3.5. CHANGES IN TEXTURAL, MICROSTRUCTURAL, AND COLOUR CHARACTERISTICS DURING RIPENING OF CHEESES MADE FROM RAW, PASTEURIZED OR HIGH-PRESSURE-TREATED GOATS' MILK.

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Changes in textural, microstructural, and colour characteristics during ripening of cheeses made from raw, pasteurized or high-pressure-treated goats' milk

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Abstract

Goats' milk cheeses were made from raw (RA), pasteurized (PA; 72°C, 15 s) or pressure-treated (PR; 500 MPa, 15 min, 20°C) milk to compare textural, microstructural, and colour characteristics in relation to ripening time. Texture, microstructure and colour were evaluated by uniaxial compression and stress relaxation tests, confocal laser scanning microscopy and Hunter colorimetry, respectively.

Raw and PR cheeses were firmer and less fracturable than PA cheese, but differences became less notable toward the end of ripening. PA and PR cheeses were less cohesive than RA cheese. Although cheeses exhibited a loss of elastic characteristics with ageing, PR cheese showed the most elastic behaviour initially. Confocal laser scanning micrographs displayed PR cheese with a regular and compact protein matrix, with small and uniform fat globules resembling the structure of RA cheese. Finally, colour evaluation demonstrated significant differences between cheeses due to milk treatments and ripening time.

Keywords: Texture; Microstructure; Colour; Goats' milk cheese; High-pressure treatment

1. Introduction

It is without doubt that for consumers texture plays an important role in food quality. Texture, particularly in cheese, is one of the most important attributes that help to determine the identity of a product. One generally accepted definition of texture is that it describes the attribute of a food material resulting from a combination of physical and chemical properties, perceived largely by the senses of touch, sight and hearing (Lewis, 1990).

Traditionally, cheeses are made from raw (RA) milk, but due to hygienic reasons most cheeses are currently made from pasteurized (PA) milk. Pasteurization eliminates the heat-sensitive raw milk microbiota, generally attenuates or activates the activity of many indigenous milk enzymes such as the plasmin/plasminogen complex, lipases or alkaline phosphatase, and also produces slight denaturation of serum proteins and slight modifications in milk rennetability (Grappin & Beuvier, 1997). Most researchers find that pasteurization of milk results in a higher cheese moisture content than in cheese made from RA milk (Lau, Barbano, & Rasmussen, 1990), which is linked in turn to lower scores for cheese firmness (Creamer & Olson, 1982). Rheological differences between Cheddar (Amantea, Skura, & Nakai, 1986) and Manchego (Gómez, Rodríguez, Gaya, Núñez, & Medina, 1999) cheeses made from RA or PA milks are attributed to interactions between whey protein and casein, to differences in the percentage of α_s-casein degradation, and to differences in dry matter content.

High-pressure treatment (HPT) of foods is being applied recently with hygienic or technological objectives, and many of these applications have focused on milk and milk products. An advantage of HPT is that it may result in large decreases in viable number of microbial contaminants without negative effects on flavour or nutritional components due to the fact that only non-covalent bonds are affected by the pressure

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treatment. However, HPT fragments the casein micelle increasing the amount of serum casein and minerals, and causing also important denaturation of whey proteins (Trujillo et al., 2000). As result of milk-modifications caused by HPT, the coagulating properties of pressure-treated (PR) milk and the rheological characteristics of milk-based gels are affected (Buffa, Trujillo, & Guamis, 2001). Subsequently, the cheese matrix, cheese yield and ripening may be altered (Trujillo, Royo, Ferragut, & Guamis, 1999a; Trujillo, Royo, Guamis, & Ferragut, 1999b).

The close relationship between cheese microstructure and cheese rheology is often highlighted, both being determined by the chemical composition of cheese (Stanley & Emmons, 1977). Although numerous studies were carried out on cheese texture and microstructure, there are only limited studies comparing the effect of different technologies applied to milk prior to cheese production. The objective of this research was to compare the textural, microstructural and colour characteristics of cheeses made from RA, PA (72°C, 15 s) or PR (500 MPa, 15 min, 20°C) goats' milk in relation to ripening time.

2. Materials and methods

2.1. Cheese manufacture

Milk was obtained from a herd of Murciano-Granadina goats from a local farm of Muntayola (Barcelona, Spain). Cheese was manufactured from RA, PA and PR goats' milk in two experiments, each on an interval of one week. In each experiment, 50 kg of RA, 50 kg of PA or 50 kg of PR milk from the same milk batch were used for cheesemaking. Pasteurized milk was obtained by using a heat exchanger (Garvia S.A., Barcelona) at 72°C with a flow rate of 200 L h^{-1} and a holding time of 15 s. High-pressure treated milk was obtained by using a semi-continuous hyperbar equipment (GEC Alsthom ACB, Nantes, France) providing direct compression of the milk with a piston. A pressure chamber of 4 L equipped with a heating/cooling system to control temperature during pressurization and decompression was used. Twenty minutes before treatment, refrigerated milk (4°C) was equilibrated at 20°C and batches of 4 L were pressurized at 500 MPa and 20 ± 1°C with a holding time of 15 min. The PR milk was stored at 4°C until cheesemaking.

Milks were heated to 31°C and a direct-to-vat lyophilized starter culture (AM Larbus, Barcelona), containing *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*, and 35% (w/w) $CaCl_2$ (food quality grade) were both added to cheese milk to a final concentration of 2% (w/w) and 0.02% (v/w), respectively. Ten minutes later, 0.02% (v/w) of calf rennet (Reniflor-15/E, Lamirsa, Barcelona) containing 780 mg chymosin L^{-1} was added. After 30 min, the coagulum was cut, and the curds drained and moulded (13.6×13.2 cm^2). A vertical press was used to press the cheeses for 12 h (1 h at 1.3 kPa and 11 h at 2.6 kPa). The cheeses were salted by immersion in brine (19% NaCl solution) for 4 h at 14°C. Finally, cheeses were ripened in a room at 14°C and 85% relative humidity for 60 days.

2.2. Compositional analysis

Cheeses from RA, PA and PR milk were analysed for total solids (TS; IDF, 1982), fat (F; IDF, 1991) and total nitrogen (TN; IDF, 1993). The cheese nitrogen was fractionated according to the method of Kunchroo and Fox (1982) and the extracts obtained were used to determine soluble nitrogen at pH 4.6 (WSN). The pH of a cheese/distilled water (1 : 1) slurry was determined. Salt was determined by chloride analysis (Corning 926 Chloride Analyzer, Sherwood Scientific Ltd., Cambridge, UK). Analyses of RA, PA and PR cheeses from the two experiments were performed in duplicate at 1, 30 and 60 days after cheesemaking.

2.3. Uniaxial compression

Cube-shaped samples (100 mm^2) of each cheese at every stage of ripening were cut and held at 20°C for 3 h before carrying out the uniaxial compression test. Cubes were compressed to 80% of their original height at a constant temperature of 20°C using a TA-TX2 Texture Analyzer (State Micro system, Survey, UK) with a 245 N load cell and a crosshead speed of 80 mm min^{-1}. The analysis was carried out six times for each cheese sample. Analyses were performed under lubricated conditions to eliminate frictional effects (Casiraghi, Bagley, & Christianson, 1985). The true stress (σ) was calculated by dividing the force applied by the surface area of the sample at a specific time (t) as illustrated in the following equation (Calzada & Peleg, 1978):

$$\sigma_{(t)} = \frac{F_{(t)}}{A_{(t)}},$$

where $\sigma_{(t)}$ = true stress at time (t); $F_{(t)}$ = force at time (t); $A_{(t)}$ = area at time (t). The true strain (ε) was calculated according to the equation of Calzada and Peleg (1978):

$$\varepsilon = \ln \frac{H_0}{(H_0 - \Delta H)},$$

where ε = true strain; H_0 = original height; ΔH = change in height. Fracture stress (σ_f) and fracture strain (ε_f) parameters were calculated from the true stress–true strain curves.

2.4. Stress relaxation

Cube-shaped samples were also subjected to 10% compression for 3 min at a crosshead speed of 200 mm min^{-1} in the conditions described. The analysis was carried out four times for each cheese sample and ripening stage. The stress–relaxation curves obtained were evaluated by the following equation from Peleg (1979):

$$\frac{t}{Y_{(t)}} = \frac{1}{(er)} + \frac{t}{e},$$

where $Y_{(t)}$ is the normalized relaxation stress; e represents the asymptotic or equilibrium residual values of $Y_{(t)}$ when $t \to \infty$ and r is reflective of the rate at which the stress relaxes.

2.5. Microstructure

Cheese samples (approximately 1 mm thick) were stained with 0.2% (w/v) Nile Blue for 5 min. After two washes with distilled water, samples were mounted on a slide with a non-fluorescent observation medium and observed with the confocal laser scanning microscope (CLSM, Leica, TC54D, Heidelberg, Germany). Nile Blue was used because it contains trace amounts of fluorescent Nile Red (Nile Blue A oxazone) which diffuses into the oil phase. A krypton/argon laser was used to excite Nile Red dye at 488 nm and Nile Blue dye at 568 nm. Analyses were performed for cheeses at 30 and 60 days of ripening.

2.6. Colour analysis

A portable HunterLab spectrocolorimeter (MiniScan XETM, Hunter Associates Laboratory Inc., Reston, Virginia, USA) was used to measure the colour of the samples. HunterLab L-, a- and b-values were read from the samples. The L-value ranges between 0 and 100 and was used as a measure of lightness. Positive or negative increases of a-value correspond to increases in red or green colour proportions. The b-value represents colour ranging from yellow (+) to blue (−). Total colour differences (ΔE) were calculated using the formula:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}.$$

Values of ΔE were calculated to compare PA and PR cheeses with RA cheese. Illuminant Fcw (cool white fluorescent) with a 10° observer was used. Measurements were taken on different points of the surface of cheeses cut in two halves. Eight consecutive measures were taken for every cheese.

2.7. Statistical analysis

Data were processed by a correlation analysis using the SAS® System for WIN™ (6.12 version). Analysis of variance (ANOVA) using the general linear models procedure of the SAS® System was also carried out. The Student–Newman–Keuls test was used for comparison of data. Evaluations were based on a significance level of $p < 0.05$.

3. Results and discussion

3.1. Compositional analyses

Moisture content evolution of RA, PA and PR cheeses during ripening is shown in Table 1. Moisture content of cheeses steadily decreased throughout ripening (nearly 23–24%) due to water surface evaporation. No differences ($p < 0.05$) in moisture content were observed between cheeses on the first day of ripening.

RA cheese exhibited the highest pH value at the beginning and at the end of ripening. The evolution of the pH was similar in all cheeses, slightly decreasing ($p < 0.05$) as the cheeses aged. No differences ($p < 0.05$) were observed in the total nitrogen or in the fat content of the 1-day-old cheeses, which represented $5.67 \pm 0.22\%$ and $54.37 \pm 0.59\%$ of the total solids, respectively. The WSN fraction of cheeses increased during ripening indicating progressive proteolysis (Table 1). No differences ($p < 0.05$) in the WSN content among cheeses were observed at the beginning nor at the end of ripening.

Salt-in-moisture content was higher ($p < 0.05$) in PA than RA or PR cheeses at the selected ripening stages. Moisture content affects the rate of salt absorption and/or salt diffusion of salt throughout the cheese (Geurts, Walstra, & Mulder, 1974). Although moisture content was initially similar, PA cheeses exhibited slightly higher moisture contents than RA or PR cheeses. The higher salt content of PA cheeses could be attributed to the greater capacity of exchange between whey retained in fresh cheese and the brine during the salting process.

3.2. Uniaxial compression

Fracture stress is the force required to fracture the cheese matrix and can be used as a fracturability index; a low numerical value indicates greater fracturability. Generally, fracture stress of RA, PA and PR milk cheeses increased from day 1 to day 60 (Table 2), indicating the significant ($p < 0.05$) effect of ripening time on fracturability. Fracture stress increased markedly from the day 1 to day 30, and from then on, the increase declined. Correlation analysis carried out between rheological and physicochemical parameters

Table 1
Composition of cheeses (mean and *standard deviation)* made from raw, pasteurized and pressure-treated goats' milk[a]

	Day 1			Day 60		
	Raw	Pasteurized	Pressure-treated	Raw	Pasteurized	Pressure-treated
pH	5.05[b] *0.03*	4.99[c] *0.02*	5.01[c] *0.06*	4.89[b] *0.02*	4.84[c] *0.04*	4.76[d] *0.02*
M (%)	52.58[b] *0.28*	53.04[b] *0.74*	52.75[b] *0.31*	29.31[b] *0.10*	28.03[c] *0.28*	29.54[b] *0.46*
S/M (%)	1.76[c] *0.52*	2.29[b] *0.58*	1.77[c] *0.41*	6.92[c] *0.41*	7.51[b] *0.40*	7.03[c] *0.54*
WSN/TN (%)	10.63[b] *1.63*	10.46[b] *2.25*	9.06[b] *0.96*	17.93[b] *1.74*	18.50[b] *0.58*	17.76[b] *1.27*

[a] M: Moisture content; S/M: Salt/Moisture; WSN/TN: Water Soluble Nitrogen/Total Nitrogen.
[b,c,d] Means within the same row and day without a common superscript are significantly different ($p<0.05$).

Table 2
Mean and *standard deviation* for uniaxial compression tests of cheeses made from raw, pasteurized and pressure-treated goats' milk

	Day 1	Day 30	Day 60
Fracture stress (kPa)			
Raw	30.09[a] *4.47*	119.21[a] *14.61*	191.44[a] *19.66*
Pasteurized	24.49[b] *2.87*	122.05[a] *10.71*	170.15[b] *25.79*
Pressure-treated	28.91[a] *2.63*	123.51[a] *10.00*	189.68[ab] *22.54*
Fracture strain			
Raw	0.76[a] *0.06*	0.27[a] *0.02*	0.25[a] *0.03*
Pasteurized	0.68[b] *0.07*	0.25[ab] *0.03*	0.23[a] *0.03*
Pressure-treated	0.67[b] *0.05*	0.24[b] *0.02*	0.24[a] *0.03*

[a,b] Means within the same column without a common superscript are significantly different ($p<0.05$).

evaluated showed that fracture stress was strongly correlated to WSN ($R^2 = 0.957$) and to moisture ($R^2 = -0.967$) contents. Creamer and Olson (1982) claimed that diminution of the fracturability of Cheddar cheese throughout the ripening period was related to the content of intact α_{s1}-casein in cheese. A similar behaviour of fracture stress was reported by other authors in Mozzarella and Cheddar-like goats' milk cheese (Yun, Hsieh, Barbano, & Kindstedt, 1994; Attaie, Richter, & Risch, 1996). On the other hand, several authors have reported that as the moisture content decreases, the firmness of the cheese increases (Creamer & Olson, 1982; Amantea et al., 1986). The diminution of cheese moisture during ripening causes a lower hydratation of protein, less freedom of movement for the protein molecules and a firmer casein matrix. In our cheeses, the increase in fracture stress values observed during ripening could be attributed to the predominant effect of moisture loss over the weakening effect of proteolysis, in a similar way to that described for Manchego cheese (Picon, Gaya, Medina, & Núñez, 1995).

Fracture stress mean values of 1-day-old PA cheeses were lower ($p<0.05$) than those of RA and PR cheeses (Table 2). Gómez et al. (1999) reported differences in the fracture stress of RA and PA Manchego cheeses and linked these to differences in dry matter content and in percentage of degradation of α_s-caseins. Accordingly, the lowest fracture stress values observed in 1-day-old PA cheese were probably due to its slightly higher moisture content (Table 1). Fracture stress values were similar in all cheeses on day 30, whereas on day 60, PA cheese again presented the lowest fracture stress value. Although no significant differences in WSN content were observed at 60 days of ripening, the slightly higher WSN content of PA cheeses indicates a higher degree of proteolysis that could affect their fracture stress values.

Fracture strain describes the deformability of cheese, which may express cohesion properties; a higher numerical value indicates greater deformability. Globally, fracture strain of all cheeses decreased ($p<0.05$) from day 1 to day 60 (Table 2) and, in the same way as observed for fracture stress, changes were stronger in the first stage of ripening than in the final stage. Similar fracture strain behaviour is reported in Manchego, Bergkäse and Gouda cheeses (Pavia, Guamis, Trujillo, Capellas, & Ferragut, 1999; Ginzinger, Jaros, Lavanchy, & Rohm, 1999; Bertora, Califano, Bevilacqua, & Zaritzky, 2000). Creamer and Olson (1982) described fracture strain decreases with ageing in Cheddar cheese and hypothesized that this decrease was due to the loss of elastic structural elements and to the decrease in the amount of water available for solvation of protein. In this work, fracture strain was well correlated with moisture and WSN: $R^2 = 0.936$ and $R^2 = -0.907$, respectively.

