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ORIGINAL PAPER

High-pressure treatment of milk in industrial and pilot-scale equipments: effect of the treatment conditions on the protein distribution in different milk fractions

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Abstract The effects of two different high-pressure (HP) equipments, operating at industrial- and pilot scales, and of the HP-release rate on the contents of non-sedimentable proteins and denatured whey proteins were investigated after treatments of skim milk—from 250 to 650 MPa. Non-sedimentable caseins and denatured whey proteins significantly increased with the pressure level. The industrial-scale equipment produced lower micellar disintegration than the pilot-scale equipment with similar degrees of whey protein denaturation. Ultracentrifugation supernatants obtained from skim milk at 100,000×*g* and 20 °C for 1 h were also HP-treated for comparative purposes, showing that, in skim milk, the presence of casein promoted the denaturation of whey proteins, although the extent of whey protein denaturation did not influence the release of casein to the soluble phase. Furthermore, most denatured whey proteins remained soluble after treatment in both equipments. In the pilot-scale equipment, the pressure-release rate influenced casein solubilization and whey protein denaturation.

Keywords HP treatment · Industrial scale · Pilot scale · Pressure-release rate · Milk proteins · Protein distribution

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Introduction

The increased consumer's demand for better quality products, which combine improved or novel sensory and nutritional characteristics with an expanded shelf life, has challenged the food industry to develop new preservation techniques and, in this respect, high-pressure (HP) processing has experienced a huge growth in the last 20 years to become an industrial reality [1].

Although milk was the first food to undergo HP treatments by Hite in 1899, up to now there are only a few commercial pressurized dairy products, such as yoghurt and colostrum [2]. These industrial applications of HP are mainly oriented to improve the safety and stability, while other uses aimed to induce structural changes that ameliorate milk protein functionality have scarcely been exploited [3]. HP treatments cause substantial modifications to milk proteins and to the mineral balance of milk. As a result, casein micelles disaggregate and reaggregate, releasing soluble casein particles, and whey proteins denature [4, 5]. Micellar disruption results in the formation of new protein structures, difficult to attain by the use of conventional processing methods, that influence rennet coagulation properties of milk, favor acid coagulation, and could act as functional units for the encapsulation and delivery of nutrients in dairy products [6]. These events depend on the intensity, duration, and temperature of the treatment as well as on the pH and protein concentration [6–13]. While the influence of most of these variables is now well documented, the effect of the type of pressure unit, and its specific operation parameters, has seldom been looked at [14].

HP equipments consist of a vessel: a cylinder filled with a pressure-transmitting fluid, usually potable water, or a water/oil mixture; one or several pumps to generate

pressure; a temperature-control device and the material-handling system [15]. HP-processing equipments differ in terms of scale, size, vertical or horizontal orientation, and other features. Product specifications include maximum vessel pressure, inner vessel diameter, inner vessel height or length at maximum pressure, rate of pressure built-up and release, pressure-transmitting fluid, and temperature range. Both pilot-scale and industrial production-scale systems are available. In the food industry, vessels with a volume of several hundred liters are in use, with typical operating pressures in the range 250–600 MPa, holding times of about 1–8 min, and fast come-up and come-down times, as the cycle time or production rate is an important consideration. Laboratory-scale HP equipments capable of reaching pressures up to 1,400 MPa are also available with either versatile or specific designs [16].

The aim of this work was to study the influence of the differential features of two pieces of HP equipment for processing at industrial and pilot scales, in particular, the pressure-transmitting fluid and the resulting treatment temperature, and the rate of pressure release, on the distribution of milk proteins between the colloidal and soluble phases in milk and on whey protein denaturation, upon pressure treatments between 250 and 650 MPa. In addition to skim milk, skim milk ultracentrifugation supernatants (100,000×*g* and 20 °C for 1 h) were also HP-treated for comparative purposes.

Materials and methods

Samples

For HP treatment experiments, both skim raw bovine milk and skim milk ultracentrifugation supernatants were used. Milk, collected from a local farm, was warmed to 37 °C during 30 min to facilitate fat separation and skimmed by centrifugation at 3,000×*g* and 20 °C for 30 min, followed by filtration through glass wool. Aliquots of skim milk were ultracentrifuged at 100,000×*g* and 20 °C for 1 h in a Beckman L70 preparative ultracentrifuge (Beckman Instruments Inc., San Ramon, CA, USA) using a type 70 Ti rotor. The ultracentrifugation supernatants were kept at 4 °C for a maximum of 18 h before the HP treatments.

