



High-pressure homogenization of raw and pasteurized milk modifies the yield, composition, and texture of queso fresco cheese

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ABSTRACT

High-pressure homogenization (HPH) of milk was studied as an alternative processing operation in the manufacturing of queso fresco cheese. Raw and pasteurized (65°C for 30 min) milks were subjected to HPH at 0, 100, 200, and 300 MPa and then used to manufacture queso fresco. The cheeses were evaluated for yield, moisture content, titratable acidity, nitrogen content, whey protein content, yield force, yield strain, and tactile texture by instrumental or trained panel analyses. The combination of HPH and thermal processing of milk resulted in cheeses with increased yield and moisture content. The net amount of protein transferred to the cheese per kilogram of milk remained constant for all treatments except raw milk processed at 300 MPa. The highest cheese yield, moisture content, and crumbliness were obtained for thermally processed milk subjected to HPH at 300 MPa. The principal component analysis of all measured variables showed that the variables yield, moisture content, and crumbliness were strongly correlated to each other and negatively correlated to the variables yield strain, protein content (wet basis), and sensory cohesiveness. It is suggested that the combination of thermal processing and HPH promotes thermally induced denaturation of whey protein, together with homogenization-induced dissociation of casein micelles. The combined effect results in queso fresco containing a thin casein-whey matrix that is able to better retain sweet whey. These results indicate that HPH has a strong potential for the manufacture of queso fresco with excellent yield and textural properties.

Key words: queso fresco, Hispanic cheese, high-pressure homogenization

INTRODUCTION

Queso fresco is a Hispanic-style cheese that has become a regular part of the Latin-American diet (Clark et al., 2004) and is increasingly popular among US consumers (USDA-NASS, 2010). Queso fresco is a fresh, soft, white, high-moisture, slightly salty cheese characterized by a crumbly texture and low meltability (Van Hekken and Farkye, 2003). It was traditionally made using raw bovine milk and consumed within 14 d of manufacture (Renyé et al., 2008). Typical moisture ranges from 46 to 57% (wet basis), NaCl is 1 to 3% (wt/wt), and pH is about 6.1 (Hwang and Gunasakaran, 2001; Tunick, 2006). Queso fresco made from raw milk provides growth-promoting conditions for pathogenic bacteria, including *Listeria monocytogenes* and *Salmonella typhimurium*, and it has been linked to several foodborne outbreaks and recalls (Altekruse et al., 1998; Cody et al., 1999; Villar et al., 1999; Hise et al., 2004; MacDonald et al., 2005; FDA, 2010a,b). Current US regulations prohibit the commercialization of queso fresco manufactured from raw milk, and only cheese made from pasteurized milk and commercial starter cultures can be sold. However, many traditional consumers consider that thermal pasteurization of milk modifies the organoleptic properties of the final product.

Several nonthermal food preservation processes are being studied (e.g., high hydrostatic pressure), with the aims of replacing thermal processing primarily in foods that are consumed fresh or minimally processed. Because of the legal requirement for pasteurization of milk for fresh cheeses such as queso fresco, alternative processing techniques are considered as additions, not substitutions, for pasteurization.

Valve homogenization at pressure <20 MPa is a nonthermal technology currently used by the dairy industry to prevent creaming in fluid milk by reducing the fat globule radius (r), thus lowering its terminal velocity (v_t ; $v_t \sim r^2$). High-pressure valve homogenization (HPH) is a similar process but with operating

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pressure up to 400 MPa, in which a combination of physical phenomena (shear stress, hydrostatic pressure, cavitation, turbulence, and temperature) modifies the physicochemical properties of the processed fluid food and promotes microbial inactivation. High-pressure homogenization has been used to inactivate pathogenic and spoilage microorganisms in fluid foods (Lanciotti et al., 1994, 1996; Kheadr et al., 2002; Wuytack et al., 2002; Thiebaud et al., 2003; Hayes et al., 2005; Pereda et al., 2007; Taylor et al., 2007) and to modify the functionality of protein and fat constituents in milk (Paquin, 1999; Hayes and Kelly, 2003a,b; Sandra and Dalgleish, 2005; Iucci et al., 2007; Roach and Harte, 2008).

In recent years, the use of HPH for cheese manufacture has been studied for its potential to reduce microbial populations in raw milk, combined with changes in yield and physicochemical properties of cheese. Lanciotti et al. (2007) found a reduction of biogenic amines in Caciotta and Pecorino cheeses obtained from milk processed by HPH. Changes in lipolytic and proteolytic patterns were found during the ripening of Italian (Lanciotti et al., 2006; Vannini et al., 2008) and goat cheeses (Guerzoni et al., 1999; Buffa et al., 2005) manufactured using HPH-treated milks.

The objective of this work was to determine yield, composition, texture, and organoleptic properties of queso fresco made from raw and pasteurized milk processed by HPH up to 300 MPa.

