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Combined effects of high-pressure treatment and storage temperature on the physicochemical properties of caprine milk

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ABSTRACT

The effects of high-pressure (HP) treatment (200–500 MPa for 25 min at 25 °C) combined with storage temperature (25 and 4 °C) on the physicochemical properties of raw caprine milk were studied. Storage of HP-treated and untreated milk samples at 25 °C considerably affected the changes in the conformation of milk proteins, which were reflected by changes in the protein sedimentation rate, gradual decreases in the soluble calcium and phosphorus contents, a slight decrease in pH, an insignificant decrease ($P > 0.05$) in viscosity, and a decrease in the casein hydration level of milk at the end of the storage time. In contrast, the HP-treated and untreated milk samples stored at 4 °C demonstrated different characteristics than the samples stored at 25 °C. These results could be due to calcium and phosphate association with caseins, which screen charges and reduce the repulsion of micelles during the storage time.

1. Introduction

Caprine milk is of increasing economic importance, and the technological qualities of this milk have received more attention. On average, caprine milk contains 3.5% protein, 3.8% fat, 4.1% lactose, 0.8% ash, 1.2 g calcium and 1 g phosphate per litre, and these concentrations are similar to those in cow milk (Lad, Aparnathi, Mehta, & Velpula, 2017).

During high-pressure (HP) treatment of milk at room temperature, several changes in milk proteins occur, such as a reduction in the size of casein micelles and denaturation of β-lactoglobulin (β-LG) and α-lactalbumin (α-LA) (Lopez-Fandino, Carrascosa, & Olano, 1996; Nassar, Mladenovic, Orlien, & Knudsen, 2016). In a casein micelle model, colloidal calcium phosphate (CCP) crosslinks sub-micelles caseins and neutralises negatively charged phosphoserine groups, allowing the formation of hydrophobic interactions between caseins (Anema, 2008; Cadesky, Walkling-Ribeiro, Kriner, Karwe, & Moraru, 2017; López-Fandiño, 2006). In line with this process, hydrophobic and electrostatic interactions between proteins are disrupted and CCP is solubilised under pressure (Knudsen & Skibsted, 2010; Orlien, Boserup, & Olsen, 2010). After HP treatments, casein sub-micelles move from the casein micelles to the serum. Consequently, there are considerable changes in the casein micelle size, a decrease in milk turbidity and colour lightness, and an increase in milk viscosity (Devi, Buckow, Singh, Hemar, & Kasapis, 2015; Kim, Kim, Choi, Min, & Kwak, 2008; Tran, Roberts, Felix, & Harte, 2018).

During prolonged pressurisation (200–300 MPa), the average casein micelle size initially decreases; however, after some time the average casein micelle size increases and exceeds the average size of casein micelles in non-pressurised milk (Huppertz, Fox, & Kelly, 2004a; Regnault, Thiebaud, Dumay, & Cheftel, 2004).

The amount of β-LG and α-LA denaturated at HP increases with longer holding time, higher temperature and pH of milk (Huppertz, Fox, & Kelly, 2004b; Keim & Hinrichs, 2004; Scollard, Beresford, Needs, Murphy, & Kelly, 2000a). During the storage period, renaturation of α-LA and β-LG occurs within 1–2 days at 20–40 °C (Goyal, Sharma, Upadhyay, Sihag, & Kaushik, 2013). In contrast, reassociation does not take place at lower temperatures (5 °C) because the mobility of atoms is too low to form hydrophobic and ionic bond (García-Risco, Olano, Ramos, & Lopez-Fandino, 2000). Furthermore, decreases in temperature increases the solubility of CCP; thus, the cooling of milk results in the dissolution of CCP, but these changes are reversible by rewarming the milk (Pierre & Brule, 1981; Regnault et al., 2004). Therefore, in the present study, the

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combined effects of high-pressure (HP) treatment, storage temperature and storage time on the physicochemical properties of raw caprine milk were evaluated.

2. Materials and methods

2.1. Caprine milk samples

Fresh caprine milk samples were obtained from a caprine farm in Hebei province (China). Broad spectrum microtabs (0.045%) were added to the milk samples as a preservative, except for the milk samples tested for the determination of turbidity, colour and size distribution. Then, the milk samples were stored in a refrigerator at 4 ± 1 °C further use.

2.2. High hydrostatic pressure treatment

The caprine milk was transferred to plastic bottles, which were filled without head-space and subsequently vacuum-packed in polyethylene bags. The caprine milk samples were subjected to high hydrostatic pressure in an HPP L2 600 thermostatted hydraulic-pressure chamber (Huatai Senmiao, Tianjing, China). Isostatic pressures of 200, 400 and 500 MPa at 25 °C were held for 25 min. After pressure release, the pressurised caprine milk samples were stored at ambient temperature, i.e., 25 ± 2 °C, and at refrigerator temperature, i.e., 4 ± 1 °C, for up to 4 days and the first measurements were taken 24 h after pressurisation of the samples.

