Effect of moderate inlet temperatures in ultra-high-pressure homogenization treatments on physicochemical and sensory characteristics of milk

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ABSTRACT

The effect of ultra-high-pressure homogenization (UHPH) on raw whole milk (3.5% fat) was evaluated to obtain processing conditions for the sterilization of milk. Ultra-high-pressure homogenization treatments of 200 and 300 MPa at inlet temperatures (T_i) of 55, 65, 75, and 85°C were compared with a UHT treatment (138°C for 4 s) in terms of microbial inactivation, particle size and microstructure, viscosity, color, buffering capacity, ethanol stability, propensity to proteolysis, and sensory evaluation. The UHPH-treated milks presented a high level of microbial reduction, under the detection limit, for treatments at 300 MPa with T_i of 55, 65, 75, and 85°C, and at 200 MPa with T_i = 85°C, and few survivors in milks treated at 200 MPa with T_i of 55, 65, and 75°C. Furthermore, UHPH treatments performed at 300 MPa with T_i = 75 and 85°C produced sterile milk after sample incubation (30 and 45°C), obtaining similar or better characteristics than UHT milk in color, particle size, viscosity, buffer capacity, ethanol stability, propensity to protein hydrolysis, and lower scores in sensory evaluation for cooked flavor.

Key words: ultra-high-pressure homogenization, milk sterilization, UHT milk

INTRODUCTION

Since its development, heat treatment has been by far the most applied technology to enhance milk shelf life, as it destroys pathogen microorganisms and most of the spoilage bacteria (Walstra et al., 2006). The cooked off-flavor, a result of the whey protein denaturation process caused by the temperature increase, is the greatest disadvantage of these treatments, along with the loss of some physicochemical and nutritional quality (Fox and McSweeney, 1998; Datta and Deeth, 2003; Walstra et al., 2006). From studies on the vari-

ous heat treatments applied to milk, UHT treatment may increase shelf life to up to 6 or even 8 mo, with the main problems associated with the treatment being cooked off-flavor development, protein gelation, and nutritional compound destruction (Datta and Deeth, 2003; Cattaneo et al., 2008; Chavan et al., 2011).

For these reasons, the food industry is developing new processes to treat milk, with the intention of reducing the use of heat, using what are known as nonthermal technologies. Within the nonthermal technologies, several applications of ultra-high-pressure homogenization (UHPH; 100–400 MPa), which has previously been used in the pharmaceutical, chemical, and biochemical industries (Popper and Knorr, 1990; Flourey et al., 2000), have already been studied by the food industry, with interesting results. The process is similar to the conventional homogenization used in the dairy industry, with improvements in both the valve material, which helps to reach pressures up to 400 MPa, and the geometry of the valve (Flourey et al., 2004; Diels and Michiels, 2006; Huppertz, 2011). The most important processing parameters are the operating pressure, inlet temperature (T_i), and number of passes (Datta et al., 2005; Diels and Michiels, 2006; Donsì et al., 2009; Dumay et al., 2013).

The UHPH process applied to milk has shown interesting results, such as the achievement of finer emulsions due to particle size reduction (Hayes et al., 2005; Zamora et al., 2007; Donsì et al., 2009), bacterial load reduction (including the reduction of pathogenic microorganisms; Diels and Michiels, 2006; Briñez et al., 2007; Pereda et al., 2007; Donsì et al., 2009), enzyme inactivation (Hayes and Kelly, 2003b; Datta et al., 2005; Hayes et al., 2005; Pereda et al., 2007), structural changes in the milk proteins through partial disassociation, and changes in the coagulation properties of milk (Datta et al., 2005; Sandra and Dalgleish, 2007; Zamora et al., 2007; Grácia-Juliá et al., 2008; Roach and Harte, 2008).

The main mechanisms involved in microbial reduction by UHPH treatment are shear stress, high-speed collisions, impingement, and cavitation (Paquin, 1999; Diels and Michiels, 2006; Donsì et al., 2009; Dumay et

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al., 2013). Even though the UHPH technology can be considered a nonthermal technology, fluid temperature elevation does occur, but for a very small fraction of time, if an appropriate cooling system is applied after the valve. This elevation is due to a variety of phenomena, such as shear stress, turbulence, cavitation, and the transformation of kinetic energy into heat during the pressure increase (Datta et al., 2005; Diels and Michiels, 2006; Donsì et al., 2009). This temperature increase makes an important contribution to microbial reduction and other changes in milk characteristics.

Several authors have suggested the use of UHPH treatment to reach similar levels of microbial load reduction as heat pasteurization. In this regard, Hayes et al. (2005) applied UHPH treatments of 250 MPa at $T_i = 45^\circ\text{C}$ to milk, obtaining a microbial load decrease of 3 log cfu/mL. Furthermore, Pereda et al. (2006), applied UHPH treatments of 300 MPa at $T_i = 30$ and 40°C and achieved microbial reductions of around 4 log cfu/mL. Smiddy et al. (2007) carried out UHPH treatments of 200 and 250 MPa at T_i of 55 and 70°C , reaching microbial reductions of above 5 log cfu/mL. More recently, Ruiz-Espinosa et al. (2013) used UHPH treatments of 250 MPa at $T_i = 25^\circ\text{C}$ with 3 passes, obtaining microbial reductions above 5 log cfu/mL. Other authors have shown that UHPH is able to produce milk with a shelf life similar to heat-pasteurized milk. Similarly, Pereda et al. (2007) attained a shelf life longer than 15 d in UHPH milk treated at 300 MPa with a T_i of 30 and 40°C .

From these studies, it is important to notice the effect of inlet temperatures on microbial load reduction when it is combined with pressure treatments above 250 MPa. Nevertheless, at the moment, only a few studies have been published regarding the use of UHPH treatments with moderate inlet temperatures (between 50 and 90°C) to obtain milk of a high microbiological quality (i.e., sterile milk) while maintaining the sensory characteristics and reducing physicochemical changes. For these reasons, the aim of this study was to explore the possibility of obtaining sterile milk by applying UHPH treatments at moderate inlet temperatures; to evaluate microbial reduction, sensory, and physicochemical changes; and to compare the results to those obtained from UHT milk.

MATERIALS AND METHODS

Milk Supply

Fresh raw bovine milk ($11.72 \pm 0.45\%$ TS and $3.37 \pm 0.09\%$ protein) was obtained from a local producer (Can Badó, La Roca del Vallès, Spain), standardized at $3.5 \pm 0.06\%$ fat, and then stored for 24 h at 4°C .

