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Effects of Ultra-High-Pressure Homogenization Treatment on the Lipolysis and Lipid Oxidation of Milk during Refrigerated Storage

JULIETA PEREDA, VICTORIA FERRAGUT, JOAN MIQUEL QUEVEDO, BUENAVENTURA GUAMIS, AND ANTONIO J. TRUJILLO*

Centre Especial de Recerca Planta de Tecnologia dels Aliments (CERPTA), XaRTA, XiT, Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

Free fatty acid (FFA) release and quantification and lipid oxidation extent of ultra-high-pressure homogenized (UHPH) milk samples were evaluated to assess the effect of UHPH on the susceptibility of milk lipids to lipolysis and oxidation. Milk was UHPH-treated at 200 and 300 MPa with inlet temperatures of 30 and 40 °C. UHPH-treated samples were compared to high-pasteurized milk (PA; 90 °C, 15 s). Results showed that all FFA increased significantly during storage only in 200 MPa samples. Lipid oxidation was measured as an accumulation of lipid hydroperoxides as the primary oxidation product and malondialdehyde and hexanal as the secondary oxidation products. Samples treated at 300 MPa presented higher malondialdehyde and hexanal content compared to 200 MPa treated-samples and to PA milk.

KEYWORDS: Milk; lipolysis; lipid oxidation; ultra-high-pressure homogenization

INTRODUCTION

The development of off-flavors in milk can reduce the shelf life and quality of pasteurized fluid milk (*1*), with lipolysis, lipid oxidation, and the growth of bacteria being the main phenomena that participate in the modification of the overall flavor. The hydrolysis of triglycerides into free fatty acids (FFA) and partial glycerides is called lipolysis in milk. This reaction can be either spontaneous or induced. The former one occurs when milk is cooled to <10 °C just after milking, while induced lipolysis is related to physical changes in the original fat globule membrane (MFGM) occurring during agitation, foaming, homogenization, and freezing (*2*).

Lipolysis in milk can be catalyzed by two enzymatic processes, one of them caused by the native milk lipoprotein lipase (LPL; EC 3.1.1.34) and the other by microbial lipases produced by psychrotrophic microorganisms. Sine LPL is relatively unstable to heat, pasteurization inactivates most of the enzyme, and therefore, lipolysis does not occur in pasteurized homogenized milk (*2*). When lipolysis occurs in milk, it produces detrimental aspects such as flavor and functionality defects, and the short- and medium-chain FFA ($C_{4:0}$-$C_{12:0}$) released are those that cause the appearance of rancid flavors (*3*). Although flavor defects are the most likely result of lipolysis in milk, other consequences may arise from an elevated level of FFA such as a decrease in milk foaming ability due to partial glycerides, which are surface active and displace the foam-stabilizing proteins at the air−water interface of the foam bubbles (*4*). However, FFA also plays an important role in providing the desirable flavor of milk products, such as cheese.

Lipid oxidation is a chemical reaction that has received much attention because of its contribution to a decrease in sensory and nutritional qualities of foods. Cholesterol, fatty acids of triacylglycerols, partial glycerides, and free fatty acids, particularly unsaturated fatty acids, are vulnerable to oxidation and give rise to unstable hydroperoxides, which decompose into a wide range of carbonyl compounds, such as saturated and unsaturated aldehydes followed by lesser amounts of unsaturated ketones, saturated and unsaturated hydrocarbons, semialdehydes, and saturated and unsaturated alcohols (*5*). The oxidative stability of milk is also affected by diverse pro-oxidative factors (such as the presence of oxygen, light, endogenous and exogenous metals, and enzymes such as xanthine oxidase and lactoperoxidase), antioxidative factors (i.e, ascorbic acid, tocopherols, carotenoids, thiols, and enzymes such as superoxide dismutase) and the balance between pro- and antioxidative factors (*5, 6*).

In general, thermal treatment improves the safety and shelf life of milk; however, the flavor and nutritional value can be negatively affected (*7*). Consumer acceptance of fluid milk is determined mainly by its sensory characteristics and by its nutritional value; therefore, alternative nonthermal technologies are being studied. One of these alternative technologies is ultra-high-pressure homogenization (UHPH), which is based on the same principle as conventional homogenization but works at higher pressures, up to 400 MPa compared to 20−50 MPa

* Corresponding author.Tel: +34. 935813292. Fax: +34 0.935812006. E-mail: Toni.Trujillo@uab.es.