MATERIALS AND METHODS

Milk Processing

Raw whole milk was collected from the University of Tennessee Institute of Agriculture dairy farm located in Knoxville, Tennessee. Milk was pasteurized by heating at 65°C for 30 min in a pilot-scale water bath. Then, 2 L of raw or pasteurized milk was subjected to HPH at 0, 100, 200, and 300 MPa using a 2-stage continuous high-pressure valve homogenizer (model FPG 12500, Stanstead Fluid Power, Essex, UK) equipped with a data logger, which recorded pressure and temperature immediately after the secondary pressure valve. An approximate 18°C increase in temperature was recorded for every 100-MPa increment in pressure. A heat exchanger connected immediately after the secondary pressure valve cooled the samples to approximately 5°C in less than 2 s. A total of 8 queso fresco treatment combinations were made from raw and pasteurized whole milk subjected to HPH from 0 to 300 MPa. Three independent queso fresco cheeses were made for each treatment combination.

Preparation of Queso Fresco

Each individual cheese was made from an initial 2-kg mass of treated milk. The cheeses were manufactured according to the method developed by Clark et al. (2001). Briefly, the processed milk was heated to 32°C and 0.26 g of starter culture (Choozit MA 11 LYC 50 DCU, Danisco Inc., Scarborough, ON, Canada) was added. Thirty minutes later, 0.26 mL of coagulant (DCI Supreme Rennet, Dairy Connection Inc., Madison, WI) was added, and the cultured milk was allowed to set for approximately 30 min. The curd was cut in approximately 10-mm³ cubes and allowed to heal for 10 min before cooking. Curd and whey were heated to 38°C over 30 min (stirring continuously), and then the temperature was maintained and curd was stirred continuously for an additional 30 min. After cooking, the first half of the whey was drained. Ten minutes later, the first one-third of total salt was added at a rate of 10 g/kg of milk, mixed briefly, and left undisturbed for 10 min. After 10 min, one-quarter of the whey was drained and the second one-third of salt was added and stirred in. After 5 min, the final amount of whey was drained and the rest of the salt was added and then stirred in for 5 min. The curd obtained was pressed in a perforated polyvinyl chloride cylinder (diameter = 11.1 cm) under 2.275 MPa pressure for 1 h at room temperature (~23°C). After the pressing step, each cheese was removed from the cylinder and crumbled. Subsequently, the crumbled cheese was pressed in the same hoop for another 20 h.

Total Protein, Moisture, and Yield

Crumbled cheese samples were analyzed in duplicate. Moisture was obtained by oven drying at 102°C following AOAC method 948.12 (AOAC, 1990) and protein content following AOAC method 990.03 (AOAC, 1990) using a Leco FP-2000 nitrogen analyzer (Leco, St. Joseph, MI). Cheese yield was calculated as the percentage ratio between mass of cheese over mass of initial milk (yield = 100 × g of cheese/g of initial milk).

SDS-PAGE

An approximately 50-g sample of each cheese was kept frozen (-20°C) for SDS-PAGE. After thawing the cheese at room temperature for 1 h, proteins were extracted using the method described by Ong et al. (2006), with modifications. Briefly, an aliquot of each cheese sample (0.05 g) was suspended in 1 mL of Tris (10 mM)-EDTA (1 mM) pH 8.0 buffer, 350 µL of 10% SDS, and 50 µL of β-mercaptoethanol. The samples

Table 1. Texture attributes definition and anchors determined by the trained panel

Category	Procedure	Attribute	Definition	Anchors
Texture before breaking	Lift the sample from plate and press the sample gently for 1 to 2 s without breaking	Firmness	Force required for compressing the cheese with the fingers	3 = Cream cheese 6 = Mozzarella 10 = Swiss 15 = Parmesan
		Springiness	Rate at which the cheese springs back to original shape and height after compressed	0 = Cream cheese 5 = Mozzarella 12 = Swiss 15 = Parmesan
Texture during breaking	Manipulate the sample using thumb and 2 fingers 5 times	Crumbliness	Ease of sample to break apart or crumble during manipulation	0 = Cream cheese 3 = Swiss 7 = Sharp Cheddar 13 = Feta
		Stickiness	How much the sample sticks to the fingers during manipulation	1 = Swiss 6 = Sharp Cheddar 9 = Feta 15 = Cream cheese
		Cohesiveness	How well the sample holds together during manipulation	2 = Feta 8 = Swiss 11 = Mozzarella 15 = Cream cheese
Residuals left after breaking	Feel any tactile residual (left over) on the fingers	Oiliness/greasiness residual	How much oil or grease residual film is left on the fingers after manipulation	4 = Swiss 6 = Sharp Cheddar 9 = Feta 11 = Mozzarella 15 = Cream cheese

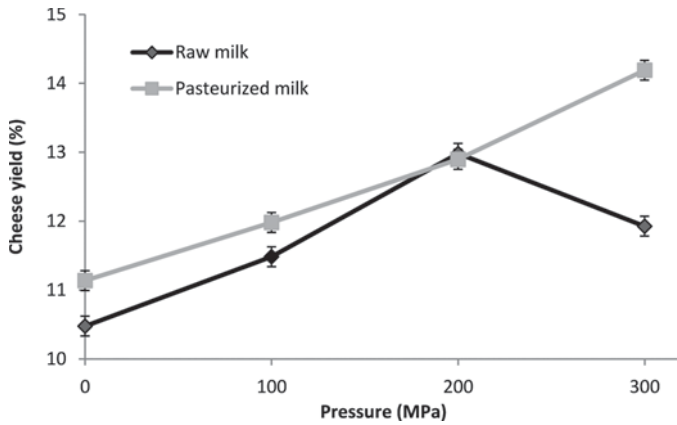


Figure 1. Yield (wt/wt %) of queso frescos made from raw and pasteurized milk subjected to high-pressure homogenization. Bars are 95% CI.

were agitated for approximately 1 h, until the cheese solids were completely dispersed.