One-day-old RA cheeses exhibited the highest ($p<0.05$) fracture strain values. However, differences between cheeses became non-significant at the end of ripening. Cheese texture is determined primarily by the pH of the cheese and the ratio of intact casein to moisture (Lawrence, Creamer, & Gilles, 1987). According to Creamer and Olson (1982), the casein molecules acquire a negative charge at higher pH values and water is partly absorbed to solvate the ionic charges formed. This has consequences for the rheological properties of cheese. These authors observed in cheese with high pH a relatively high deformability. Thus, differences in fracture strain found in RA cheese, as compared to PA and PR cheeses, at the beginning of ripening can possibly be attributed to its higher pH.

3.3. Stress relaxation

Stress relaxation can be described as the ability of a cheese to reduce an imposed stress over time at a constant strain (Konstance & Holsinger, 1992). The *r*-value is a reflection of the rate at which the stress relaxes. An *r*-value near to zero corresponds to a more elastic solid, while higher values are indicative for a viscous behaviour. An increase ($p<0.05$) in *r*-value was observed through the ripening period of all cheeses (Table 3). This increase indicates the loss of elastic characteristics of cheeses toward the end of ripening, an effect that could be also indirectly observed by the decrease of fracture strain values of cheeses during ripening. Increases in *r*-value through time were also reported by Nolan (1987) and by Pavia et al. (1999) in stirred Cheddar curd and in Manchego-type cheese, respectively. Changes in *r*-value of cheeses could be explained by the weakening of the cheese matrix due to proteolysis consistent with the increase of WSN values during ripening (Table 1), and by the moisture loss of cheeses. In agreement, statistical analysis of data revealed a strong correlation between *r* and WSN ($R^2 = 0.872$) and moisture ($R^2 = -0.905$).

The *e*-value represents the asymptotic or equilibrium residual values of normalized relaxation stress when $t \to \infty$. Throughout ripening, *e*-value decreased significantly in RA and PR cheeses, while PA cheese exhibited no changes (Table 3). Pavia et al. (1999) reported no changes of *e*-value of Manchego cheese with time. However, Yun et al. (1994) observed a decrease in the *e*-value with storage of Mozzarella cheese attributed to the breakdown of some cross-links which maintained the cheese structure.

Cheeses from PR milk exhibited lower ($p<0.05$) *r*-values than RA and PA cheeses at the beginning of ripening, but these differences were not significant towards the end of the experiment (Table 3). In the same way, PR cheese generally exhibited the highest *e*-values throughout ripening indicating a more elastic behaviour. Buffa et al. (2001) reported that rennet curds obtained from PR goats' milk were firmer than those from PA and RA milks. Differences in rennet coagulation properties of PR milk were attributed to numerous changes produced by HPT, such as changes in size and distribution of casein micelles, denaturation of whey proteins and solubilization of caseins and minerals from casein micelles leading to a more continuous and closely gelled network. Thus, an even distribution of the protein network caused by the HPT could be responsible for the values found in the stress relaxation tests.

3.4. Microstructure

Fig. 1 shows the CLSM images of the protein matrix and fat globules of 30-day-old cheeses made from RA, PA and PR goats' milk. The appearance of protein matrix observed was a sponge-like structure that contained numerous fat globules of variable size and shape distributed within the protein matrix, as described by several authors (Crites, Drake, & Swanson, 1997; Pavia et al., 1999). The PA cheese exhibited an open structure with numerous and irregular cavities, while the PR cheese exhibited the most regular and closed protein matrix. Fat globules of PR cheese were more homo-

Table 3
Mean and *standard deviation* for stress relaxation tests of cheeses made from raw, pasteurized and pressure-treated goats' milk

	Day 1		Day 30		Day 60	
r (s^{-1})						
Raw	0.020[a]	*0.002*	0.031[a]	*0.002*	0.033[a]	*0.003*
Pasteurized	0.021[a]	*0.003*	0.030[a]	*0.003*	0.032[a]	*0.002*
Pressure-treated	0.018[b]	*0.001*	0.031[a]	*0.003*	0.033[a]	*0.002*
e						
Raw	5.56[b]	*0.29*	4.86[b]	*0.65*	4.62[b]	*0.71*
Pasteurized	5.41[b]	*0.46*	5.16[b]	*0.73*	5.29[a]	*0.50*
Pressure-treated	6.24[a]	*0.35*	6.09[a]	*0.41*	5.19[ab]	*0.33*

[a,b] Means within the same column without a common superscript are significantly different ($p<0.05$).

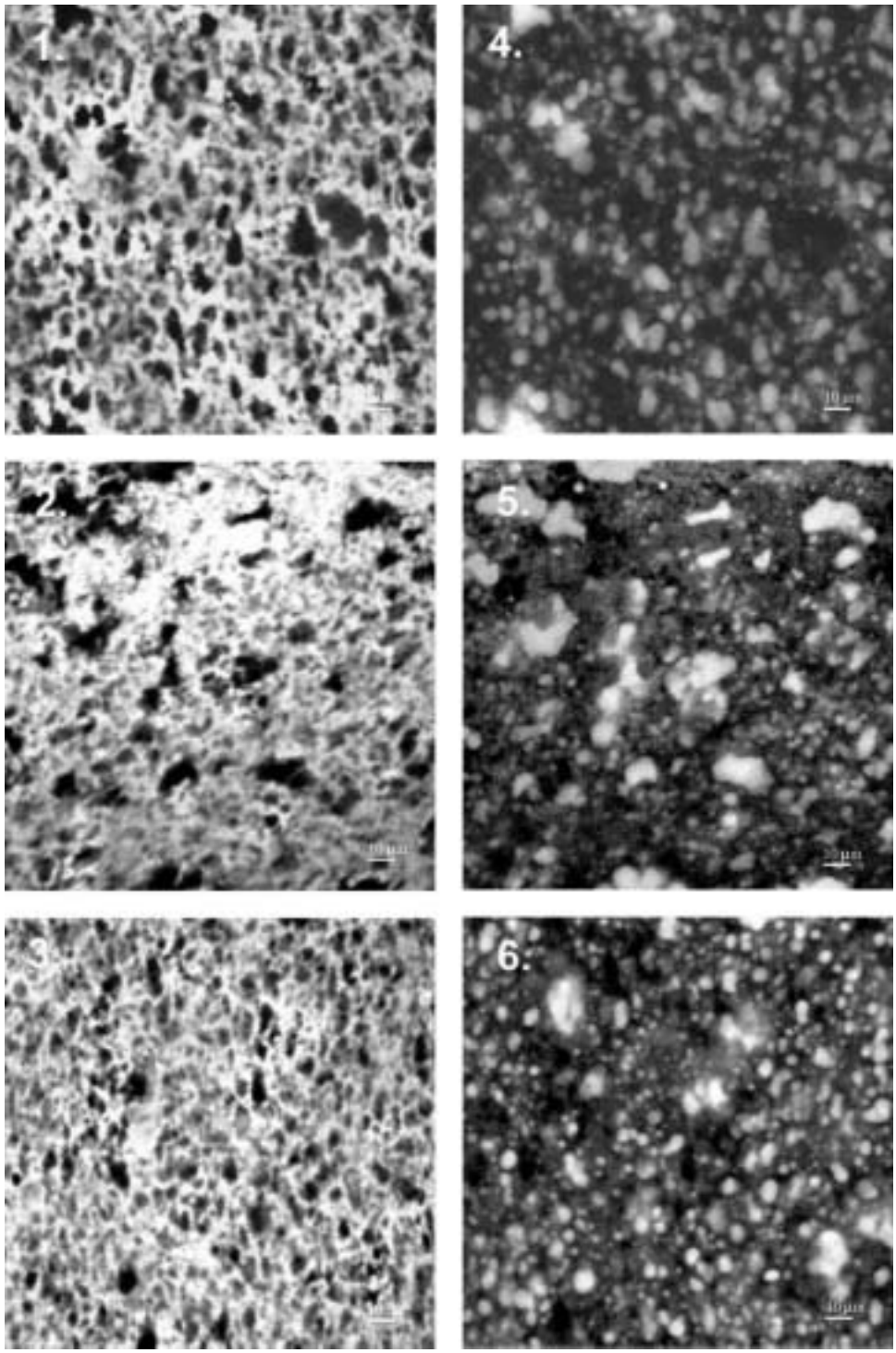

Fig. 1. Confocal laser scanning micrographs showing the structure of cheeses made from raw (1,4), pasteurized (2,5) and pressure-treated (3,6) goats' milk. Images 1–3 and 4–6 correspond to the protein matrix of cheeses and to the cheese fat-globules, respectively.

genically distributed in the protein network than in RA or PA cheeses. In PR cheese, fat globules were smaller and more uniform in size and shape than those in PA cheese, and resembled the structure of those in RA cheese. These results agree with the observations of Drake, Harrison, Asplund, Barbosa-Canovas, and Swanson (1997), that the microstructure of Cheddar cheese made from RA, PA or PR cows' included a more similar overall microstructure between PR and RA cheese. As cheeses aged, the protein matrix appearance

became much more dense and compact, probably due to water evaporation, and differences of microstructure between 60-day cheeses were less evident (not shown).

Studies with goats' milk report that HPT (400–500 MPa) at room temperature results in micelle disintegration into small particles with a concomitant increase in the amount of serum casein and minerals as well as denaturation of whey proteins, especially β-lactoglobulin (Law et al., 1998). According to various authors (Johnston, Austin, & Murphy, 1992; Needs et al., 2000) pressure applied to milk results in suppression of hydrophobic interaction between the caseins within the micelles, releasing free caseins and small casein clusters. When pressure is released interactions can reform but the original structure is not regained and the reformed micelles are very small. During the reformation of micelles, denatured β-lactoglobulin could form disulfide bonds with κ- and/or α_{s2}-caseins, or form self-aggregated polymers, which may appear associated or trapped within casein micelles. This could lead to micelles with altered structure and/or composition producing rennet gels which are firmer than gels obtained from RA or PA milks (Buffa et al., 2001). This is a direct result of an increase in the number of protein particles and of the number of potential cross-linking sites. This suggests that the changes caused to milk by HPT, whey protein denaturation and disintegration/reforming of micelles, could lead to a more homogeneous and uniform protein matrix and could explain the initial elastic behaviour of PR cheeses.

Heat treatment of milk leads to a slight denaturation of serum proteins with the formation of a complex which particularly involves κ-casein and β-lactoglobulin at the micelle surface. Park, Nakamura, and Niki (1996), observing rennet gels from PA milk with transmission electron microscopy, reported the presence of filamentous attachments consisting of denatured β-lactoglobulin bound with κ-casein on the micelle surface. This may be expected to affect cheese microstructure and could explain the more open structure and the irregular cavities observed in PA cheeses.

3.5. Colour analysis

The analysis of variance identified the significant ($p<0.05$) effect of ripening time on Hunter values of RA, PA and PR cheeses (Table 4). Although the *a*-value did not show a definite trend throughout ripening, the *L*-value decreased and the *b*-value increased as the cheese aged. Previous research on cheese colour as a function of ripening time by Rohm and Jaros (1996a) reported a decrease of *L*-value and an increase of *a*- and *b*-values during ripening of Emmental cheese. Ginzinger et al. (1999) reported that yellowness index, a one-dimensional measure of cheese colour highly correlated with *b*, increased as cheese aged. As presented in Table 4, total colour differences of RA and PR cheeses were small, which almost correspond to the sensory difference threshold (Rohm & Jaros, 1996b). However, greatly different values of ΔE were found for RA and PA cheeses at 30 and 60 days of ripening. The almost identical colour values found in RA and PR cheeses could be attributed to their similar structure.

4. Conclusions

Uniaxial compression and stress relaxation tests showed textural differences between cheeses, especially at the beginning of ripening, which probably were derived from the physicochemical and microstructural characteristics of the rennet curds. However, these differences became less pronounced as cheeses aged.

Raw and PR cheeses were firmer and less fracturable than PA cheeses, while PA and PR cheeses were less cohesive. Microstructural analysis displayed that PR cheeses had the most regular and close protein matrix, with small and uniform (in size and shape) fat globules, explaining the more elastic behaviour of these cheeses. Microstructure of PR cheese resembled that of RA cheese, whereas PA cheese had an open structure with numerous and irregular cavities. Colour evaluation showed significant differences between cheeses related to milk treatment and/or ripening time, although due to

Table 4
Mean and *standard deviation* for colour parameters of cheeses made from raw, pasteurized and pressure-treated goats' milk

	Hunter values									
	L		*a*		*b*		ΔE^a			
	Day 30	Day 60	Day 30	Day 60	Day 30	Day 60	Day 30	Day 60		
Raw	92.85[b] *0.40*	91.56[b] *0.21*	−0.95[d] *0.09*	−1.08[d] *0.15*	9.98[b] *0.28*	11.05[b] *0.23*				
Pasteurized	91.53[c] *0.20*	89.08[d] *0.44*	−0.55[b] *0.14*	−0.48[b] *0.06*	8.51[d] *0.22*	9.73[d] *0.21*	2.02	2.86		
Pressure-treated	92.91[b] *0.46*	91.03[c] *0.43*	−0.69[c] *0.09*	−0.85[c] *0.05*	9.03[c] *0.13*	10.13[c] *0.29*	0.97	1.08		

[a] Values of ΔE (total colour differences) were calculated to compare pasteurized and pressure-treated milk cheeses with raw milk cheese.
[b,c,d] Means within the same column without a common superscript are significantly different ($p<0.05$).

the similar structure the cheeses, the more identical values were found for RA and PR cheeses.

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3.6. CHANGES IN WATER BINDING DURING RIPENING OF CHEESES MADE FROM RAW, PASTEURIZED OR HIGH-PRESSURE-TREATED GOAT MILK.

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Note

Changes in water binding during ripening of cheeses made from raw, pasteurized or high-pressure-treated goat milk

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Abstract – The different types of water contained in the matrix of cheeses made from raw (RA), pasteurized (PA; 72 ºC, 15 s) or pressure-treated (PR; 500 MPa, 15 min, 20 ºC) goat milk were studied throughout ripening. Water content was qualitatively and quantitatively assessed by thermogravimetry. Thermogravimetric curves showed that water is lost in two successive steps (W_1 and W_2), depending on the temperature required for water to leave the cheese network. Although water content in W_1 and W_2 of all cheeses followed similar trends, decreasing towards the end of ripening, large relative decreases were observed in W_1. The highest decrease was observed in PA milk cheese, while PR milk cheese showed behavior similar to that made from RA milk. Differences in water binding could be attributable to changes in the cheese-matrix structure due to the technological treatment applied to milk, and/or physicochemical or biochemical differences (NaCl, proteolysis, lipolysis, ...).

Water binding / thermogravimetry / goat cheese / high-pressure treatment

Résumé – Changements de l'eau liée des fromages faits à partir de lait de chèvre cru, pasteurisé ou traité par haute pression. Les différents types d'eau contenus dans la matrice des fromages faits à partir de lait de chèvre cru (RA), pasteurisé (PA ; 72 ºC, 15 s) ou traité par haute pression (PR ; 500 MPa, 15 min, 20 ºC) ont été étudiés durant la maturation. La teneur en eau a été qualitativement et quantitativement évaluée par thermogravimétrie. Les courbes thermogravimétriques ont montré que l'eau a été perdue en deux étapes consécutives (W_1 et W_2), dépendant de la température que l'eau nécessite pour quitter le réseau du fromage. Bien que la teneur en eau dans W_1 et W_2 de tous les fromages ait suivi les mêmes tendances, en baissant vers la fin de la maturation, des baisses relatives élevées ont été observées dans W_1. La décroissance la plus accentuée a été observée dans le fromage obtenu à partir du lait PA, alors que le fromage obtenu à partir du lait PR a montré un comportement semblable à ceux faits à partir du lait RA. Les différences en eau liée peuvent être attribuées aux changements dans la structure de la matrice du fromage due au traitement technologique appliqué au lait, et/ou aux différences physico-chimiques ou biochimiques (NaCl, protéolyse, lipolyse, ...).

Eau liée / thermogravimétrie / fromage de chèvre / traitement de haute pression

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1. INTRODUCTION

The ripening of cheese is a complex process that involves chemical and biochemical reactions, water loss, salt diffusion, and changes in pH and in the microbial population. During ripening, water has a predominant role because it is the medium where these reactions take place. Water is also essential for the development of cheese microbiota, and through interaction with the cheese matrix, it contributes to the texture of cheese.

Although goat milk cheeses have been traditionally made from raw (RA) milk, aspects related essentially to microbial safety have increased the use of pasteurization treatments. However, most researchers have found that cheese made from pasteurized (PA) milk has higher moisture content than that made from RA milk, which in turn could cause differences in cheese firmness or in the cheese component degradation during ripening [4, 9].

