Influence of High-Intensity Ultrasound and Heat Treatment in Continuous Flow on Fat, Proteins, and Native Enzymes of Milk

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The effect of continuous flow high-intensity ultrasound (with and without heat generation) on alkaline phosphatase, γ-glutamyltranspeptidase, lactoperoxidase, whey proteins (α-lactalbumin and β-lactoglobulin), casein, and fat was studied in milk. Results were compared with those obtained using a conventional heating system having similar processing conditions. Hardly any effect on enzymes was observed when ultrasound was applied without heat generation. The highest denaturation of enzyme and whey proteins was found in samples subjected to ultrasound and heat. At 61, 70, and 75.5 °C a synergistic effect between ultrasound and heat was observed for the inactivation of alkaline phosphatase, γ-glutamyltranspeptidase, and lactoperoxidase, respectively. A noticeable synergism between ultrasound and heat was detected for α-lactalbumin and β-lactoglobulin denaturation. No changes in the casein were observed after any of the conditions assayed. As a consequence of ultrasound effects, a substantial reduction (up to 81.5%) in the size of the fat globule was observed. When 70 and 75.5 °C were achieved during high-intensity ultrasonic homogenization, a better particle distribution was observed as compared to that obtained at lower temperatures. This work describes the influence of continuous flow high-intensity ultrasound on important milk components as a first step for future processing applications.

Keywords: *Milk; ultrasound; continuous flow; native enzymes; proteins; fat globule*

INTRODUCTION

Due to the clear consumer demand for high-quality foods, new safe and effective methods of food processing and preservation have to be developed. One such alternative technology proposed is high-intensity ultrasound. High-intensity ultrasound (≤0.1 MHz, 10−1000 W cm^{-2}) in combination with heat has been used in the dairy industry for cleaning of equipment and homogenization of milk with acceptable results (Villamiel et al., 1999). It has been proven that it will be difficult for ultrasonic treatment to become a commercial process on its own, but in combination with other treatments such as heat it has more potential as a minimal processing method in the industry (Sala et al., 1995). In general, the studies reported in the literature about the use of this technique as a preservation method for dairy products are in batch conditions, and no detailed experiments on the influence of continuous flow ultrasound on milk components have been performed (Villamiel et al., 1999).

In general terms, although many aspects of ultrasound mechanisms remain obscure, most of them can be related to cavitation (formation and violent collapse of bubbles), heating (specific absorption of acoustic energy), dynamic agitation and shear stresses, turbulence (microstreaming), and others (Floros and Liang, 1994).

The effects of ultrasonic waves on enzymes are very complex, and activation or inactivation can take place depending on the conditions and intensity of the treatment (McClements, 1995), as well as on the nature of the enzyme (Sala et al., 1995). The most recent investigations have studied the increase of effectiveness of batch ultrasound enzyme inactivation using heat and pressure (mano-thermo-sonication, MTS) (Sala et al., 1995). The effect of MTS on lipoxygenase, peroxidase, and polyphenol oxidase, as well as heat-resistant lipase and protease from *Pseudomonas fluorescens*, has been studied in whey and different model systems (López et al., 1994; López and Burgos, 1995a,b; Vercet et al., 1997). These authors have pointed out that this combined treatment is a synergistic phenomenon.

The effect of ultrasound on some enzymes involved in the coagulation of milk (chymosin, pepsin, and several fungal enzymes) has been studied in model systems using batch processes. In general, after long (several minutes) ultrasonic treatments, the proteolytic activity of the studied enzymes decreased. However, when a mixture of milk and chymosin was subjected to ultrasound, hardly any enzyme inactivation was observed (Raharintsoa et al., 1977, 1978). No data on native milk enzyme inactivation by ultrasound have been previously reported.