2.3. Determination of pH

The pH values of untreated and HP-treated goat milk samples were determined with a pH meter (Hanna Instruments pH 211 Microprocessor pH Meter) with a glass electrode (Hanna HI1230).

2.4. Determination of soluble calcium and phosphorus

The concentrations of soluble Ca and P in the control and pressurised caprine milk samples were determined using an inductively coupled plasma mass spectrophotometer (Agilent, Santa Clara, CA, USA). The samples were prepared following the methods of Frederiksen et al. (2011) with slight modifications. In addition, the samples were separated into soluble and colloidal phases by using high-speed centrifugation. Ten millilitres of sample were centrifuged at 100,000×*g* for 1 h using a T4-TI-70 rotor in a Beckman-Coulter Optima L-80XP Ultracentrifuge (Beckman Coulter Inc., Brea, CA, USA). The supernatant was carefully collected for analysis. All samples were measured in triplicate.

2.5. Determination of the hydration of ultracentrifuged pellet

The casein hydration level of the control and HP-treated caprine milk samples were determined according to the methods of Gaucheron et al. (1997). After ultracentrifugation at 77,000×*g* (Beckman Coulter Inc., T4-TI-70 rotor) for 2 h at 4 °C, the supernatants were carefully separated from the pellets. Then, the pellets were weighed and dried at 103 °C for 7 h to remove the water of hydration. The hydration values (grams of water g^{-1} of dry pellet) were calculated by the difference in the weight of the samples between before and after drying. All samples were measured in triplicate.

2.6. Nitrogen analysis

The total nitrogen (TN) fraction was determined for the control and pressurised caprine milk samples by macro-Kjeldahl (IDF, 2014; 20–1:2014; AOAC 2010; method 991.20, while the nonprotein nitrogen (NPN) was measured using the AOAC 2010 method 991.21), both as described by Barbano, Lynch, and Fleming (1991). Moreover, the noncasein nitrogen (NCN) and NPN contents were determined from the nitrogenous compounds that were soluble at pH 4.6 and in a 15% trichloroacetic acid solution, respectively. Nitrogen fractions were calculated by the difference in the experimental data obtained as previously described, i.e., casein nitrogen (CN) = TN-NCN; whey protein nitrogen (WPN) = NCN-NPN, and:

$$(DWPD\ \%) = \frac{(WPN\ Control - WPN\ HP\ treated) * 100}{WPN\ Control}, \quad (1)$$

where DWPD % is the degree of whey protein denaturation, WPN Control is the whey protein nitrogen in the control milk sample, and WPN HP-treated is the whey protein nitrogen in the pressurised milk samples.

2.7. Turbidity

The turbidity (absorbance value) of untreated and HP-treated caprine milk samples were measured after 24 h in storage at different temperatures, i.e., 25 °C and 4 °C, with ELISA-like spectrophotometer assay (spark 20 m multimode micro plate reader from TECAN, Mannedorf, Switzerland) by the transmission of light at 860 nm (Shanmugam, Chandrapala, & Ashokkumar, 2012). After centrifugation of milk samples at 15,000×*g* for 1 h at 4 °C, the supernatants were carefully separated from the pellets and fat. All samples were measured in triplicate.

2.8. Determination of colour parameters

Colour measurements of the HP-treated and untreated caprine milk samples were made using a portable Hunter Lab Colour Quest XE Spectrophotometer (Birstall, Leicestershire, UK) after calibrating the original value with a standard plate. Three measurements were taken from each sample. Ten millilitres of each sample were warmed to 20 °C before analysis and analysed for colour coordinate values with the following variables: L*, lightness; a*, red/green coordinates; b*, yellow/blue coordinates.

2.9. Viscosity

The viscosity values of the samples (20 mL) were measured at 25 °C and 4 °C using a Rheometer (Physica MCR 301, Anton Paar, Graz, Austria) with a CC27-SN13078 probe at 100 rpm every 300 s. All samples were measured in triplicate.

2.10. Particle size distribution

The Z-average particle sizes of the unpressurised and pressurised whole caprine milk samples were determined by using a Zetasizer Nano ZS -Zeta potential analyser (Malvern Instruments Ltd, Worcestershire, UK) after the milk was diluted with distilled water (1:50, v/v). All measurements were performed at 25 °C using the refractive index of the milk protein, i.e., 1.45.