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for conventional homogenization. Cavitation, friction, turbulence, and shear stress are the forces encountered during UHPH (*8*). In recent years, applications of UHPH have been described in milk (*9*), cheese (*10*), and yogurt (*11*) production. Reports on the effect of UHPH on milk have demonstrated its ability to reduce microbial counts (*9, 12*), denaturate whey proteins (*10, 13*), inactivate indigenous milk enzymes such as plasmin and alkaline phosphatase (*13, 14*), and drastically reduce fat globule size (*9, 15, 16*). The initial step in lipid oxidation in emulsions takes place at the interface between the oil and water phases (*17*). It is important to note that high-pressure homogenization reduces the droplet size and consequently increases the total interfacial area of the emulsion. One would therefore expect the rate of lipid oxidation to increase as the droplet size of emulsion decreases because a greater amount of lipid would be exposed to the aqueous phase. However, some studies in emulsions support this hypothesis, while other studies have found no dependence of the lipid oxidation rate on droplet size (*18*).

Until now, it is known that UHPH reduces fat globule size and also that UHPH appears to enhance the activity of LPL compared to a thermal treatment (*19*); however, it is not known whether this reduction of fat globule size and residual LPL activity is detrimental for lipid oxidation and lipolysis of milk. Therefore, the aim of the present work has been to study the FFA profile and lipid oxidative changes during storage at 4 °C in milk submitted to UHPH treatments and in thermally processed milk.

MATERIALS AND METHODS

Milk Supply. Fresh raw whole milk (11.6 ± 1.0% total solids and 3.29 ± 0.03% protein) was obtained from a local dairy farm (Can Badó, Barcelona, Spain). Milk was standardized at 3.5 ± 0.2% fat, and then it was kept overnight at 4 °C.

Ultra-High-Pressure Homogenization and Heat-Pasteurization of Milk. UHPH treatments were applied to the milk samples using a Stansted high-pressure homogenizer (model FPG11300, Stansted Fluid Power Ltd., Essex, UK). This high-pressure machine comprises two intensifiers, driven by a hydraulic pump and a high-pressure valve made of resistant ceramics capable of supporting 350 MPa. It also comprises a second pneumatic valve capable of supporting 50 MPa located behind the first one. The flow rate in the machine during the process was 120 L/h. Milk was treated at 200 and 300 MPa with inlet temperatures (Ti) of 30 and 40 °C. The inlet temperatures of milk were reached by passing milk through a heat exchanger located behind the feeding tank. During UHPH treatments an increase in milk temperature (∼19 °C per 100 MPa) is produced as a consequence of the adiabatic heating generated in the machine in addition to the high turbulence and shear and cavitation forces that the fluid suffers in the homogenization valve. Throughout the experiment, the range of milk temperature was 34 to 44 °C before the first valve and 74 to 101 °C before the second valve. In order to minimize temperature retention after treatment, two spiral type heat-exchangers (Garvía, Barcelona, Spain) located behind the second valve were used; therefore, the holding time at the highest temperature was very short, around half-second.

Ultra-high-pressure-treated samples were compared with a high-pasteurized milk (PA). Two-stage homogenization (18 MPa plus 2 MPa) and pasteurization (90 °C for 15 s) of raw milk were carried out with a Niro Soavi homogenizer (model X68P, Parma, Italy) and a Finamat heat exchanger (model 6500/010, Gea Finnah Gmbh, Ahaus, Germany), respectively.

Milk samples were collected and stored at 4 °C in the dark. Immediately after treatment and at each day of sampling (7, 14, and 18 days), milk samples were frozen at − 80 °C until lipolysis and oxidation analyses were performed. Three replications were performed.

Lipolysis. *Free Fatty Acids Analysis.* Free fatty acids were extracted according to the method described by de Jong and Badings (*20*). Ten milliliters of milk was mixed in a screw-capped tube with 1 g of anhydrous Na_2SO_4, 10 mL of ethanol, 1 mL of H_2SO_4 (2.5 M), and 30 μL of internal standard solution (heptanoic acid 36.8 mg mL^{-1}). Dry diethyl ether/heptane (10 mL 1:1 v/v) was added, and the mixture was shaken for 3 min using a vortex mixer. After centrifugation at 2500 rpm for 2 min, the supernatant was transferred to a screw-capped tube containing 1 g of anhydrous Na_2SO_4. Extraction was repeated twice but with 4 mL of dry diethyl ether/heptane.