Colloidal milk proteins and fat globules could absorb or reflect ultrasound energy (Paci, 1953; Huhtanen, 1966). Hueter et al. (1953) stated that probably the major contribution to ultrasound attenuation is made by the structure of casein, the most heat-stable protein in milk. Taylor and Richardson (1980) suggested that the increase of antioxidant activity of skim milk subjected to sonication is attributable to the disruption by ultrasound energy of the quaternary and tertiary structure of the casein. Wrigley and Llorca (1992) indicated that after ultrasonication of skim milk in a water bath at temperatures up to 50 °C, the soluble protein

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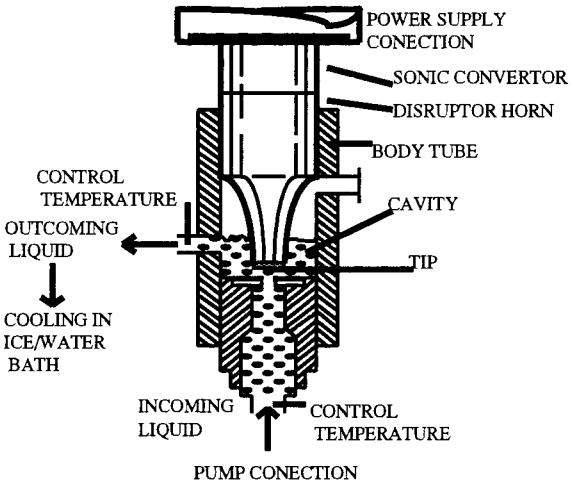

Figure 1. Scheme of the continuous flow ultrasonic treatment system.

concentrations were not significantly different from the unsonicated samples.

The aim of this research was to study the influence of continuous flow high-intensity ultrasound treatment (with and without heat generation) on native milk enzymes [alkaline phosphatase (AP), γ-glutamyltranspeptidase (GGTP), and lactoperoxidase (LPO)], proteins (whey proteins and casein), and milk fat.

MATERIALS AND METHODS

Milk Samples. Raw milk was purchased from different local farms. Milk was kept refrigerated for up to 2 h and heated in a water bath at 23.5 ± 1 °C until it was processed. Centrifugation at 4 °C and 3800*g* for 15 min was used to produce skim milk.

Ultrasonic and Conventional Heat Treatments. Ultrasound irradiation of milk samples (20 kHz frequency, 120 μm amplitude) was carried out in duplicate in a 450 Sonifier II (Branson Ultrasonic Corp.), 150 W full power. The equipment cavity (18.76 mL) (Figure 1) was connected to a peristaltic tube pump (A. F. Verder-Vleuten N.V.) to provide different residence times (102.3−40.2 s) in the ultrasonic cavity according to the flow rate variations (11−28 mL/min). Inlet (23.5 ± 1 °C) and outlet temperatures were continuously monitored using two thermocouples positioned outside the ultrasonic cavity and connected to a PM 8237 multipoint data recorder (Philips). Several experiments were performed at a maximum temperature of 30 °C to study the effect of ultrasound without the influence of the heat treatment. In this case, the ultrasonic cavity was immersed in an ice/water bath. Other treatments were carried out by insulating this cavity to reach different outlet temperatures (55−75.5 °C) depending on the flow rate (28−11 mL/min). The outlet milk was immediately cooled in a silicone coil (0.35 × 200 cm) immersed in an ice/water bath. Before samples were taken for analysis, sufficient milk was run to achieve constant outlet temperatures. Samples were collected once the steady state was achieved, and outlet temperatures were maintained with coefficients of variations <1%. In a typical experiment, once the system was equilibrated, 80 mL of milk was collected for analysis.

Comparative conventional heating experiments were performed in a stainless steel tubing (with the same volume, 18.76 mL, as the ultrasound cavity) immersed in a temperature-controlled water bath. Under the same flow conditions used during the ultrasonic treatment, similar outlet temperatures were also achieved by adjusting the temperature of the water bath. Experiments were performed in duplicate, and samples were cooled and collected as described for the ultrasonic treatments.

Analysis. The effect of the treatments on AP, GGTP, and LPO activities, denatured whey proteins (in the casein fraction), and the fat globule size distribution was estimated. The quantitative determinations of the activities of the three native milk enzymes were performed in a UV−visible spectrophotometer (Varian Cary 1E). AP, GGTP, and LPO activities were determined at 610, 540, and 413 nm, according to the methods of the IDF (1971), McKellar et al. (1991), and Shindler et al. (1976), respectively. Results were expressed as percentage of remaining activities relative to appropriate unexposed controls to correct for variations between batches (which may be attributed to natural variation of enzyme levels in the milk).

The denatured whey proteins in the casein fraction precipitated at pH 4.6 were determined by capillary zone electrophoresis (CZE) following the method of Recio and Olieman (1996). The analyses were carried out in duplicate. The data were calculated by dividing the normalized area of each peak (peak area/migration time) by the total normalized peak area.