Electrophoresis was done following the procedure described by Laemmli (1970) using a mini-protean II gel electrophoresis unit (Bio-Rad, Hercules, CA). One hundred microliters of protein dispersion was diluted in 400 μ L of sample buffer (62.5 mM Tris-HCl, pH 6.8, 20% glycerol, 2% SDS, 5% β -mercaptoethanol, and 0.025% bromophenol blue) and then heated for 5 min at 95°C. A working volume of 15 μ L of each sample and 10 μ L of standards (α -CN, β -CN, and β -LG; Sigma-Aldrich, Milwaukee, WI) were loaded into the wells of 15% polyacrylamide ready-gel (Bio-Rad) and run at a constant 200 V for 40 min at room temperature. Proteins were stained using 0.25% (wt/vol) Coomassie Brilliant Blue R-250 [50% methanol, 10% acetic acid, 0.25% (wt/vol) Coomassie Blue] during 10 min and then destained with

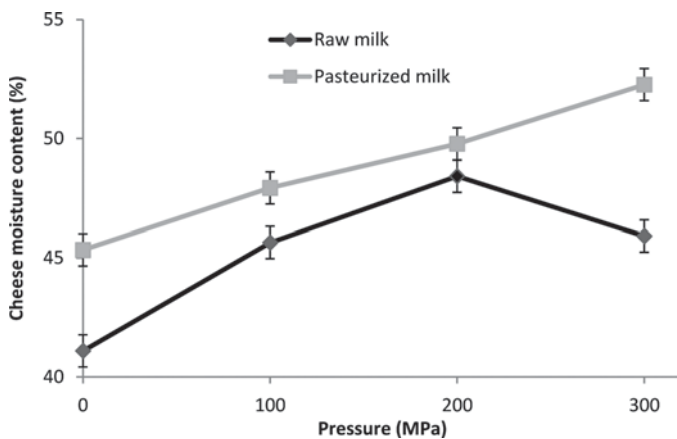


Figure 2. Moisture content (wt/wt %) of queso frescos made from raw and pasteurized milk subjected to high-pressure homogenization. Bars are 95% CI.

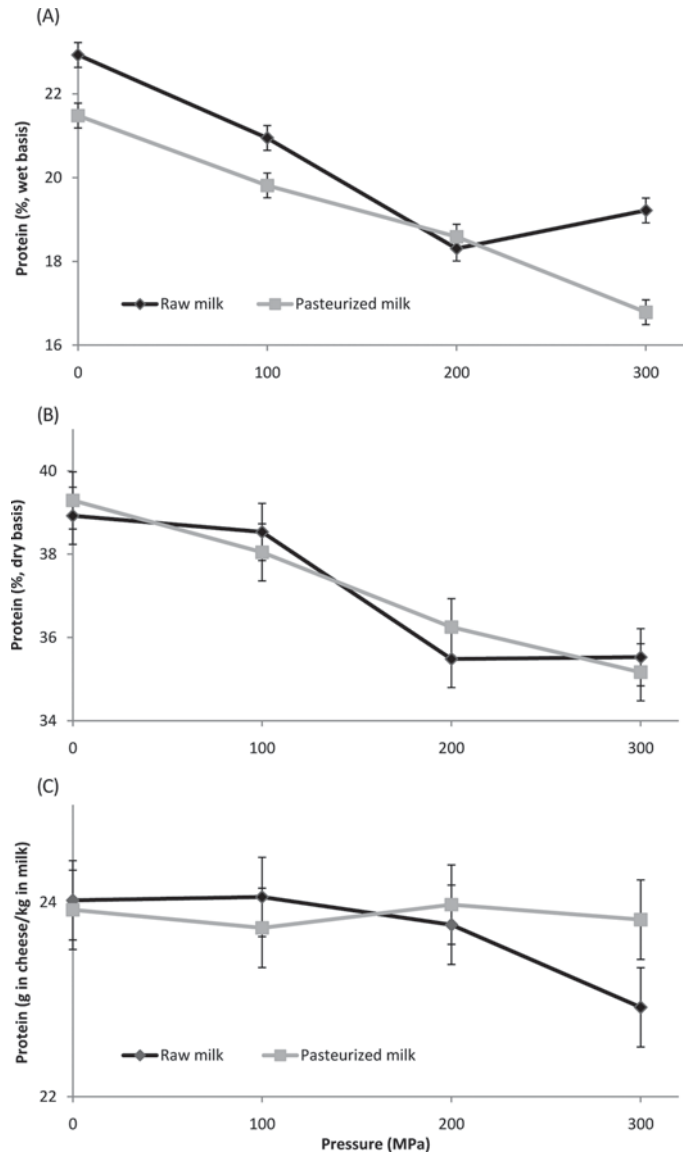


Figure 3. Protein content of queso frescos made from raw and pasteurized milk subjected to high-pressure homogenization: (A) weight percent dry basis; (B) weight percent wet basis; (C) grams of protein in cheese per kilogram of protein in milk. Bars are 95% CI.

deionized water containing 15% (vol/vol) methanol and 10% (vol/vol) acetic acid.