2.11. Statistical analysis

Statistical analysis of data was performed by applying two-way ANOVA (for colour, size distribution and turbidity), three-way ANOVA (for the rest of the parameters) and multiple comparisons of the means of each treatment using Tukey's HSD at a confidence level of 95%. Data analysis was carried out with SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. pH

The changes of pH over the storage period (four days) at 25 °C and 4 °C was a consequence of the HP. Fig. 1A shows, as the storage period increased, small variations in the milk pH were measured. All pH values were in the range 6.54–6.63. However, the caprine milk treated at 500 MPa had a higher pH than the milk subjected to the other treatments from the first to the fourth day of storage. Meanwhile, the significant decrease in pH in the pressurised milk samples over the four-day during storage period at 25 °C might have been caused by a conversion of Ca and P from the serum phase to the colloidal phase ($CaHPO_4$) through the reaction shown in equation (2) (De La Fuente, Olano, Casal, & Juárez, 1999; Nassar et al., 2016; Tsioulpas, Lewis, & Grandison, 2007).

$$Ca^{2+} + H_2PO_4^- \rightarrow CaHPO_4 + H^+ \quad (2)$$

According to Fig. 1B, over the whole storage period at 4 °C, there were no marked differences in the pH values of the HP-treated and untreated raw goat milk samples. However, the pH values of all control and HP-treated milk samples increased significantly ($P < 0.05$) in the range 6.65–6.76. This significant change is related to dissolution of CCP due to the effects of cold temperature during storage time. The main phosphate species, $H_2PO_4^-$, is generally unprotonated when present as insoluble calcium phosphate. Thus, as the concentration of H^+ ions decreases, the protons in the of HP-treated and control milk samples are absorbed by the phosphate ions dissolved in the milk serum (De La Fuente, Requena, & Juárez, 1997; Huppertz, Kelly, & Fox, 2002).

3.2. Hydration

Fig. 2 shows the effects of HP at 25 °C for 25 min in combination with storage at 25 °C or 4 °C on the hydration of the ultra-centrifuged pellets. The samples stored at 25 °C (Fig. 2A) and

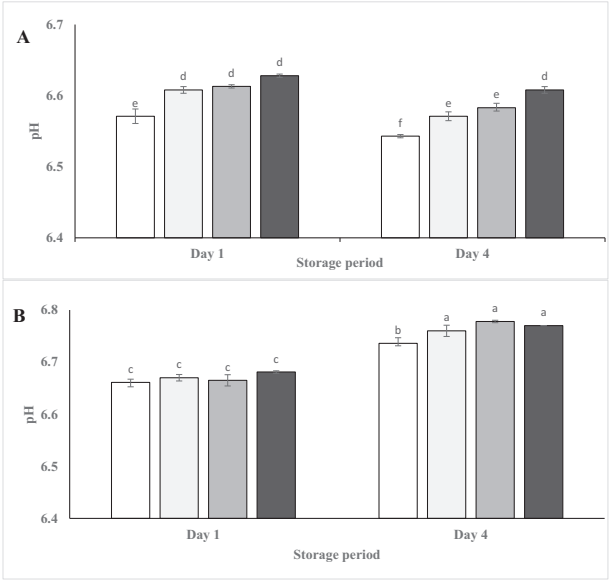

Fig. 1. Effect of high-pressure processing combined with storage temperatures (A) of 25 °C, and (B) of 4 °C on pH of (□) untreated and HP-treated at (▒) 200, (■) 400 and (■) 500 MPa caprine milk. Data points are averages of triplicate measurements, and error bars represent ±1 SD. Different letters within days indicate significant differences ($P < 0.05$).

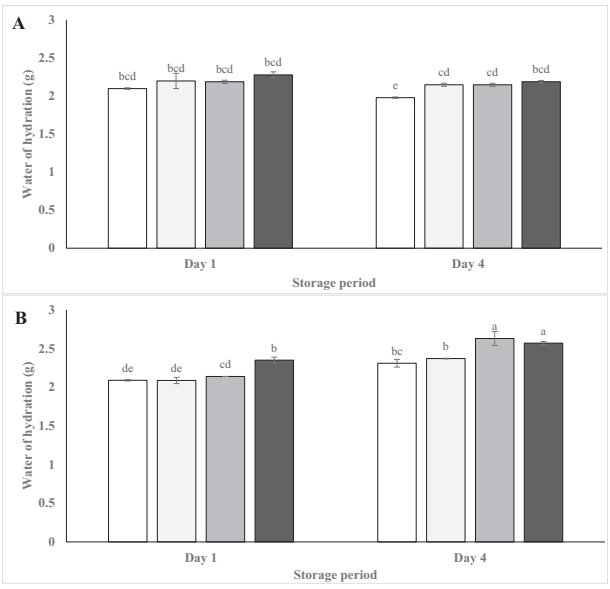

Fig. 2. Effect of high-pressure processing combined with storage temperatures (A) of 25 °C, and (B) of 4 °C on hydration of (□) untreated and HP-treated at (▒) 200, (■) 400 and (■) 500 MPa caprine milk. Data points are averages of triplicate measurements, and error bars represent ±1 SD. Different letters within days indicate significant differences ($P < 0.05$).