The size of the fat globule was determined using a laser light-scattering instrument (Malvern MasterSizer X, Malvern Instruments Ltd.). Before the measurements were performed, the milk samples were diluted as required using distilled water to eliminate multiple scattering effects. Different parameters afford suitable information about the fat globule size and size distribution. Thus, $D[3,2]$ can be defined as volume/surface average diameter, and it is inversely proportional to the specific surface area of the globules. $D[v,0.5]$, $D[v,0.9]$, and $D[v,0.1]$ are direct measurements of the size globule and correspond, respectively, to the average diameters of 50, 90, and 10% of the total volume. Span is calculated dividing by $D[v,0.5]$ the difference between $D[v,0.9]$ and $D[v,0.1]$ and indicates the width distribution. Results of the size reduction were expressed as percentage with respect to appropriate unexposed controls to correct for variations between batches. Optical microscopy was used to check if homogenization clusters or other abnormalities were formed. The samples were warmed to 40 °C and shaken before the analysis if they were stored in refrigeration. Therefore, changes in globule size due to the sampling were avoided (Walstra, 1975).

RESULTS AND DISCUSSION

Native Milk Enzymes. Tables 1 and 2 show the remaining activity (percent) of AP, GGTP, and LPO observed after 56.3, 70.3, and 102.3 s of continuous flow ultrasonic, ultrasonic and heat, and conventional heat treatments of whole and skim milk, respectively. The treatments carried out during 40.2 s, even with heat (55 °C), did not cause any inactivation in the three enzymes studied (results not shown).

AP. The initial average content of AP was higher in whole milk (4030 μg of phenol/mL) than in skim milk (2196 μg of phenol/mL) due to the enzyme absorption on fat globules. These data are within the range reported by Rosakis and Anifantakis (1982) (1872−4740 μg of phenol/mL) for different milk samples taken during the lactation period. The value of the ratio between the content of AP in whole and skim milk was close to 2, a result that is in agreement with the value previously reported by Kosikowski (1988). Conventional heating similar to thermization (61 °C for 56.3 s) reduced the activity of the enzyme in both types of milk by ≈24%, a value that is in agreement with the suggested levels for thermization treatments (10−20%) (IDF, 1990). Hardly any activity (≤3.5%) was observed after 70.3 s of conventional heating at 70 °C, and complete inactivation of AP was achieved at temperatures of 75.5 °C for 102.3 s in whole and skim milk. The ultrasonic treatments carried out without heating did not inactivate the enzyme, even after 102.3 s. When the ultrasonic treatments were performed with heating to 61 °C, considerable enzyme inactivation was found, and these inactivations were similar in whole (49%) and

Table 1. Remaining Activity (Percent)[a] of the Alkaline Phosphatase (AP), γ-Glutamyltranspeptidase (GGTP), and Lactoperoxidase (LPO) after Continuous Flow Ultrasonic and Conventional Heat Treatments of Whole Milk under Different Conditions of Processing

	ultrasound				ultrasound and heat				heat		
tr[b] (s)	AP	GGTP	LPO	*T* (°C)[c]	AP	GGTP	LPO	*T* (°C)	AP	GGTP	LPO
56.3	100	96.5 (6.2)	100	61	51.0[d] (9.2)	96.9 (6.3)	100	61	76.5 (8.7)	87.5 (6.9)	100
70.3	100	85.5 (4.9)	87.2 (14.7)	70	0.7 (0)	24.6[d] (3.2)	77.9[e] (11.2)	70	0.5 (0)	55.0 (5.4)	86.0 (13.5)
102.3	98.2 (6.7)	77.9 (2.8)	85.6 (2.1)	75.5	0	0	30.8[d] (2.6)	75.5	0	7.7 (2.4)	63.5 (1.5)

[a] Mean values of two independent treatments expressed as percentage of the enzyme activity in the raw milk. Standard deviation in parentheses. [b] Residence time of milk in the ultrasonic cavity or in the stainless steel tubing (conventional treatment). [c] Outlet temperature. [d] Synergistic effect. [e] Additive effect.