Isolation of FFA from the ether/heptane extract was done using a solid phase extraction technique with aminopropyl column Spe-ed NH_2 500 mg·3 mL^{-1} (Applied Separations, Allentown, PA, USA). The aminopropyl column was conditioned with 10 mL of heptane before the lipid extract was applied to the column. Then hexane/2-propanol (20 mL; 3:2 v/v) was used to eliminate glycerides, and finally, the FFA were eluted with 5 mL of dry diethyl ether containing 2% formic acid. All organic solvents were purchased from Panreac (Barcelona, Spain), while formic acid and sulfuric acid were acquired from Sigma Aldrich (St Louis, MO, USA). All chemical used were of analytical grade.

Analysis of FFA was carried out with an HP 6890 Series II gas chromatograph (Hewlett-Packard Inc., Wilmington, DL, USA) using a fused silica capillary column, (30 m × 0.32 mm i.d.), coated with DB-FFAP with a film thickness 0.25 μm (J&W Scientific, Folsom, California, USA) and a flame-ionization detector. Helium was the carrier gas with a constant flow of 2 mL min^{-1}. The initial column temperature of 75 °C was maintained for 1 min, then raised to 240 °C at a rate of 5 °C min^{-1}, and then held at 240 °C for 21 min. Injection was done in split mode with a 7:1 split ratio and an injector temperature of 250 °C. The detector temperature was 300 °C. Each fatty acid was identified with reference to the retention time of the analytical grade standards supplied by Sigma Aldrich (St Louis, MO, USA), and quantified with respect to the internal standard ($C_{7:0}$).

Indicators of Lipid Oxidation. *Hydroperoxide Value Determination.* Lipid hydroperoxides in milk were determined by using the method described by Ostdal et al. (*21*). Milk (2 mL) was mixed with 2 mL of methanol. Then, 4 mL of chloroform was added and vortexed for 30 s. After centrifugation for 10 min at 12000*g*, 1 mL of the chloroform phase was transferred to a test tube, was mixed with 1 mL of Fe (II)/thiocyanate in methanol/chloroform, and was kept at room temperature for 5 min to allow the reaction. This reaction consists of the oxidation of ferrous to ferric ion by hydroperoxides in the presence of ammonium thiocyanate to produce ferric thiocyanate whose absorbance can be measured at 500 nm. Milk samples were analyzed in quadruplicate, and data were expressed as units of absorbance.

Malondialdehyde−Thiobarbituric Acid (MDA-TBA) Determination. Secondary oxidation products such as MDA were determined with the spectrophotometric method described by Fenaille et al. (*22*). Milk (3 mL) was mixed with 2.4 mL of 5% trichloroacetic acid (Sigma Aldrich, St Louis, USA) and 2.4 mL of 0.8% butylated hydroxytoluene (Sigma Aldrich, St Louis, USA) in ethanol. The mixture was centrifuged at 2700*g* for 5 min, and then the upper phase was filtered through Whatman paper No. 1 and heated at 70 °C for 1 h. After cooling, the third derivative spectrum of MDA-TBA solutions was recorded from 400 to 650 nm at a scanning speed of 800 nm/min against the blank reaction mixture. Peak height measurements were performed at 521.5 nm. During this TBA analysis, besides MDA other substances such as ketones, ketosteroids, acids, esters, sugars, imides and amides, amino acids, oxidized proteins, and pyridines and pyrimidines can react with the TBA, and are called TBARS (substances that react with TBA) (*22*). The concentration of these TBARS in samples was determined using an external calibration curve of malondialdehyde and was expressed as μg MDA/mL milk. Three replicates of each sample were analyzed.

Hexanal Content. Hexanal was analyzed using a solid phase microextraction technique with a 50/30 μm DVB/Carboxen/PDMS fiber (Supelco Inc., Bellefonte, PA, USA) in combination with gas chromatography−mass spectrometry according to the method described by Pereda et al. (*23*). In order to quantify hexanal content, a standard addition of 0 to 15 ppb of hexanal (Sigma Aldrich, Steinheim, Germany) was made in a milk sample with a low level of this compound.