UHPH and UHT Milk Treatments

The UHPH treatment of milk tested in this study was performed in a Stansted high-pressure homogenizer (model FPG11300; Stansted Fluid Power Ltd., Harlow, UK). The equipment comprises a high-pressure ceramic valve able to support 350 MPa and a second pneumatic valve, located after the first, able to support up to 50 MPa. The high-pressure system consists of 2 intensifiers driven by a hydraulic pump. The flow rate of the milk in the homogenizer was approximately 120 L/h. To minimize temperature retention after treatment and its effect on milk properties, 2 spiral-type heat-exchangers were located behind the second valve, as described by Guamis et al. (2010). The T_i and the temperature both before the first homogenization valve (**T1**) and after the homogenization process (**T2**) were monitored throughout the experiment. In all experiments, the outlet temperature was approximately 15°C . Milk was UHPH treated under the following conditions: 200 and 300 MPa at the first valve with T_i of 55, 65, 75, and 85°C without using the second valve.

Before UHT treatment, a traditional milk homogenization process was carried out in a double-stage Niro Soavi homogenizer (model X68P; GEA Niro Soavi, Parma, Italy) at $18 + 4$ MPa (first valve pressure + second valve pressure) at 65°C . A UHT milk treatment was then applied in a tubular indirect Finamat heat exchanger (model 6500/010; GEA Finnah GmbH, Ahaus, Germany) at 138°C for 4 s. Samples of both treatments were manually collected in sterile bottles (50 mL), in a laminar flow clean air bench cabinet (Mini V/PCR; Telstar, Terrassa, Spain), previously sterilized to obtain aseptic conditions.

Collected samples were immediately cold stored at 4°C , except for those incubated for sterility control. Three replicates of each experiment were conducted.

Compositional Analysis

Protein and fat content were determined by duplicate using the Dumas and Gerber methods, respectively (IDF, 1981, 2002). The TS and ashes analyses were carried out according to the International Dairy Federation (IDF, 1987) and AOAC International (2000; method 945.46) standards, respectively. The milk pH was evaluated at d 1 by electrode immersion with a micro pH-potentiometer (model 2001; Crison Instruments SA, Alella, Spain).

Microbiological Analysis

To determine the microbial load reduction obtained, before treatments the following microbial groups were

enumerated in duplicate: total bacteria count was determined by the pour-plate method in plate count agar (Oxoid Ltd., Basingstoke, UK) and incubated for 48 h at 30°C. To determine the total spore load, 5 mL of milk was heated for 5 min at 80°C and then enumerated by the pour-plate method in plate count agar (Oxoid Ltd.) and incubated for 48 h at 30°C. Lactobacilli and lactococci were enumerated in de Man, Rogosa, and Sharpe (MRS) and M17 agar (Oxoid Ltd.) respectively, pour plated, and incubated for 72 h and 48 h respectively, at 30°C. Coliform bacteria were enumerated on violet red bile glucose (VRBG) agar (Oxoid Ltd.) by pour plating, and incubated for 48 h at 37°C. Coagulase-positive *Staphylococcus* spp. were enumerated on Baird-Parker agar (Oxoid Ltd.) with rabbit plasma fibrinogen (RPF) supplement and incubated for 24 h at 37°C.

To evaluate the sterility of the milk, UHPH samples were incubated at 45 and 30°C for 7 and 15 d, respectively, evaluating the total bacteria count and visually observing the samples. Microbial load reduction was calculated with the difference between the logarithms of colony counts of the untreated (N_0) and treated (N) samples ($\log_{10} N_0 - \log_{10} N$).

Particle Size Determination

The particle size determination of milk samples were carried out using a Beckman Coulter laser diffraction particle size analyzer (LS 13 320 series; Beckman Coulter Inc., Fullerton, CA). Samples were diluted in distilled water until an appropriate obscuration was obtained in the diffractometer cell. 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, and pump speed: 20%. The size distribution was characterized by the diameter below which 50 or 90% of the volume of particles were found (D_{50} and D_{90} , respectively), the Sauter diameter (surface-weighted mean diameter, $D_{3,2}$), and the volume-weighted mean diameter value ($D_{4,3}$). Each sample was measured in triplicate.

Viscosity Determination

The milk sample viscosity was measured in duplicate at 20°C in a rotational rheometer (Haake RheoStress 1; Thermo Electron Corp., Karlsruhe, Germany), using an internal cylinder Z34 (DIN53019) and an external cylinder Z34 (DIN53018). Samples were subjected to a shear rate, increasing from 0 to 140 s^{-1} in 180 s and the experimental flow curves were fitted to the Oswald-de Waele rheological model.

Color Determination

The color values of the milk samples were determined using a Hunter laboratory colorimeter (mini Scan XETM; Hunter Associates Laboratory Inc., Reston, VA), based on the CIE $L^*a^*b^*$ color space [where L^* = lightness ($L^* = 0$ yields black and $L^* = 100$ indicates diffuse white), a^* = position between red/magenta and green (negative values indicate green and positive values indicate magenta), and b^* = position between yellow and blue (negative values indicate blue and positive values indicate yellow)], with an illuminant of D65 and a standard observer of 10° being the colorimeter calibrated against white and black tile standards. Five milliliters of each milk sample was warmed to 20°C before analysis.

Buffering Capacity and Ethanol Stability

The buffering capacity in the milk samples was evaluated adding 100 μL of 0.5 M HCl every 30 s in a 100-mL milk sample. The final buffering capacity was counted when a constant pH of 4.5 was reached (Huppertz et al., 2004a). The ethanol stability in milk samples was determined by the standard alcohol test, according to the Food and Agriculture Organization of the United Nations (FAO, 2000).

Milk Protein Hydrolysis

To evaluate the possible proteolysis by residual milk enzymes after processing, UHT and UHPH milk samples were subjected to incubation at 37°C with a preservation agent (0.05% thimerosal) for 7 d. The pH 4.6-soluble nitrogen fraction in milk was prepared by precipitation of milk with a buffer made of 1 M sodium acetate and 10% acetic acid (pH 4.6), followed by centrifugation at $4,500 \times g$ for 15 min at 4°C. The supernatant containing the pH 4.6-soluble nitrogen fraction was filtered through Whatman 40 paper and kept frozen until used. Proteolysis was calculated as the percentage increase in the pH 4.6-soluble nitrogen fraction in relation to the total nitrogen content of milk, and determined by the Dumas combustion method (IDF, 2002) in raw, UHT, and UHPH milk on d 0 and 7, in duplicate.