The interest in non-thermal technologies, such as high-hydrostatic pressure, in milk has recently increased. Additionally to microbial destruction, high-pressure treatments induce numerous effects on the technological properties and milk components: fragmentation of casein micelles, aggregation of whey proteins and modifications of the mineral equilibrium [20], which cause changes in the rennet coagulation and cheese yield properties of pressure-treated (PR) milk [2, 18, 19]. The higher yields obtained from cheeses made from PR milk could be mainly attributed to the higher moisture content in these cheeses, a fact that alludes to the level of denatured whey proteins retained in these curds, and to the fragmentation of casein micelles produced by the pressure treatment [19].

Thermogravimetry (TG) is the branch of thermal analysis that examines the change in mass of a sample as a function of temperature in the scanning mode or a function of time in the isothermal mode [11], and it has been successfully applied in food analysis (studies of proteins, carbohydrates and fats) [7]. Measurement of the amount of moisture in foods is an obvious application for TG. Moisture can be present in the food matrix as bound or unbound water, and this degree of binding is reflected by the temperature at which mass is lost. When the matrix is heated, water is lost in successive stages, depending on the temperature required to break the bonds (hydrogen bonds, Van der Waals forces, London forces, etc.) formed between water and the cheese matrix [5].

The aim of this study was to quantify the different types of water contained in the matrix of cheeses made from RA, PA (72 ºC, 15 s) or PR (500 MPa, 15 min, 20 ºC) goat milk, and to compare their behaviors in relation to ripening time.

2. MATERIALS AND METHODS

2.1. Cheese manufacture

Goat cheese was manufactured from RA, PA (72 ºC, 15 s) and PR milk in two independent experiments, within an interval of one week. In each experiment, 50 kg of RA, 50 kg of PA and 50 kg of PR milk, from the same milk batch, were used for cheese-making.

High-pressure treated milk was obtained by using a semi-continuous hyperbar equipment (GEC Alsthom ACB, Nantes, France) by direct compression of the liquid with a piston. Batches of 4 L of milk were pressurized at 500 MPa and 20 ± 1 ºC with a holding time of 15 min. The pressure-chamber temperature was determined by means of a thermoregulation system that circulated heating cooling fluid (water) within the walls of the vessel. The increase in temperature caused by the adiabatic compression in the equipment was in the order of 2 ºC per 100 MPa, which was rapidly compensated for by the

thermoregulation system. The PR milk was kept at 4 ºC until cheese-making.

Milk was heated to 31 ºC and then a starter culture (AM Larbus, Barcelona, Spain) containing *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*, and 35% (w/w) $CaCl_2$ (food quality grade) were both added to cheese milk to a final concentration of 2% (w/w) and 0.02% (v/w), respectively. Ten minutes later, 0.02% (v/w) of calf rennet (Reniflor-15/E, Lamirsa, Barcelona, Spain), containing 780 mg L^{48} chymosin was added. After 30 min, the coagulum was cut, and the curds drained and moulded (13.6 Δ 13.2 cm).

Due to the different technological treatments and in order to obtain cheeses with comparable moisture in non-fat material (M/NFM), which markedly influences the ripening of cheese [15], pressing time was established at 12 h (1 h at 1.3 kPa and 11 h at 2.6 kPa). The pressing time used in this study was based on the experience gained from previous experiments.

After that, cheeses were salted by immersion in brine (19% NaCl solution) for 4 h at 14 ºC. Finally, cheeses, each one of approximately 1.31 ± 0.03 kg, were ripened in a room at 14 ºC and 85% relative humidity for 60 d.

2.2. Compositional analysis

Cheeses were analyzed for total solids and fat according to standard methods [12, 13]. Salt was determined by chloride analysis (Corning 926 Chloride Analyzer, Sherwood Scientific Ltd., Cambridge, UK). Liberation of free amino-acids was determined on the water-soluble cheese extract [14] by the Folkertsma and Fox cadmium-ninhidrin method [8]. The pH was measured in a cheese/distilled water (1:1) slurry.

For each of the two experiments, analyses of RA, PA and PR cheeses were performed in duplicate at 1, 30 and 60 d after cheese-making.

2.3. Cheese weight-loss assessment

A representative sample of the cheese batches (three cheeses from each batch of six) made from RA, PA and PR milk, in both experiments, was weighed weekly for nine weeks. Then, drying curves were drawn from the cheese-weight measurements.

2.4. Thermal analysis

Evaluation of the water contained in the matrix of cheeses was performed by thermogravimetry, using a TGA/SDTA851e thermobalance (Mettler-Toledo GMdH analytical, Schwerzenbach, Switzerland). Approximately 20 mg of grated cheese was placed in the thermobalance sample pan and heated from 25 to 250 ºC at a scanning rate of 5 ºC min^{-1}, in a flow of nitrogen of 60480 mL min^{-1}.

For each of the two experiments, analyses of RA, PA and PR milk cheeses were performed in triplicate at 1, 30 and 60 d after cheese-making. The output signal from the thermobalance was evaluated using the Mettler-Toledo STARe software.

2.5. Statistical analysis

Data were processed by analysis of variance (ANOVA) using the general linear models procedure of SAS™ System for WIN⊗ (8 version). The Student-Newman-Keuls test was used for comparison of sample data. Evaluations were based on a significance level of $P < 0.05$.

3. RESULTS AND DISCUSSION

No differences ($P < 0.05$) in M/NFM content were observed between cheeses on the first day of ripening (Tab. I). M/NFM of all cheeses decreased as they aged

Table I. Composition of cheeses (mean and *standard deviation*) made from raw (RA), pasteurized (PA) and pressure-treated (PR) goat milk.

		Day 1		Day 30		Day 60	
pH	RA	5.05[a]	*0.03*	4.80	*0.03*	4.89[a]	*0.02*
	PA	4.99[b]	*0.02*	4.80	*0.01*	4.84[b]	*0.04*
	PR	5.01[b]	*0.06*	4.79	*0.05*	4.76[c]	*0.02*
M/NFM	RA	2.45	*0.05*	1.33	*0.05*	1.01[a]	*0.02*
(%)	PA	2.47	*0.08*	1.29	*0.05*	0.92[b]	*0.01*
	PR	2.43	*0.05*	1.36	*0.04*	0.98[a]	*0.02*
Salt/M	RA	1.76[b]	*0.52*	5.00	*0.56*	6.92[b]	*0.41*
(%)	PA	2.25[a]	*0.53*	5.30	*0.68*	7.51[a]	*0.40*
	PR	1.77[b]	*0.41*	5.00	*0.50*	7.03[b]	*0.54*
Free AA	RA	0.45	*0.10*	2.16[a]	*0.08*	4.56[a]	*0.69*
(mg Leu g^{-1} cheese)	PA	0.45	*0.07*	1.09[b]	*0.37*	3.07[b]	*0.98*
	PR	0.41	*0.04*	1.87[a]	*0.14*	4.03[a]	*0.57*

M/NFM: moisture in non-fat material; S/M: salt in moisture; AA: amino acids.
[a,b, c] Means within the same column without a common superscript are significantly different ($P < 0.05$).

(approximately 23–24%) due to water surface migration-evaporation. However, after two months of ripening, PA milk cheese presented the lowest M/NFM values, suggesting that it had a greater water evaporation rate than RA or PR milk cheeses. On the other hand, salt in-moisture (S/M) content was higher ($P < 0.05$) in PA milk cheese than in that made from RA or PR milk.

Buffa et al. [3], using confocal laser scanning microscopy, observed that PA milk cheeses exhibited a less continuous matrix (open and porous) with more numerous and irregular spaces compared to RA or PR milk cheeses, which in turn each presented a more regular and closed protein network. However, as cheeses aged, the protein matrix became much more dense and compact, and differences between cheeses were less evident.

The microstructure described for PA milk cheese could explain its level of S/M, as well as the M/NFM content found at 60 d. In relation to S/M contents, when salting conditions are standardized (salting time, cheese geometry, brine temperature), the quantity of salt absorbed will depend mainly on the intrinsic properties of cheese. A relatively narrow pore width of the protein matrix exerts a frictional effect on the diffusing NaCl and H_2O molecules, reducing their relative diffusion rates [10]. Therefore, the open microstructure of PA milk cheese could facilitate the NaCl diffusion into the cheese matrix, thus explaining the high S/M content observed in comparison to RA and PR milk cheeses.

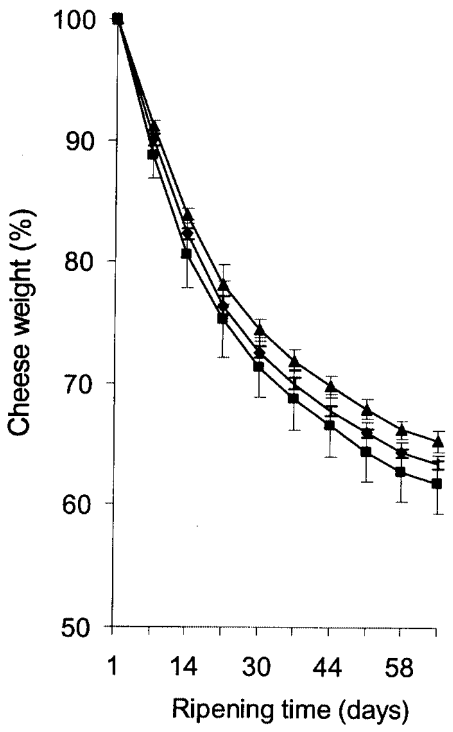

Figure 1. Drying curves (n=6) of cheeses made from raw (u), pasteurized (<) and pressure-treated (s) goat milk during ripening.

Microstructure could also play an important role in the low M/NFM found in PA milk cheeses at the end of ripening. In the first stages of ripening, moisture might be located in the large interstitial spaces described for PA milk cheese. However, when proteolysis advances and the protein matrix becomes more homogeneous, large amounts of water may be released.

Drying curves of all cheeses also showed differences (Fig. 1). In agreement with M/NFM content data, PA milk cheese showed the fastest rate of weight-loss ($y = 410.925\ln(x) + 103.16$; $R^2 = 0.9415$), while in the cheeses made from PR milk this process was significantly slower ($y = 49.928\ln(x) + 03.32$; $R^2 = 0.9237$). RA milk cheeses, in turn, showed an intermediate behavior ($y = 410.499\ln(x) + 103.27$; $R^2 = 0.9319$). These results suggest that the water loss of cheeses during ripening is also controlled by the internal profiles of water in the cheeses, which in turn is related to the cheese-matrix microstructure, and not only by the external conditions of ripening.

Examination of curves from the TG, and their first derivatives, showed two partially overlapping weight-loss steps within the range 25–200 ºC (Fig. 2). The presence of water over this range has been confirmed by De Angelis-Curtis et al. [5] by means of IR analysis. Each step is the result of the convolutions of a series of subprocesses corresponding to interactions between the water and different components of the matrix [5]. The first slight step corresponds to the water retained with less energy to the matrix (W_1), which is lost in the temperature range of 30 to 90–110 ºC. The second step (110–200 ºC) corresponds to the water more strongly linked to the cheese network (W_2), which requires more energy to break the bonds with the matrix. According to De Angelis-Curtis et al. [5], IR analysis of the gas produced indicates that other substances (CO_2, amines) also escape around 150 ºC. However, these losses of substances could be considered negligible compared to water desorption.

At the beginning of ripening, the W_1 amount, which showed no differences ($P > 0.05$) between RA, PA and PR milk cheeses, represented nearly 60% of the total water of the system. The W_1 content of all cheeses declined ($P < 0.05$) for the first 30 d of ripening, but thereafter this decrease was not significant (Fig. 3). The behavior of W_1 is affected by a much larger series of parameters than W_2, such as the transformation of W_2 into W_1, migration-evaporation processes, salt distribution, etc. [5]. During ripening of cheese, the liquid phase could be forced out easily by the diffusion gradient produced by water evaporation from the cheese body to the surface. However, the decrease in water of the system as cheese ages, brings down the diffusion gradient,

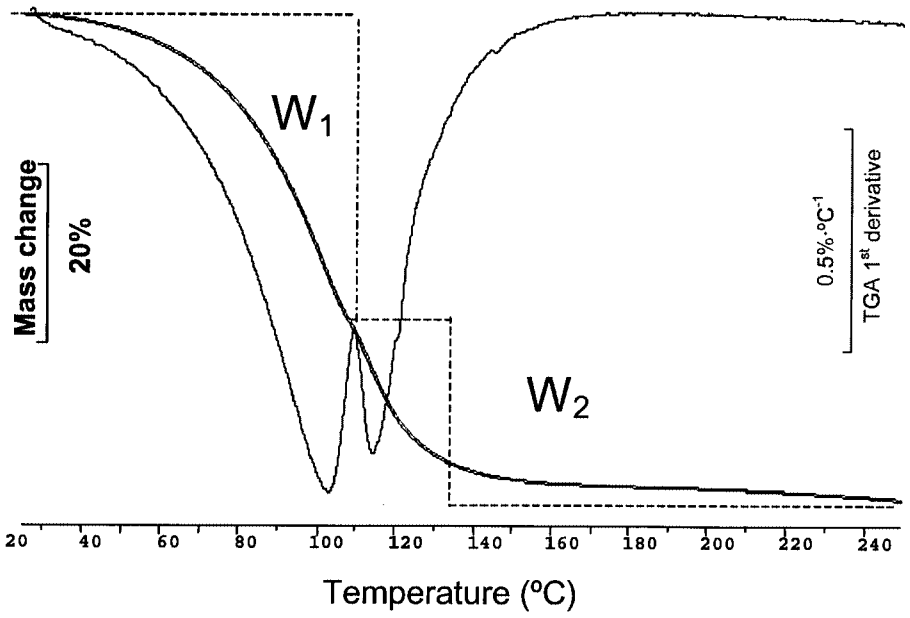

Figure 2. TG trace (bold line) and its corresponding first derivative (thin line) of a goat cheese. W_1 and W_2 indicate the two weight-loss steps.

consequently restricting the liquid phase movement. Additionally, the microstructure of a high moisture cheese (e.g. at the beginning of ripening) has high porosity, so that the liquid phase could be forced out easily by the diffusion gradient produced by the water evaporation [16]. As cheese water content decreases, cheese microstructure becomes more compact [17]. Thus, the cheese-matrix compaction may also restrict the liquid phase movement, decelerating in turn the migration-evaporation process.

The 30 and 60-d-old PA milk cheeses had lower ($P < 0.05$) amounts of W_1 than RA or PR cheeses, which both showed similar values (Fig. 3). As we commented previously, S/M content was lower ($P < 0.05$) in cheeses made from RA or PR milk than in PA milk cheese (Tab. I). In this way, the lower salt concentration observed in both RA and PR milk cheeses leads to a reduction in the hydrophilic ions capable of binding water, with a consequent increase available "free water" [5]. Additionally, these results suggest that the rapid decrease in the W_1 amount of PA milk cheeses could also be facilitated by their more open and porous microstructure [3].

As shown in Figure 3, no differences ($P > 0.05$) were found in the W_2 amount of the 1-d-old RA, PA and PR cheeses, which decreased towards the end of ripening. The 30-d-old PA milk cheeses showed a higher ($P < 0.05$) percentage of W_2 than RA or PR cheeses, whereas at the end of ripening no differences were found between cheeses.

The behavior of W_1 and W_2 in cheese is also affected by many other parameters, such as the effect of water migration-evaporation, salt diffusion, and proteolysis and lipolysis phenomena. Part of the system water is situated among the protein chains, becoming less free to move away. Thus, higher temperatures are required for the water to leave the cheese network.

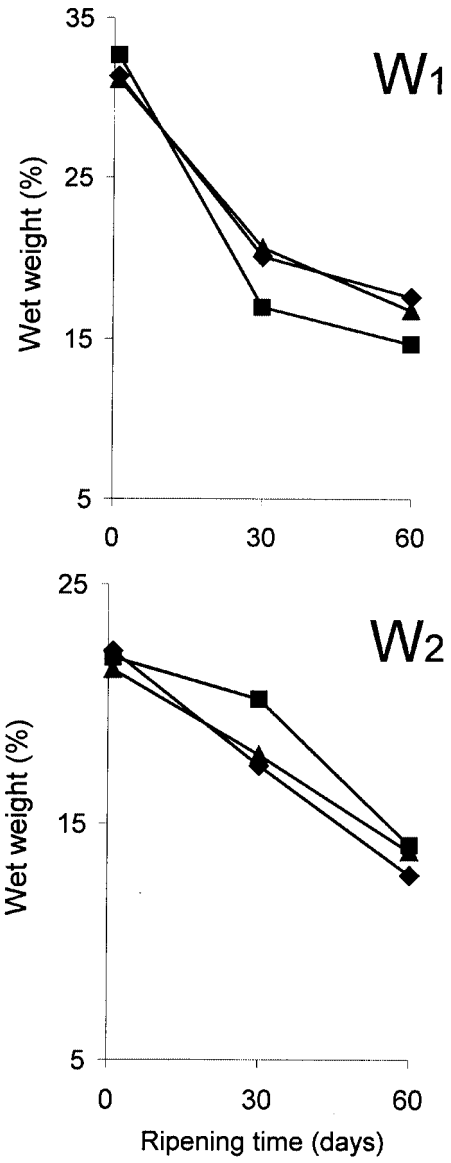

Figure 3. Evolution of W_1 and W_2 content of cheeses made from raw (u), pasteurized (<) and pressure-treated (s) goat milk during ripening. W_1 is the water retained with less energy in the matrix, while W_2 is the more strongly linked water.