Table 2. Remaining Activity (Percent)[a] of the Alkaline Phosphatase (AP), γ-Glutamyltranspeptidase (GGTP), and Lactoperoxidase (LPO) after Continuous Flow Ultrasonic and Conventional Heat Treatments of Skim Milk under Different Conditions of Processing

	ultrasound				ultrasound and heat				heat		
tr[b] (s)	AP	GGTP	LPO	T(°C)[c]	AP	GGTP	LPO	T(°C)	AP	GGTP	LPO
56.3	100	100	100	61	56.1[d] (5.7)	100	100	61	76.2 (9.1)	100	100
70.3	98.6 (12.7)	94.2 (11.4)	100	70	0.9 (0)	28.6[d] (5.2)	100	70	3.5 (0.4)	64.4 (8.4)	90.1 (11.7)
102.3	100	82.8 (5.3)	100	75.5	0	0	47.2[d] (3.6)	75.5	0	8.5 (0.9)	62.9 (3.0)

[a] Mean values of two independent treatments expressed as percentage of the activity of the raw milk. Standard deviation in parentheses. [b] Residence time of milk in the ultrasonic cavity or in the stainless steel tubing (conventional treatment). [c] Outlet temperature. [d] Synergistic effect.

skim milk (44%). These values were higher than those observed for similar conventional heating (24%). Hardly any enzyme activity (≤0.9%) in whole or skim milk was observed after 70.3 s of ultrasonic treatments at 70 °C, and complete inactivation was achieved at 75.5 °C for 102.3 s.

GGTP. Due to the enzyme distribution in milk, the initial activity of GGTP was higher in whole than in skim milk. In the skim milk fraction values in the range 67−72% of the total activity were found. These results were similar to those reported by McKellar et al. (1991) for raw skim milks used in different laboratory pasteurization treatments. After conventional heating at 61 °C, some enzyme inactivation (12.5%) was observed in whole milk but not in the case of skim milk. However, after heating at temperatures >70 °C, no differences were found between the two types of milk. These results are in agreement with the data previously reported by McKellar et al. (1991), who noted no significant differences in GGTP activities between skim and whole milk after heating at ≥67 °C. Ultrasonic processes without heating produced up to 22% of enzyme inactivation after 102.3 s of treatment. This decrease in GGTP activity was higher when heating was produced during ultrasonic treatment. Treatments with ultrasound for 102.3 s at 75.5 °C completely inactivated the GGTP, whereas, after the comparable conventional process, some remaining activity was found (≤8.5%). In general, similar values of remaining enzyme activity were observed for whole and skim milk after all treatments.

LPO. The enzyme activity in raw milk did not greatly vary among the control samples, with an average of 0.90 ± 0.13 unit/mL. This value is similar to that reported by Griffiths (1986) (0.73 ± 0.14 unit/mL) for 26 samples of raw milk. Conventional heating of whole and skim milk carried out at 75.5 °C resulted in a decrease of the enzyme activity close to 37%. This value is not very high for the thermostability of this enzyme; nevertheless, Griffiths (1986) demonstrated that this thermostability was less for samples heated by a plate−heat exchanger than for laboratory-treated samples. As found for AP and GGTP, higher enzyme inactivation was achieved when heating was considered during ultrasonic treatments. In the case of LPO this was mainly observed for whole and skim milk treated with ultrasound for 102.3 s at 75.5 °C. In skim milk no inactivation of the LPO was found after any of the ultrasonic treatments carried out without heating. However, in whole milk the activity of the enzyme decreased ∼14% after 102.3 s of treatment with ultrasound. Because similar values of remaining enzyme activity were observed during the conventional treatments at 75.5 °C of whole (63.5%) and skim milk (62.9%), the differences found between the two types of milk when ultrasound and heating were considered (30.8 and 47.2% for whole and skim milk, respectively) seem to be due to the different behavior of the milks under ultrasonic treatment.

The three enzymes studied do not present the same sensitivities to ultrasonic inactivation. In general, the effect of ultrasound without heating is very low, and long exposure times are required for enzyme degradation. In some cases, ultrasonic treatments that alone did not inactivate the enzymes caused considerable decrease in enzyme activity when the treatments were carried out with heating, this effect being additive or synergistic (see Tables 1 and 2). Although the mechanisms of enzyme inactivation by ultrasound are very complex, ultrasound might act by increasing the susceptibility of the active centers of the enzymes to heating inactivation (or vice versa). Additionally, the increase in the temperature diminishes the viscosity of the liquid, favors the penetration of the waves (Earnshaw et al., 1995), and decreases the violence of implosion of the formed bubbles (Sala et al., 1995). Nevertheless, cavitation is not always necessary to produce enzyme inactivation, and the shear stress and turbulence produced

by the effect of ultrasound could also be responsible for enzyme degradation (Price, 1990).