Sensory Evaluation

A group of 9 students was selected from the Food Science and Technology Department at the University of Tennessee-Knoxville and given 6 training sessions (1 h each) on tactile evaluation of a range of commercially available cheeses used as anchors. The sensory evaluation of queso fresco was performed after 2 d of manufacturing. Six cheese attributes, grouped into 3 catego-

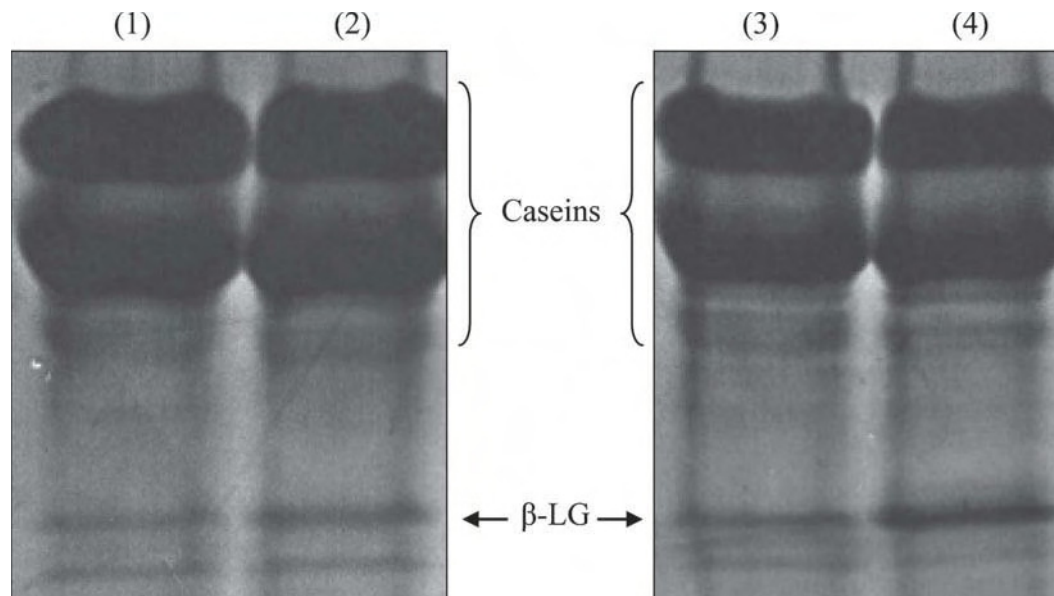


Figure 4. The SDS-PAGE profiles of queso fresco made from (1) raw milk, (2) pasteurized milk, (3) raw milk homogenized at 300 MPa, (4) pasteurized milk homogenized at 300 MPa.

ries, were evaluated: texture before breaking (firmness and springiness), texture during breaking (crumbliness, stickiness, and cohesiveness), and oiliness/greasiness residual after crumbling. The texture terms, anchors, and cheese standards selected for the hand evaluation are shown in Table 1. A 16-point scale based on Sandra et al. (2004b) was used, where 0 = none, 3 = slight, 7 = moderate, and 15 = extreme. Experimental cheese samples were cut in $\sim 1.5\text{-cm}^3$ cubes and allowed to equilibrate at room temperature for 30 min before sensory evaluation. The cheese cubes were identified using randomly generated 3-digit codes and randomly presented to the trained panelists. The panelists were provided with 3 cheese cubes per test evaluation for descriptive cheese texture analysis.

Uniaxial Compression Test

Cheeses were stored for 2 d at 4°C and then cut into 12-mm^3 cubes. The cubes were kept in plastic containers to prevent water loss and equilibrated to room temperature for 30 min before analysis. The compression test was done using a texture analyzer (TA-XT Plus, Texture Technology Corp., Stable Microsystems, Scarsdale, NY) with a 5-kg load cell. The cheese cubes were compressed to 80% of their original height using a 3.5-cm-diameter cylindrical probe moving at a displacement speed of 0.4 mm/s. The inflection point in the force versus strain curve was used to calculate the cheese initial force and yield strain fracture. Overall means of 18 determinations (6 determinations per

cheese, 3 cheeses per treatment) are reported in this manuscript.

Statistical Analysis

Experiments were analyzed as a completely randomized design with 3 replicates. Analysis of variance was done using SAS software (version 9.2, SAS Institute Inc., Cary, NC). In cases where a significant treatment effect was found, Student's least significant difference multiple comparison test ($\alpha = 0.05$) was done to test for differences among individual treatment means. Principal component analysis of average values for sensory determinations, texture, yield, moisture, and protein content was done to illustrate the relationship among the various parameters using the XLSTAT-Pro 2010 Excel add-in (Addinsoft, New York, NY).