Statistical Analysis. Results were analyzed by an analysis of variance ANOVA using the general linear models procedure of Statistical Analysis System (SAS, 2004). Data are presented as least-

Table 1. Means ± Standard Deviation of Free Fatty Acids (mg FFA/L milk) Found in Raw, Ultra-High-Pressure Homogenized (200 MPa 30 °C, 200 MPa 40 °C, 300 MPa 30 °C, and 300 MPa 40 °C), and High-Pasteurized Milk Samples during Refrigerated Storage at 4 °C[a]

		treatment					
	day	raw	200 MPa 30 °C	300 MPa 30 °C	200 MPa 40 °C	300 MPa 40 °C	PA[b]
C4:0	1	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.1
	7		1.7 a ± 0.7	0.2 b ± 0.0	0.3 b ± 0.1	0.2 b ± 0.0	0.3 b ± 0.1
	18		4.3 a ± 3.5	0.3 b ± 0.1	0.6 b ± 0.2	0.3 b ± 0.1	0.4 b ± 0.3
C6:0	1	0.8 a ± 0.2	0.7 a,b ± 0.4	0.6 a,b ± 0.2	0.5 b ± 0.1	0.5 a,b ± 0.1	0.5 a,b ± 0.2
	7		2.6 a ± 0.7	0.5 b ± 0.1	0.6 b ± 0.3	0.4 b ± 0.0	0.5 b ± 0.1
	18		6.0 a ± 5.2	0.5 b ± 0.1	1.3 b ± 0.2	0.5 b ± 0.1	0.6 b ± 0.3
C8:0	1	0.8 b ± 0.1	1.0 a ± 0.3	0.7 b,c ± 0.2	0.5 c,d ± 0.0	0.5 d ± 0.0	0.6 c,d ± 0.1
	7		3.5 a ± 1.4	0.6 b ± 0.1	0.7 b ± 0.3	0.5 b ± 0.0	0.5 b ± 0.2
	18		7.5 a ± 6.3	0.6 b ± 0.2	1.6 b ± 0.4	0.6 b ± 0.1	0.6 b ± 0.3
C10:0	1	1.9 b ± 0.1	2.6 a ± 0.8	1.8 b ± 0.4	1.3 b ± 0.2	1.4 b ± 0.1	1.6 b ± 0.3
	7		9.0 a ± 4.4	1.5 b ± 0.1	1.8 b ± 0.8	1.3 b ± 0.1	1.5 b ± 0.3
	18		19.3 a ± 14.3	1.5 b ± 0.5	4.4 b ± 1.1	1.5 b ± 0.2	2.0 b ± 1.0
C12:0	1	3.1 b ± 0.3	4.8 a ± 1.7	3.1 b ± 0.6	2.6 b ± 0.2	2.5 b ± 0.2	3.4 b ± 1.1
	7		13.4 a ± 5.8	2.6 b ± 0.3	3.4 b ± 1.7	2.2 b ± 0.3	3.3 b ± 1.2
	18		27.5 a ± 20.0	2.9 b ± 0.7	6.5 b ± 2.1	2.8 b ± 0.4	3.6 b ± 1.7
C14:0	1	6.6 b,c ± 0.6	11.9 a ± 2.5	6.9 b,c ± 1.7	5.8 c ± 0.8	5.6 c ± 0.4	8.5 b ± 2.0
	7		35.9 a ± 19.0	6.0 b ± 0.2	7.8 b ± 2.7	5.1 b ± 0.3	8.1 b ± 2.2
	18		62.6 a ± 32.5	6.5 b ± 1.0	14.7 b ± 3.2	6.4 b ± 1.7	8.7 b ± 2.8
C16:0	1	20.8 c ± 3.0	45.9 a ± 5.1	23.3 c ± 6.6	20.5 c ± 4.6	19.6 c ± 2.8	31.5 b ± 6.0
	7		138.1 a ± 80.9	20.2 b ± 2.3	26.2 b ± 7.3	17.2 b ± 1.8	29.8 b ± 6.7
	18		215.8 a ± 84.0	22.6 b ± 2.0	51.2 b ± 9.9	22.4 b ± 8.9	32.1 b ± 7.7
C18:0	1	4.6 b ± 0.8	9.2 a ± 1.3	5.8 b ± 2.8	4.1 b ± 1.3	4.0 b ± 0.5	6.1 b ± 1.8
	7		25.4 a ± 10.5	4.1 b ± 0.5	4.8 b ± 1.2	3.6 b ± 0.5	5.3 b ± 1.2
	18		46.3 a ± 26.1	4.6 b ± 0.5	10.3 b ± 3.1	4.2 b ± 1.4	6.3 b ± 1.3
C18:1	1	13.1 b ± 2.8	24.4 a ± 2.0	15.2 b ± 6.4	12.0 b ± 4.9	11.3 b ± 3.4	15.7 b ± 1.3
	7		103.4 a ± 50.9	12.6 b ± 2.4	16.3 b ± 4.6	10.7 b ± 2.0	14.4 b ± 0.6
	18		193.5 a ± 101.3	14.3 b ± 3.0	27.0 b ± 13.0	13.2 b ± 5.8	18.4 b ± 4.8
C18:2	1	0.6 b ± 0.2	0.9 a ± 0.3	0.5 b ± 0.1	0.5 b ± 0.1	0.5 b ± 0.1	0.6 b ± 0.1
	7		5.3 a ± 2.4	0.5 b ± 0.0	0.7 b ± 0.3	0.6 b ± 0.3	0.6 b ± 0.1
	18		7.8 a ± 6.0	0.6 b ± 0.1	1.8 b ± 0.3	0.6 b ± 0.2	0.8 b ± 0.4
total	1	52.7 b,c ± 6.7	101.7 a ± 14.6	58.2 b,c ± 7.7	48.2 c ± 6.6	46.0 c ± 6.3	68.8 b ± 9.9
(∑C4−C18:2)	7		338.4 a ± 47.8	48.9 b ± 6.6	62.6 b ± 8.5	41.9 b ± 5.6	64.3 b ± 9.4
	18		590.8 a ± 79.2	54.5 b ± 7.4	119.4 b ± 16.0	52.5 b ± 7.2	73.5 b ± 10.3