De Angelis-Curtis et al. [5, 6] claimed that protein hydrolysis, and in a minor extent lipolysis, lead to release the water more strongly linked to the cheese matrix.

Cheese proteolysis, evaluated by the amount of free amino-acids, increased in all cheeses ($P < 0.05$) during ripening. However, proteolysis was more intense ($P < 0.05$) in both RA and PR milk cheeses than in PA milk cheese (Tab. I). Furthermore, it has been reported that cheeses made from PR milk showed a similar level of lipolysis to RA cheeses, whereas the level in PA milk cheese was lower, due to the thermal inactivation of native milk lipase [1]. Thus, the higher values of W_2 observed in PA milk cheeses could account for their low proteolysis and lipolysis levels.

4. CONCLUSIONS

No differences were detected in the M/NFM content of RA, PA and PR goat milk cheeses on the first day of ripening, however, PA cheeses showed a large rate of weight-loss during ripening.

Total water content, W_1 and W_2 of all cheeses decreased as cheese aged, indicating the significant effect of the ripening stage on cheese water binding. Large relative decreases were observed in the W_1 content, which was the predominant type of water of 1-d-old cheeses. The highest decreases of W_1 corresponded to PA milk cheese. During ripening, the W_1 and W_2 values of PR milk cheese showed a similar behavior to that of RA milk cheese, a fact that could be attributable to their similar cheese-matrix structure, and/or to the similarity of their main physicochemical or biochemical characteristics.

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3.7. CHANGES IN ORGANIC ACIDS DURING RIPENING OF CHEESES MADE FROM RAW, PASTEURIZED OR HIGH-PRESSURE-TREATED GOATS' MILK.

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Changes in organic acids during ripening of cheeses made from raw, pasteurized or high-pressure-treated goats' milk[1]

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Organic acids of cheeses made from raw (RA), pasteurized (PA; 72 ºC, 15 s) or pressure-treated (PR; 500 MPa, 15 min, 20 ºC) goats' milk were qualitatively and quantitatively assessed during ripening. Nine organic acids (citric, pyruvic, malic, lactic, formic, acetic, uric, propionic and butyric) were analysed in each sample by HPLC.

Milk treatment did not affect the total organic acids content of 1-day-old cheeses, which increased steadily from day 1 to day 60. At the end of ripening, RA and PR milk cheeses both exhibited higher concentration of organic acids than in those made from PA milk.

Lactic acid was found in higher concentration in PR milk cheese from 30 days of ripening. The RA milk cheese, that showed the highest non-starter lactic acid bacteria counts, were characterised by an elevate amount of propionic and acetic acids. These cheeses also were negatively correlated with both pyruvic and citric acid contents. The PA milk cheese showed a high level of malic acid, and was clearly differentiate from RA and PR milk cheeses by its low level of butyric acid.

Keywords*:* organic acids, goat cheese, high-pressure treatment

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Introduction

Goats' milk cheese is greatly appreciated by its organoleptic properties. Their complex flavour depends on the well balance between a large number of volatile and non-volatile compounds, such as organic acids, sulphur compounds, lactones, methyl ketones, alcohols, etc.

Organic acids are the major products of carbohydrate catabolism of lactic acid bacteria (González-de Llano *et al.*, 1996). They contribute to cheese quality, playing an integral role in flavour (Califano and Bevilacqua, 2000). Organic acids have been used as classification parameters for different cheeses, and in models to predict ripening time or the glycolytic age. These compounds can reflect the status of microbial metabolism, they are important secondary carbon sources for many microorganisms and are intermediates and metabolites of a variety of biochemical processes (Bevilacqua and Califano, 1992; Lues, 2000).

Although many goats' milk cheeses have been traditionally made from raw (RA) milk, aspects related essentially to the microbial safety have popularised the use of pasteurization treatments. However, pasteurization induces numerous changes in milk relevant to cheese making, such as destruction of the heat sensitive microbiota, inactivation/activation of enzymes and partial denaturation of whey proteins. In this way, the drastic reduction in the number of propionibacteria and facultatively heterofermentative lactobacilli produced by milk pasteurization, modifies the catabolism of many organic acids, such as lactate and citrate (Grappin and Beuvier, 1997; McSweeney and Sousa, 2000).

There is currently an increased interest in food processing with high hydrostatic pressure as a viable alternative to heating. High-pressure treatment (HPT) of milk can produce a large decrease in microbial numbers without negative effects on flavour or nutritional components. It has been described that HPT can reduce the natural milk microbiota with similar efficiency to pasteurization treatment did (Drake et al., 1997; Buffa et al., 2001b). Furthermore, Buffa *et al.* (2001b) compared the microbial groups most relevant during ripening of goat cheeses made from RA, pasteurized (PA) or pressure-treated (PR) milk, reporting that cheeses made from PR

(500 MPa, 20ºC, 15 min) milk had similar microbiological characteristics to PA milk cheeses. Although most works have been focused on the use of HPT to inactivate microorganisms, other modifications, such as fragmentation of casein micelles or aggregation of whey proteins, and on mineral equilibrium result from HPT (Trujillo et al., 2002; Huppertz et al., 2002). These modifications alter the milk aptitude to cheesemaking and are important factors in cheese manufacture and ripening (Molina et al., 2000; Trujillo et al., 2000).

Numerous studies have been carried out on organic acid concentration in cheeses, but there are only limited studies comparing the effect of different technologies applied to milk for cheese production. The objective of this work was to assess (qualitatively and quantitatively) the organic acids amount of cheeses made from RA, PA (72 ºC, 15 s) or PR (500 MPa, 15 min, 20 ºC) goats' milk in relation to ripening time.

Materials and methods

Cheese manufacture

Milk was obtained from a herd of Murciano-Granadina goats from a local farm of Muntayola (Barcelona, Spain).

Cheese was manufactured from RA, PA and PR goats' milk in two experiments, each on an interval of one week. In each experiment, 50 kg of RA, 50 kg of PA and 50 kg of PR milk from the same milk batch were used for cheese making.

Pasteurized milk was obtained by using a heat-exchanger (Garvia S.A., Barcelona) at 72 ºC at a flow rate of 200 L h^{-1}, with a holding time of 15 s.

High-pressure treated milk was obtained by using a semi-continuous hyperbar equipment (GEC Alsthom ACB, Nantes, France) by direct compression of the liquid with a piston. A pressure chamber of 4 L equipped with a heating/cooling system to control temperature during pressurisation and decompression was used. Twenty minutes before pressure-treatment, milk

portions (4 L) were successively taken from the lot of 50 kg of refrigerated milk (4 ºC), and equilibrated at 20 ºC. Then, portions were pressurised at 500 MPa and 20 ± 1 ºC with a holding time of 15 min. The PR milk was kept at 4 ºC until cheese making.

Milks were heated to 31 ºC and then a starter culture (AM Larbus, Barcelona) containing *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*, and 35 g/100 g $CaCl_2$ (food quality grade) were both added to cheese milk to a final concentration of 2 g/100 g and 0.02 ml/100 g, respectively. Ten minutes later, 0.02 ml/100 g of calf rennet (Reniflor-15/E, Lamirsa, Barcelona), containing 780 mg chymosin L^{-1} was added. After 30 min, the coagulum was cut, and the curds drained and moulded (13.6 Δ 13.2 cm).

A vertical press was used for 12 h (1 h at 1.3 kPa and 11 h at 2.6 kPa) in order to press the cheeses. After that, cheeses were salted by immersion in brine (19 g/100 ml NaCl solution) for 4 h at 14 ºC. Finally, cheeses were ripened in a room at 14 ºC and 85% relative humidity for 60 days.

Compositional analysis

Cheeses from RA, PA and PR milk were analysed for total solids according to IDF (IDF, 1982). The pH of a cheese/distilled water (1:1) slurry was measured. Salt was determined by chloride analysis (Corning 926 Chloride Analyzer, Sherwood Scientific Ltd., Cambridge, UK).

Analyses of cheeses from the two experiments were performed in duplicate 1, 30 and 60 days after cheese making.

Microbiological analysis

The microbiological quality of the different cheeses was assessed by enumerating the total bacteria on Plate Count Agar medium (Oxoid Ltd, Basingstoke, Hampshire, England) incubated for 72 h at 30 ºC. *Enterobacteriaceae* were counted on Violet Red Bile Glucose Agar medium (Biokar Diagnostics, Bioser SA, Barcelona, Spain), incubated for 24 h at 37 ºC in microaerophylia. The growth of non-starter lactic acid bacteria (NSLAB) was assessed on Rogosa Agar medium (Biokar Diagnostics), incubated for 72 h at 30 ºC in microaerophylia.

The detection limit was 10 cfu g^{-1} cheese. Analyses of RA, PA and PR goats' milk cheeses were performed in duplicate at 1, 30 and 60 days after cheese making.

Organic acid analysis

Sample extraction

About 150 g of a representative sample from each cheese was grated. Fifteen mL of 0.1 N phosphoric acid were added to 5 g of grated cheese and dispersed at 1500 rpm in a homogenizer (Heidolph Diax 900, Schwabach, Germany) for 5 min, essentially following the method of Lues *et al.* (1998). Then, cheese homogeneizates were centrifugated at 7000 rpm for 15 min. Finally, the supernatant was filtered through a 0.2 σm PVDF membrane filter (Teknokroma, Barcelona, Spain) before the chromatographic analysis.

HPLC analysis

Organic acids were separated by HPLC using an automated system (LCM1, Waters Corp., Milford, MA, USA). All separations were carried out on a Supelcogel C-610H (300 Δ 7.8 mm) ion-exchange chromatography column (Supelco Inc., Bellefonte, PA, USA), at a constant temperature of 30 ºC.

The mobile phase was 0.1 N phosphoric acid, prepared by diluting HPLC-grade H_3PO_4 (Panreac, Barcelona, Spain) with Milli-Q water (Millipore Corp., Bedford, MA, USA) and then filtered through 0.2 σm PVDF membrane filter. The flow rate of the mobile phase was 1 mL min^{-1} and the volume of sample injected was 40 σL.

Citric, pyruvic, malic, lactic, formic, acetic, propionic and butyric acids were detected at 210 nm, while uric acid was detected at 290 nm. Individual organic acids were identified and quantified using standards (purity >99% by HPLC) supplied by Sigma (Sigma Chemical Company, St Louis, MO, USA).

Organic acids analysis of cheeses from the two experiments were performed at 1, 30 and 60

days after cheese making. Two separate extractions were performed for each cheese sample, and two injections were made from each extracted sample. Results are presented as mg kg^{-1} dry matter of cheese.

Statistical analysis

Results were processed by a two-way analysis of variance (ANOVA) using the general linear models procedure of SAS™ System for WIN (8 version). The Student-Newman-Keuls test was used for comparison of sample data. Evaluations were based on a significance level of $P < 0.05$. To visualize the data matrix, a principal component analysis was performed using Statistica™ software (5.0 version; StatSoft Inc., Tulsa, OK, USA).

Results

Compositional and microbiological analyses

Composition of RA, PA and PR milk cheeses at 1 and 60 days of ripening is showed in Table 1. No differences ($P < 0.05$) in moisture content were observed between cheeses, which declined regularly during ripening due to evaporation of water from the surface. At the end of ripening, PA milk cheeses showed the lowest moisture content. Salt-in-moisture content was higher ($P < 0.05$) in PA than RA or PR cheeses. The evolution of the pH was similar in all cheeses, slightly decreasing ($P < 0.05$) as the cheeses aged. At the beginning and at the end of ripening RA milk cheeses showed the highest pH values.

Non-differences in the number of total microorganims were found between the 1-day-old cheeses, approximately $4.3 \Delta 10^9$ cfu g^{-1} cheese. This number declined throughout the 60 days of ripening, reaching final values of 10^7 cfu g^{-1} cheese. Cheeses made from PA or PR milks contained almost 10^3 cfu g^{-1} of *Enterobacteriaceae,* while RA milk cheeses contained the

highest ($P < 0.05$) number ($2.9 \Delta 10^5$ cfu g^{-1} cheese). However, counts of *Enterobacteriaceae* fall to non-detectable levels by 30 days of ripening in all cheeses. The number of NSLAB found in our study was similar for PA and PR milk cheeses (approximately 10^3 cfu g^{-1}), but lower ($P < 0.05$) than RA milk cheeses, which showed approximately 10^6 cfu g^{-1} cheese. In all cases counts increased further during ripening (Fig 1).

Organic acid analysis

Milk treatment did not affect the total organic acids content of 1-day-old cheeses (Table 2). Total organic acid content of cheeses increased from day 1 to day 60, indicating the significant ($P < 0.05$) effect of the ripening time on their production. However, at the end of ripening, cheeses made from RA or PR milk exhibited higher concentration of organic acids than in those made from PA milk.

Lactic acid was the main organic acid of all cheeses, representing approximately 74 % of the total organic acids content in 1-day-old cheeses (Table 2). The PR milk cheeses showed the highest ($P < 0.05$) concentrations of this compound from 30 days of ripening, while RA and PA milk cheeses had similar values.

The concentration of citric acid in PA and PR milk cheeses increased ($P < 0.05$) during ripening (Table 2). Nevertheless, the concentration of citric acid in RA milk cheeses showed a different trend; it sharply decreased ($P < 0.05$) by 30 days increasing therefrom, but its final concentration was lower than in either PA or PR milk cheeses.

The pyruvic and acetic acid contents both increased as cheeses aged (Table 2). At the end of ripening PA and RA milk cheeses showed the highest and lowest ($P < 0.05$) concentrations of pyruvic acid, respectively, while in PR milk cheese it showed and intermediate behaviour. Cheese made from RA milk showed the highest ($P < 0.05$) concentration of acetic and propionic acids at 60 days of ripening.

A very low, but similar ($P > 0.05$), concentration of uric acid was detected in 1-day-old cheeses

(Table 2). However, it was not detected, or detected in negligible quantities, in the following ripening stages evaluated.

Malic acid increased its concentration as cheeses ageing. Sixty days after cheese making, PA milk cheeses had the highest concentration of malic acid, whereas RA and PR milk cheeses showed comparable quantities.

Butyric acid showed two opposite trends in cheeses throughout ripening: it increased in RA and PR milk cheeses, while in cheeses from PA milk it declined.

A visualisation of the complex data matrix was performed by principal component analysis (PCA), with 60-day-old RA, PA and PR milk cheeses as objects, and organic acids, NSLAB NaCl, pH and data as variables. Results from the PCA showed two interpretable factors that described about 70.63% of the total variation of sample, 45.66% and 24.97% for factor 1 and factor 2, respectively. Factor 1 was heavily loaded on pH, NSLAB, citric, pyruvic, acetic and propionic acids, while factor 2 was loaded on malic and lactic acids.

The PCA analysis showed three clusters, which corresponded with the different treatments applied to milk (Fig 2). The clustered samples with more elevate NSLAB counts (RA milk cheese) were characterised by a high amount of propionic and acetic acids. These cheeses also were negatively correlated with both pyruvic and citric acids.

On the other hand, PA milk cheese was characterised by a high level of malic and pyruvic acids, and was clearly differentiated from RA and PR milk cheeses by its low level of butyric acid. Pyruvic acid values were highly correlated with the salt concentration of cheeses.

Lactic acid was found in higher concentration in PR milk cheeses. The elevate amount of lactic acid was associated with the lower pH value observed in these cheeses.

Discussion

Under normal circumstances, lactose that remains in the curd after cheese making, is quickly metabolized by the starter bacteria. According to McSweeney and Sousa (2000) the metabolism

of lactose to lactate is essential to the production of most cheese varieties, because it is necessary for both proper manufacture and normal ripening.

The PR milk cheese showed at 60 days of ripening higher lactic acid values than those found in either RA or PA milk cheeses (Table 2). It is well know that the drastic reduction in the number of raw milk microbiota produced by milk pasteurization, modifies the catabolism of many organic acids (Grappin and Beuvier, 1997). Figure 1 shows identical NSLAB counts for PA and PR milk cheeses, while in RA milk cheese they were higher. Buffa *et al.* (2001b) compared the microbial groups most relevant during ripening of RA, PA or PR goats' milk mini-cheeses, reporting similar characteristics between PA and PR milk cheeses. In agreement, main differences found in that work were the higher counts of non-starter microorganisms in cheeses made from RA milk.