Transmission Electron Microscopy

To examine the changes in milk microstructure, raw, UHT, and UHPH milk samples were observed using transmission electron microscopy, preparing the samples as described by Cruz et al. (2007). Milk samples

were mixed with glutaraldehyde (3% final concentration) and then mixed with 2% low-temperature gelling agarose (1:1 ratio). After gelling, the gel obtained was cut into 1-mm³ cubes. After that, the cubes were washed with 0.1 M sodium cacodylate buffer (pH 7.2) for 30 min, which was repeated again after 1 h. Afterward, the cubes were left for 1 h and replaced with a 1-mL solution containing 50% osmium tetroxide (2% solution) and a 50% cacodylate/HCl buffer. The cubes were left for 2 h before being replaced with 1 mL of uranium acetate for 30 min. After that, the sample cubes were washed with water before the dehydration process was applied. Sample dehydration consisted of washing with 50, 70, and 90% ethanol (vol/vol) for 5, 30, and 180 min respectively. The 100% ethanol was changed after 30 and 60 min; EPON resin was then added (to preserve the histological structure) and the sample was laminated in 0.03 to 0.05 slides, which were dyed with acetate uranium and lead citrate to enable observation. Examination was performed with a Philips 201 transmission electron microscope at an acceleration voltage of 60 kV (NL-5600 MD; Philips, Eindhoven, the Netherlands).

Sensory Evaluation

The sensory evaluation of milk samples was carried out by a direct comparative test between UHPH and UHT milk, based on the IDF 99C Standard (IDF, 2009). A panel of 10 trained judges was asked to evaluate sensory attributes, such as color, mouth feel, and a variety of flavors, such as bitter, cooked, salty, rancid, and metallic. The evaluation was carried out using a scale of 0 to 4 (where 0 = no differences compared with UHT milk, 1 = minimal differences, 2 = noticeable differences, 3 = considerable differences, and 4 = extreme differences). The sensory evaluation was undertaken between 2 and 4 d after milk treatments.

Statistical Analysis

The results were analyzed by ANOVA, using the software Minitab 15 (Minitab Inc., State College, PA).

The data were presented as mean $\pm$ standard deviation. The Tukey test was used to compare sample data. Evaluations were based on a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Temperature Increase During UHPH Treatments

It is well known that the temperature of fluid increases after its transit through the homogenization valve in the UHPH treatment. This increase is due to various phenomena, such as shear stress, turbulence, cavitation, and the transformation of kinetic energy into heat during the pressure increase (Datta et al., 2005; Diels and Michiels, 2006; Donsi et al., 2009). Table 1 presents the temperature increase during the UHPH treatments applied in the current study, with measurements of the temperature of the fluid before (T1) and after (T2) it had passed through the homogenization valve. A linear temperature increase was calculated plotting T2 at different pressure treatments (200 and 300 MPa), obtaining a linear increase of 21.12, 19.7, 18.3, and 17.82°C/100 MPa at T_i of 55, 65, 75, and 85°C, respectively.

A temperature increase in milk due to UHPH treatment has been reported by several authors using UHPH equipment with the same valve design (Stansted Fluid Power Ltd.). Pereda et al. (2007) reported an increase rate of 19.5°C/100 MPa in UHPH treatments of 200 and 300 MPa at 40°C. Hayes et al. (2005) described an increase rate of 16.6°C/100 MPa in UHPH treatments from 150 to 250 MPa at 45°C. Furthermore, Hayes and Kelly (2003a) obtained an increase of 17.6°C/100 MPa, applying 50 to 200 MPa at 6 to 10°C. Thiebaud et al. (2003) presented an 18.5°C/100 MPa increase, using 100 to 300 MPa at 4, 14, and 24°C.

As discussed above, the UHPH process generates an increase in fluid temperature. To reduce the effect of this temperature increase on the physicochemical and sensory characteristics of milk, heat exchangers were placed after the homogenization valve, which rapidly

Table 1. Temperature control¹ chart in ultra-high-pressure homogenization treatments²

Homogenization pressure (MPa)	Inlet temperature (°C)	Temperature before the homogenization valve T1 (°C)	Temperature after the homogenization valve T2 (°C)
200	55	63.7 $\pm$ 0.6	108.3 $\pm$ 0.6
	65	74.0 $\pm$ 1.0	115.0 $\pm$ 1.0
	75	80.0 $\pm$ 0.0	117.0 $\pm$ 0.0
	85	84.5 $\pm$ 0.5	120 $\pm$ 0.1
300	55	69.0 $\pm$ 1.0	129.3 $\pm$ 0.6
	65	78.3 $\pm$ 0.6	135.0 $\pm$ 0.0
	75	79.0 $\pm$ 2.7	133.3 $\pm$ 3.1
	85	85.3 $\pm$ 0.2	139 $\pm$ 2.7

¹T1 = temperature before the first homogenization valve; T2 = temperature after the homogenization process.

²Values are means $\pm$ SD (n = 3 independent experiments).

reduced the fluid temperature to 15 to 17°C after treatment. Moreover, it is important to notice that even though a significant increase in the fluid temperature occurred after the homogenization valve (i.e., $T_i = 85^\circ\text{C}$ at 300 MPa with $T_2 = 139^\circ\text{C}$), the residence time was very short (<0.5 s).