[a] Different letters in the same row indicate significant differences ($P < 0.05$). [b] PA = High-pasteurized milk (90 °C for 15 s).

square means. The Tukey test was used for the comparison of sample data. Evaluations were based on a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Free Fatty Acids. At the first day of storage, total FFA content was approximately double ($P < 0.05$) in a UHPH-treated sample at 200 MPa 30 °C compared with the other UHPH samples and PA milk (**Table 1**), with $C_{18:0}$, $C_{18:1}$, and $C_{16:0}$ being the major FFA detected in milk samples. Esterification of principal fatty acids in milk is not random: palmitic acid ($C_{16:0}$) and stearic acid ($C_{18:0}$) are sterified mainly at position sn-1 of triglycerides, while unsaturated fatty acids are esterified mainly at positions sn-1 and sn-3 of triglycerides. Lipolysis in milk can be produced by natural lipases, such as LPL, which is completely inactivated by an HTST heat treatment (72 °C × 15 s) or by microbial lipases from psychrotrophic bacteria, which are thermoresistant at the temperatures of pasteurization and even to UHT treatments (*4*). The LPL hydrolyses preferentially positions 1 and 3 of long-chain triglycerides (*4*), which is related to the higher level of $C_{16:0}$, $C_{18:0}$, and $C_{18:1}$ FFA. In treatment at 200 MPa 30 °C, temperature achieved after the high-pressure valve was around 75 °C, while higher temperatures (from 85 to 100 °C depending on the combination of pressure-temperature) were reached in the other UHPH treatments. Probably, 75 °C combined with the short time at which the fluid is maintained at this temperature was not high enough to inactivate milk LPL completely. As a consequence of the damage to the original MFGM and the reduction of fat globule size after homogenization treatments, this enzyme could easily gain access to the fat and could find greater interfacial fat surface area on which to act, generating lipolysis in the milk. Hayes and Kelly (*15*) working with UHPH at pressures from 50 to 200 MPa observed a decrease in pH after refrigeration at 4 °C for 24 h, and they suggested thatLPL was probably not completely inactivated by the conditions of pressure−temperature used in the study. Several authors have studied the effect of UHPH on the inactivation of milk enzymes such as plasmin, lactoperoxidase, and alkaline phosphatase (*9, 14, 19*). However, the effect of UHPH on LPL is not well understood. Datta et al. (*19*) studied the inactivation of milk lipase by UHPH, and they observed that UHPH did not cause more inactivation of LPL than the corresponding thermal treatment; on the contrary,

pressure homogenization treatment seems to enhance the activity of the enzyme.