The results obtained in this work, performed in continuous flow with native enzymes of milk, confirm previous data obtained after batch treatments of different enzymes in whey and model systems (Sala et al., 1995; Vercet et al., 1997).

The effectiveness of ultrasound for enzyme inactivation depends not only on the type of enzyme, equipment, and conditions used but also on the nature of the sonicating medium (Sala et al., 1995). Thus, some differences found in this study between whole and skim milk for the inactivation of the enzymes could be due to factors related to the composition of the medium. In general, other authors have demonstrated that the effect of ultrasonic waves increases with solids concentration (Santamaría and Castellani, 1952; Berliner, 1984; Sala et al., 1995) and diminishes with increasing enzyme concentration (Raharintsoa et al. 1977; Sala et al., 1995). In skim milk, the concentration of solids is lower than in whole milk (less effect). However, the concentration of enzyme in skim milk (AP and GGTP) is also lower (more effect) than in whole milk because these enzymes (linked to fat globule membrane) can be liberated by ultrasound effect to the serum phase, increasing its concentration. Therefore, no important differences are expected in these cases. LPO is located in the whey, and the main cause of the higher decrease of enzyme activity in whole milk than in skim milk by the effect of ultrasound and heat (75.5 °C; 102.3 s) could be the higher concentration of solids in the former than in the latter.

Milk Proteins. Figure 2 shows the electropherograms of whey proteins [α-lactalbumin (α-La) and *β*-lactoglobulin (*β*-Lg)] in the casein fraction corresponding to raw skim milk (A) and ultrasonicated skim milk (B) during 102.3 s at 75.5 °C. The same peaks of milk proteins were found in both cases. Table 3 shows the denaturation of α-La and *β*-Lg observed in whole and skim milk after 102.3 s of treatment in continuous flow by ultrasound, ultrasound and 75.5 °C, and conventional heat treatment at the same temperature. The ultrasound was able to denature both whey proteins, and this effect was higher in whole milk than in skim milk. In agreement with the results reported for the enzyme inactivation, the denaturation of α-La and *β*-Lg was higher when the ultrasonic treatment was performed with heat as compared to the same treatment carried out without heating. This effect is synergistic and seems to be more important in the case of whole milk than in skim milk. This result can be probably due to higher solids concentration and lower protein concentration in the former than in the latter and/or binding locations of whey proteins on fat globule membrane during the homogenization process. No changes in the peak areas of any of the casein were observed after the treatment conditions assayed (data not shown). This result is in agreement with the theory suggested by Taylor and Richardson (1980), who stated that sonication can modify the quaternary and/or tertiary structure of the casein but does not disrupt fully micelle casein.

Fat Globule. According to Gopal (1968) and Tornberg and Lundh (1978) the cavitation (due to high-pressure gradient arising from the collapse of bubbles) caused by ultrasound is the primary cause of fat globule disruption. Table 4 shows the effect of continuous flow ultrasonic treatment with and without the influence of the heating (55−75.5 °C) on the reduction of the milk fat globule size calculated from *D*[*v*,0.5]. In general, a substantial reduction (up to 81.5%) with respect to the control was found after all treatments. The main effect was due to ultrasound, although a higher reduction was obtained when the treatments were performed with heat. These results might be attributed to the fact that the viscosity of milk can be reduced when the liquid is