Microbial Inactivation

The initial bacterial load of raw milk is shown in Table 2. In this study, a significant reduction in bacteria count was achieved in all treatments applied (~ 5 log cfu/mL), presenting some surviving microorganisms (about 5 cfu/mL) in milks treated at 200 MPa at 55, 65, and 75°C . In contrast, in all treatments applied at 300 MPa and 200 MPa with $T_i = 85^\circ\text{C}$, microbial counts under the detection level were obtained. The differences between the bacteria count inactivation found in the present work and other published studies may be explained by either the use of higher T_i or the use of different numbers of homogenization passes (Diels and Michiels 2006; Donsì et al., 2009; Maresca et al., 2011; Dumay et al., 2013). In this way, the results of the current study were higher than those observed by Picart et al. (2006), using similar pressure levels (100–300 MPa), but applying a T_i of 24°C . In the study of Picart et al. (2006), the bacteria count inactivation achieved was 0.7, 1.7, 2.3, and 2.9 log cfu/mL in UHPH treatments at 100, 200, 250, and 300 MPa, respectively. Furthermore, Thiebaud et al. (2003) obtained lower microbial reductions (1 to 1.15 log cfu/mL) by applying UHPH treatments at 200 MPa and $T_i = 24^\circ\text{C}$. Kheadr et al. (2002) obtained a bacteria inactivation count of 2 log cfu/mL in whole bovine milk (3.4% fat) after treatments at 200 MPa at $T_i = 28^\circ\text{C}$, using 5 passes through the homogenization valve. Moreover, Hayes et al. (2005) reached inactivation levels of 1.83 and 3 log cfu/mL, using 200 and 250 MPa at T_i 45°C , respectively. More recently, Ruiz-Espinosa et al. (2013) applied a response surface method to describe a combination of pressure levels and number of passes at 1 inlet temperature to achieve a 5-log cfu/mL reduction. The results showed that 2 optimized UHPH processes of five 202-MPa passes and four 232-MPa passes at 25°C would obtain a reduction in bacteria counts and *Staphylococcus aureus* (a baroresistant pathogen) of 5 log cfu/mL.

It is relevant to highlight the effect of pressure and inlet temperature on the inactivation obtained. Smiddy et al. (2007) evaluated the effect of pressure/temperature and temperature by itself on the level of inactivation achieved. First, UHPH treatments were applied to milk (200 and 250 MPa at $T_i = 55$ and 70°C), with the effect of temperature then being examined by passing the milk at the inlet temperature (T_i

Table 2. Initial microbial load (log cfu/mL) in raw milk storage at 4°C ¹

Microbiological group	Load (log cfu/mL)
Total bacteria count	5.8 ± 0.3
Spore count	1.0 ± 0.4
Lactococci	5.6 ± 0.3
Lactobacilli	4.3 ± 0.5
Coagulase-positive <i>Staphylococcus</i> spp.	ND ²
Coliforms	4.1 ± 0.4

¹Values are means $\pm$ SD ($n = 3$ independent experiments).

²ND = not detected.

= 55 and 70°C), but without applying pressure. The results showed the significance of combining both effects, as heat treatments by themselves had previously accomplished a maximum reduction of 2 log cfu/mL. However, when UHPH treatments were applied, the initial load decreased to under the detection level (~ 5.3 log cfu/mL). Similarly, Picart et al. (2006) inoculated *Listeria innocua*, *Micrococcus luteus*, and *Pseudomonas fluorescens* in whole milk and evaluated the effect of the UHPH process (100–300 MPa at 24°C), comparing it to the inactivation obtained by heat treatments (from 53 to 87°C for 4 s), based on the temperature of the fluid once it has left the homogenization valve (T_2). After treatments, the inactivation curves for both heat-treated and UHPH treated samples were plotted and the curves intercepted at 70, 65, and 64°C for *L. innocua*, *M. luteus*, and *P. fluorescens*, respectively. Below these intercepting points, the inactivation levels obtained for the 3 respective microorganisms were higher for UHPH treatments than heat treatment. In this section of the curve, those authors suggested that the microbial inactivation in UHPH was essentially due to the mechanical effects [such as shear stress, cavitation and (or) high-speed impacts] generated in the UHPH valve rather than the inactivation mechanisms associated with heat. Above the intercepting point (64 – 70°C), the microbial inactivation achieved was lower in the UHPH treatments than in the heat treatment applied in the milk. In this case, the authors assumed that these results were based on the residence time after the fluid had passed through the homogenization valve (~ 215 ms), being much less than the residence time for heat treatment applied (4 s). Nevertheless, those authors considered that heating the milk for 215 ms probably had no effect by itself in the inactivation achieved; but, it may induce some modifications in the cell membrane. These modifications in the membrane could lead to a synergistic effect with the mechanical effects taking place in the UHPH process.

Moreover, the effect of the heat component of the UHPH treatments on the inactivation achieved is evi-

dent when comparing the results from the current study with those from Pereda et al. (2007), as both studies were carried out using the same equipment. Pereda et al. (2007) evaluated the inactivation obtained through the effect of pressure/ T_i during the UHPH process, with the objective of comparing the effectiveness of UHPH technology with that of the heat pasteurization process. Using UHPH conditions of 200 and 300 MPa, but with less intense T_i (30 and 40°C), no significant difference in the inactivation level was found among treatments (3.2 and 3.5 log cfu/mL, respectively). However, when the results were compared with those from the current study (about 5.8 log cfu/mL), the difference was found to be due to the higher T_i , especially so in the treatments applying more severe T_i (65, 75, and 85°C).

In the current study, no coagulase-positive *Staphylococcus* spp. were found in raw milk samples, whereas other studies (Smiddy et al., 2007) have shown the complete inactivation of *Staph. aureus* in similar treatments (200 and 300 MPa, $T_i = 55$ and 70°C). Ruiz-Espinosa et al. (2013) obtained 2.7 and 6 log cfu/mL of inactivation by using UHPH treatments of 250 MPa at 25°C with 1 and 5 passes, respectively.

Coliforms are used as an index of food contamination. In milk, high counts of coliforms indicate a deficiency in good manufacturing practice during milking, collection, or manipulation. In the current study, the coliform counts in the raw milk were 4.1 log cfu/mL; however, after treatment, counts were under the detection limit. Similar results regarding the complete inactivation of coliform counts were presented by Pereda et al. (2007), Smiddy et al. (2007), and Hayes et al. (2005). Other bacterial groups, such as lactobacilli and lactococci, have different resistance to UHPH, with the latter being more resistant to the UHPH process (Pereda et al., 2007). Nevertheless, in the current study, both microbial groups were inactivated to below the detection limit in all treatments applied. In this regard, Smiddy et al. (2007) reported lactobacilli inactivation rates of 3.8 log cfu/mL in milk treated by UHPH at 200 and 250 MPa with T_i of 55 and 70°C.