treated by HP had a higher average hydration than the untreated milk samples. Subsequently, by the fourth day of storage, there were nonsignificant decreases in the hydration of all HP-treated samples. These decreases may be due to the effect of the storage temperature because at a higher storage temperature (25 °C), the contents of all the individual casein micelles in the soluble phase decreased over the storage time, as clearly shown in the control samples over the whole storage period (Ali, Andrews, & Cheeseman, 1980; Pierre & Brule, 1981; Regnault et al., 2004).

For storage at 4 °C (Fig. 2B), the average hydration of the HP-treated and untreated caprine milk samples increased significantly over the whole storage period (four days). On the first day of storage, the control milk and milk pressurised at 200 MPa had the same hydration value (2.09 g water g^{-1} dry pellet), whereas the milk treated at 500 MPa had a significantly higher hydration value (2.35 g water g^{-1} dry pellet). These increases in the average hydration values were caused by pressure treatment-induced ionisation (Table 1) (Gaucheron et al., 1997; Sood, Sidhu, & Dewan, 1976). Furthermore, the hydration of casein micelles increases with decreasing micelle size (Table 2). On the fourth day of storage, there was a significant increase in the hydration for all treatments samples; milk samples treated at 400 MPa and 500 MPa had significantly higher hydration values than the milk treated at 200 MPa and the control milk sample. This may be caused by the storage temperature, which plays an important role in casein micelle hydration. The hydration of casein micelles occurs as a result of the distribution of casein molecules between the micellar and soluble phases in milk. As seen in Table 1, at all storage temperatures, the CN level in HP-treated milk samples decreased over storage. Thus, the soluble casein content is increased by lowering the temperature, which consequently increases the hydration of casein micelles (Ali et al., 1980; Huppertz et al., 2004b; Pierre & Brule, 1981).

3.3. Nitrogen compounds

On the first day of storage at 25 °C and 4 °C, the content of NPN in the serum phase of milk pressurised at 200, 400 and 500 MPa

Table 1
Effect of high-pressure treatment at 200, 400 and 500 MPa for 25 min at 25 °C and subsequent storage for up to 4 d at 25 and 4 °C, on nitrogen compounds and calcium and phosphorous content in the serum phase of HP-treated and untreated caprine milk samples.[a]

Treatments	Parameters	Days of storage at 25 °C		Days of storage at 4 °C		Statistical analysis		
		1	4	1	4	SEM	R	CV
Untreated	NPN (%)	0.031^{a}	0.033^{a}	0.032^{a}	0.035^{a}	0.024	0.94	3.94
	NCN (%)	0.094^{a}	0.094^{a}	0.093^{ab}	0.094^{a}	0.001	0.94	3.53
	Whey nitrogen (%)	0.061	0.061	0.061	0.059	–	–	–
	CN (%)	0.381^{g}	0.380^{g}	0.380^{g}	0.381^{g}	0.001	0.94	0.76
	Calcium (mM)	8.83^{fghi}	8.62^{ij}	8.68^{hij}	8.96^{efg}	0.104	0.94	1.73
	Phosphorous (mM)	13.65^{def}	12.06^{h}	12.74^{g}	13.87^{cde}	0.125	0.87	1.55
200 MPa	NPN (%)	0.032^{a}	0.048^{a}	0.034^{a}	0.042^{a}	0.024	0.94	3.94
	NCN (%)	0.083^{cd}	0.090^{abc}	0.084^{cd}	0.08^{bcd}	0.001	0.94	3.53
	DWPD (%)	16.01^{h}	7.09^{j}	18.03^{g}	9.97^{i}	0.206	0.99	1.40
	CN (%)	0.392^{ef}	0.384^{fg}	0.391^{ef}	0.389^{efg}	0.001	0.94	0.76
	Calcium (mM)	9.37^{abc}	8.72^{ghij}	9.03^{defg}	9.35^{abc}	0.104	0.94	1.73
	Phosphorous (mM)	15.11^{b}	13.26^{fg}	14.04^{cd}	15.50^{ab}	0.125	0.87	1.55
400 MPa	NPN (%)	0.034^{a}	0.047^{a}	0.039^{a}	0.041^{a}	0.024	0.94	3.94
	NCN (%)	0.071^{ef}	0.080^{d}	0.071^{ef}	0.078^{de}	0.001	0.94	3.53
	DWPD (%)	39.40^{e}	32.89^{f}	47.50^{c}	39.30^{e}	0.206	0.99	1.40
	CN (%)	0.404^{abc}	0.395^{de}	0.405^{abc}	0.396^{cde}	0.001	0.94	0.76
	Calcium (mM)	9.32^{abcd}	8.78^{ghij}	9.11^{cdef}	9.51^{ab}	0.104	0.94	1.73
	Phosphorous (mM)	15.24^{ab}	13.48^{ef}	14.35^{c}	15.69^{a}	0.125	0.87	1.55
500 MPa	NPN (%)	0.040^{a}	0.050^{a}	0.040^{a}	0.047^{a}	0.024	0.94	3.94
	NCN (%)	0.067^{f}	0.079^{de}	0.068^{f}	0.071^{ef}	0.001	0.94	3.53
	DWPD (%)	55.70^{a}	45.50^{d}	54.10^{b}	47.60^{c}	0.206	0.99	1.40
	CN (%)	0.410^{ab}	0.396^{cde}	0.413^{a}	0.404^{bcd}	0.001	0.94	0.76
	Calcium (mM)	9.60^{a}	8.51^{j}	9.23^{bcde}	9.63^{a}	0.104	0.94	1.73
	Phosphorous (mM)	15.49^{ab}	13.35^{ef}	15.13^{b}	15.72^{a}	0.125	0.87	1.55