In this way, the low content of lactic acid found in RA milk cheeses could be explained by (1) the heterofermentative metabolisms of non-starter microbiota, which can produce formic, acetic and ethanol from lactose and/or (2) the indigenous cheese microbiota that may use lactic acid in different metabolic pathways, such as to produce propionate, acetate or CO_2, (Eliskases-Lechner et al., 1999; Califano and Bevilacqua, 2000).

On the other hand, the activity of the starter and its ability to ferment residual lactose is influenced by the S/M content of cheese (Thomas and Pearce, 1981). As is shown in Table 1, PA milk cheese had higher S/M content than both RA and PR milk cheeses. Buffa *et al.* (2001c) using confocal laser scanning microscopy observed that PA milk cheeses exhibited a less continuous matrix (open and porous) with more numerous and irregular spaces compared to RA or PR milk cheeses, which in turn each presented a more regular and closed protein network. Thus, the open microstructure of PA milk cheese could facilitate the NaCl diffusion into the cheese matrix. The high S/M content observed in PA milk cheese could affect in some extend the starter activity during cheese ripening, and in turn the lactic acid production, resulting in lower lactic acid content than in PR milk cheeses at the end of ripening.

Acetic acid, an important flavour compound in many cheeses, in addition to being formed from citrate mainly by *Leuconostoc* spp., it could be also result from various metabolic routes such as

from lactate or from amino acids by lactobacilli (Fox et al., 1990). In this way, acetic acid provides an indication of the degree of heterofermentative metabolism that may have taken place in cheese (Bouzas et al., 1993). Propionic acid, an essential compound for the eye and flavour development of Swiss-type cheeses, is mainly formed from lactate by *Propionibacterium* spp., but it could be also formed from casein amino-acids (Califano and Bevilacqua, 2000). Because of the lower number of propionibacteria, some researchers have found that acetic and propionic acid concentrations in cheeses made from PA milk were lower than in those made from RA milk (Bouton and Grappin, 1995; Beuvier et al., 1997). Thus, pasteurization and pressure treatments, which both reduced the number of non-starter microbiota, could lead to a large reduction in the acetic and propionic acid amounts in cheese.

The importance of citrate is highlighted since it may be metabolized by certain mesophilic microorganisms, such as lactococci, leuconostocs, and lactobacilli (strains of *Lactobacillus casei*, *L. plantarum*, and *L. brevis*) to produce acetic acid, ethanol and various carbonyl compounds (diacetyl, acetoin, 2,3-butanediol) (Peterson and Marshall, 1990; Bouzas et al., 1993; McSweeney and Sousa, 2000). As is shown in Figure 2, NSLAB were presented in a much higher number in RA milk cheeses than in PA or PR milk cheeses. Thus, the lower amount of citrate found in RA milk cheese could be attributed to its elevate NSLAB population, which could used citrate as metabolic substrate. In addition, RA milk cheese also showed the lowest concentration of pyruvate. It has been suggested that high levels of pyruvate during ripening might be an indicator of accumulation and/or reduction of metabolic activity (Skeie *et al.*, 2001). In this way, the low concentration of pyruvate in RA milk cheese seems indicate a more intense metabolism, and may probably be associated to the elevate NSLAB number.

Butyric acid is mainly formed by lipases from milk, starter bacteria and non-starter bacteria. The lowest concentration of butyric acid found in cheeses made from PA milk, compared with those of RA and PR milk cheeses, confirms the results obtained by Buffa *et al.* (2001a), who attributed the low lipolysis of PA milk cheeses to the heat-sensitive but partial pressure-resistant characteristics of the endogenous milk lipase.

Conclusions

Milk treatment did not affect the total organic acids content of 1-day-old cheeses, which increased steadily from day 1 to day 60. Both RA and PR milk cheeses exhibited higher ($P < 0.05$) concentration of organic acids than in those made from PA milk at the end of ripening. However, cheeses made from RA milk were characterised by an elevate amount of propionic and acetic acids, and lower pyruvic and citric acids concentrations, whereas lactic acid was found in higher concentration in PR milk cheese. PA milk cheese was characterised by high level of malic acid, and the lowest level of butyric acid.

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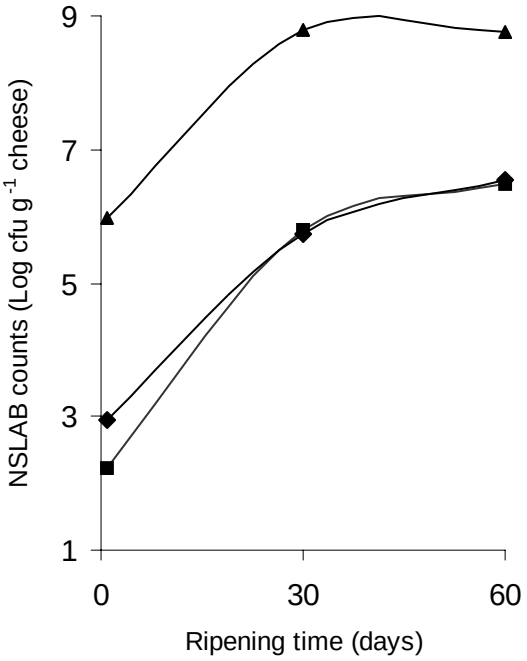

Fig. 1 Non-starter lactic acid bacteria (NSLAB) counts of cheeses made from raw (≠), pasteurized (<) or high-pressure treated (É) goats' milk.

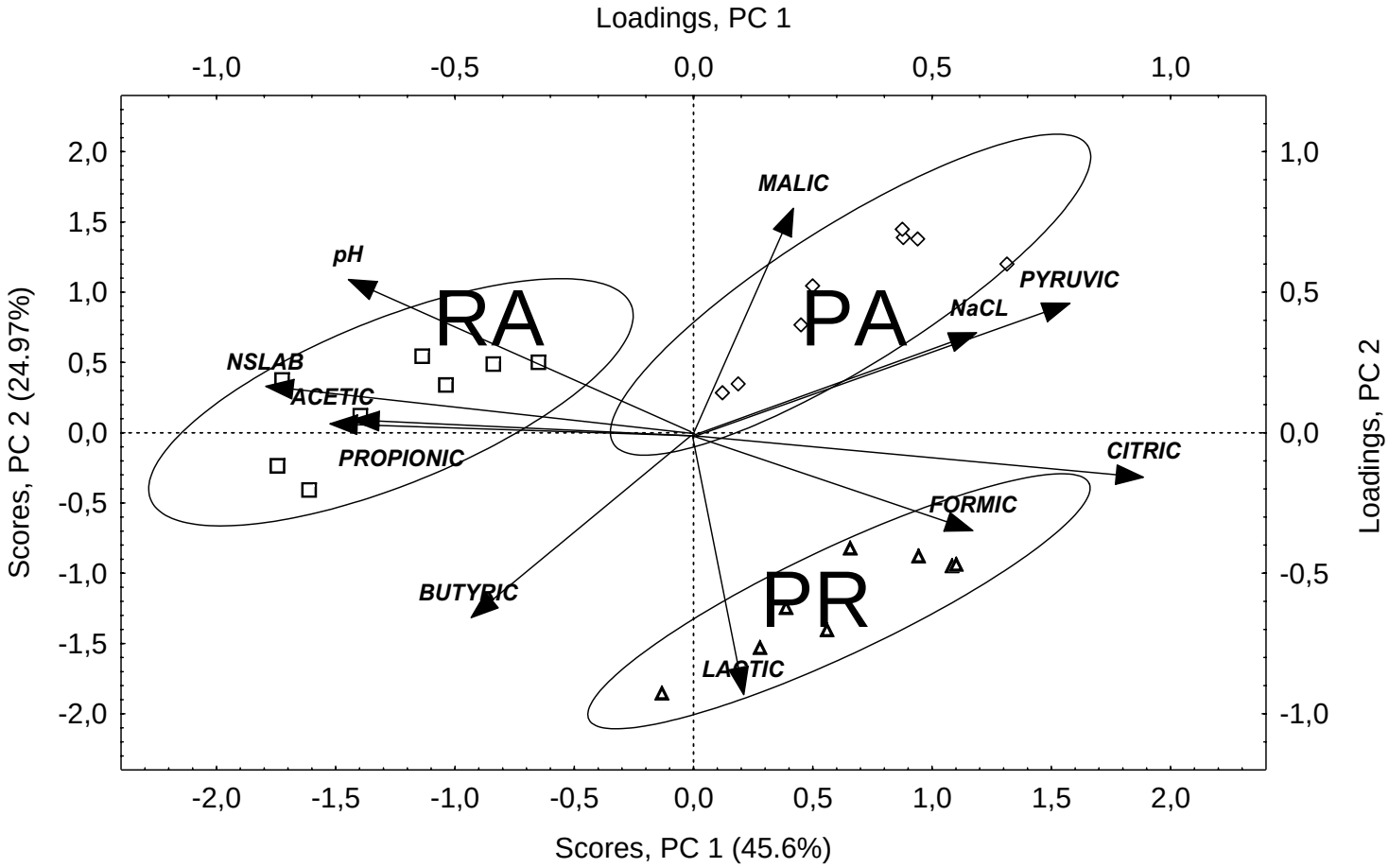

Fig. 2 Score plot and loading vectors obtained by principal component analysis of 60-day-old cheeses made from raw (RA), pasteurized (PA) or pressure-treated (PR) goats' milk. Results of each treatment are surrounded by an ellipse, which includes the members of this group ($P < 0.05$).

Table 1 Composition of cheeses (mean ∂ *standard deviation)* made from raw (RA), pasteurized (PA) and pressure-treated (PR) goat milk.

		Ripening time (days)	
		1	60
pH			
	RA	5.05 ∂ *0.03*[a]	4.89 ∂ *0.02*[a]
	PA	4.99 ∂ *0.02*[b]	4.84 ∂ *0.04*[b]
	PR	5.01 ∂ *0.06*[b]	4.76 ∂ *0.02*[c]
Moisture (g/100 g)			
	RA	52.58 ∂ *0.28*	29.31 ∂ *0.10*[a]
	PA	53.04 ∂ *0.74*	28.03 ∂ *0.28*[b]
	PR	52.75 ∂ *0.31*	29.54 ∂ *0.46*[a]
Salt/Moisture (g/100g)			
	RA	1.76 ∂ *0.52*[b]	6.92 ∂ *0.41*[b]
	PA	2.29 ∂ *0.58*[a]	7.51 ∂ *0.40*[a]
	PR	1.77 ∂ *0.41*[b]	7.03 ∂ *0.54*[b]

Physicochemical values are means of duplicate analysis from the two independent experiments. [a,b,c] Means within the same column without a common superscript are significantly different ($P < 0.05$).

Table 2 Organic acid concentration of cheeses made from raw (RA), pasteurized (PA) and pressure-treated (PR) goat milk.

	Organic acids (mg kg^{-1} dry matter)									
	Citric	Pyruvic	Malic	Lactic	Formic	Acetic	Uric	Propionic	Butyric	Total
	Day 1									
RA	37,2^{ab}	0,5	2,6^{b}	303,4	3,6^{b}	9,5	0,5	10,2^{a}	45,0^{b}	412,2
PA	39,5^{a}	0,5	2,8^{ab}	310,1	5,3^{a}	11,2	0,4	7,5^{ab}	55,9^{a}	433,2
PR	35,4^{b}	0,5	3,1^{a}	313,0	4,6^{ab}	11,0	0,5	6,2^{b}	39,9^{c}	414,4
	Day 30									
RA	16,1^{b}	1,7^{a}	15,6	294,5^{a}	5,9^{a}	36,0^{a}	-	20,5^{a}	44,6^{a}	434,9
PA	30,5^{a}	1,3^{ab}	16,3	276,3^{b}	3,9^{b}	20,0^{b}	-	20,9^{a}	37,0^{b}	406,1
PR	29,6^{a}	0,7^{b}	14,7	322,4^{a}	6,2^{a}	16,1^{b}	-	15,3^{b}	39,1^{b}	424,0
	Day 60									
RA	28,0^{b}	2,6^{c}	39,6^{b}	315,7^{b}	8,9^{b}	59,3^{a}	-	87,1^{a}	61,1^{a}	602,3^{a}
PA	48,3^{a}	4,3^{a}	47,8^{a}	302,4^{b}	10,6^{b}	39,3^{b}	0,1	69,4^{b}	25,2^{b}	541,0^{b}
PR	49,2^{a}	3,3^{b}	34,2^{c}	368,6^{a}	12,6^{a}	37,7^{b}	0,2	66,0^{b}	59,0^{a}	630,8^{a}

^{a,b,c} Means within the same row and day without a common superscript are significantly different ($P<0.05$).

3.8. PER SE *EFFECT OF HIGH-PRESSURE-TREATMENT OF MILK ON CHEESE PROTEOLYSIS.*

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Per se effect of high-pressure-treatment of milk on cheese proteolysis

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Running ahead: proteolysis in goat cheeses

The extent of primary and secondary proteolysis of cheeses made from raw (RA), pasteurized (PA, 72ºC, 15s) or pressure-treated (PR, 500 MPa, 15 min, 20ºC) goats' milk was assessed. Modifications in cheese making technology were introduced in order to obtain cheeses with the same moisture, and thus studied *per se* the effect of milk treatment on cheese proteolysis.

The PR milk cheese samples were differentiated from RA and PA milk cheeses by their elevate η-LG content, and by the higher degradation of ζ_{s2}-CN and η-CN throughout ripening.

Non-significant differences were found in either WSN or TCA values of cheeses. However, milk pasteurization depressed the FAA liberation in cheese. The RA milk cheeses had the highest amount of proline and the lowest concentrations of serine, tyrosine, arginine and AABA, whereas PR milk cheese showed higher levels of arginine.

Keywords: proteolysis, ripening, goat cheese, high-pressure treatment

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Europe has a significant tradition in goats' milk production, and most of this milk is actually used for cheese making. Although many goats' milk cheeses have been traditionally made from raw (RA) milk, aspects related essentially to microbial safety have increased the use of pasteurization treatments. The destruction of some desirable indigenous milk microbiota by the thermal treatment has been linked to minor aroma or typical flavour development of cheeses, in relation to those made from RA milk (Grappin & Beuvier 1997; McSweeney et al. 1993). Likewise, pasteurization attenuates or activates the activity of many indigenous milk enzymes, such as plasmin/plasminogen complex, lipases or alkaline phosphatase, and it also produces slight denaturation of serum proteins and slight modifications in milk rennetability (Grappin & Beuvier 1997).

The interest in non-thermal technologies, such as high-hydrostatic pressure, on milk has recently increased mainly due to the possibility to reduce the microbial number without minor effects on flavour or nutritional components. Additionally to microbial destruction, high-pressure treatments induce numerous effects on milk components: fragmentation of casein micelles, aggregation of whey proteins, modifications of the mineral equilibrium, etc (Felipe et al. 1997; Law et al. 1998; López-Fandiño et al. 1998; Needs et al. 2000), which affect the technological properties of milk (Huppertz et al. 2002; Trujillo et al. 2002b).

Most researchers have found that pasteurization of milk results in higher cheese moisture content than in cheese made from RA milk (Grappin & Beuvier. 1997). In the same way, Drake et al. (1997) reported that Cheddar cheese made from pressure-treated (PR) milk showed a higher moisture content than RA or pasteurized (PA) milk cheeses. Utilizing PR goats' milk for cheese making, Trujillo et al. (1999a) increased cheese yield basically due to higher moisture retention. This property of PR milk has been successfully used for Molina et al. (2000) to make reduced-fat cheeses, where high moisture retention is usually pursued to improve quality.

Nevertheless, moisture in non-fat substance (M/NFS) influences markedly the ripening of cheese, affecting the cheese component degradation (Lawrence et al. 1987). Trujillo et al. (2002a) studied primary and secondary proteolysis of goat cheese made from RA, PA (72ºC,

15 s) and PR (500 MPa, 15 min, 20ºC) milks. During ripening, the hydrolysis of ζ_{s1}-CN in cheeses was in the order PA>PR>RA and PA>PR=RA for η-CN. Analysis of secondary proteolysis showed differences between cheeses, especially in PR milk cheeses, which contained considerably higher concentrations of free amino acids than either PA or RA milk cheeses throughout ripening. According to the authors, the high moisture content of PR and PA milk cheeses, related to RA milk cheeses, and the effects of technological treatments on milk compounds (native enzymes i.e. plasmin, casein micelles, whey proteins, etc.), could explain the differences in proteolysis found in cheeses.

In this work, with aim to assess *per se* the effect of milk treatment on primary and secondary proteolysis, cheeses from RA, PA (72ºC, 15 s) or PR (500 MPa, 15 min, 20ºC) goats' milk were made. However, when experimental cheeses are made is very difficult to have the same M/NFS in RA, PA and PR milk cheeses without modifying the technological parameters of cheese making (Grappin & Beuvier 1997). So, modifications in cheese making technology were introduced in order to obtain cheeses with the same M/NFS.