During refrigerated storage at 4 °C, total FFA content increased in all samples; however, this increase was statistically significant ($P < 0.05$) between days only for samples treated at 200 MPa at both inlet temperatures. As was explained before, the signs of lipolysis in 200 MPa and 30 °C were observed from the first day of storage, total FFA concentration of this sample being approximately 100 mg FFA/L compared to 50 mg FFA/L in the other milk samples. In the case of 200 MPa and 40 °C, the increase in FFA started to be noticeable from the seventh day of storage.

In a previous study, Pereda et al. (*9*) reported the presence of *Pseudomonas* spp. at the end of storage (day 18) in milk treated at 200 and 300 MPa at Ti = 40 °C, which could produce microbial lipases. The delay in the increase of FFA content toward the end of storage in the milk sample treated at 200 MPa 40 °C combined with temperature achieved in this treatment (85 °C), able to inactivate LPL, and the presence of *Pseudomonas* spp., suggests that lipolysis could be a consequence of the microbial lipases but not caused by LPL. However, although *Pseudomonas* spp. were present both in samples treated at 200 and 300 MPa at Ti = 40 °C, 300 MPa at 40 °C-treated milk did not experience such an intense lipolysis at the end of storage as 200 MPa 40 °C milk did. This difference could be explained by the presence of clusters of fat globules that have been observed by different authors (*9, 13, 16*) in milk samples treated at 300 MPa, which could produce an increase in the fat droplet particle size and therefore less interfacial area on which the *Pseudomonas* spp. lipases can act.

Figure 1 shows the evolution of short-chain FFA ($C_{4:0}$-$C_{8:0}$; SCFFA), medium-chain FFA ($C_{10:0}$-$C_{14:0}$; MCFFA), and long-chain FFA ($C_{16:0}$-$C_{18:2}$; LCFFA) at each sampling time for the different treated samples. The SCFFA represented approximately 2% of the total FFA content in all milk samples, while MCFFA and LCFFA represented 20 and 78%, respectively. As can be seen in **Table 1**, myristic acid ($C_{14:0}$) was the predominant FFA in the MCFFA group, while palmitic ($C_{16:0}$), stearic ($C_{18:0}$), and oleic ($C_{18:1}$) acids were the main FFA in the LCFFA group and also the major FFA in milk samples. This is in accordance with the FFA composition detected in pasteurized milk by de Jong and Badings (*20*); however, concentration of these FFA were lower in our study. These differences in concentration could be related to the experimental chromatographic conditions and especially to the mode of injection (split).

The relative increase in the concentration of SCFFA during storage was higher than that of MCFFA and LCFFA, and was more pronounced in samples treated at 200 MPa. This increase in SCFFA could be explained by the specificity of LPL to act on primary ester bonds with some preference for the *sn*-1 and the *sn*-3 positions of triglycerides (*4*). In general, position *sn*-3 is occupied by SCFFA. Free fatty acids, particularly SCFFA and MCFFA have strong flavors that in elevated concentrations are described as rancid, butyric, and astringent (*3*). LCFFA have little contribution to the development of off-flavors; however, unsaturated fatty acids such as oleic $C_{18:1}$ and linoleic $C_{18:2}$ are vulnerable to oxidation and to producing a metallic flavor.

