] ± 0.031
300 MPa at 40°C	2.385[a] ± 0.059	2.505[b] ± 0.026	2.651[a] ± 0.082	2.624[a] ± 0.055	2.727[a] ± 0.146

[a–c]Different superscripts in the same column indicate significant differences ($P < 0.05$).

[1]Data are means ± standard deviations of duplicate analysis from three different productions.

[2]Pasteurized = high-pasteurized milk, 90°C for 15 s.

except for milk treated at 300 MPa at 40°C, which showed a marked change at d 14 of storage. Regardless of the differences in instrumental viscosity measurements, they were not visibly obvious.

Viscosity can be influenced by particle size (Floury et al., 2000), casein micelle aggregates (Walstra and Jenness, 1984), and denaturation of whey proteins by heat (Clare et al., 2005). The UHPH treatments reduced particle size; however, milks treated at 300 MPa were characterized by the formation of large particles or fat aggregates, whereas this behavior was not observed at 200 MPa. The formation of these clusters could explain the higher viscosity values from milks treated at 300 MPa. To date, studies of viscosity on UHPH-treated milks from 200 to 300 MPa have not been published. Floury et al. (2000), working with oil in water emulsions in a range of pressures from 20 to 150 MPa, found that as pressure increased, the mean droplet diameters, the droplet size distributions, and viscosity of emulsions decreased. Recoalescence was observed at pressures of 300 MPa; however, and in disagreement with our results, at this pressure the lowest value of viscosity was measured.

Rate of Creaming

The effect of storage time on the rate of creaming is shown in Figure 2. After the first 4 h of storage at 4°C, untreated raw milk showed an initial creaming of 4 mL/100 mL of milk. The rate of creaming showed a rapid increase until 120 h where it reached a cream layer of approximately 8 mL/100 mL of milk, which remained more or less constant until 168 h of storage. According to Stokes' law, the cream separation rate, which is due to the density difference between the fat and aqueous phases, can be decreased by reducing milk fat globule size. Due to the reduction in particle size achieved, no creaming was observed in UHPH-treated milks during refrigerated storage. In spite of the presence of aggregates in milks treated at 300 MPa at inlet temperatures of 30 and 40°C, creaming was not observed during storage at 4°C. The same effect was observed by Thiebaud et al. (2003), and they supposed that these particles might correspond to fat clusters formed with protein adsorbed at the surface rather than through coalescence of fat globules. The larger fat globules detected for conventional homogenized milk could be the cause of the reduced rate of creaming observed (0.5 mL/100 mL of milk), which agrees with the results obtained by Hayes et al. (2005). However, differences between PA- and UHPH-treated milks were not significant.

CONCLUSIONS

In general, the microbial results of this study were very promising. Milk treated at 200 MPa at 30°C had

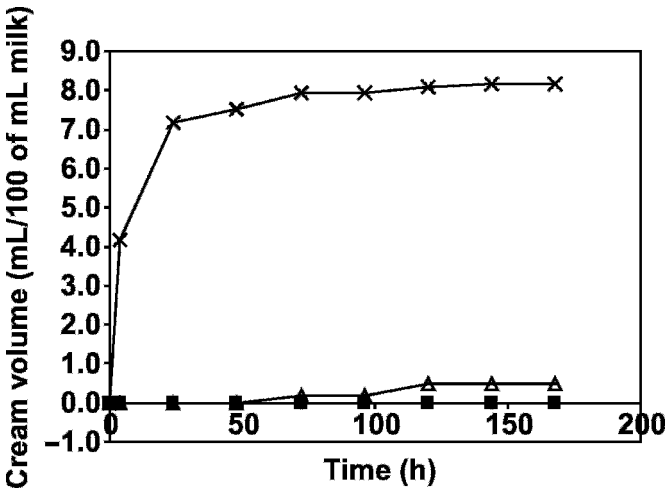

Figure 2. Effect of refrigerated storage time (h) on the rate of creaming of raw milk (×) and treated milks: 200 MPa at 30°C (■), 200 MPa at 40°C (□), 300 MPa at 30°C (▲), 300 MPa at 40°C (○), and high-pasteurized at 90°C for 15 s (△). Results for all ultra-high pressure homogenized samples (■, □, ▲, and ○) were identical and therefore the symbols are overlaid.

the longest microbial shelf life (approximately 21 d) and achieved an outlet temperature of ~80°C for 0.7 s, which means that the thermal effect on milk was less than that of the high pasteurization treatment. However, at 200 MPa and 30°C, a marked decrease of milk pH was observed. With the other UHPH treatments, a microbial shelf life between 14 and 18 d, similar to that observed for high-pasteurized milk, was obtained. Therefore, the microbial data indicate the possibility of obtaining UHPH-treated milk with equal or better microbial shelf life than high-pasteurized milk.

The UHPH treatment, besides achieving a reduction in microbial counts, generated changes in the physicochemical properties such as color, viscosity, pH, and acidity. Color, texture, and mouthfeel are important signals that determine consumer perception of freshness of milk. Nevertheless, differences in instrumental color and viscosity measurements between UHPH-treated milks and PA milks were not visually or sensorially obvious. The UHPH treatment also significantly affected fat globule size, with the formation of aggregates on some occasions; however, no creaming was observed in any UHPH sample.

The treatment performed at 300 MPa at 30°C could be a good option to be used as a one-step homogenization and pasteurization treatment to produce commercial milk with a microbial and physicochemical shelf life equal to that of high-pasteurized milk, and probably with reduced heat effects due to the short holding time needed during treatment. However, further research, which is being carried out currently, is required to evaluate the effects of UHPH on the sensory and nutritional aspects of milk.

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