HP treatments

Skim milk and skim milk ultracentrifugation supernatants equilibrated to 15 °C were subjected to 250, 350, 450, 550, and 650 MPa in an industrial-scale equipment, consisting of a 120-L vessel with water as pressure-transmitting fluid and three hydraulically driven pressure generating units (Wave 6000/120 model, NC Hyperbaric, Burgos, Spain), and in a pilot-scale equipment, consisting of a water-jacketed (5–90 °C) 1-L vessel with silicon oil as pressure-transmitting fluid and a mechanically driven pressure generating unit (TE 9000, Thiot Ingenierie, NC Hyperbaric, Bretenoux, France-Burgos, Spain). The pilot-scale equipment allowed the time setting for pressure increase and release, while the industrial-scale one did not. The pressure was raised at a rate of 3.04 and 6 MPa/s in the industrial-scale and in the pilot-scale equipment, respectively, maintained for 5 min in both equipments and released in 1.5 s in the industrial-scale equipment (IF) and either in 30 s or 5 min in the pilot-scale equipment (respectively, PF—pilot-scale equipment with fast depressurization and PS—pilot-scale equipment with slow depressurization).

The temperature of the pressure-transmitting fluid was recorded during the pressure treatments. The initial temperature was 14 ± 2 °C and it increased during pressure build-up, so that, during the holding phase, it was 25.9 and 39.5 °C at 350 MPa or 32.5 and 46.9 °C at 650 MPa, in the industrial and pilot-scale equipments, respectively.

Two independent IF, PF, and PS experiments were carried out using the same milk batch. Following the HP treatments, the samples were stored overnight at 4 °C before the analyses that were carried out at least in duplicate.

Protein fractions for analysis

Fractions soluble at pH 4.6 were obtained by drop-wise addition of 2 M HCl under continuous stirring, followed by a 20-min standing period at room temperature, centrifugation at 4,000×*g* and 20 °C for 30 min, and filtration through Whatman no. 40 filter paper.

Ultracentrifugation supernatants were obtained from HP-treated skim milk samples by ultracentrifugation at 100,000×*g* and 20 °C for 1 h, as described above.

Analysis of milk and protein fractions

Determination of the protein content

The total protein content of samples was determined by the Kjeldahl method, according to the reference procedure published by the International Dairy Federation [17] to determine total nitrogen. The total nitrogen was multiplied by a factor of 6.38 to obtain the protein content. Samples were digested using a DK-20 Heating Digester (Velp Scientifica Srl., Usmate, Italy) and distilled using a UDK 142 Automatic Distillation Unit (Velp Scientifica Srl., Usmate, Italy).

Capillary electrophoresis

Capillary electrophoresis separations were performed using a Beckman P/ACE System 2050 and a TSP-coated fused-silica capillary (BGB Analytik Vertrieb, Schlossboeckelheim, Germany) of 57 cm (effective length of 50 cm), 0.50 μm i.d., and slit opening of 100 × 800 μm, at 45 °C with a linear gradient from 0 to 25 kV in 3 min, followed by a constant voltage of 25 kV during 47 min. The injection time was 60 s, and detection was at 214 nm. Protein identification was carried out according to Recio et al. [18].

Statistical analysis

Results from two independent experiments were expressed as mean values ±95 % confidence intervals and were analyzed by a two-way analysis of variance (ANOVA) to test the influence of the two studied factors (pressure level and type of treatment, that is, HP unit or pressure-release time) and their interaction. In addition, in order to compare both HP units and to assess the effect of the pressure-release time within the pilot-scale unit and the effect of the pressure level within each treatment, data were also analyzed by the least significant difference test (LSD), considering confidence levels of 95 %. The Statgraphic Plus 5.0 for Windows was used for data processing (Statistical Graphics Corporation, Washington, USA, www.statgraphics.com).

Results

Non-sedimentable caseins and whey proteins in HP- treated skim milk

The total protein contents of the ultracentrifugation supernatants of HP-treated skim milk samples are shown in Fig. 1. The statistical analysis revealed that the pressure level and the type of treatment (IF, PF and PS) significantly affected the concentration of soluble proteins and that there were interactions between both factors ($P < 0.01$).

There was more soluble protein in the ultracentrifugation supernatants of the skim milk samples treated in the pilot-scale equipment (PF and PS) than in those treated in the industrial-scale equipment (IF) at all pressure levels ($P < 0.01$). The highest contents of non-sedimentable proteins were found at 550, 450, and 250 MPa in the IF, PF, and PS treatments, respectively.