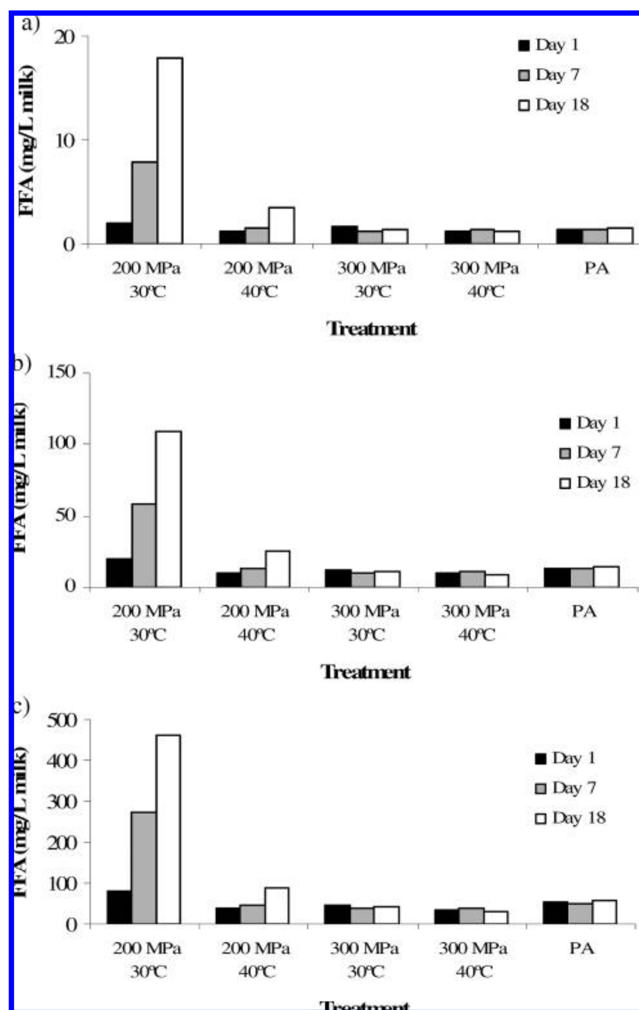

Figure 1. Evolution of (**a**) short-chain FFA, (**b**) medium-chain FFA, and (**c**) long-chain FFA in ultra-high-pressure homogenized (200 MPa 30 °C, 200 MPa 40 °C, 300 MPa 30 °C, and 300 MPa 40 °C) and high-pasteurized (PA) samples during storage.

Lipid Oxidation. Lipid oxidation is a free-radical chain reaction involving initiation, propagation, and termination stages. The initial step in the autoxidation of unsaturated fatty acids is the formation of free radicals, which then react with molecular oxygen to form a peroxide free radical. This free radical reacts with another unsaturated molecule to continue the reaction and generate a hydroperoxide. Hydroperoxides are the primary oxidation products generated during the lipid oxidation reaction, which decompose mainly into aldehydes and unsaturated ketones (secondary oxidation products) (*5*).

Analytical values obtained for the secondary oxidation products, TBARS and hexanal, and for the primary oxidation product hydroperoxide enabled a clear discrimination between samples. As can be seen in **Table 2**, immediately after treatments 300 MPa-treated samples presented lower ($P < 0.05$) hydroperoxide value and higher TBARS and hexanal content compared to that of UHPH samples treated at 200 MPa and PA milk. The lower hydroperoxide value in combination with the higher levels of TBARS and hexanal in 300 MPa milk samples indicates the progression of oxidation from a primary to a secondary state. The present results showed that 300 MPa treatments resulted in emulsions that were less stable against lipid oxidation compared to 200 MPa treatments, which could be attributed to various factors. On the one hand, O'Brien and O'Connor (*24*) reported that heat treatment promotes the formation of alkyl radicals, which are extremely reactive and can propagate the lipid oxidation reaction. During UHPH treatment at 300 MPa, milk achieved temperatures around 100 °C, which could explain the difference in lipid oxidation between these samples and 200 MPa and PA milk. On the other hand, homogenized milk has been reported to be less susceptible to oxidation than raw milk because after homogenization, the new

Table 2. Means ± Standard Deviation of Hydroperoxide (Absorbance Value), Malondialdehyde (μg MDA/mL Milk) and Hexanal (ppb) Content in Raw, Ultra-High-Pressure Homogenized (200 MPa 30 °C, 200 MPa 40 °C, 300 MPa 30 °C, and 300 MPa 40 °C), and High-Pasteurized Milk Samples during Refrigerated Storage at 4 °C[a]

		treatment					
	day	raw	200 MPa 30 °C	300 MPa 30 °C	200 MPa 40 °C	300 MPa 40 °C	PA[b]
hydroperoxide (Abs)	1	0.068 a ± 0.004	0.055 b ± 0.005	0.039 c ± 0.003	0.049 b ± 0.007	0.035 c ± 0.006	0.055 b ± 0.005
	18		0.051 a ± 0.008	0.038 b ± 0.001	0.051 a ± 0.007	0.035 b ± 0.006	0.051 a ± 0.006
TBARS (μg MDA/mL)	1	0.0105 b ± 0.0004	0.0114 b ± 0.0006	0.0132 a ± 0.0003	0.0116 b ± 0.0004	0.0139 a ± 0.0015	0.0106 b ± 0.0002
	18		0.0119 b ± 0.0002	0.0134 a ± 0.0002	0.0119 b ± 0.0004	0.0135 a ± 0.0002	0.0107 b ± 0.0001
hexanal (ppb)	1	2.175 b ± 0.74	5.102 b ± 2.244	15.347 a ± 8.205	6.386 b ± 2.859	15.844 a ± 6.921	2.460 b ± 0.615
	18		5.985 b,c ± 3.298	10.239 a,b ± 0.87	4.601 b,c ± 1.233	12.844 a ± 7.871	2.211 c ± 0.283