To evaluate the sterility of the samples obtained by applying different UHPH treatments (300 MPa at 55, 65, 75, and 85°C and 200 MPa at 85°C), they were incubated at 30 and 45°C for 15 and 7 d, respectively. The microbial presence was evaluated based on protein coagulation (visual observation) and microbial count in plate count agar. The results showed that milks treated at 300 MPa at 75 and 85°C were sterile during the incubation. On the other hand, the samples treated under less intense conditions presented protein coagulation and high microbial counts.

Particle Size Evaluation

Figure 1 shows the effect of UHPH and UHT treatments on particle size distribution with respect to raw milk. A bimodal characteristic distribution was found in raw milk: a 0.2- μ m peak, corresponding to casein micelles, and a second peak of 3.7 μ m, corresponding to milk fat globules. After the treatments were applied, significant changes in particle size distribution could be observed. One of them was the change in the particle distribution from bimodal to monomodal when treatments of 200 MPa were applied, with a considerable increase in the peak of around 0.2 μ m. Moreover, the traditional homogenization carried out in the UHT process showed a change in the typical bimodal distribution of raw milk, with a diminution in the second peak intensity and a displacement of the first peak. In the samples treated at 300 MPa, no change was observed in the bimodal distribution, but a reduction in the intensity in the first peak and the presence of the tail at the end of the second peak (~ 3.7 μ m) could be observed, with the last phenomena caused by the formation of fat aggregates or larger particles. Similar results in the particle distribution of whole milk were presented by Pereda et al. (2007), who applied UHPH treatments at 200 and 300 MPa at T_i of 30 and 40°C.

With regard to the D_{50} and D_{90} parameters, both were reduced by conventional homogenization in UHT treatment from 2.68 and 4.90 μ m in raw milk to 0.45 and 3.2 μ m, respectively (Table 3). The UHPH treatments were capable of a significant reduction in both parameters, with the exception of the 300 MPa/85°C treatment, which produced an increase in the D_{90} parameter. Samples treated at 200 MPa always showed lower values for both parameters than those treated at 300 MPa. Moreover, the T_i was seen to affect the D_{50} and D_{90} parameters in samples treated at 300 MPa, with a progressive increase in both parameters when the temperature increased from 55 to 85°C. This increase in the D_{50} and D_{90} parameters in milk samples treated at 300 MPa was also reported by Pereda et al. (2007) using a lower range of T_i (30 and 40°C).

Moreover, among the UHPH treatments applied, significant differences ($P < 0.05$) were found with regard to the pressure used in the $D_{3,2}$ and $D_{4,3}$ parameters. At the same T_i , $D_{3,2}$ and $D_{4,3}$ values were always lower in samples treated at 200 MPa than in those treated at 300 MPa. Related to the T_i used and for each pressure applied, no significant differences ($P > 0.05$) were found in the $D_{4,3}$ and $D_{3,2}$ parameters, except for the treatments performed at 85°C, in which a significant increase was observed. Pereda et al. (2007) obtained similar results in the particle size distribution param-

Figure 1. Particle size distribution in milk samples. Color version available in the online PDF.

eters in milk treated at 200 and 300 MPa ($T_i = 30$ and 40°C), with significant differences in $D_{3,2}$ between the homogenized-pasteurized milk (18 ± 2 MPa at 90°C for 15 s) and UHPH treatments. Similarly, Thiebaud et al. (2003) presented an increase in both parameters in whole milk when the temperature was increased from 4 to 24°C at 300 MPa, reporting an increase from 0.20 to $1.27 \mu\text{m}$ and 0.73 to $2.31 \mu\text{m}$ for $D_{3,2}$ and $D_{4,3}$, respectively. In addition, Hayes et al. (2005) found similar values in parameter $D_{4,3}$ in UHPH milk treated at 250 MPa and 45°C and commercial pasteurized whole milk (0.86 and $0.88 \mu\text{m}$, respectively).

Variations in parameter $D_{4,3}$ are due to the aggregation of small particles and their presence may be useful

as a means of charting the phenomena of aggregation in food emulsions (Cortés-Muñoz, et al., 2009; Zamora et al., 2012). In UHPH milk samples treated at 300 MPa, these aggregation phenomena can be corroborated by the presence of a tail in the second peak (in the 1.9 - to $2.1\text{-}\mu\text{m}$ range), a consequence of many simultaneous events in the homogenization valve, such as high speed collision between particles, cavitation, and turbulence, among others (Thiebaud et al., 2003; Diels and Michiels, 2006). This behavior has been described in several studies applying UHPH in the range of 250 to 300 MPa, regardless of the food fluid material pressurized, such as soy milk (Cruz et al., 2007; Poliseli-Scopel et al., 2012) or bovine milk (Hayes et al., 2005; Pereda et al., 2007; Zamora et al., 2007). Furthermore, the sensibility of milk protein to aggregation depends on UHPH factors such as pressure and T_i applied, and can lead to severe changes in functional properties by affecting the stability of the micelle components (κ -CN, calcium phosphate, and hydrophobic interactions; Sandra and Dalgleish, 2007; Roach and Harte, 2008). Another explanation for the aggregation phenomena presented at 300 MPa can be the mechanism by which fat coalesces and creams. Fat globules are stabilized by casein micelles, which are the last limiting component in this mechanism (Paquin, 1999; Hayes et al., 2005). The UHPH causes a rupture in the native fat globules, creating more fat globules in the process and, thus, increasing the amount of casein adsorbed into the fat globules to stabilize the system. In this regard, Cano-Ruiz and Richter (1997) reported a significant increase in the amount of casein adsorbed into fat globules when the pressure was elevated from 30 to 90 MPa. However, at pressures above 200 MPa, the amount of available casein may become limiting, leading to the partial ag-