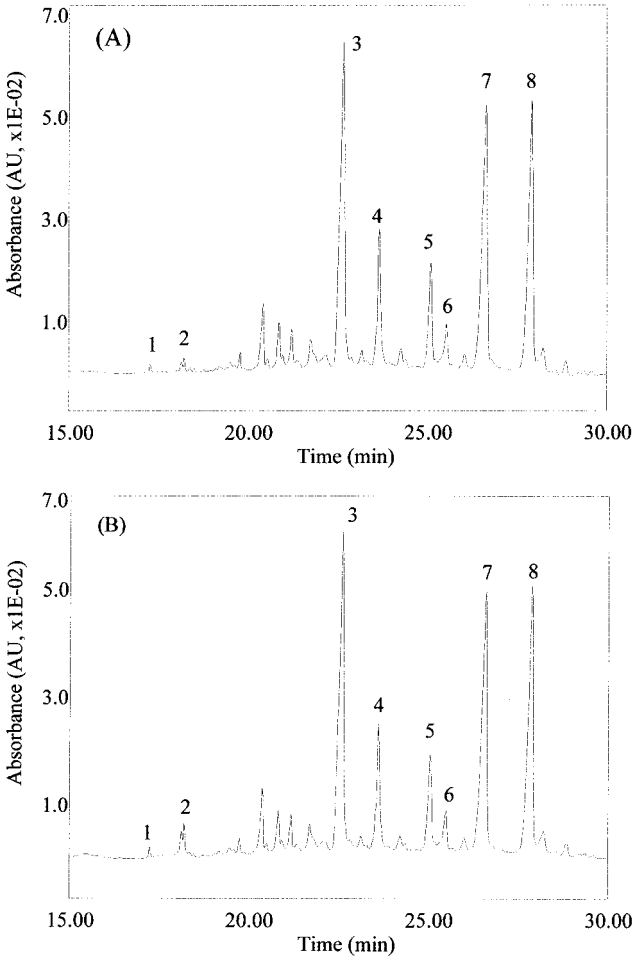

Figure 2. Electropherogram of casein fraction of raw skim milk (A) and of skim milk subjected to continuous flow ultrasonic treatment for 102.3 s at 75.5 °C (B). Peaks: 1, α-lactalbumin; 2, *β*-lactoglobulin; 3, α_{s1}-casein; 4, α_{s0}-casein; 5, *κ*-casein; 6, *β*-casein B; 7, *β*-casein A1; 8, *β*-casein A2.

Table 3. Denatured Whey Proteins [α-Lactalbumin (α-La) and *β*-Lactoglobulin *β*-Lg)][a] in the Casein Fraction Precipitated at pH 4.6 of Whole and Skim Milk Subjected to Continuous Flow Ultrasonic, Ultrasonic and Heat (75.5 °C), and Conventional Heat (75.5 °C) Treatments for 102.3 s

	whole milk		skim milk	
sample	α-La	*β*-Lg	α-La	*β*-Lg
control	2.10	8.29	2.24	11.73
	(0.12)	(0.19)	(0.57)	(2.55)
ultrasound	4.21	18.80	2.29	14.93
	(0.32)	(1.56)	(0.08)	(0.21)
ultrasound and heat	6.17	41.75	3.59	29.10
	(0.14)	(0.44)	(0.23)	(4.78)
heat	2.58	18.00	2.92	17.38
	(0.18)	(0.22)	(0.32)	(0.38)

[a] Mean values multiplied by 10^3 and expressed as normalized area of each peak (peak area/migration time)/total normalized area. Standard deviation multiplied by 10^3 in parentheses (n = 4).

Table 4. Effect of Continuous Flow Ultrasonic (US) and Heat Treatment of Milk after 40.2, 56.3, 70.3, and 102.3 s of Residence Time in the Ultrasonic Cavity on Some Homogenization Parameters (See Materials and Methods)[a]

parameter	40.2 s		56.3 s			70.3 s			102.3 s		
(μm)	control	US + 55 °C	control	US	US + 61 °C	control	US	US + 70 °C	control	US	US + 75.5 °C
D[3,2]	1.95	0.68	1.90	0.83	0.56	1.90	0.70	0.53	2.42	0.61	0.48
D[v,0.5]	2.79	0.95	2.68	1.47	0.72	2.69	1.04	0.64	3.08	1.05	0.57
		(65.9)[b]		(45.1)	(73.1)		(61.3)	(76.2)		(65.9)	(81.5)
D[v,0.9]	5.93	3.81	5.37	4.18	3.13	5.54	3.67	2.08	5.86	4.10	2.08
D[v,0.1]	0.99	0.31	0.98	0.36	0.27	0.98	0.31	0.28	1.33	0.25	0.24
span	1.78	3.68	1.64	2.61	4.03	1.71	3.24	2.84	1.47	3.65	3.21

[a] Standard deviation values in the range 0−0.29. [b] Reduction with respect to the control (%).