[a] Abbreviations are: SEM, standard error of mean; R, R-square; CV, coefficient of variance; NPN, nonprotein nitrogen; NCN, noncasein nitrogen; DWPD, degree of whey protein denaturation; CN, casein nitrogen. Results are the means of data from triplicate experiments on individual milk samples; different superscript letters indicate significant differences between means ($P < 0.05$).

Table 2
Changes in turbidity (absorbance value), particle size (d = average particle diameter nm), viscosity (mPa s) and colour parameters following pressure treatment at 200, 400 and 500 MPa at 25 °C for 25 min and storage at 25 or 4 °C for 4 days after treatment.[a]

Treatments	Storage temperature (°C)	Storage time (days)	Absorbance	d (nm)	Viscosity (mPa s)	Colour parameters		
						$^{a}L^{*}$	$^{b}a^{*}$	$^{c}b^{*}$
Untreated	25	1	0.1372 ± 0.0024^{a}	328.66 ± 1.13^{a}	1.78 ± 0.04^{fg}	95.34 ± 0.03^{a}	-1.74 ± 0.17^{a}	3.80 ± 0.46^{a}
		4	nd	nd	1.59 ± 0.01^{9}	nd	nd	nd
	4	1	0.1064 ± 0.0004^{c}	302.50 ± 5.42^{bc}	3.31 ± 0.01^{e}	95.12 ± 0.07^{ab}	-1.66 ± 0.03^{a}	3.56 ± 0.17^{a}
		4	nd	nd	3.56 ± 0.02^{cde}	nd	nd	nd
200 MPa	25	1	0.1175 ± 0.0024^{b}	313.06 ± 3.15^{ab}	1.93 ± 0.01^{fg}	94.87 ± 0.00^{bc}	-1.50 ± 0.01^{a}	4.48 ± 0.16^{a}
		4	nd	nd	1.68 ± 0.03^{fg}	nd	nd	nd
	4	1	0.0931 ± 0.0007^{d}	278.50 ± 1.55^{d}	3.52 ± 0.02^{de}	94.78 ± 0.21^{bcd}	-1.62 ± 0.01^{a}	4.54 ± 0.69^{a}
		4	nd	nd	3.90 ± 0.01^{bc}	nd	nd	nd
400 MPa	25	1	0.0805 ± 0.0014^{e}	289.23 ± 2.1^{cd}	2.04 ± 0.02^{f}	94.44 ± 0.07^{cd}	-1.34 ± 0.17^{a}	4.93 ± 0.12^{a}
		4	nd	nd	1.92 ± 0.01^{f}	nd	nd	nd
	4	1	0.0723 ± 0.0007^{fg}	230.35 ± 2.49^{e}	3.73 ± 0.03^{cd}	94.43 ± 0.17^{cd}	-1.60 ± 0.20^{a}	4.67 ± 0.07^{a}
		4	nd	nd	4.27 ± 0.02^{b}	nd	nd	nd
500 MPa	25	1	0.0735 ± 0.0019^{ef}	211.73 ± 2.24^{f}	2.08 ± 0.01^{f}	94.57 ± 0.22^{cd}	-1.23 ± 0.46^{a}	4.97 ± 0.83^{a}
		4	nd	nd	2.05 ± 0.01^{f}	nd	nd	nd
	4	1	0.0665 ± 0.0006^{g}	201.60 ± 3.63^{f}	4.25 ± 0.04^{b}	94.40 ± 0.04^{d}	$-1\ 0.35 \pm 0.48^{a}$	4.88 ± 0.37^{a}
		4	nd	nd	5.37 ± 0.09^{a}	nd	nd	nd