The analysis by CE of the individual caseins released to the soluble phase in both pieces of equipment with similar depressurization rates (Fig. 2; Table 1) showed that the IF treatment led to a significantly lower solubilization of all caseins than the PF treatment ($P < 0.05$). At 250 MPa, the

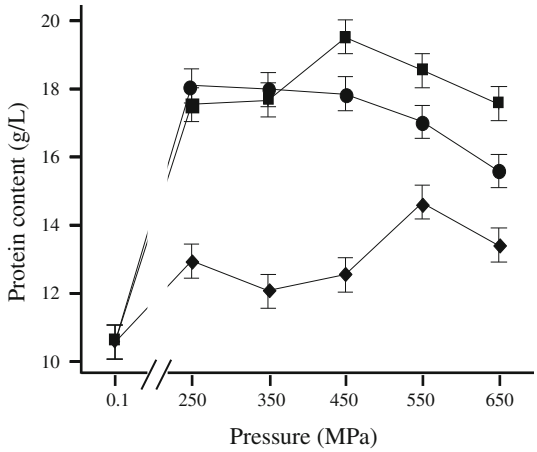

Fig. 1 Protein content (g/L) of the ultracentrifugation supernatants of control skim milk (0.1 MPa) and milk pressurized at 250, 350, 450, 550, and 650 MPa and 15 °C, during 5 min, in an industrial-scale (IF, *diamond*) and a pilot-scale equipment with fast (PF, *square*) and slow depressurization (PS, *circle*). The *bars* represent 95 % confidence intervals ($n = 2$)

levels of α_S-caseins (α_{S1}- 9P, α_{S1}- or α_{S2}-casein) increased from 12.4 to 15.0 % of the content of each individual protein in the control milk to 17.3–29.6 % and 34.1–45.2 % after the IF and PF treatments, respectively, while β-caseins (βA_1-, βA_2- or βB-casein) increased from 10.5–18.4 % to 21.8–25.4 % (IF) and 35.7–30.3 % (PF). At this pressure, the soluble κ-casein amounted to 28.8 and 50.8 % of its content in milk after IF and PF treatments. Following treatments at higher pressures, in general terms, in the industrial-scale equipment (IF), non-sedimentable individual caseins increased slightly or leveled off at 350 and 450 MPa, with maximum solubilization occurring after treatments at 550 and 650 MPa. This trend was similar in the pilot-scale equipment with fast depressurization although, in this case, the contents of non-sedimentable caseins reached their maximum after pressure treatments at 450 and 550 MPa.

Non-sedimentable whey proteins present in the ultracentrifugation supernatants, particularly β-Lg, gradually decreased with the pressure level in both pieces of equipment (Fig. 2; Table 1), what probably accounted for the reductions in the total soluble protein content observed at the highest pressures (mainly at 550 and 650 MPa, after IF and PF treatments, respectively, Fig. 1). Although soluble β-Lg decreased at a somehow slower rate after IF than after PF treatments, the differences were only significant at 350 MPa.

Denaturation of whey proteins in HP- treated skim milk

The total protein contents of the fractions soluble at pH 4.6 of HP-treated skim milk samples are shown in Fig. 3. In all treatments, the amount of protein significantly decreased