Table 3. Particle size parameters (μm) in raw and treated milks¹

Sample	Diameter ²			
	D_{50}	D_{90}	$D_{3,2}$	$D_{4,3}$
Raw	2.68 ± 0.31^a	4.90 ± 0.38^b	0.43 ± 0.08^a	3.00 ± 0.91^a
UHT ³	0.45 ± 0.02^b	3.20 ± 0.60^c	0.39 ± 0.03^b	1.35 ± 0.38^c
55°C and 200 MPa	0.16 ± 0.04^c	0.33 ± 0.14^f	0.14 ± 0.03^d	0.20 ± 0.09^f
65°C and 200 MPa	0.14 ± 0.01^e	0.23 ± 0.00^f	0.13 ± 0.01^d	0.15 ± 0.01^f
75°C and 200 MPa	0.15 ± 0.01^e	0.24 ± 0.01^f	0.13 ± 0.01^d	0.16 ± 0.01^f
85°C and 200 MPa	0.32 ± 0.07^c	2.78 ± 0.58^d	0.23 ± 0.08^c	0.88 ± 0.01^d
55°C and 300 MPa	0.23 ± 0.02^d	0.82 ± 0.35^e	0.19 ± 0.01^c	0.40 ± 0.06^e
65°C and 300 MPa	0.25 ± 0.03^d	1.46 ± 0.63^d	0.21 ± 0.03^c	0.51 ± 0.10^{de}
75°C and 300 MPa	0.27 ± 0.03^d	2.39 ± 0.64^d	0.22 ± 0.02^c	0.72 ± 0.17^d
85°C and 300 MPa	0.40 ± 0.02^c	7.16 ± 0.29^a	0.23 ± 0.01^c	2.33 ± 0.27^b

^{a-f}Different superscript letters within a column indicate significant differences ($P < 0.05$).

¹Values are means $\pm$ SD ($n = 3$ independent experiments).

² D_{50} = diameter below which 50% of the volume of particles are found; D_{90} = 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.

³UHT = treatment at 138°C for 4 s.

glomeration of very small fat particles not stabilized by casein.

Viscosity Determination

Viscosity is an important property in milk that may change the flow conditions and is also related to the mouthfeel sensory attribute. Significant differences ($P < 0.05$) in viscosity values were found between UHPH samples treated at 300 MPa at 65, 75, and 85°C, with respect to raw, UHT, and UHPH milks treated at 200 and 300 MPa at 55°C (Table 4). Moreover, an increase in viscosity was measured in UHPH-treated milks when the pressure was increased from 200 to 300 MPa. In this case, the sample that achieved the highest viscosity value was the sample treated at 300 MPa with $T_i = 85^\circ\text{C}$. The viscosity increase in UHPH milks treated at higher T_i (75–85°C) and pressure (300 MPa) can be related to the presence of large particles or aggregates due to the insufficient covering by the milk proteins (casein and whey proteins) of the new particles created. When protein is limited, there is no longer sufficient protein to fully stabilize the fat droplet interface and, therefore, larger particles or aggregates may be formed as a result of coalescence or bridging flocculation, increasing the milk viscosity. In this regard, Walstra et al. (2006) described that the presence of larger particles or aggregates increases the viscosity of the milk by increasing the volume of the dispersed phase. Furthermore, the increase in the viscosity when the UHPH samples were treated at the same pressure with an increment in the T_i (from 55 to 65, 75, and 85°C) could be related to the denaturation of whey proteins. This increase in temperature can lead, in cases when the temperature is extremely high, to the insolubility of serum proteins, increasing the viscosity of skim milk

about 10%, due to the increment in the voluminosity of the serum proteins (Walstra et al., 2006). However, these differences in the viscosity measurements in milk samples made by rheological analysis were not detected in the sensory evaluation. Similar results in the increase in viscosity when the pressure in the treated product was augmented were reported by Pereda et al. (2007) in a study applying similar pressure levels (200 and 300 MPa) to milk but at $T_i = 30$ to 40°C ; by Boeneke et al. (2009), who used 50, 125, and 200 MPa; and also by Cortés-Muñoz et al. (2009) in oil-in-water emulsions UHPH treated at 100, 200, and 300 MPa at 24°C .

Color Determination

With regard to the color parameter L^* , the lowest value was for raw milk (89.27 ± 0.75), whereas the UHT and UHPH treatments showed a significant increase (Table 5). Although significant differences ($P < 0.05$) in the parameter L^* between raw and homogenized milk samples were detected, no differences were detected between UHT and UHPH milks. Similar results were reported by Hayes and Kelly (2003a), Hayes et al. (2005), and Pereda et al. (2007), showing an increment in this parameter in treatments of 50, 100, 150, and 200 MPa at 5°C ; 150, 200, and 250 MPa at 45°C ; and 200 to 300 MPa at T_i of 30 and 40°C . According to Walstra et al. (2006), this increase in the parameter L^* is due to an increase in the number of fat globules, which can diffract light more effectively.

With regard to the other color parameters (a^* and b^*), no significant differences ($P > 0.05$) were found among raw, UHT, and UHPH milks. The color parameter a^* showed a small increase with the UHPH process, which was higher in 200 MPa-treated milks. The color parameter b^* showed a reduction when UHPH

Table 4. Viscosity, ethanol stability, and buffering capacity in raw and treated milks¹

Sample	Viscosity (mPa × s)	Ethanol stability (% ethanol)	Buffering capacity ² (mL of 0.5 M HCl)
Raw	1.47 ± 0.2 ^c	80.47 ± 2.17 ^a	ND ³
UHT ⁴	1.42 ± 0.2 ^c	69.17 ± 1.44 ^{gh}	21.55 ± 0.21 ^a
55°C 200 MPa	1.27 ± 0.00 ^c	73.33 ± 1.44 ^{bc}	18.05 ± 0.07 ^{cd}
65°C 200 MPa	1.26 ± 0.1 ^c	74.17 ± 1.44 ^b	17.60 ± 0.28 ^d
75°C 200 MPa	1.29 ± 0.1 ^c	70.83 ± 1.44 ^{ef}	18.75 ± 0.07 ^b
85°C 200 MPa	1.83 ± 0.1 ^b	70.00 ± 0.00 ^{fg}	18.70 ± 0.14 ^{bc}
55°C 300 MPa	1.80 ± 0.1 ^b	72.50 ± 0.00 ^{cd}	18.55 ± 0.07 ^{bc}
65°C 300 MPa	2.17 ± 0.4 ^a	71.67 ± 1.44 ^{de}	19.10 ± 0.14 ^b
75°C 300 MPa	2.10 ± 0.2 ^{ab}	70.00 ± 0.00 ^{fg}	21.10 ± 0.14 ^a
85°C 300 MPa	2.39 ± 0.02 ^a	68.75 ± 1.76 ^h	21.25 ± 0.21 ^a

^{a-h}Different superscript letters within a same column indicate significant differences ($P < 0.05$).

¹Data are means ± SD (n = 3 independent experiments).

²Milliliters needed to reduce the pH to 4.6.

³ND = not determined.

⁴UHT = treatment at 138°C for 4 s.