RESULTS AND DISCUSSION

The combined thermal and HPH processing of milk resulted in better cheese yield and queso frescos containing higher moisture content compared with cheeses made with raw or HPH-only milks (Figures 1 and 2). In cheeses made from thermally pasteurized milk, the increase in yield was linearly related to the homogenization pressure from 0 to 300 MPa, with a 1% increment in final yield for every 100-MPa increment in homogenization pressure [yield = $11.04\% + (1.01 \times \text{homogenization pressure})$; $r^2 = 0.98$; $P < 0.01$]. However, cheeses made from raw milk showed an increase

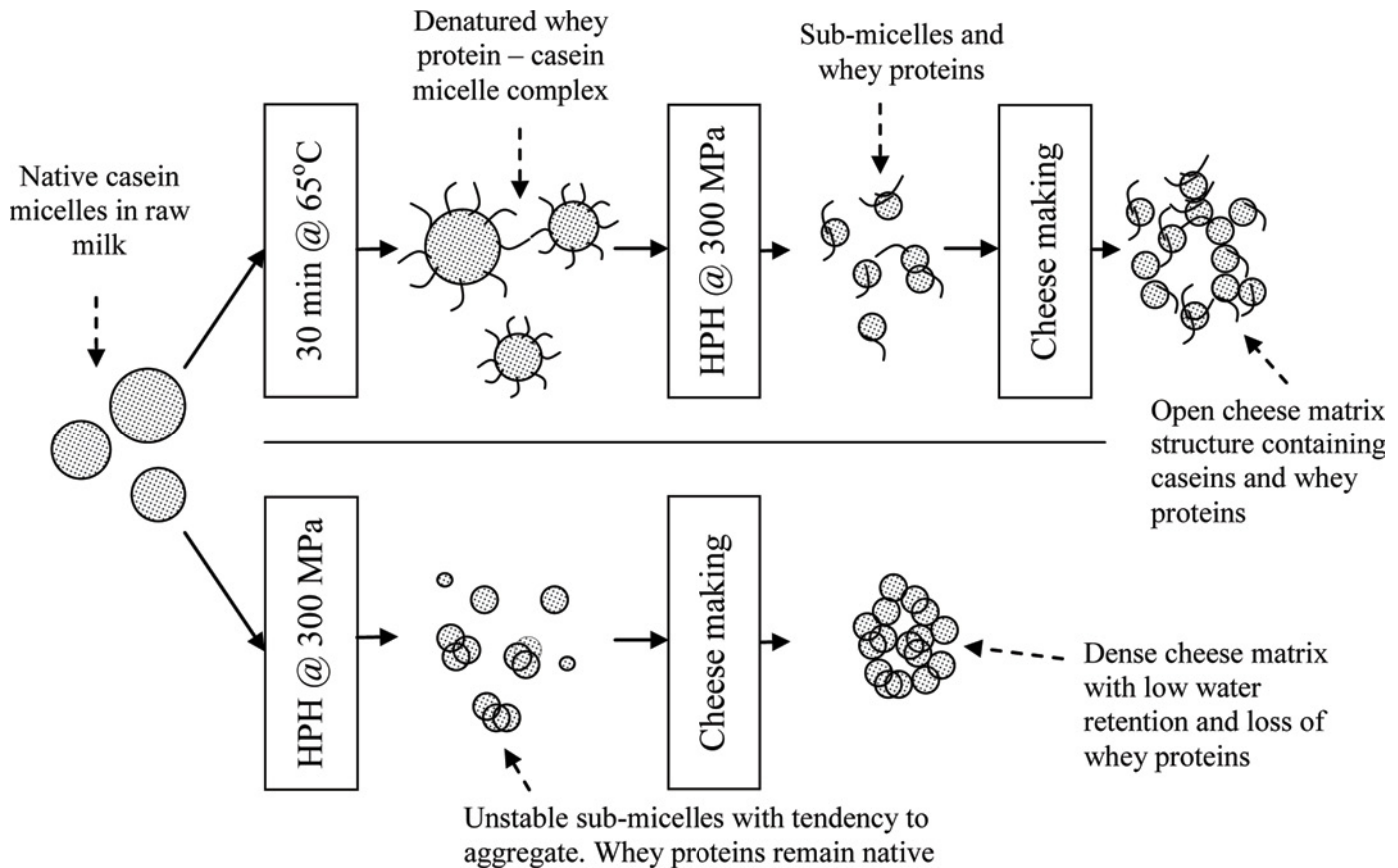


Figure 5. Diagram showing suggested changes in the structure of queso fresco made from raw and pasteurized milk homogenized at 300 MPa (HPH = high-pressure homogenization).

in yield and moisture content that was proportional to the homogenization pressure only up to 200 MPa, and a sharp decrease in both yield and moisture content when raw milk was subjected to homogenization at 300 MPa (Figures 1 and 2). The increased cheese yield and moisture content in cheeses made from raw milk processed with homogenization up to 200 MPa was consistent with previous reports in cheeses made with caprine and bovine milks homogenized up to 100 MPa (Guerzoni et al., 1999; Lanciotti et al., 2004, 2006; Zamora et al., 2007; Burns et al., 2008; Vannini et al., 2008). These authors attributed the better yield to an increase in the water-holding capacity of proteins and to higher whey protein retention during curd formation.