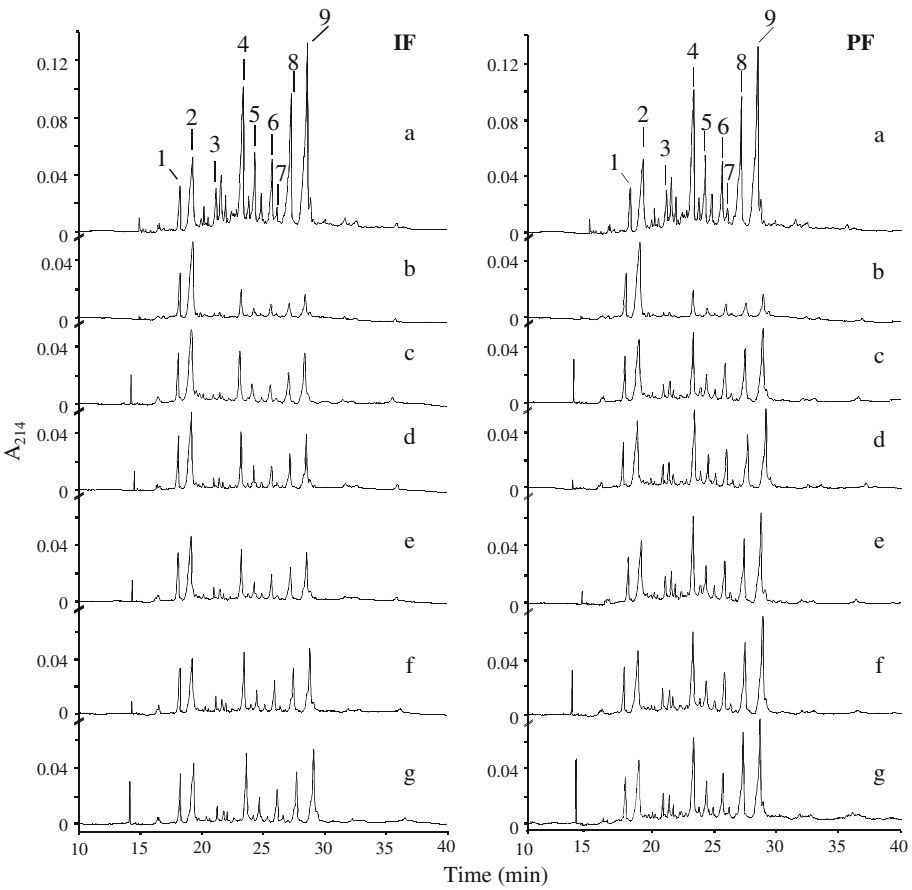

Fig. 2 Electropherograms of skim milk (*a*) and ultracentrifugation supernatants of: control skim milk (0.1 MPa, *b*) and milk pressurized at 250, 350, 450, 550, and 650 (*c*–*g*) and 15 °C, during 5 min, in an industrial-scale (IF) and a pilot-scale equipment with fast depressurization (PF). *1*: α-La; *2*: β-Lg; *3*: α_{S2}-CN; *4*: α_{S1}-CN; *5*: α_{S1}-CN 9P; *6*: κ-CN; *7*: βB-CN; *8*: βA_1-CN; *9*: βA_2-CN

with the pressure level ($P < 0.01$) due to the denaturation of whey proteins. There were significant differences between both equipments, IF and PF, and the pilot-scale equipment at slow depressurization rate, PS, at pressures ≥450 MPa, with whey protein denaturation following the order PS > PF > IF.

The loss of solubility of the individual whey proteins, α-La and β-Lg, at pH 4.6, as determined by CE, is illustrated in Fig. 4. Approximately 10 % of α-La was denatured after IF and PF treatments at pressures ≥450 MPa, increasing to 25 % at 650 MPa. Denaturation of α-La was significantly higher after PS treatments at similar HP levels, reaching approximately 40 % at 650 MPa. β-Lg denaturation progressively increased with pressure ($P < 0.01$), amounting to, approximately, 90 % at 650 MPa. There were significant differences among the three types of treatment at pressures ≥350 MPa ($P < 0.01$). The order of β-Lg denaturation, as estimated by the loss of solubility at pH 4.6, was as follows: PS > PF > IF.

Non-sedimentable and denatured whey proteins in HP-treated skim milk ultracentrifugation supernatants

Figure 5 shows the soluble protein contents of HP-treated milk ultracentrifugation supernatants. The type of pressure unit and the pressure-release rate within the pilot-scale equipment did not influence significantly the solubility of whey proteins. In the industrial-scale equipment (IF) and in the pilot-scale equipment with either fast (PF) or slow pressure-release rate (PS), these values remained constant up to 550 MPa, decreasing by 8.3–13.1 % at 650 MPa ($P < 0.05$).

As shown in Fig. 6, the content of whey proteins soluble at pH 4.6 started to decrease at pressures ≥350 MPa. There were significant differences between the two pieces of equipment ($P < 0.05$) and the depressurization rate at pressures ≥550 MPa ($P < 0.01$), following the order PS > PF > IF.

Discussion

Effect of the HP equipment and depressurization rate on the release of non-sedimentable casein to the soluble phase of milk

The results of this study showed that the type of HP equipment and the pressure-release rate influenced the composition of the colloidal and soluble fractions of milk. The contents of non-sedimentable caseins and denatured

Table 1 Levels of individual proteins present in the ultracentrifugation supernatants of control skim milk (0.1 MPa) and milk pressurized at 250, 350, 450, 550, and 650 and 15 °C, during 5 min, in an industrial-scale (IF) and a pilot-scale equipment with fast depressurization (PF)