[a] Abbreviations are: L*, lightness (L* = 0 yields black and L* = 100 indicates diffuse white), a*, position between red/magenta and green (negative values indicate green and positive values indicate magenta), b*, position between yellow and blue (negative values indicate blue and positive values indicate yellow), nd, not determined. Data are means ± SD (n = 3 independent experiments); different superscripts within a column indicate significant differences ($P < 0.05$).

increased with increasing pressure compared with that of the control milk sample (Table 1). Caprine milk samples pressurised at 500 MPa and stored at 25 °C and 4 °C had higher NPN (0.040%) than at the samples HP-treated at 400 and 200 MPa. In addition, these results agree with an earlier study on the effect of HP on nitrogen compounds in milk (Kiełczewska, Czerniewicz, Michalak, & Brandt, 2004). In contrast, when the treatment pressure was increased to 500 MPa, the NCN content decreased, and these decreases in the NCN content were related to denaturation of whey proteins, which are denatured at pressures higher than 200 MPa. In addition, these results agree with Scollard et al. (2000a) and Huppertz et al. (2002). Consequently, samples treated at 500 MPa and stored at 25 °C and 4 °C had a significantly higher percentage of whey protein denaturation than the other milk samples (55.7% and 54.1%, respectively) (Table 1) (Considine, Patel, Anema, Singh, & Creamer, 2007; Huppertz et al., 2004b; Nassar et al., 2016).

On the final day of storage day at 25 °C or 4 °C, a decrease in CN and an increase in NPN were noted (Table 1). These differences, i.e., the decrease in CN and increase in NPN, could be explained by the presence of other minor nitrogen compound such as proteose-

peptones (Upadhyay, McSweeney, Magboul, & Fox, 2004) that, due to the increased proteolysis of milk treated at HPs, is probably related to the HP-induced disruption of casein micelles (Devi et al., 2015; Kim et al., 2008; Scollard et al., 2000a). Additionally, the effects of storage temperature on CN may be due to the activation of plasmin, which is resistant to inactivation by pressure (García-Risco, Recio, Molina, & López-Fandiño, 2003; Huppertz, Fox, & Kelly, 2004d; Scollard, Beresford, Murphy, & Kelly, 2000b). The disruption of casein micelles (increasing the protein surface area available) facilitates the proteolysis of caseins by residual active plasmin, which readily hydrolyses β-casein and, more slowly, α_{S1}- and α_{S2}-caseins (Bastian & Brown, 1996). It was observed that HP-treated samples stored at 25 °C had lower values of CN than the HP-treated samples stored at 4 °C.

In addition, the degree of whey protein denaturation for all HP-treated milk samples decreased significantly. The decreasing degree of denaturation was greater for the HP- treated samples stored at 25 °C than those stored at 4 °C (10.2% and 6.5%, respectively), for samples HP-treated at 500 MPa (calculated from Table 1). In addition, possibly due to the renaturation of β-Lg and α-La at 25 °C (Goyal et al., 2013), at 4 °C, the mobility (energy) of atoms is too low to form hydrophobic and ionic bonds. In addition, at low temperature, the strength of hydrophobic interactions is very low; thus, reassociation does not take place or occurs at low levels (García-Risco et al., 2000).

3.4. Calcium and phosphorus in the serum phase

In the HP-treated and control raw caprine milk samples, there was a gradual decrease in the soluble Ca and P contents from the first to the fourth day of storage period at 25 °C (Table 1). On the first day of storage at 25 °C, the control sample showed the lowest concentration of Ca and P in the serum phase. However, raw caprine milk treated at 500 MPa had higher concentrations of Ca and P in the serum phase than the other HP-treated milk samples (Table 1). This could be due to the HP dissolution of CCP into hydrated calcium phosphate and partial further dissociation into charged ions in the serum (Huppertz & Kruif, 2007; López-Fandiño, 2006; Nassar et al., 2016; Orlien et al., 2010).

Toward the end of storage at 25 °C, gradual decreases in the Ca and P contents in the serum phase were measured in milk HP-treated at 200, 400 or 500 MPa compared with the control milk. These decreases could be due to reassociation and aggregation of casein micelles, because the association of Ca and P with casein molecules screens charge and reduces the repulsion of micelles (Huppertz, Fox, & Kelly, 2004c; Nassar et al., 2016).