The cheese protein content on a wet basis followed an inverse relationship with homogenization pressure (Figure 3A) and was negatively correlated to cheese moisture content ($r = -0.94$, $P < 0.01$) and cheese yield ($r = -0.97$, $P < 0.01$), which was consistent with better cheese yields explained by higher moisture retention. However, the cheese protein content on a dry basis exhibited a negative simple linear regression with respect to homogenization pressure ($r^2 = 0.99$, $P < 0.01$;

Figure 3B) for both raw and pasteurized milks, with an average 1.4% reduction in total protein content on a dry basis for every 100-MPa increment in homogenization pressure. The relative reduction of total protein

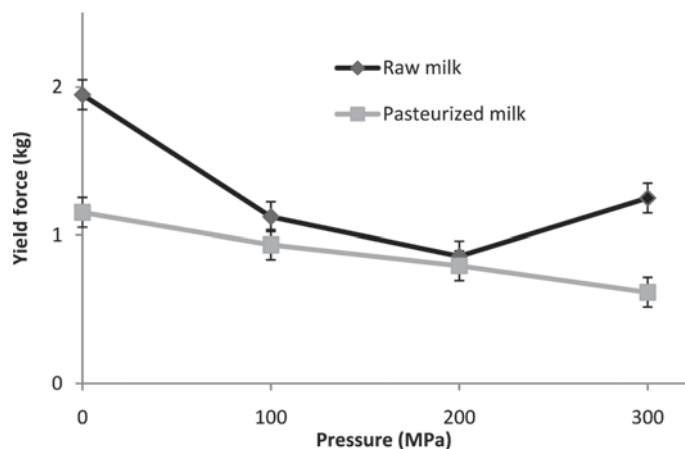


Figure 6. Force at first peak during uniaxial compression of queso fresco made from raw and pasteurized milk subjected to high-pressure homogenization. Bars are 95% CI.

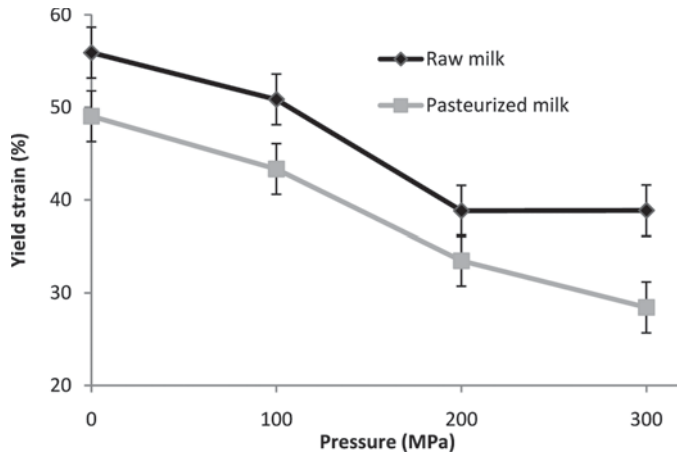


Figure 7. Strain at first peak during uniaxial compression of queso fresco made from raw and pasteurized milk subjected to high-pressure homogenization. Bars are 95% CI.

content on a dry basis suggests that the homogenization process promoted better lactose and fat retention in the cheese. This is hypothetical, because residual lactose and fat contents were not measured in the cheeses. However, sweet whey has a high lactose content and HPH is known to improve the emulsifying properties of the casein micelles, which could improve fat retention in cheese (San Martín-González et al., 2009).

The net amount of protein in milk that was transferred to the cheese (g of protein in cheese/kg of protein in milk) remained essentially constant for all processing conditions, with the exception of raw milk processed at 300 MPa (Figure 3C), in which a sharp decrease in the net amount of protein transferred to cheese was observed. This was an indication that more protein was lost in the sweet whey during curd formation, when extreme homogenization conditions were used in raw milk.

The protein patterns from the SDS-PAGE gels revealed a stronger retention of whey proteins (β -LG and α -LA) in cheese when a combined thermal and 300 MPa homogenization pressure was used to process the milk (Figure 4). However, electrophoretic bands corresponding to whey proteins in cheeses made from raw milk processed with only HPH at 300 MPa (Figure 4) showed relatively lower concentrations than the combined HPH and thermal treatment. Although the homogenization of milk resulted in shear-induced increases of temperature (approximately 18°C for every 100-MPa increase in homogenization pressure), the use of a heat exchanger immediately after the homogenization valve promoted rapid cooling of the milk (<2 s), and extensive denaturation of whey protein was prevented. The SDS gels of cheeses made from raw milk processed at 300 MPa exhibited β -LG bands similar to those of control

cheeses made from raw milk. Furthermore, even though the milk processed at 300 MPa reached about 80°C immediately after the homogenization valve, exposure was so limited that cheese made from raw milk processed at 300 MPa exhibited evidence of enzymatic rancidity due to active lipase enzymes. These observations were consistent with reports by Pereda et al. (2008, 2009), in which limited shear-induced damage was observed in various milk components, including whey proteins.

The constant amount of total protein transferred from milk to cheese and the evidence of increased whey retention in cheeses made from pasteurized and homogenized milk would suggest that some casein may be lost in the sweet whey and some whey proteins are retained in the cheese curd. Roach and Harte (2008) found a decrease in the apparent casein micelle size from 0 to 200 MPa but an increase in micelle diameter in the range from 250 to 350 MPa due to instability and coalescence. It was hypothesized that the pressure- and shear-induced dissociation of the casein micelles at pressure >200 MPa was masked by rapid aggregation of casein submicelles due to temperature-induced hydrophobic interactions, resulting in relatively larger and unstable reformed casein micelles.