Proteins	HP equipments	Pressure (MPa)						Pooled SD
		0.1	250	350	450	550	650	
α-La	IF	$^{A}117.7^{b}$	$^{A}116.9^{b}$	$^{A}116.2^{b}$	$^{B}112.8^{b}$	$^{A}109.0^{ab}$	$^{A}94.9^{a}$	4.0
	PF	$^{A}117.7^{c}$	$^{A}107.4^{b}$	$^{A}99.6^{ab}$	$^{A}99.0^{ab}$	$^{A}101.3^{ab}$	$^{A}93.4^{a}$	2.9
β-Lg	IF	$^{A}106.2^{d}$	$^{A}100.8^{d}$	$^{B}91.8^{c}$	$^{A}76.4^{b}$	$^{A}71.1^{ab}$	$^{A}65.48^{a}$	2.4
	PF	$^{A}106.2^{d}$	$^{A}93.3^{c}$	$^{A}77.5^{b}$	$^{A}70.3^{ab}$	$^{A}71.3^{ab}$	$^{A}63.6^{a}$	2.6
α_{S2}-CN	IF	$^{A}14.4^{a}$	$^{A}17.3^{a}$	$^{A}17.2^{a}$	$^{A}19.8^{ab}$	$^{A}28.4^{b}$	$^{A}29.3^{b}$	1.5
	PF	$^{A}14.4^{a}$	$^{B}34.1^{b}$	$^{B}39.7^{c}$	$^{B}46.2^{de}$	$^{B}50.8^{e}$	$^{B}43.5^{cd}$	1.4
α_{S1}-CN	IF	$^{A}12.4^{a}$	$^{A}24.1^{bc}$	$^{A}21.1^{b}$	$^{A}22.7^{b}$	$^{A}27.5^{c}$	$^{A}33.5^{d}$	0.8
	PF	$^{A}12.4^{a}$	$^{B}39.7^{b}$	$^{B}40.5^{b}$	$^{B}42.7^{b}$	$^{B}44.3^{b}$	$^{B}43.6^{b}$	1.5
α_{S1}-CN 9P	IF	$^{A}15.0^{a}$	$^{A}29.6^{c}$	$^{A}23.2^{b}$	$^{A}26.2^{bc}$	$^{A}30.3^{cd}$	$^{A}35.0^{d}$	2.3
	PF	$^{A}15.0^{a}$	$^{B}45.2^{b}$	$^{B}47.2^{b}$	$^{B}49.1^{bc}$	$^{B}57.4^{c}$	$^{B}46.0^{bc}$	2.5
κ-CN	IF	$^{A}19.0^{a}$	$^{A}28.2^{bc}$	$^{A}24.9^{b}$	$^{A}28.5^{c}$	$^{A}36.4^{d}$	$^{A}39.2^{d}$	1.0
	PF	$^{A}19.0^{a}$	$^{B}50.8^{b}$	$^{B}51.5^{b}$	$^{B}55.1^{b}$	$^{B}56.3^{b}$	$^{B}55.8^{b}$	2.1
β-CN B	IF	$^{A}18.4^{a}$	$^{A}25.4^{b}$	$^{A}23.6^{b}$	$^{A}23.1^{b}$	$^{A}30.5^{c}$	$^{A}34.2^{c}$	1.0
	PF	$^{A}18.4^{a}$	$^{B}37.0^{b}$	$^{B}42.1^{b}$	$^{B}42.2^{b}$	$^{B}46.0^{b}$	$^{A}39.3^{b}$	2.2
β A_1-CN	IF	$^{A}10.5^{a}$	$^{A}21.8^{b}$	$^{A}18.4^{b}$	$^{A}19.6^{b}$	$^{A}27.2^{c}$	$^{A}31.2^{c}$	1.0
	PF	$^{A}10.5^{a}$	$^{B}35.7^{b}$	$^{B}37.7^{b}$	$^{B}40.9^{b}$	$^{B}45.2^{b}$	$^{B}46.4^{b}$	2.2
β A_2-CN	IF	$^{A}13.5^{a}$	$^{A}25.2^{c}$	$^{A}22.6^{b}$	$^{A}22.9^{b}$	$^{A}30.1^{c}$	$^{A}34.9^{c}$	1.7
	PF	$^{A}13.5^{a}$	$^{B}39.3^{b}$	$^{B}41.0^{bc}$	$^{B}44.4^{bc}$	$^{B}46.6^{bc}$	$^{B}47.7^{c}$	2.0

Values ($n = 2$) are expressed as percentages of the content of each individual protein in raw skim milk estimated by capillary electrophoresis