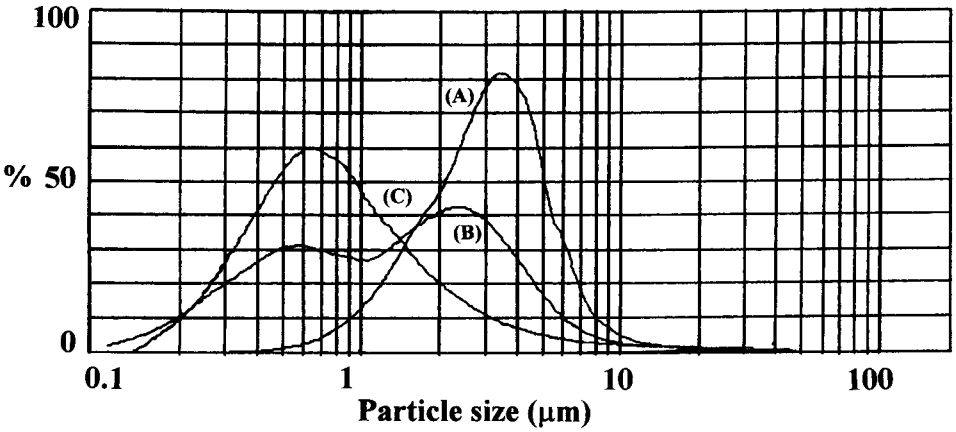

Figure 3. Models of distribution of the particle size of the fat globule in raw milk (A), ultrasonicated milk during 70.3 s (B), and ultrasonicated milk during the same time at 70 °C (C).

heated and ultrasound stands a better chance of penetration (Earnshaw et al., 1995).

In general terms, the reduction of fat globule size increased with the residence time; however, from 70.3 to 102.3 s of residence time, only a slight increase in this reduction (~5%) was achieved. In an homogenization process, the size of the fat globule is influenced by the flow rate of the equipment (Walstra, 1975). To determine the correlation between the two parameters in our system, the log D[3,2] and −log flow rates were calculated (data not shown). In the relation found the slopes were 0.50 ($r^2 = 0.96$) and 0.37 ($r^2 = 0.95$) for the treatments without and with effect of temperature, respectively. These data, especially 0.37, are similar to the experimental (0.39) and theoretical (0.40) values reported by Walstra (1969) for continuous flow ultrasonic homogenization in a Branson sonifier at flow rates approximately in the range 2.6−19.0 mL/min. This author did not specify if the treatments were carried out with heating. According to these results, too low flow rates are less efficient for ultrasonic fat globule disruption.

When ultrasonic treatments were performed with heating, the fat globule sizes obtained (D[v,0.5]) were in the range 0.57−0.95 μm. When the diameter is reduced below 0.8 μm, the creaming effect during the storage of the milk is very slow, as previously described by Muir (1992). Schmidt (1985) observed that after batch ultrasonic homogenization of milk at 60 °C, fat globules of ≤1 μm diameter could be obtained. Martínez et al. (1987) homogenized human milk at temperatures of 45 and 55 °C using a batch ultrasonic method and achieved fat globules of ≤1.2 μm diameter. The possible differences with respect to our results could be due to the different parameters of measurement, equipment, and conditions used for the homogenization of milk by ultrasound in each study. Walstra (1975) reported values of D[3,2] approximately in the range of 0.30−0.90 μm for homogenization of milk with classical high pressure and valve homogenizer.

Although since the first work on ultrasonic homogenization (Chambers, 1937) ultrasonic treatment has been considered as an effective system for the reduction of fat globule size (Schmidt, 1985; Martínez et al., 1987), data are not available about the shape of size distribution. When in an homogenization process the particle size distribution is markedly variable, partial homogenization has been produced and the process cannot be considered as acceptable (Walstra, 1975). Table 4 also shows the span parameter, which can afford some information about the distribution width. For all of the samples submitted to ultrasound and ultrasound and heat, high values of span were obtained. Moreover, different models of distribution were observed (Figure 3), results that are in agreement with the observations performed by optical microscopy (Figure 4). A bimodal distribution was obtained after all ultrasonic treatments carried out without heating and after ultrasonic treatments performed at 55 and 61 °C. However, treatments of ultrasound and heat at 70 and 75.5 °C resulted in particle distributions with only one maximum but with high span values. During ultrasonic treatments without heat or at low temperatures, clusters of fat globules with casein could be found. Also, Müller (1992) suggested that the application of ultrasound for milk homogenization before cheese-making could improve the yield of the cheese due to an increase in binding locations for proteins links on the fat globule membrane.