Figure 5 summarizes the suggested structural differences between cheeses made from raw and thermally pasteurized milk processed at 300 MPa. Thermal processing promotes extensive whey protein denaturation and interaction with κ -casein in the surface of the casein micelle (Fox and McSweeney, 1998). It is hypothesized that denatured whey proteins would protect the casein micelles from excessive HPH dissociation and would also constitute physical barriers against the re-formation of dense casein aggregates during curd formation, resulting in queso fresco with a relatively open structure and high moisture retention. In the case of cheeses made from raw milk homogenized at 300 MPa, little whey protein denaturation is achieved and the dissociated micelles re-form into dense aggregates, resulting in cheeses with higher density and relatively lower moisture and whey protein retention.

The yield force, defined as the force exerted at the point where the cheese samples started breaking during uniaxial compression, is shown in Figure 6. In the case of cheeses made from thermally pasteurized milk, the yield force decreased linearly with increasing homogenization pressure. For queso fresco made from HPH-treated raw milk, a similar decrease in yield force was observed, but with homogenization pressure up to 200 MPa. The overall yield force for all cheeses exhibited a strong negative correlation with cheese moisture content ($r < -0.96$; $P < 0.001$) and cheese yield ($r < -0.86$; $P < 0.01$) and a positive correlation with protein content on a wet basis ($r > 0.86$; $P < 0.01$).

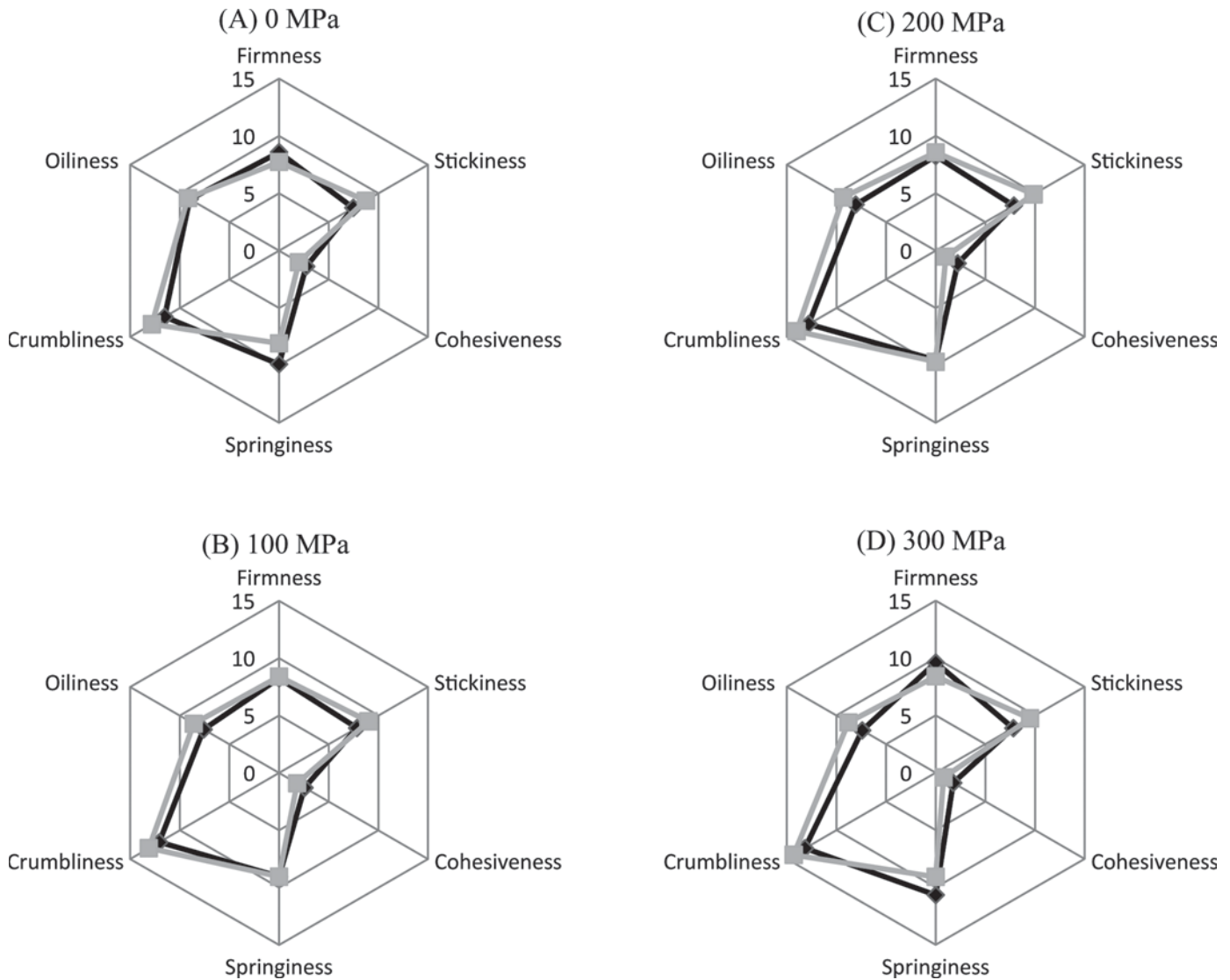


Figure 8. Scores by trained panelists for 6 texture descriptors of queso fresco made from raw (black line) and pasteurized (gray line) milk processed at 0 to 300 MPa homogenization pressure.