$^{a–d}$ Different superscripts, on the right and in rows, indicate significant differences ($P < 0.05$) produced by the pressure treatment and $^{A–B}$different superscripts, on the left in columns for each protein, mean significant differences ($P < 0.05$) produced by the high-pressure equipment

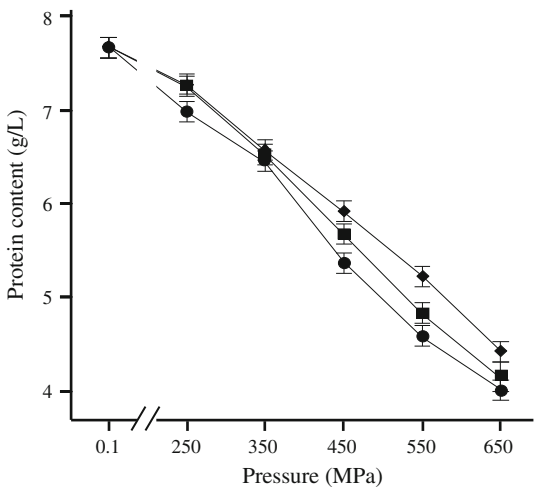

Fig. 3 Soluble protein at pH 4.6 (g/L) in control milk (0.1 MPa) and milk pressurized at 250, 350, 450, 550, and 650 MPa and 15 °C during 5 min, in an industrial-scale (IF, *diamond*) and a pilot-scale equipment with fast (PF, *square*) and slow depressurization (PS, *circle*). The *bars* represent 95 % confidence intervals ($n = 2$)

whey proteins significantly increased with the pressure level under the three experimental conditions considered, although differences were found between the industrial and the pilot-scale equipments and between the two HP-release times assayed.

During HP treatment of milk, the casein micelles disintegrate due to disruption of intermolecular electrostatic interactions and solubilization of colloidal calcium phosphate [10, 19]. In-situ measurements of the light transmission of milk show that casein disruption occurs at pressures from 100 MPa, although at intermediate pressures of 250 and 300 MPa, initial disintegration of caseins during the first 5 min of treatment is followed by a progressive aggregation in the course of prolonged pressure treatment, due to solvent-mediated interactions or increased hydrophobic interactions at these intermediate pressures [10, 20]. The resulting casein micelles are markedly more hydrated and either casein fragments or individual caseins solubilize, accounting for increases of non-sedimentable proteins at pressures ≥100 MPa [11, 13]. This implies that the level of non-sedimentable casein is inversely correlated with casein micelle size except for the aggregated samples [8].

In general terms, the levels of soluble caseins found (Figs. 1, 2) were in the range of those previously reported in HP-treated milk, with some differences that could be attributed to the use of different ultracentrifugation speeds

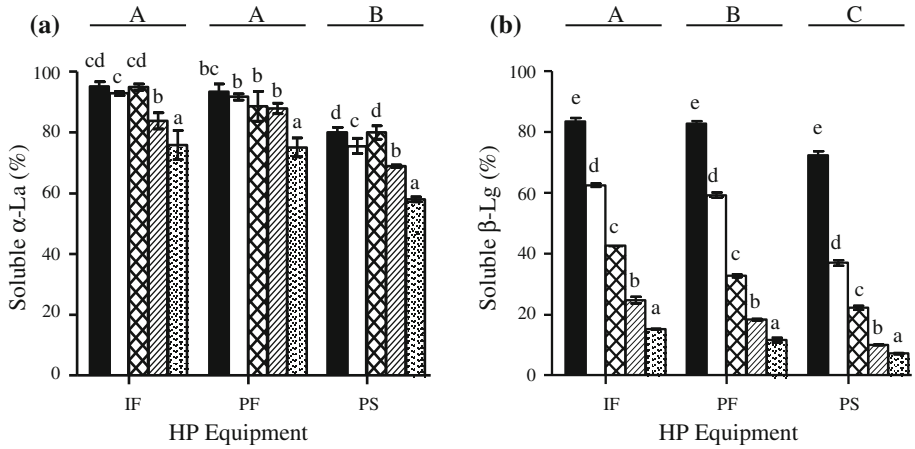

Fig. 4 Contents of pH 4.6 soluble α-La (**a**) and β-Lg (**b**), as measured by capillary electrophoresis, of milk pressurized at 250 (■), 350 (□), 450 (⊠), 550 (▨), and 650 (⊡) MPa and 15 °C during 5 min in an industrial-scale (IF) and a pilot-scale equipment with fast (PF) and slow depressurization (PS). The *bars* represent standard deviation ($n = 2$). *Capital letters* indicate significant differences ($P < 0.05$) due to the type of treatment (HP unit or pressure-release rate, IF, PF, and PS) while *low-case letters* indicate significant differences ($P < 0.05$) due to the pressure level