Table 1 shows the changes in the mineral balance (Ca and P in the serum phase) at 4 °C in the control and pressurised raw caprine milk samples over 4 days of storage. The soluble Ca and P concentrations increased during the first day of cooling. The raw caprine milk sample HP-treated at 500 MPa had the highest concentrations of Ca and P in the serum phase, with concentrations of 9.23 and 15.13 mM, respectively. This result may be due to the migration of CCP to the liquid phase as a result of HP. After that, the contents of C and P in all HP-treated and control milk samples gradually increased until the end of the storage period at 4 °C. This increase could be a consequence of the solubilisation of Ca and P during cold storage (De La Fuente et al., 1997; Raynal & Remeuf, 2000; Regnault et al., 2004).

In contrast, the concentrations of Ca and P in the micellar phase of HP-treated milk samples showed have the opposite behaviour with respect to the concentrations of Ca and P in the serum phase (data not shown). In the HP-treated and untreated caprine milk samples, there was a gradual increase in the micellar Ca and P contents from the first to the fourth day of storage at 25 °C (data not shown).

3.5. Viscosity

The viscosity of the HP-treated milk samples ranged from 1.93 to 2.80 mPa s at 25 °C after the application of HP (data not shown). Variation in milk viscosity during storage is mainly dependent on milk components such as casein, whey protein and milk fat. Table 2 shows the changes in the viscosity of HP-treated and control raw caprine milk samples over storage at 25 and 4 °C.

During the first day of storage at 25 °C (Table 2), an increase in viscosity was measured in the HP-treated samples when the pressure increased from 200 to 500 MPa compared with untreated milk (1.93, 2.08 and 1.78 mPa s, respectively). The raw caprine milk sample pressurised at 500 MPa had the highest viscosity, with a value of 2.08 mPa s. In addition, the viscosity of milk changed with the milk components, and this change was mainly because an increase in pressure leads to increased denaturation of whey proteins (Table 1), which tend to form whey protein/casein complexes. Furthermore, the dissolved CCP as a result of HP increased the soluble calcium level (as seen in Table 1), which reduced the repulsion among negatively charged casein molecules and affected viscosity. All of these reasons could lead to an increase in the milk viscosity (Altuner, Alpas, Erdem, & Bozoglu, 2006; Ting, Liu, Li, & Hua, 2016; Tran et al., 2018).

At the end of the storage period at 25 °C, nonsignificant decreases ($P > 0.05$) in the viscosity values were measured for the milk samples HP-treated at 200, 400 and 500 MPa compared with those of the control milk samples. This result could be due to the reassociation and aggregation of casein micelles as the associations of Ca and P with casein molecules screen charges and reduce the repulsion of micelles. This decrease is related to the effect of the storage temperature (Huppertz et al., 2004c; Knudsen & Skibsted, 2010; Orlien et al., 2010).

According to Table 2, the viscosity of the HP-treated and untreated caprine milk samples over the whole storage period increased under cold storage conditions compared with that under warm storage conditions.

The viscosity values of HP-treated milk ranged from 3.51 to 4.25 mPa s during the first day of storage at 4 °C. Notably, the samples HP-treated at 500 MPa (for 25 min at 25 °C) and stored at 4 °C had the significantly highest viscosity value at all sampling times, while the control milk samples showed the lowest values of viscosity (Table 2).

On the fourth day of storage at 4 °C, significant increases in the viscosity were measured in the milk HP-treated at 200, 400 and 500 MPa compared with those in the control milk. This could be a consequence of the solubilisation of Ca and P during cold storage and the distribution of casein between the micellar and soluble phases of milk (De La Fuente et al., 1997; Raynal & Remeuf, 2000; Ting et al., 2016). Meanwhile, there was a strong positive correlation ($r = 0.87$) between pH and viscosity of milk samples (Fig. 3A). The pH value of HP-treated milk samples may indicate the dissolution of CCP into hydrated calcium phosphate, which consequently resulted in a change in the viscosity.