Sandra et al. (2004a) found that queso fresco made from milk subjected to high hydrostatic pressure (400 MPa, 20 min) needed less maximum force to compress during 80% compression and exhibited less perceived sensory firmness compared with cheeses made from raw milk. Whereas high hydrostatic pressure and HPH are different processes, it is known that the processing of milk with high hydrostatic pressure at 250 to 400 MPa for >1 min promotes extensive casein dissociation (Harte et al., 2007). Figure 7 shows the relationship between yield strain (strain where the cheese begins to crumble during uniaxial compression) and homogenization pressure for cheese made from raw and thermally processed milks. The behavior was similar to the observed yield force, with a negative correlation with cheese moisture

content ($r < -0.92$; $P < 0.01$) and cheese yield ($r < -0.94$; $P < 0.01$), and a positive correlation with protein content on a wet basis ($r > 0.97$; $P < 0.01$).

Average scores for the tactile sensory evaluation of queso frescos made from raw and pasteurized milks processed by HPH are shown in Figure 8. All cheeses followed similar sensory attribute patterns, resulting in comparable radar charts.

The sensory attribute crumbliness, which is a key quality attribute for queso fresco, exhibited a linear regression in response to the homogenization of milk for both raw milk (crumbliness = $11.5 + 0.0056 \times \text{HPH}$; $R^2 = 0.952$) and pasteurized milk (crumbliness = $12.7 + 0.0054 \times \text{HPH}$; $R^2 = 0.987$). A constant difference of about 1 in the 15-point sensory scale was observed

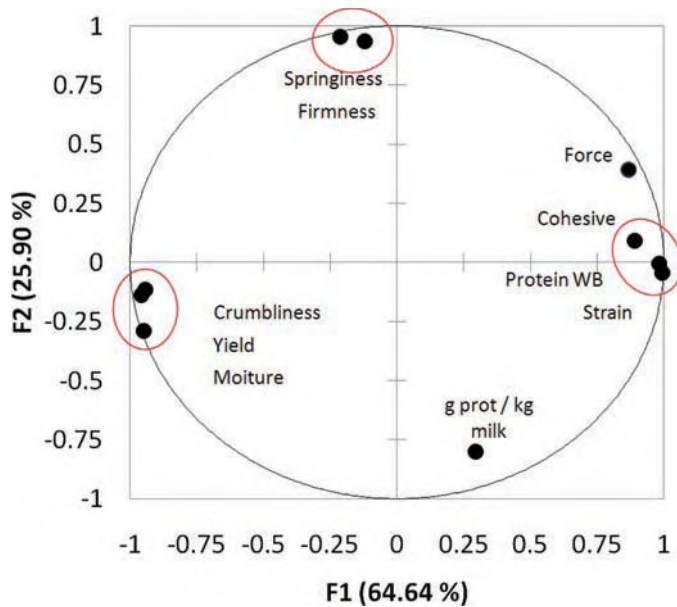


Figure 9. Principal component analysis (PCA) of the various measured parameters in cheeses made from raw and pasteurized milk processed with high-pressure homogenization. Prot = protein; WB = wet basis. Color version available in the online PDF.

for cheeses from pasteurized versus raw milk, regardless of the homogenization pressure. The best crumbliness score was observed in cheeses made from pasteurized milk processed at 300 MPa. In this case, some judges considered that crumbliness was “out of scale” and above the maximum allowable score of 15. The sensory attribute cohesiveness exhibited a strong negative correlation ($r < -0.98$; $P < 0.01$) with the attribute crumbliness.

These results further highlight the differences between processing using HPH versus high hydrostatic pressure, because most reports suggest that cheeses made from milk processed using high hydrostatic pressure exhibit lower crumbliness scores compared with controls made from raw milk (Sandra et al., 2004a; Hnosko, 2007).

A principal component analysis was done using all measured variables exhibiting significant correlations in cheeses made from raw and pasteurized milk subjected to 0 to 300 MPa homogenization pressure (Figure 9). Two principal components explained 90.5% of the observed variability. The group integrated by the variables sensory crumbliness, yield, and moisture content showed strong correlation with each other and negative correlation with the group integrated by the variables protein on a wet basis, yield strain, and sensory cohesiveness. On the other hand, the variables sensory springiness and sensory firmness were grouped together and exhibited negative correlation with the variable grams of protein in cheese per kilogram of milk. The

crumbly nature of queso fresco also resulted in a lack of correlation between the sensory perception of firmness and the yield force measured by a texture analyzer ($r < 0.19$; $P > 0.05$), contrary to data reported by Hort et al. (1997) in Cheddar cheese.

CONCLUSIONS

The combined processing of milk using thermal pasteurization and HPH from 100 to 300 MPa promotes modifications in the constituents of milk, leading to strong changes in yield, moisture content, and sensory properties of queso fresco. Our results and previous reports suggest that the better yield and moisture retention properties of cheeses made from thermally processed milk subjected to 300 MPa pressure are the result of thermal-induced denaturation of whey proteins, together with homogenization-induced dissociation of casein micelles. These combined effects result in queso fresco containing a thin casein–whey protein matrix structure able to better retain sweet whey. The combined use of HPH and thermal processing of milk offers a potential to increase yield, moisture content, and textural quality of queso fresco.

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