Probably during ultrasonic treatment carried out at higher temperatures (70 and 75.5 °C) more spreading of casein from the micelles occurs, as previously reported by Walstra and Oortwijn (1982) for conventional homogenization processes. The high distribution width found in this study might be related to variations in the

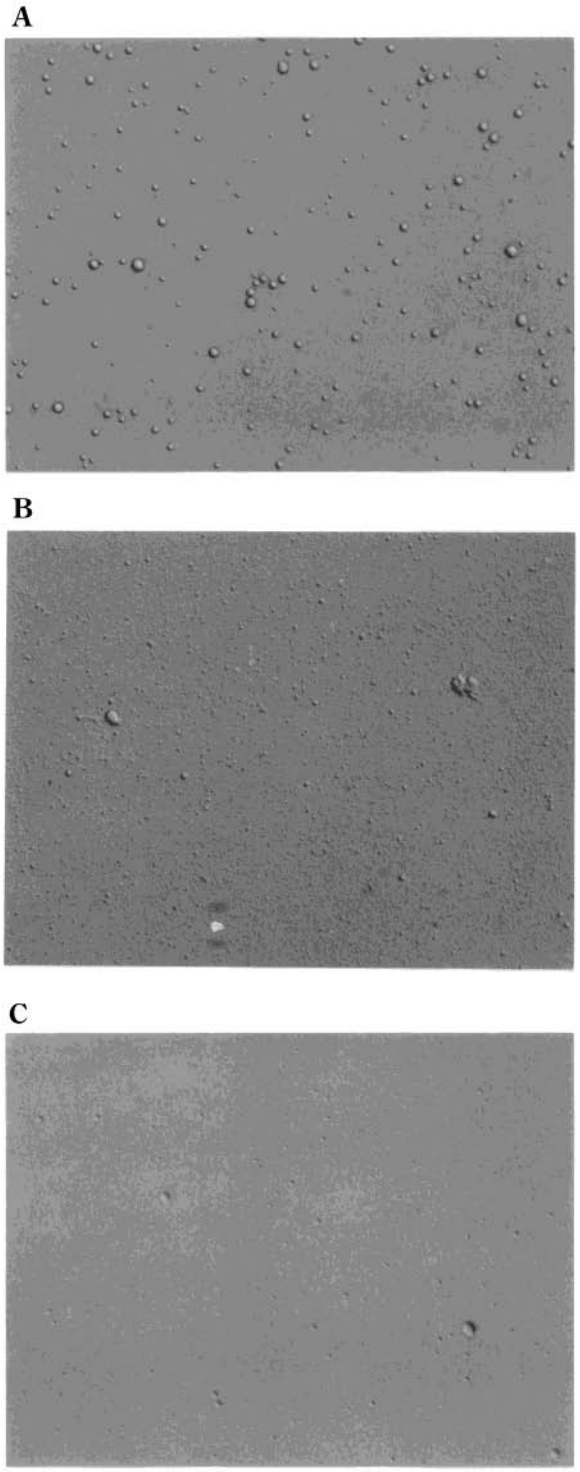

Figure 4. Pictures taken from optical microscopy analysis (×400) corresponding to raw milk (A), ultrasonicated milk during 70.3 s (B), and ultrasonicated milk during the same time at 70 °C (C). Figure is reproduced here at 80% of the original.

residence time in the cavity as well as to the stochastic nature of fat globule disruption by turbulent eddies. One of the mechanisms involved in high-intensity ultrasound effects is the production of strong eddies within the liquid that is subjected to the ultrasound action (Floros and Liang, 1994; Earnshaw et al., 1995). Repeated homogenization/sonification could help to improve the distribution width, as previously reported by Walstra (1975) for conventional homogenization.

Conclusions. Although more research is needed to develop new processing equipment with improved conditions that provide less variation in the residence time, the results presented in this work indicate that high-intensity ultrasound applied with heating in continuous flow might be a useful method to reduce the fat globule size. The observed results of this new technique on proteins and native milk enzymes afford important information about the chemical changes produced during the treatment of milk with ultrasound and heat.

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