MATERIALS AND METHODS

Cheese manufacture

Milk was obtained from a herd of Murciano-Granadina goats (Muntanyola, Barcelona, Spain). Goat cheese was manufactured from RA, PA (72ºC, 15 s) and PR milk in two independent experiments, within an interval of one week. In each experiment, 50 kg of RA, 50 kg of PA and 50 kg of PR milk from the same milk batch were used for cheese making.

Pasteurized milk was obtained by using a heat-exchanger (Garvía S.A., Barcelona, Spain) at 72ºC at a flow rate of 200 L h^{-1} with a holding time of 15 s.

High-pressure treated milk was obtained by using a semi-continuous hyperbar equipment (GEC

Alsthom ACB, Nantes, France) by direct compression of the liquid with a piston. Batches of 4 L of milk were pressurized at 500 MPa and 20 ± 1 ºC with a holding time of 15 min. The PR milk was kept at 4ºC until cheese making.

Milk was heated to 31ºC and then a starter culture (AM Larbus, Barcelona) containing *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*, and 35% (w/w) $CaCl_2$ (food quality grade) were both added to cheese milk to a final concentration of 2% (w/w) and 0.02% (v/w), respectively. Ten minutes later, 0.02% (v/w) of calf rennet (Reniflor-15/E, Lamirsa, Barcelona), containing 780 mg chymosin L^{-1} was added. After 30 min, the coagulum was cut, and the curds drained and moulded (13.6 Δ 13.2 cm).

Due to the different technological treatments, and in order to obtain comparable cheese moisture content, pressing time was prolonged up to 12 h (1 h at 1.3 kPa and 11 h at 2.6 kPa). The pressing condition used in this study was based on the experience gained for previous experiments.

After that, cheeses were salted by immersion in brine (19% NaCl solution) for 4 h at 14ºC. Finally, cheeses, each of one of approximately 1.31 ± 0.03 kg, were ripened in a room at 14ºC and 85% relative humidity for 60 days.

Compositional analysis

Analyses of RA, PA and PR milk cheeses from the two experiments were performed in duplicate for total solids (TS; (IDF 1982)), fat (F; van Gulik method (ISO 1975)) and total nitrogen (TN; (IDF 1987)). Salt was determined by chloride analysis (Corning 926 Chloride Analyzer, Sherwood Scientific Ltd., Cambridge, UK). The pH was measured in a cheese/distilled water (1:1) slurry.

Preparation of cheese extracts

Water-soluble fractions (WSF) of cheeses were prepared following the method of Kuchroo & Fox (1982). The soluble nitrogen at pH 4.6 (WSN) and the soluble nitrogen in 12% trichloracetic (TCA) acid were determined from WSF.

Capillary electrophoresis

The water-insoluble fractions recovered during the water soluble extraction were washed three times with 1 M sodium acetate buffer (pH 4.6) and the remaining fat was eliminated by washing with diclhlorometane-sodium acetate buffer (1:1, v/v). Then, the final protein precipitate was lyophilized.

Capillary electrophoresis (CE) analysis were performed following the method of Recio & Olieman (1996). Sample buffer (pH 8.6) contained 167 mM Tris(hydroxymethyl)aminometane (Amresco, Solon, Ohio, USA), 42 mM 3-morpholino-propanesulfonic acid (Biohemika MicroSelect, Fluka, Buchs, Switzerland), 67 mM ethylendinitritetra-acetic acid disodium salt dihydrate (Tritiplex III; Merck, Darmestadt, Germany), 17 mM DL-dithiothreitol (Sigma, St. Louis, MI 63178, USA), 6 M urea (BioChemika MicroSelect, Fluka) and methylhydroxypropyl cellulose (0.5 g/L, Sigma).

The separation buffer (pH 3.0) contained 0.32 M citric acid, 20 mM sodium citrate, 6 M urea and 0.5 g/L of methylhydropropyl cellulose.

Lyophilized water insoluble fractions from cheese (18 mg) were dissolved in 1 mL of diluted sample buffer (sample buffer/water 1.5: 1). After incubation for 1 h at room temperature, 20 σL lactic acid 18% (v/v) were added (final pH 7.0) and injected in the CE apparatus.

Separation were carried out using a BioFocus 2000 capillary electrophoresis instrument (Bio-Rad Laboratories Ltd., Hercules, CA 94547, USA) controlled by BioFocus System operating Software. The separations were performed using a hydrophilic-coated fused-silica capillary

column (CElect P1, Supelco, Bellefonte, PA 16823, USA) of 0.47 m Δ 50 σm i.d. (effective length 0.40 m). Electrophoresis was run at 40 ºC and a final voltage of 20 kV and UV-detection was performed at 214 nm.

The area of each peak was integrated using BioFocus System Integration Software (version 5.0).

Free amino acids analysis

The concentrations of free amino acids (FAA) were analysed by ion exchange chromatography with postcolumn ninhydrin derivatisation and detection at 570 and 440 nm for primary and secondary amino acids according to the procedure of Spackman et al (1958).

The WSF of cheeses were centrifuged at 13000 rpm by 15 min. Then, 250 σL of WSF were taken from the supernatant of centrifugation and 25 σL of 1 mM L-norleucine (Sigma) were added as internal standard. Samples were filtered throughout a 10 kDa membrane filter (Ultra Free, Millipore, France), and then 40 σL were injected in the amino acid analyser (Alpha Plus series II, Pharmacia LKB Biotechnology, Sweden). Separation was carried out on a sulphonated polystyrene divinilbenzene column (200 × 4 mm, Biochrom Ltd., Cambridge, UK) using lithium citrate buffer as mobile phase. Individual FAA were identified and quantified by using a mixture of amino acids standards (Sigma).

Statistical analysis

Data were processed by analysis of variance (ANOVA) using the general linear models procedure of SAS™ System for WIN⏐ (8 version). The Student-Newman-Keuls test was used for comparison of sample data. Evaluations were based on a significant level of $P<0.05$.

Principal component analysis (PCA) was performed using Statistica™ software (5.0 version;

StatSoft Inc., Tulsa, OK, USA). Kaiser's criterion (an eigenvalue greater than 1) was employed to establish the number of final factors from the general parameters considered. Orthogonal rotation of the factors using the Varimax method was used to interpret these results.

RESULTS AND DISCUSSION

Physico-chemical analyses

The composition of RA, PA and PR milk cheeses is shown in Table 1. No differences (P<0.05) in M/NFS content and F were observed between cheeses, but at the end of ripening the PA milk cheese presented the lowest M/NFM suggesting that it had a greater water evaporation rate than RA or PR milk cheeses. In this way, Buffa et al. (2003) studied the water binding properties of cheeses made from RA, PA and PR goats' milk. In that study, PA milk cheeses showed large decreases of water retained with less energy to the cheese-matrix, whereas PR and RA milk cheeses retained more water. These authors suggested that the water loss of cheeses during ripening is also controlled by the internal profiles of water in the cheeses, which in turn is related to the cheese-matrix microstructure, and not only by the external conditions of ripening.

The pH of PA and PR milk cheeses was lower than in cheeses made from RA milk (Table 1). Salt-in-moisture content was higher (P<0.05) in PA than in RA or PR cheeses. Buffa et al. (2001b) using confocal laser scanning microscopy observed that PA milk cheeses exhibited a less continuous matrix (open and porous) with more numerous and irregular spaces compared to RA or PR milk cheeses, which in turn each presented a more regular and closed protein network. Thus, the open microstructure of PA milk cheese could facilitate the NaCl diffusion into the cheese matrix during salting.

Primary proteolysis

Proteolytic phenomena take place in two steps that are usually called primary and secondary proteolysis. The first of each may be defined as those changes in caseins (ζ_{s1}-CN, ζ_{s2}-CN, η-CN and ν-CN) and peptides, which can be detected by electrophoretic methods (Fox 1989).

The water-insoluble fractions at pH 4.6 of RA, PA and PR milk cheeses were analysed by capillary electrophoresis. Designation of CE peaks of intact caseins (ζ_{s1}-CN, ζ_{s2}-CN and η-CN) was carried out by comparing the electrophoregrams of skimmed milk and 1-day-old cheeses with those of isolated pure proteins (Trujillo et al. 2000). The relative migration time of peaks was in concordance with previous reports (Recio et al. 1997).

Electrophoretic profiles of 1-day-old RA, PA and PR milk cheeses are shown in Fig. 1.

The main difference observed between cheeses corresponded to the presence of η-LG in PA and PR milk cheeses, which as we expected was much higher in the later. It has been reported that η-LG, additionally to their heat-sensitive behaviour, is the whey protein most easily denatured by the high-pressure treatment of milk (Felipe et al. 1997). Whereas in normal conditions of PA (72ºC 15 s) about 7% of whey proteins are denatured (Lawrence 1991), almost 50% of whey proteins are irreversible aggregated above 400 MPa (Felipe et al. 1997). These facts could explain the presence, as well as the quantity, of η-LG found in cheeses. The η-LG was resistant to proteolysis in PA and PR milk cheese after 60 days of ripening.

Excepting for η-LG peak, little qualitative differences were observed between the electrophoretic profiles of cheeses at day 1. Nevertheless, significant quantitative differences were evident during ripening (Fig. 2).

An intense decrease in the area of peaks corresponding to #ζ_{s1}-CN was observed at 30 days of ripening (Fig. 1 and Fig. 2). The #ζ_{s1}-CN represented the main target for proteolysis, showing at the end of ripening approximately 50% of their initial values. Sixty-day-old PR milk cheeses exhibited higher (P<0.05) #ζ_{s1}-CN degradation than both RA and PA milk cheeses. The

hydrolysis of #ζ_{s1}-CN was coincident with the appearance of new peaks with a high electrophoretic mobility, which corresponded to the breakdown product#ζ_{s1}-I. These peaks increased as cheeses ageing, and after 30 days of ripening cheeses made from PR milk showed the highest values for ζ_{s1}-I, fact that agrees with the elevate degradation of #ζ_{s1}-CN in these cheeses.

In all the cheeses analysed,#ζ_{s1}-CN was degraded to a major extent than η-CN, which in turn was slowly hydrolysed, especially in RA and PA milk cheeses (Fig. 2). At the end of ripening, cheeses made from PR milk exhibited higher η-CN degradation (approximately 19%) than both RA and PA milk cheeses. When animal rennets are used, ζ_{s1}-CN undergoes considerable proteolysis during ripening of hard and semi-hard bacterially ripened cheeses, but η-CN remains unchanged until and advanced stage of ripening (Fox et al. 1993).

Plasmin, the main endogenous proteinase of milk, acts mainly on η-CN forming the ν-CN (Fox et al. 1993). The area of ν-CN peaks increased approximately 50% during ripening, but not differences (P>0.05) in their values were observed between cheeses.

The ζ_{s2}-CN, like η-CN, is susceptible to plasmin attack. The ζ_{s2}-CN showed a marked decrease (P<0.05) throughout ripening. Cheeses made from PR and RA milk showed the highest and lowest degradation of ζ_{s2}-CN, respectively, whereas PA milk cheeses exhibited an intermediate behaviour.

Para-ρ-CN showed a decrease (approximately 20%) in all cheeses during ripening, but non-differences were observed between cheeses at 60 days of ripening.

Actually, the effect of high-pressure treatments of milk on cheese proteolysis is not well known. Trujillo et al. (1999a) evaluated the proteolysis of PA and PR goat milk cheeses by SDS-PAGE electrophoresis, reporting no major qualitative differences in the casein degradation pattern of cheeses. On the other hand, Molina et al. (2000) reported that low fat cheeses made from PR milk showed a faster rate of protein breakdown (ζ_{s1}- and η-CN) than those made from PA milk. This fact was explained by the higher level of residual rennet retained in PR milk curds,

attributed in turn to their higher moisture content. According to these authors, the major moisture content of PR milk cheeses could directly boosted proteolysis.

Recently, Trujillo et al. (2002a) found that, even PR milk cheeses showed the highest moisture content compared to PA and RA counterparts, cheeses made from PA milk showed the highest degradation of ζ_{s1}-, ζ_{s2}- and η-CN. These findings were explained by greater rennet retention and by the activation of plasminogen in plasmin and/or inactivation of plasmin inhibitors following pasteurization. In that work, PR milk cheeses showed an intermediary degradation of ζ_{s1}-CN (PA>PR>RA). Furthermore, PR and PA milk cheeses displayed similar degradation of η-CN at 15 days of ripening, but higher than those found in RA milk cheeses.

Considering the essential role of moisture on cheese proteolysis, we introduced modifications in cheese making technology in order to obtain cheeses with the same M/NFS. As is shown in Table 1, cheeses had similar (P<0.05) M/NFS values, but at the end of ripening the PA milk cheese showed a minor value. Additionally, PA milk cheeses showed higher S/M content (Table 1), which also could affect the proteolytic processes. Thus, lower M/NFS at the end of ripening and elevate S/M content both probably affected the protein degradation of PA milk cheeses.

Although PR and RA milk cheeses had similar S/M and M/NFS contents, the protein breakdown was higher in the former. This finding could be attributed to changes in the cheese matrix, imputed in turn to the high-pressure treatment applied on cheese milk. When pressure is applied to milk, the hydrophobic interactions between the micelles of casein are partly suppressed, releasing free caseins and small casein clusters. Once pressure is released, a reforming process of milk micelles happens, but its morphology as well as its size are altered. During the reforming micelle process, denatured η4LG could form disulfide bonds with ρ4 and/or ζ_{s2}-CN or form self-aggregated polymers, which might appear associated or trapped within casein micelles (Needs et al. 2000). This could lead to reformed micelles with altered structure and/or composition, which may alter the susceptibility of milk proteins to the proteolytic action. Then, the pressure-treatment applied on milk may change the casein micelle structure exposing

a larger surface area of protein for proteolytic attack, which in turn could explain the elevate proteolysis of PR milk cheeses.

Secondary proteolysis

The degree of secondary proteolysis was evaluated by the fractionation of nitrogen fractions, pH 4,6 soluble-nitrogen (WSN), 12% trichloracetic soluble-nitrogen (TCA) and by the determination of individual free amino acids (FAA). The WSN fraction includes proteins (excluding caseins), all peptides, FAA and smaller nitrogen compounds, while TCA fraction contains medium and small-size peptides, FAA and smaller nitrogen compounds (Ardö 1999).

In all cheeses, both WSN and TCA fractions increased (P<0.05) by 60 days, indicating the significant effect of the ripening time on their concentration (Table 2). However, non-significant differences were found in either WSN or TCA values of RA, PA or PR milk cheeses in any stage of ripening.

In the same way, total FAA content of cheeses also increased (P<0.05) by time (Fig. 3). Nevertheless, both RA and PR milk cheeses showed higher (P<0.05) amount of total FAA than that found in cheeses made from PA milk from 30 days onward.

It has been described that, as general rule, RA milk cheeses show higher levels of total FAA than cheeses made from PA milk (Grappin & Beuvier 1997). Those findings were linked to the amino peptidase activities of raw milk bacteria. The elevate concentration of FAA found in PR milk cheeses, compared to PA milk cheese, agrees with the results of Trujillo et al. (1999b; 1999a).

The release of individual FAA is shown in Fig. 4. Twenty-three amino acids were identified and quantified from the cheese samples. The main FAA observed throughout ripening in the three types of cheeses were leucine, glutamic, proline and phenylalanine, representing together approximately 41% of total FAA content.

Visualisation of the complex data matrix was performed by principal component analysis (PCA), with RA, PA and PR milk cheeses as objects and individual FAA data as variables. Results from the PCA showed three interpretable factors that described about 85.1% of the total variation of sample, 61.3, 13.8 and 9.9% for factor 1, 2 and factor 3, respectively. Factor 1 was loaded on aspartic acid, threonine, asparagine, glutamic acid, glutamine, proline, glycine, alanine, valine, methionine, isoleucine, leucine, phenylalanine, ornithine, lysine and histidine. The first principal component expressed cheese age, reflecting that the amount of most amino acids increased in all cheeses during ripening. Factor 2 was loaded on serine, ζ-aminobutyric acid (AABA), tyrosine and arginine, and seems to be linked to cheese milk treatment. The thirst factor explains the behaviour of cysteine and tryptophan, which both increased by 30 days of ripening and then decreased toward the end of the experiment.

The PCA analysis showed three partially overlapped clusters, which corresponded with the different treatments applied to milk. As is shown in Fig. 5, the clustered RA milk cheese samples differed from PA and PR milk cheeses at the end of ripening. The RA milk cheeses were differentiated by its elevate amount of proline, and by their low values of serine, AABA, tyrosine and arginine. The PR milk cheeses showed higher levels of arginine than those found in either RA or PA milk cheeses.

According to Polo et al*.* (1985) and Freitas et al*.* (1999) the pattern of FAA releasing depends on the metabolic routes followed by the enzymatic degradation of peptides by various microorganisms, as well as from the amino acids interconversion, excresion or degradation. The released amino acids from protein network are precursors for several amino acids not presents in casein, such as glutamic for AABA and ν-aminobutyric acid (GABA), arginine for ornithine and citrulline, etc (Bütikofer & Fuchs 1996). Other reaction that usually takes place in cheese is the decarboxylation of tyrosine to compounds like tyramina (Skeie & Ardö 2000).