Table 5. Color parameters for raw and treated milks¹

Sample	Color ²		
	<i>L</i> [*]	<i>a</i> [*]	<i>b</i> [*]
Raw	89.27 ± 0.75 ^c	−2.10 ± 0.38 ^a	6.56 ± 1.02 ^a
UHT ³	92.54 ± 0.22 ^b	−2.10 ± 0.89 ^a	6.79 ± 1.15 ^a
55°C and 200 MPa	92.13 ± 0.69 ^b	−2.67 ± 0.26 ^a	4.95 ± 1.26 ^a
65°C and 200 MPa	92.40 ± 0.26 ^b	−2.64 ± 0.14 ^a	5.38 ± 0.58 ^a
75°C and 200 MPa	92.46 ± 0.28 ^b	−2.57 ± 0.09 ^a	5.39 ± 0.55 ^a
85°C and 200 MPa	93.83 ± 0.35 ^a	−2.62 ± 0.02 ^a	5.40 ± 0.01 ^a
55°C and 300 MPa	92.19 ± 0.24 ^b	−2.36 ± 0.11 ^a	5.50 ± 0.30 ^a
65°C and 300 MPa	92.48 ± 0.24 ^b	−2.33 ± 0.08 ^a	6.03 ± 0.25 ^a
75°C and 300 MPa	92.18 ± 0.31 ^b	−2.36 ± 0.05 ^a	5.74 ± 0.67 ^a
85°C and 300 MPa	92.75 ± 1.20 ^{ab}	−2.37 ± 0.03 ^a	5.79 ± 0.01 ^a

^{a-c}Different superscripts within a column indicate significant differences ($P < 0.05$).

¹Data are means ± SD ($n = 3$ independent experiments).

²*L*^{*} = lightness (*L*^{*} = 0 yields black and *L*^{*} = 100 indicates diffuse white), *a*^{*} = position between red/magenta and green (negative values indicate green and positive values indicate magenta), and *b*^{*} = position between yellow and blue (negative values indicate blue and positive values indicate yellow).

³UHT = treatment at 138°C for 4 s.

technology was applied (fewer yellow tones); nevertheless, among the UHPH samples analyzed, no differences were found ($P > 0.05$). These results for both *a*^{*} and *b*^{*} parameters are contrary to those reported by other authors who have tested UHPH. Pereda et al. (2007) applied both UHPH treatments of 200 and 300 MPa at 30 and 40°C and heat treatment (90°C for 15 s), showing only small changes, with an increase in the *a*^{*} parameter and a decrease in the *b*^{*} parameter, depending on the UHPH treatment applied. Hayes and Kelly (2003a) and Hayes et al. (2005), with relatively less intense UHPH treatments (50, 100, 150, and 200 MPa at 7 and 45°C), presented small changes in both parameters. Nevertheless, even though significant differences existed in the instrumental evaluation of the samples, no visual differences were perceived among UHT and UHPH milks.

Buffering Capacity Determination

With regard to buffering capacity in milk samples, significant differences ($P < 0.05$) can be observed between some UHPH samples and UHT milk (Table 4). First, among UHT milk and UHPH treatments at 300 MPa at 65, 75, and 85°C, no significant differences were detected ($P > 0.05$), requiring similar amounts of 0.5 *M* HCl to reduce their pH to 4.6. However, between UHT and UHPH treatments of 300 MPa at 55°C and 200 MPa at 55, 65, 75, and 85°C, significant differences were detected ($P < 0.05$), presenting lower amounts of acid (from 17.5 to 19 mL of 0.5 *M* HCl) to achieve the same final pH value. Only small differences between the UHPH and UHT milks were found in the first acidification stages (pH 6.5 to 5.5), indicating similar buffering capacity of milks at this pH range. However, in the

latter stages of acidification (pH 5.5 to 4.6), UHPH milks treated at 300 MPa with *T*_i of 75 and 85°C and UHT milks required higher amounts of acid, indicating a higher buffering capacity for these milks compared with the other UHPH milks in this pH range (Figure 2).

The milk components that contribute to buffering capacity are soluble phosphate, colloidal calcium phosphate, citrate, bicarbonate, caseins, and whey proteins (McCarthy, 2002). Although slight differences exist between reports with regard to the contribution of milk components to buffering capacity, the most common distribution is 40, 35, 20, and 5% for soluble minerals, caseins, colloidal calcium phosphate, and whey proteins, respectively (Salaün et al., 2005). According to Huppertz et al. (2004a), the buffering capacity of milk is determined by the calcium phosphate solubilization speed, with an inverse relationship between these parameters. This increase in the buffering capacity of milk that corresponds inversely with decreasing pH at values <5.5 is the result of the solubilization of colloidal calcium phosphate, which takes place during milk acidification. It is well known that heat treatment leads to a decrease in soluble calcium and inorganic phosphate, due to the precipitation of calcium phosphate, causing an increase in the mineral level in the colloidal phase, which may be reversed, depending on the intensity of the treatment (Gaucheron, 2005). Moreover, Zamora et al. (2007) observed that the amount of minerals in rennet whey from milk UHPH treated at 300 MPa was lower than that produced by other treatments that applied lower pressures, suggesting a mineral transfer from the soluble to the colloidal phase caused by heat during UHPH treatment. The higher buffering capacity of milk samples treated at 300 MPa with *T*_i of 75 and

Figure 2. Buffering capacity in UHT milk and ultra-high-pressure homogenized (UHPH) milk. Color version available in the online PDF.

85°C and UHT milks in the pH range 5.5 to 4.6 may be related to the solubilization of their colloidal calcium phosphate, which increases the levels of soluble calcium and phosphate even further compared with the other milk samples, thus providing greater buffering capacity.