3.6. Turbidity and size distribution

The destabilisation of casein micelles is known to be caused by HP treatment, which results in a more translucent appearance, reduction in turbidity, and smaller average casein micelle diameter (Gaucheron et al., 1997; Needs, Stenning, Gill, Ferragut, & Rich, 2000; Orlien et al., 2010). The results presented in Table 2 indicate that the turbidity (value of absorbance at 860 nm after centrifugation at 15,000×g for 1 h at 4 °C) and particle size decreased with increasing pressure due to the disruption of casein micelles, which form small micelles (Knudsen & Skibsted, 2010). In

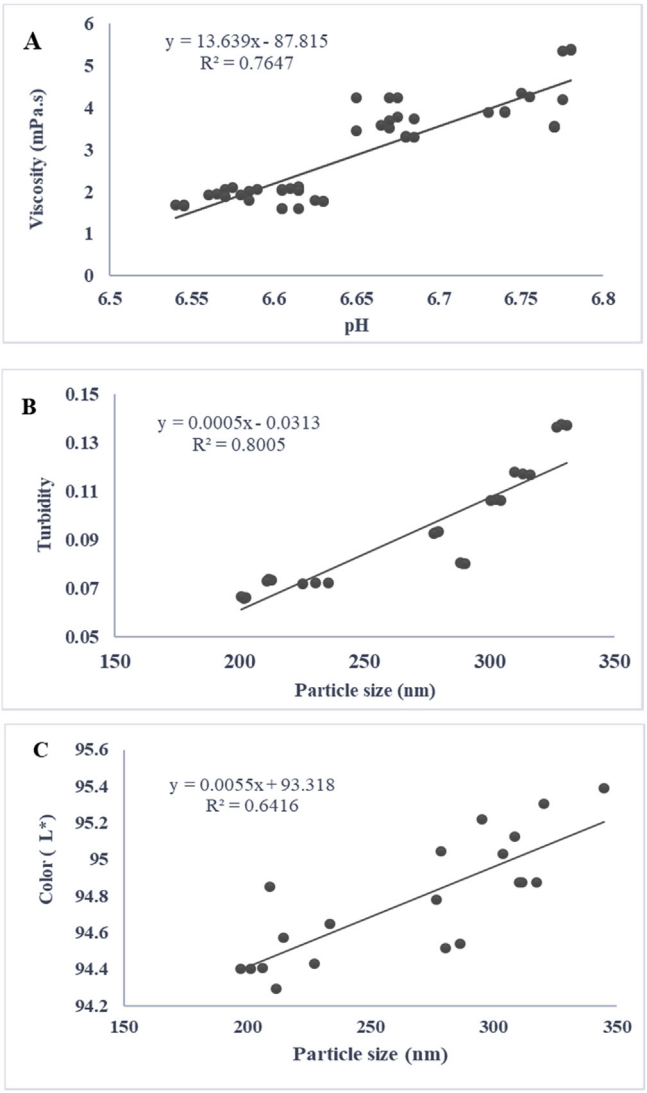

Fig. 3. Correlations between (A) pH and viscosity, (B) particle size and turbidity and (C) particle size and colour (L*) for untreated and HP-treated caprine milk samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

addition, an increase in storage temperature (25 °C) has been found to reduce the fraction of soluble caseins, the hydration of micelles (Orlien et al., 2010), and the return of soluble calcium and phosphate to the micellar structure (seen in Table 1). The turbidity and particle size values for all HP samples stored at 25 °C were higher than the pressurised caprine milk samples stored at 4 °C (Table 2). Moreover, in the present study, there was a strong positive correlation (r = 0.89) between size distribution and turbidity of caprine milk samples (Fig. 3B). The samples pressurised at 500 MPa had the lowest turbidity and particle size distribution compared with those of the other HP-treated and untreated milk samples when stored at 25 °C and 4 °C with turbidity values of 0.0735 and 0.0665, respectively, and size values of 211.73 and 201.60 nm, respectively.

3.7. Colour

Table 2 compares the colour of the HP-treated milk and raw caprine milk after 24 h of storage at 25 °C and 4 °C. The L values of the HP-treated caprine milk samples decreased with increasing pressure compared with those of the control milk sample, whereas the a values increased in the HP-treated samples. The b values showed the same general trend as the a values, and these results are consistent with a previous study by Kim et al. (2008). Regarding the colour parameter L*, the lowest value was for the 500 MPa/25 °C sample after 25 min of storage at 4 °C (94.40). According to Desobry-Banon, Richard, and Hardy (1994), HP treatment reduces the L value of milk. This result is mainly due to the disruption of casein micelles, which form small fragments that increase the translucence of milk. Also, there was a strong positive correlation (r = 0.74) between particle size and L value of milk samples (Fig. 3C). Additionally, these results are understandable in terms of size distribution and turbidity, as shown by the earlier results in this study.

4. Conclusions

High pressure treatment reduced casein micelle size of raw caprine milk, resulting in decreases in milk turbidity and colour lightness and increases in the denaturation of the serum protein, the levels of nonprotein nitrogen compounds, the degree of hydration micelles, the solubilisation of CCP and the viscosity. Thus, HP treatment combined with storage temperature and time induces a dynamic equilibrium disturbance in milk proteins and consequently may exert an influence on the stability of these proteins with consequent implications for milk processing.

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