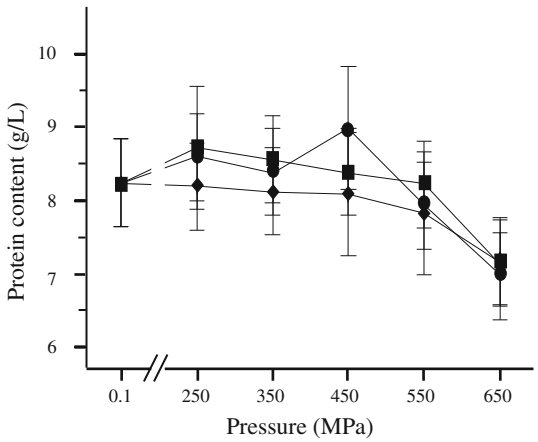

Fig. 5 Protein content (g/L) of control skim milk ultracentrifugation supernatants (0.1 MPa) and ultracentrifugation supernatants pressurized at 250, 350, 450, 550, and 650 MPa and 15 °C during 5 min in an industrial-scale (IF, *diamond*) and a pilot-scale equipment with fast (PF, *square*) and slow depressurization (PS, *circle*). The *bars* represent 95 % confidence intervals

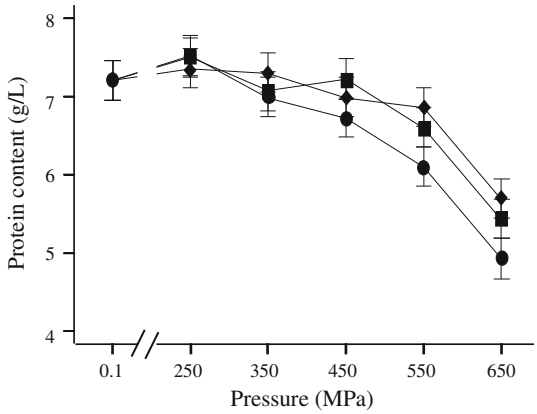

Fig. 6 Soluble protein at pH 4.6 (g/L) in control skim milk ultracentrifugation supernatants (0.1 MPa) and ultracentrifugation supernatants pressurized at 250, 350, 450, 550, and 650 MPa and 15 °C during 5 min in an industrial-scale (IF, *diamond*) and a pilot-scale equipment with fast (PF, *square*) and slow depressurization (PS, *circle*). The *bars* represent 95 % confidence intervals ($n = 2$)

[7, 8]. The maximum content of non-sedimentable casein reported by other authors, up to 40 or 50 % of the total casein content, is usually achieved at 250–300 MPa applied for 30 min at 20 °C [7, 8, 11, 13]. In the present study, and after the treatments with fast depressurization -IF and PF-, the maximum levels of non-sedimentable proteins were attained at pressures higher than those previously reported. This could be attributed to the comparatively shorter treatment time used since, even if the formation of non-sedimentable casein is very quick during the first 5 min of pressure treatment, it continues to increase gradually up to 60 min [7, 8]. In addition, the higher the pressure, the more rapid is the disruption of the casein micelles [20].

As compared with the pilot-scale treatment, the industrial-scale treatment led to a significantly lower content of non-sedimentable proteins, and the maximum release of casein to the soluble phase was achieved at higher pressures. These differences could be attributed, at least partially, to the different pressurization temperatures reached in both systems. During pressurization, and assuming that compression is uniform and there are no thermal losses, there is a temperature increase that depends on factors such as pressure level, holding time, composition of the pressure-transmitting fluid, insulating materials used, etc. [21–24]. The temperature increase as a result of adiabatic heating during pressure build-up re-equilibrates during the

holding phase. Under our experimental conditions, a treatment time of 5 min was chosen to resemble a commercial situation where a high product output is important. This holding time was too short for temperature dissipation, particularly in the industrial-scale equipment with a volume 150 times bigger than the pilot-scale one (which in turn was water-jacketed at 15 °C). While this would have resulted in a higher effective temperature during the 5-min IF treatments, the actual temperature was, for instance, 13.6 and 14.4 °C higher during PF treatments at 350 and 650 MPa. This is because of the use of silicon oil as pressure-transmitting fluid, which has higher compressibility, lower heat capacity, and lower thermal conductivity than water [21, 23]. A higher temperature would have reduced the solubility of colloidal calcium phosphate and promoted hydrophobic bonding, stabilizing casein micelles against disruption [20]. However, previous results on the effect of temperature on casein disintegration indicate that an increase in the treatment temperature, in the range of 10–40 °C, increases the rate at which casein was liberated to the supernatant [8]. However, the differences reported by Anema et al. [8] in the levels of non-sedimentable casein between 30 and 40 °C, during the first 5 min of treatment at 200–600 MPa, were less than 10 %, and, therefore, the existence in our case of an additional effect cannot be discarded (Table 1).