As is shown in Fig. 6, cheeses made from RA milk showed lower (P<0.05) values of arginine than PA and PR milk cheeses. It has been reported that some RA milk cheeses contain lower amount of arginine than their counterparts made from PA milk (Krause et al. 1997; Ordóñez et

al. 1999). Laht et al. (2002) observed that the growth of non-starter lactic acid bacteria (NSLAB) in Swiss-type cheese was accompanied by the decrease of arginine concentration and formation of ornithine, NH_3 and citrulline. In this way, correlation analysis carried out between amino acid data of RA, PA and PR milk cheeses showed that arginine was significantly correlated to ornithine. Because of arginine conversion into ornithine generates ATP, Laht et al. (2002) supposed that arginine could be one of the main energy sources for NSLAB in ripening cheese. Buffa et al. (2001a) compared the microbial groups most relevant during ripening of RA, PA or PR goats' milk cheeses, reporting similar characteristics between PA and PR milk cheeses. The main difference between cheeses was the counts of lactobacilli (main microbial group of NSLAB), which were higher (~3 log) in RA milk cheese in all the ripening stages studied. Thus, pasteurization and high-pressure treatments, which both reduced the endogenous raw milk microbiota in a similar extent, could drop the arginine degradation during ripening, and then to explain the accumulation of this amino acid in PA and PR milk cheeses.

Cheeses made from PA and PR milk had lower (P<0.05) amount of proline than RA milk cheese (Fig. 4). This finding seems to be also linked to the NSLAB population, because of some of them have specific peptidase for hydrophobic peptides rich in proline, such as prolidases, aminopeptidases P, prolyldipeptidases, etc (Fresno et al. 1997).

Pasteurization and high-pressure treatments of milk seem to exert a distinct effect on the serine amounts throughout ripening, which were higher than in RA milk cheeses. Higher levels of serine in PA milk cheese have been observed by other researchers in Idiazabal cheese (Ordóñez et al. 1999) and in model cheese (Skeie & Ardö 2000). Kieronczyk et al. (2001) studied the metabolism of amino acid by *Lactobacillus casei* and *Lb. paracasei* subsp. *paracasei* under cheese like conditions, finding a decrease in the serine amount for the two strains. According to Skeie & Ardö (2000), some lactobacilli may oxidize serine under anaerobic conditions (like in ripening cheese), because it contains an –OH group, fact that could explain the lower amounts of serine in RA milk cheeses.

The RA milk cheeses had lower amount of tyrosine than cheeses made from PA or PR milk.

Krause et al. (1997) in semi-hard and in soft cheeses and Skeie & Ardö (2000) in model cheese found lower concentration of tyrosine in RA milk cheeses in relation to those made from PA milk, probably by limiting the raw milk microbiota action. Thus, tyrosine could have been decarboxylated to tyramine by some microorganisms of the NSLAB, but unfortunately tyramine was not quantified in this work.

Milk pasteurization seems depress the total FAA liberation, while PR and RA milk cheeses, even showed similar total FAA value, differed in the concentration of some amino acids. It has been reported that cheese made from either PA or PR milk have a comparable microbiological quality (Buffa et al. 2001b; Drake et al. 1997). However, total FAA values of PR milk resemble to those found in RA milk cheese, which would be assumed to have a more heterogeneous microbiota. It is possible that changes caused to milk by HPT can alter, in some way, the susceptibility to the proteolytic action, may be increasing the substrates for enzymatic attack.

CONCLUSIONS

The PR milk cheese were differentiated from RA and PA milk cheeses by their elevate η-LG value, and by the higher degradation of ζ_{s2}-CN and η-CN throughout ripening.

Non-significant differences were found in either WSN or TCA values of cheeses. However, milk pasteurization seems depress the FAA liberation in cheese, while total FAA values of PR milk resemble to those found in RA milk cheese. The RA milk cheeses had the highest amount of proline and the lowest concentrations of serine, tyrosine, arginine and AABA. Cheeses made from PR milk showed the most elevate amount of arginine at the end of ripening.

The PR milk cheese showed the faster rate of casein breakdown, fact that could be due to changes caused to milk by high-pressure treatment, which can alter the susceptibility of milk proteins to the proteolytic action. It is possible that the rise in primary proteolysis, which could

increase the number of substrates for enzymatic attack, also facilitated the FAA releasing of PR milk cheese.

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Table 1. Composition of cheeses (mean and standard deviation) made from raw (RA), pasteurized (PA) and pressure-treated (PR) goat milk.

		Age of cheese, d		
	Milk	1	30	60
pH	RA	5.05 ∂ 0.03[a]	4.80 ∂ 0.03	4.89 ∂ 0.02[a]
	PA	4.99 ∂ 0.02[b]	4.80 ∂ 0.01	4.84 ∂ 0.04[b]
	PR	5.01 ∂ 0.06[b]	4.79 ∂ 0.05	4.76 ∂ 0.02[c]
Moisture in non-fat substance, %	RA	2.45 ∂ 0.05	1.33 ∂ 0.05	1.01 ∂ 0.02[a]
	PA	2.47 ∂ 0.08	1.29 ∂ 0.05	0.92 ∂ 0.01[b]
	PR	2.43 ∂ 0.05	1.36 ∂ 0.04	0.98 ∂ 0.02[a]
Salt-in-moisture, %	RA	1.76 ∂ 0.52[b]	5.00 ∂ 0.56	6.92 ∂ 0.41[b]
	PA	2.25 ∂ 0.53[a]	5.30 ∂ 0.68	7.51 ∂ 0.40[a]
	PR	1.77 ∂ 0.41[b]	5.00 ∂ 0.50	7.03 ∂ 0.54[b]
Fat in dry matter, %	RA	54.28 ∂ 0.32	56.08 ∂ 0.43	57.96 ∂ 0.90
	PA	54.30 ∂ 0.74	55.95 ∂ 0.44	57.59 ∂ 0.64
	PR	53.97 ∂ 0.35	56.07 ∂ 0.72	57.14 ∂ 0.87

[a,b,c] Means within a row with a different superscript were significantly different ($P<0.05$)

Table 2: Water-soluble N at pH 4.6 (WSN) and soluble N in 12% trichloracetic (TCA) values as % of total N (mean and standard deviation) of cheeses made from raw (RA) pasteurized (PA) and pressure-treated (PR) goat milk

	WSN/TN, %	TCA/TN, %
Day 1		
RA	10.63 ∂ 1.63	5.42 ∂ 0.48
PA	10.46 ∂ 2.25	5.44 ∂ 0.73
PR	9.06 ∂ 0.96	4.70 ∂ 0.76
Day 60		
RA	17.93 ∂ 1.74	9.15 ∂ 2.17
PA	18.10 ∂ 1.09	11.26 ∂ 2.51
PR	17.76 ∂ 1.27	11.20 ∂ 3.65

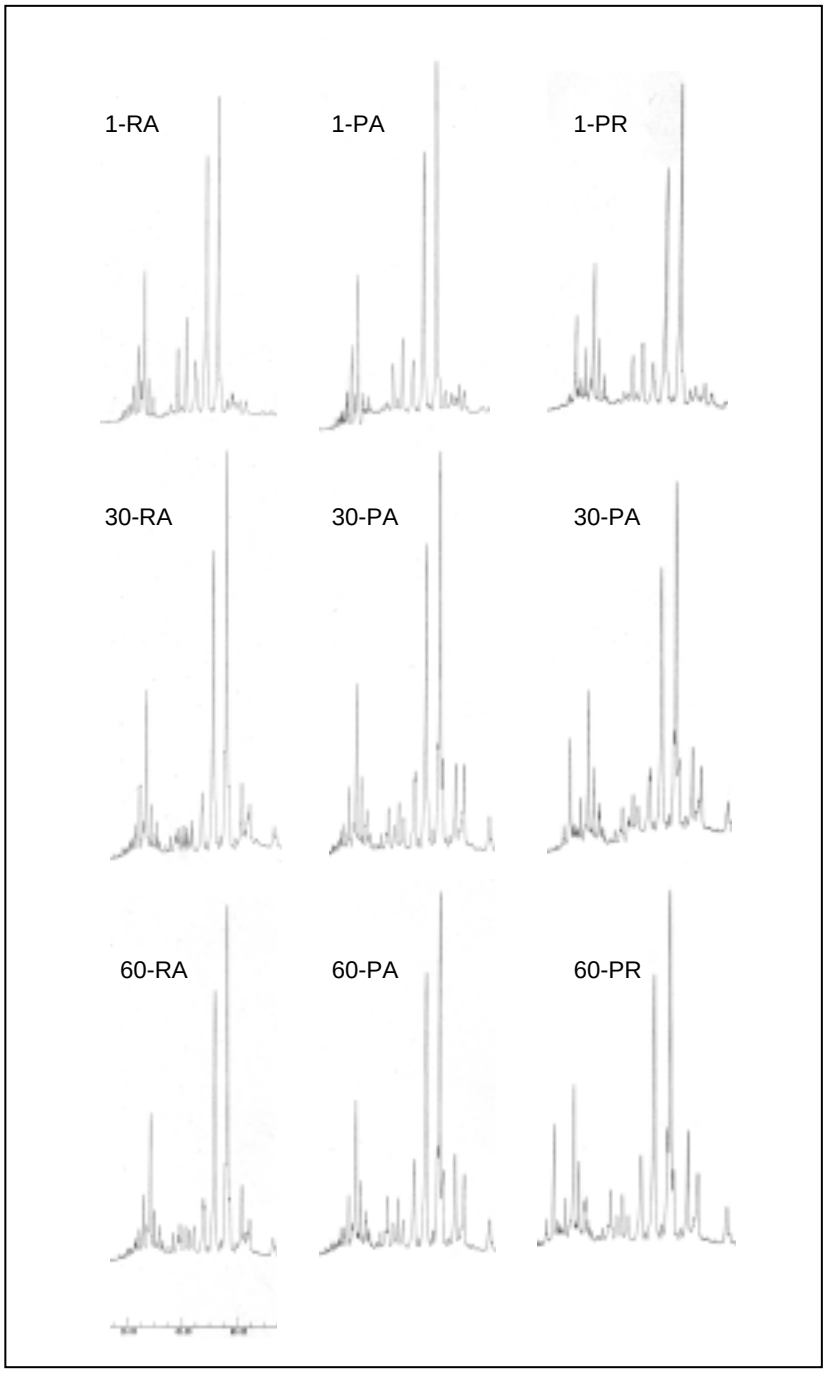

Figure 1. Capillary electrophoresis analyses of the pH 4.6 insoluble fractions of cheeses made from raw (RA) pasteurized (PA) and pressure-treated (PR) goat milk at 1, 30 and 60 d of ripening

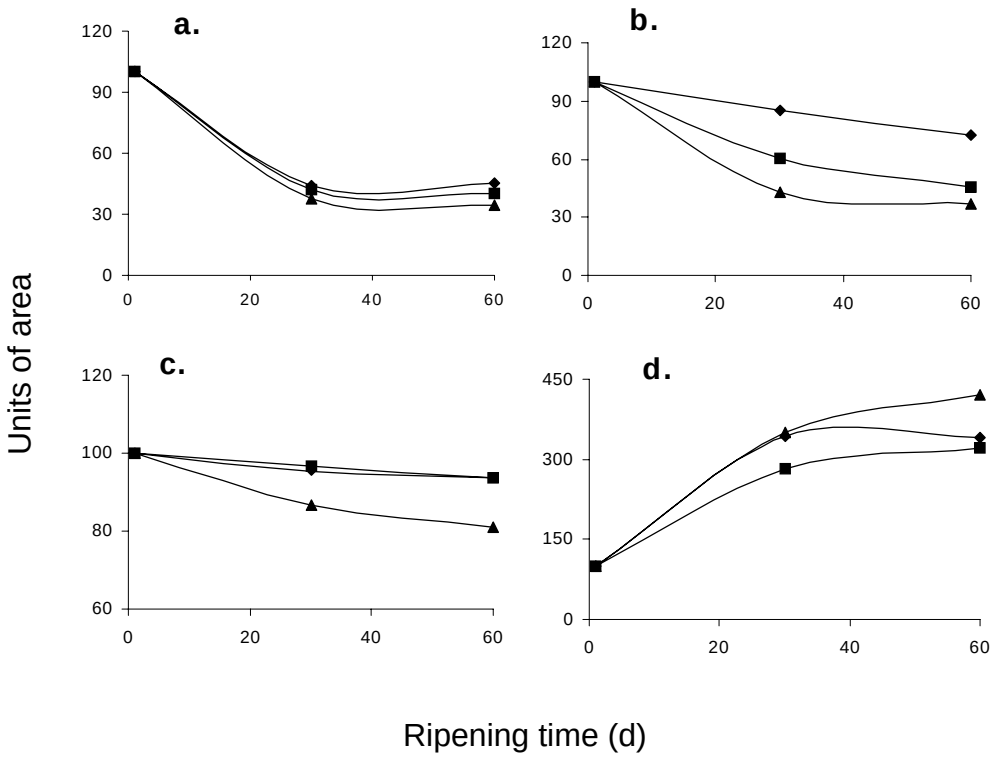

Figure 2: Residual ζ_{s1}-CN (a), ζ_{s2}-CN (b), η-CN (c) and ζ_{s1}-I (d) during ripening of cheeses made from raw (É) pasteurized (<) and pressure-treated (ź) goat milk

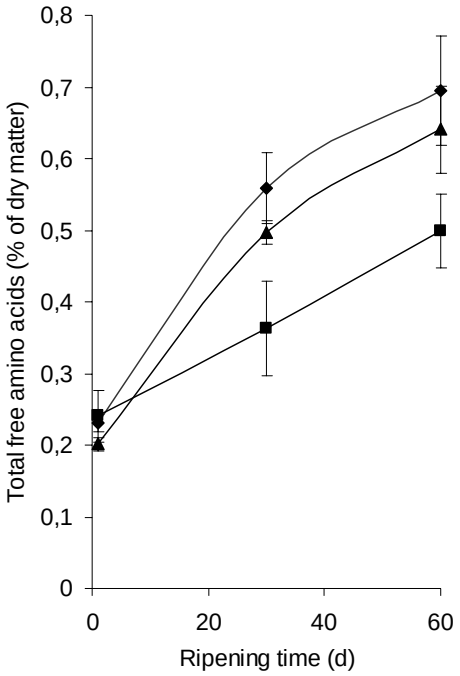

Figure 3: Free amino acid amount (% dry matter) during ripening of cheeses made from raw (É) pasteurized (<) and pressure-treated (ź) goats' milk.