[a] Different letters in the same row indicate significant differences ($P < 0.05$). [b] PA = High-pasteurized milk (90 °C for 15 s).

milk fat droplets are resurfaced with casein and also because phospholipids from the MFGM, which are highly unsaturated, are partially transferred to the aqueous phase (*25, 26*). However, in this work, samples with the most intense homogenization treatment (300 MPa) suffered more secondary oxidation. This contradictory result could be related to the presence of clusters of fat droplets that were observed by different authors at pressures over 200 MPa (*9, 13, 16*). Hayes et al. (*13*) suggested that at higher pressures there would be more exposed fat interface. The amount of casein may become limiting, resulting in insufficiently covered fat droplets that can aggregate. If the milk fat droplet is not completely protected by proteins, it could be more accessible to oxidation. Caseins have been shown to possess significant antioxidant activity, which may be related to their hydrophobic nature and orientation of potential antioxidant side chains at the lipid interface; however, they can also bind prooxidant metals to phosphoseryl residues (*24*). Probably, the presence of protective caseins at the surface of the tiny fat globules of 200 MPa milk samples is the reason why less oxidation was produced compared to that of 300 MPa samples, in spite of the higher amounts of free unsaturated oleic and linoleic acids present at 200 MPa (**Table 1**).

Aldehydes are common secondary oxidation products in milk, with flavor thresholds generally lower than those of alcohols and ketones, and for that reason, they have a large impact on milk flavor when present at concentrations over the flavor threshold. Although hexanal content in 300 MPa samples was around 15 ppb compared to 5 ppb in 200 MPa samples and 2 ppb in PA milk, the flavor threshold value of this compound in homogenized milk is 0.049 ppm (*27*); hence, it should not necessarily be related directly as a flavor problem. During storage, in general no changes in the oxidation degree of milk samples were observed, although a small, but not significant, increase in TBARS value was observed at the end of storage. Mean TBARS quantities were around 10−11 μg/L milk in 200 MPa and PA milk and around 13 μg/L milk in 300 MPa samples. These values are higher than those found by Fenaille et al. (*28*) in UHT (4 μg/L) and pasteurized (8 μg/L) milk types; however, techniques used to determine MDA were different. It must be taken into account that although results from our TBA analysis are expressed as μg MDA/ mL milk, besides the aldehydes, MDA included other substances such as ketones, ketosteroids, acids, esters, sugars, imides and amides, amino acids, oxidized proteins, pyridines, and pyrimidines can react with the TBA (TBARS) (*22*), whereas mass spectrometry used by Fenaille et al. (*28*) enables good selectivity to characterize and quantify MDA. In a previous work, Pereda et al. (*23*) characterized the profile of volatile compounds of UHPH-treated samples, and they observed an increase in 2-heptanone and 2-nonanone in milk samples during storage. These ketones can be formed during heat treatment by β-oxidation of saturated fatty acids followed by decarboxilation or by decarboxilation of β-ketoacids present in milk fat (*29*), and hence, they can interfere in TBA results.

In conclusion the results of this study clearly show that whereas lipolysis was produced in samples treated at 200 MPa, oxidation took place in samples treated at 300 MPa. Nevertheless, a sensory analysis will be carried out in order to confirm whether the concentration of hexanal in 300 MPa samples and the increase of FFA in 200 MPa and 40 °C are perceived by consumers.

ABBREVIATIONS USED

UHPH, ultra-high-pressure homogenization; PA, high-pasteurized milk; Ti, inlet temperature; FFA, free fatty acids; MFGM, milk fat globule membrane; LPL, lipoprotein lipase; MDA, malondialdehyde; TBA, thiobarbituric acid; TBRAS, TBA reacting substances; SCFFA, short-chain free fatty acids; MCFFA, medium-chain free fatty acids; LCFFA, long-chain free fatty acids.

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