Regarding the effect of the pressure-release rate, it should be noted that comparable levels of non-sedimentable proteins were found after both treatments in the pilot-scale equipment, although the maximum release of casein to the soluble phase was achieved at lower pressures after the longest pressure-release time (PS) (Fig. 1). This suggests that a slow pressure-release rate could favor casein disintegration, which agrees with previous results showing that, at 250 and 350 MPa, the slower the pressure-release rate, the higher the level of soluble casein [25]. The observation that at pressures higher than those leading to the maximum release of casein to the soluble phase the level of non-sedimentable casein is lower (Figs. 1, 2; Table 1) has also been made by other authors [8, 11, 13] and could be attributed to a restricted reformation of the solubilized micellar particles, even if an extensive reformation of casein aggregates, such as that occurring at intermediate pressures of 250–300 MPa, requires that pressure-induced disruption is neither limited nor very intensive [10]. In addition, at 550 and 650 MPa, the decrease in soluble solids (Fig. 1) could also be partially attributed to the loss of solubility of β-Lg and even that of α-La (Table 1; Fig. 2), that could account for a subsequent reduction in the total non-sedimentable protein content in the ultracentrifugation supernatants.

Influence of whey protein denaturation

The present results show that pressurization of skim milk ultracentrifugation supernatants, that contain whey proteins virtually in the absence of casein micelles, reduced the levels of both soluble and native proteins at pressures ≥550 MPa, although comparison of the fractions soluble at the normal milk pH and at pH 4.6 (Figs. 5, 6) indicated the existence of soluble denatured whey proteins. The loss of solubility of α-La and β-Lg at normal milk pH and at pH 4.6 was much more important in pressure-treated skim milk (Figs. 3, 4). These results corroborate that whey proteins are more affected by pressure when present in a milk system as compared with a serum system as, in the former, they could associate with caseins and precipitate with them at their isoelectric point [26, 27]. Nevertheless, in pressurized milk, most denatured whey proteins also remained soluble at the normal milk pH (Table 1), in agreement with Anema [7] and López-Fandiño et al. [13]. This suggests that soluble denatured whey proteins preferably self-associate or interact with the non-sedimentable casein particles released from the casein micelles upon pressure-induced disruption, and thus, they are not likely to take part in micellar changes occurring during HP treatments [25]. In this respect, although it has been suggested that association of denatured whey proteins with casein micelles could offset the HP-induced size decrease [26], pressurization experiments with casein micelles in the absence and presence of whey proteins indicated that whey protein denaturation does not interfere with micellar dissociation or reassociation [8, 28, 29].

The extent of denaturation is known to depend on the pressure level, treatment time, pH, and temperature [5]. The present study also shows that, for equivalent pressures and holding times, other factors such as the type of equipment or the depressurization rate could influence the results, although their effects were only significant when pressures of around 350–450 MPa or higher were applied to milk (Figs. 3, 4), or pressures ≥550 MPa were applied to milk ultracentrifugation supernatants (Fig. 6). Despite the higher treatment temperature, the lowest denaturation corresponded to the industrial-scale equipment. Similarly, in the pilot-scale equipment, the long pressure-release time (PS) increased whey protein denaturation over the short pressure-release time (PF) although, in agreement with previous results [25], most whey protein denaturation was shown to occur during the pressure holding time.

The results obtained suggest that HP treatments in the industrial and pilot-scale equipments studied produced different protein distributions due to a distinct disintegration of the casein micelles with similar levels of whey protein denaturation. While the presence of casein

promoted denaturation of the whey proteins, the extent of whey protein denaturation did not show any influence in micellar disruption. Thus, the industrial-scale equipment led to lower micellar disintegration than the pilot-scale equipment, but most denatured whey proteins, whether attached or not to the non-sedimentable casein particles, remained soluble in both systems. This highlights the need for adapting the process conditions to the operation characteristics of each particular type of equipment if HP is used to induce protein changes aimed to tailor protein functionality.

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