Ethanol Stability Determination

Table 4 shows the ethanol stability of milks, which is the highest ethanol concentration that can be applied without causing protein coagulation. In general, the UHPH milks presented higher ethanol stability than UHT milk, except those treated at 75 and 85°C at 300 MPa, and at 85°C at 200 MPa, which presented similar values. In general, no great differences were found among samples treated at the same T_i (55, 65, 75, and 85°C) and with the same pressures applied, but a decrease in ethanol stability could be observed when the pressure was increased from 200 to 300 MPa. Even though the mechanisms involved in the UHPH and high hydrostatic pressure are different, some points can be compared with regard to the pressure (only for a short period of time in UHPH) and the elevated temperature, which is quickly reduced by a heat exchanger. Similar results were reported by Huppertz et al. (2004b), who used high hydrostatic pressure on milk (250, 400, and 600 MPa at 20°C for 30 min) and evaluated the ethanol stability of the samples both immediately and 24 h after treatment. The results showed a reduction in the ethanol stability when high hydrostatic pressure was applied, decreasing from approximately 83% in untreated milk to approximately 64% in milk treated at 600 MPa. The decrease in ethanol stability was related to an increase in the soluble calcium present in high pres-

surized milk and the possible disassociation of κ -CN, due to micelle disintegration during treatment, causing a reduction in the steric stability of the casein micelles (Horne and Muir, 1990; Huppertz et al., 2004b). The effect of heat treatment on the ethanol stability of milk has been demonstrated by the molecular rearrangement of the soluble calcium to colloidal calcium, which then affects the steric stability of the micelle (Horne and Muir, 1990; Fox and McSweeney, 1998). In the case of the UHPH treatments, the mechanism that reduces the ethanol stability in milk could be explained by the combined effect of the mechanisms involved in the high hydrostatic pressure and heat treatments. Sandra and Dalgleish (2005) evaluated the modifications in the micelle casein structures in milk after UHPH treatment in the range of 41 to 186 MPa, and found a significant decrease in the micelle size with an increase of non-sedimentable caseins in the milk serum, such as κ -CN, which may cause a reduction in the steric stability of the casein micelles. Zamora et al. (2007) evaluated the amount of calcium in the serum in UHPH treatments of 200 and 300 MPa at 30°C and measured a decrease in the level of calcium in the serum, suggesting a mineral transfer from the soluble to the colloidal phase of milk. The lower ethanol stability of UHPH-treated milks, in comparison with raw milk, may be related to the higher calcium values and the dissociation of κ -CN in micelles produced by the UHPH treatment.

Milk Protein Hydrolysis

Plasmin and its inactive precursor plasminogen are the main proteolytic enzymes in milk that can survive severe heat treatments (Enright and Kelly, 1999). Their activity and proteolysis, in general, generate sensory problems in commercial sterile milks, because of the partial hydrolysis of β - and α_{s2} -CN (and more slowly in α_{s1} -CN), which leads to bitter and astringent flavor changes (Fajardo-Lira and Nielsen, 1998; Enright and Kelly, 1999; Santos et al., 2003). Psychrotrophic bacteria are another highly heat resistant proteolytic enzyme source in milk. These enzymes can survive UHT treatments and often cause a loss of quality during storage (Muir and Banks, 2000).

Among the treatments applied in this study, no significant differences ($P > 0.05$) were found between the soluble nitrogen found in milk samples treated by UHPH and UHT and then incubated for 7 d at 37°C with a preservation agent (data not shown). From this result, one can conclude that native or microbial milk proteases are inactivated by these treatments (UHPH or UHT) under the conditions that were used. Pereda et al. (2008) studied the proteolysis developed in milk treated by UHPH, applying similar pressure levels (200

Figure 3. Transmission electron micrographs of raw (A) and UHT (B) at 10,000 $\times$ magnification, and ultra-high-pressure homogenized (UHPH) milks treated at 200 MPa at 55°C (C) and at 85°C (E), and 300 MPa at 55°C (D) and at 85°C (F) at 10,000 $\times$ and 30,000 $\times$ magnification (bars represent 1 and 0.5 μ m, respectively).

and 300 MPa) but with lower T_i (30 and 40°C) during cold storage at 4°C, observing an increase in the inactivation of plasmin as the homogenization pressure increased. During storage, UHPH milk samples showed an increase in soluble nitrogen at pH 4.6, in which the sample at 200 MPa at 40°C obtained the highest increase, due to residual plasmin activity.

Transmission Electron Microscopy

The images obtained by transmission electron microscopy confirmed that the particle size results from the different treatments applied. Micrographs of raw milk (Figure 3A) show the typical native milk microstructure, with the characteristic sizes in both main components of milk (fat globule and casein micelle). In the UHT milk sample (Figure 3B), the fat globule size was significantly reduced and surrounded by submicelles or small micelle fractions with some aggregation phenomena, as can be observed in a conventional homogenization process. With regard to raw and UHT milk samples, UHPH-treated milk samples (Figure 3C–F) presented minor particle sizes (being more evident at 200 MPa) and different behavior, depending on the pressure and T_i used. In relation to the pressure applied, the samples treated at 200 MPa (Figure 3C and E) presented more homogenous particles ($\sim 0.2 \mu$ m) compared with the samples treated at 300 MPa (Figure 3D and F), in which an aggregation phenomena can be observed, as constituted by small fat globules, casein micelles, and possibly whey proteins undergoing a bimodal distribution. In relation to the T_i applied,

some differences still remain among UHPH treatments, where they form more aggregates with more intense treatments. This behavior was more evident between 55 and 85°C treatments, where considerable aggregates could be observed.

Sensory Evaluation

Results from sensory tests showed no significant differences ($P > 0.05$) in color, mouthfeel, or flavor defects such as bitterness, rancidity, or acid among the UHPH treatments applied compared with UHT milk as a reference (data not shown). Furthermore, UHPH milks presented less intense cooked flavor and slightly less salty flavor compared with UHT milk. In this regard, Boeneke et al. (2009) also reported no differences in flavor, appearance/color, and mouthfeel between whole milk treated by UHPH at 200 MPa and heat treated, evaluated over a period of 3 wk.

CONCLUSIONS

The UHPH treatments at different T_i proved to be efficient in achieving significant microbial inactivation close to the detection limit (200 MPa) or under the detection limit (300 MPa). During UHPH milk incubation at 30 and 45°C, treatments performed at 300 MPa with T_i of 75 or 85°C seem to be the most adequate processing conditions to obtain milk sterilization with UHPH technology. The UHPH reduced particle size and produced milk with better ethanol stability compared with UHT milk, while maintaining a similar buffering capacity. Furthermore, these treatments were able to produce milk with less intense cooked flavor than the UHT milk, with no difference in color, mouthfeel, rancidity, or acidity being found, either. Even though more studies of sterile UHPH milk after its aseptic packaging and during its storage are needed to understand changes related to the UHPH process and milk storage, the results have shown that UHPH treatments at 300 MPa with T_i of 75 or 85°C are a real alternative to heat processing.

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