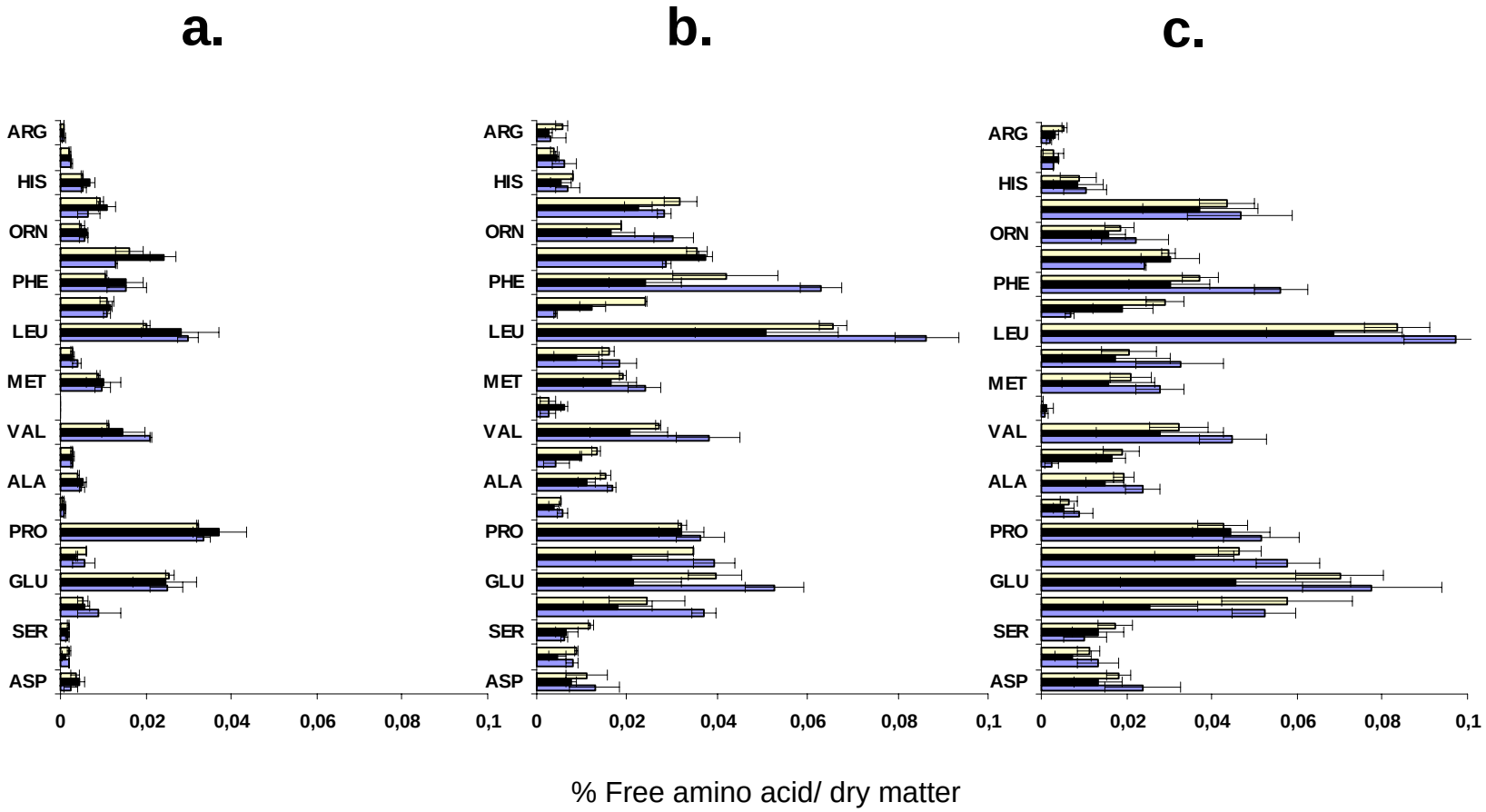

Figure 4: Free amino acid profile of cheeses made from raw (□), pasteurized (■) or pressure-treated goats' milk (□) at 1 (a), 30 (b) and 60 (c) d of ripening

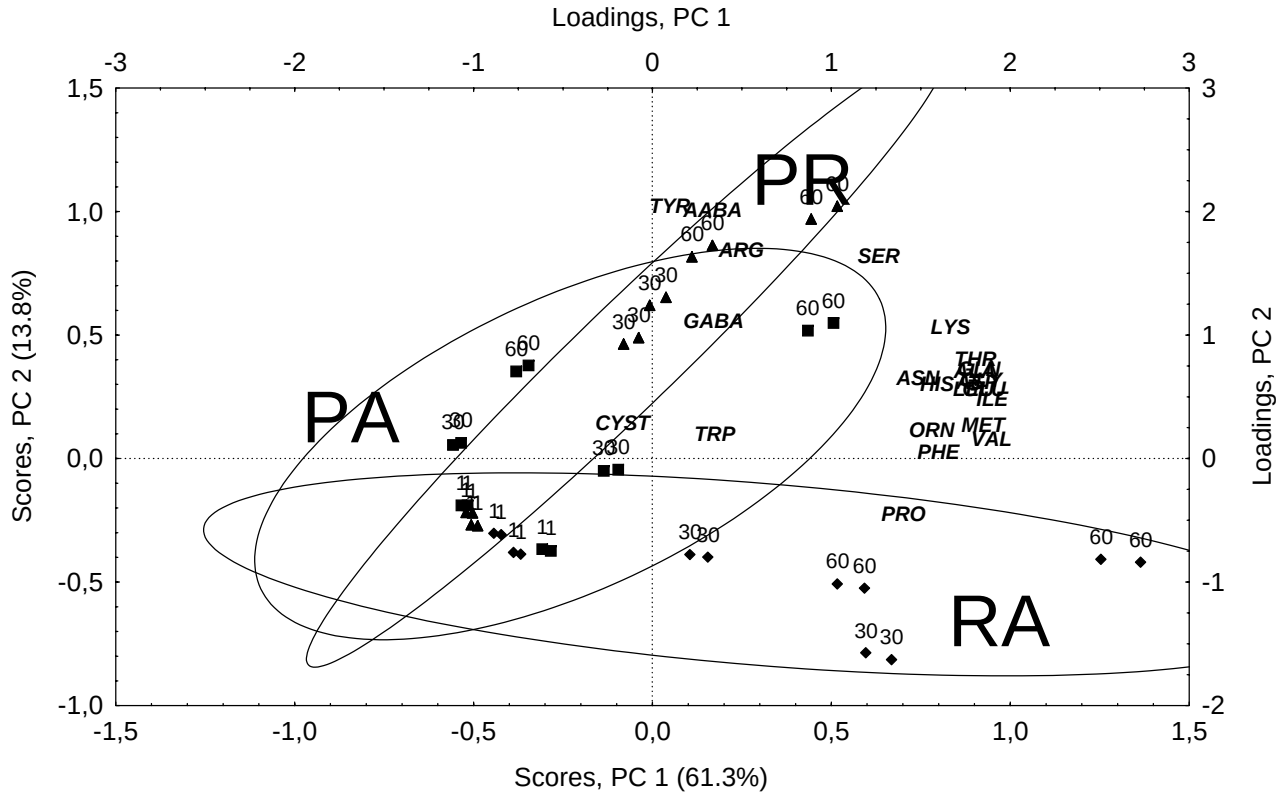

Figure 5: Score plot and loading vectors obtained by principal component analysis cheeses made from raw (É) pasteurized (<) and pressure-treated (ź) goats' milk at 1, 30 and 60 d of ripening. Results of each treatment are surrounded by an ellipse, which includes the members of this group ($P < 0.05$).

4. RESULTADOS Y DISCUSIÓN

Las experiencias llevadas a cabo durante la realización del presente trabajo fueron realizadas con dos modelos de quesos:

- una primera experiencia, en la que se realizaron cuatro producciones de quesos de pequeño formato, aproximadamente 250 g
- y una segunda experiencia en la que se efectuaron dos producciones de quesos de mayor formato (aproximadamente 1,3 kg), y de similar extracto seco inicial.

En la primera experiencia, el lote de leche se dividió en tres partes de 18 kg cada una, con el primer tercio se elaboraron los quesos de leche CR, mientras que con las otras dos partes se realizaron las producciones de leche PA y PR.

Para realizar los tratamientos de alta presión de la leche en la primer experiencia se utilizó un prototipo de máquina de alta presión discontinua (ACB, Nantes, Francia) de dos litros de capacidad y con una presión máxima de trabajo de 500 MPa. El tratamiento de la leche se efectuó en botellas de 1 litro de polietileno flexible de baja densidad con tapón de rosca de polipropileno (AZLON, Bibby Sterilin Ltd., Stone, UK). El tiempo de cada tratamiento fue de 15 min, que sumado a los tiempos de carga y descarga, llevó a un tiempo total de más de 6 h para obtener los 18 kg de leche PR necesarios para elaborar los quesos. La leche PR se mantuvo en refrigeración a 4ºC hasta el momento de fabricación del queso.

En estas miniproducciones de queso se estudiaron las características microbiológicas y físico-químicas de las leches y los quesos, la aptitud a la coagulación enzimática de las leches y la proteólisis primaria y secundaria de los diferentes quesos durante el madurado.

Las diferencias encontradas entre los quesos de leche CR, PA o PR fueron parcialmente atribuidas, como veremos a continuación, a los diferentes valores de humedad de estos quesos y/o a las modificaciones producidas en las leches tras los tratamientos tecnológicos. Así, se planteó la realización de una segunda experiencia en la que los quesos tuvieran el mismo extracto seco inicial para minimizar el efecto de la variabilidad en humedad. Aprovechando la cesión por ACB a Danone S.A. de una máquina prototipo semicontinua de 4 L de capacidad, planteamos la segunda experiencia realizando una serie de producciones de queso de mayor formato (~1,3 kg/pieza de queso) para poder realizar determinaciones que requiriesen gran cantidad de muestra, como el análisis organoléptico, ensayos de textura, etc.

Todos los quesos de mayor formato, al igual que en las producciones de la primera experiencia, fueron elaborados con un mismo lote de leche y siguiendo los mismos criterios de división del lote, utilizándose 50 kg de leche para la elaboración de cada tipo de queso.

El proceso de presurización de la leche se llevó a cabo por cargas de ~4 L. Tras las sucesivas descargas, la leche fue refrigerada hasta el momento de la elaboración del queso. El tiempo total de tratamiento (incluidos los de carga y descarga) para obtener los 50 kg de leche PR, necesarios para la elaboración de los quesos, fue de ~4 h.

Con estas producciones de queso se pudo estudiar, además de los análisis microbiológicos y de composición de las leches y quesos, las curvas de desecación, grado de interacción del agua en el queso, proteólisis primaria y secundaria, lipólisis, propiedades mecánicas, microestructura, concentración de ácidos orgánicos y análisis sensorial.

Esta sección de la tesis está dividida en dos partes; en la primera de ellas se discuten los resultados más relevantes obtenidos en la primera experiencia (quesos de pequeño formato) y en la segunda parte se discuten los resultados de la segunda experiencia (quesos de mayor tamaño y de similar humedad inicial). Los métodos utilizados en las determinaciones analíticas se detallan en el Anexo I.

4.1. PRIMERA EXPERIENCIA (PRODUCCIONES DE QUESOS DE PEQUEÑO FORMATO)

4.1.1. Composición de las leches y sueros

La composición de las leches utilizadas para la elaboración de las cuatro producciones de quesos de pequeño formato se muestra en la Tabla 6.

Los tratamientos de pasteurización (72ºC, 15 s) y de alta presión (500 MPa, 15 min, 20ºC) no provocaron cambios en los valores de pH, extracto seco y grasa de las leches. Tampoco se vieron afectados los valores de nitrógeno total (NT) o de nitrógeno soluble en ácido tricloroacético al 12%, pero se pudo observar un descenso significativo en la cantidad de nitrógeno no caseínico (NNC) en las leches PR respecto a las leches CR y PA.

TABLA 6. COMPOSICIÓN DE LAS LECHES DE CABRA[1]

	ES (%)		MG (%)		pH		NT (%)		NNC (%)		NSTCA (%)	
Cruda	13,85	*0,51*	4,84	*0,54*	6,64	*0,10*	0,56	*0,02*	$0,14^{A}$	*0,01*	0,03	*0,01*
Pasteurizada	13,50	*0,47*	4,69	*0,40*	6,63	*0,09*	0,55	*0,01*	$0,13^{A}$	*0,02*	0,03	*0,01*
Presurizada	14,07	*0,57*	4,86	*0,48*	6,69	*0,11*	0,56	*0,02*	$0,10^{B}$	*0,01*	0,03	*0,02*

ES: extracto seco; **MG**: materia grasa; **NT**: nitrógeno total; **NNC**: nitrógeno no caseínico; **NSTCA**: nitrógeno soluble en ácido tricloroacético al 12%

[1] *Promedio de cuatro experimentos independientes analizados por duplicado (n=8).*
[A,B] *Diferentes superíndices indican diferencia significativa (P<0,05).*

El análisis de las fracciones nitrogenadas de las leches mostró que los tratamientos de pasteurización y de alta presión produjeron una disminución del contenido de NNC (8,9 y 27,6%, respectivamente) de la leche. Es conocido que los tratamientos de alta presión causan una disminución en la solubilidad de las proteínas del suero a pH 4,6, especialmente de la η-LG (Felipe *et al.*, 1997). El nivel de desnaturalización de la η-LG inducido por presiones de 400-500 MPa únicamente es alcanzado tras la aplicación de tratamientos térmicos severos como por ejemplo 80ºC durante 10 min (Felipe *et al*., 1997). Así, la pérdida de solubilidad de parte de las proteínas del suero, que precipitarían a pH 4,6 junto con la fracción caseínica, explica el descenso observado en el NNC de las leches PR.

La composición de los sueros de quesería se muestra en la Tabla 7. Aquellos obtenidos durante la fabricación de queso a partir de leche CR mostraron un incremento significativo (P<0,05) en los valores de extracto seco y de materia grasa en comparación a los de leche PA y PR. Este hecho podría ser explicado en base a la ausencia de complejos ρ4CN4η4LG en la

leche CR, formando un gel con una red proteica que permitiría una mayor pérdida de grasa.

TABLA 7: COMPOSICIÓN DE LOS SUEROS DE QUESERÍA[1]								
	ES (%)		MG (%)		pH		NT (%)	
Crudo	7,70^{A}	*0,13*	1,08^{A}	*0,21*	5,84	*0,84*	0,16^{A}	*0,01*
Pasteurizado	7,22^{B}	*0,19*	0,75^{B}	*0,15*	5,97	*0,60*	0,15^{B}	*0,01*
Presurizado	7,26^{B}	*0,40*	0,79^{B}	*0,20*	6,16	*0,22*	0,14^{B}	*0,01*
ES: extracto seco; **MG**: materia grasa; **NT**: nitrógeno total								

[1] Promedio de cuatro experimentos independientes analizados por duplicado (n=8).
[A,B] Diferentes superíndices indican diferencia significativa (P<0,05).

Asimismo, como consecuencia de la mayor retención de proteína en las leches PA y PR, se observó una disminución del NT en los sueros de quesería obtenidos a partir de estas leches con respecto al suero de la leche CR (10,5 y 15%, respectivamente).

4.1.2. Coagulación enzimática

Las propiedades de coagulación de las leches CR, PA y PR fueron evaluadas directamente en la cuba de quesería durante la elaboración del queso mediante la determinación del tiempo de floculación, velocidad de agregación micelar y firmeza del gel, tal como se describe en el trabajo presentado en la sección 3.1 (Buffa, Trujillo y Guamis, 2001c).

Los tratamientos tecnológicos de pasteurización y alta presión aumentaron el tiempo de floculación de las leches PR y PA (PR>PA) en relación con la leche CR. El proceso de formación del gel enzimático ocurre en dos etapas; la primera de naturaleza enzimática, en la que las enzimas coagulantes actúan sobre la ρ4caseína hidrolizándola, y etapa no enzimática que comprende la agregación de las micelas modificadas. Por efecto del tratamiento térmico y/o el de alta presión, las proteínas del suero desnaturalizadas formarían complejos con la ρ4CN, o bien se depositarían sobre la superficie de las micelas, retardando por impedimentos estéricos la acción de los enzimas coagulantes, y como consecuencia aumentando el tiempo de floculación.

Se pudo observar también que la velocidad de agregación y firmeza del gel fueron mayores en la leche PR. Cuando la leche es sometida a presión, las micelas de caseína se disgregan, dando lugar a una gran cantidad de pequeñas submicelas (Shibauchi *et al.*, 1992;

Needs *et al.*, 2000). Tanto la disminución en el tamaño de las partículas como el aumento del número de éstas ocasionadas por la fragmentación micelar, explicarían la mayor velocidad de agregación del gel, así como su mayor firmeza final.

4.1.3. Rendimiento quesero y composición

La Figura 3 muestra los rendimientos queseros obtenidos en las diferentes producciones. El queso elaborado a partir de leche PR presentó un rendimiento de aproximadamente 5 y 12% superior a los obtenidos con leche PA y CR, respectivamente. El mayor rendimiento quesero observado en los quesos elaborados con leche PR coincide con los resultados obtenidos por Drake *et al.* (1997) para queso Cheddar elaborado a partir de leche CR, PA o PR, y con los de Trujillo *et al.* (1999b) en queso de cabra elaborado con leches PA o PR.

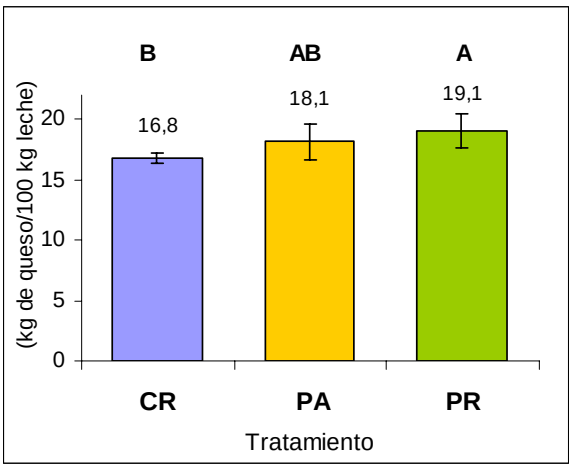

Figura 3: *Rendimientos queseros (n = 4) obtenidos en las fabricaciones de queso de leche cruda (CR), pasteurizada (PA) y tratada por altas presiones (PR).*
^{A,B} Diferentes superíndices indican diferencias significativas (P<0,05).

Las diferencias de rendimiento quesero encontradas se deberían fundamentalmente a la incorporación de las proteínas del suero, especialmente η4LG, ocasionando una mayor retención de agua y así aumentando el rendimiento.

En la Tabla 8 se muestran los valores de composición de los quesos (día 1) elaborados a partir de leche CR, PA y PR. No se observaron diferencias significativas en los valores de materia grasa, pH, nitrógeno total ni en el contenido en sal de los diferentes quesos.

Sin embargo, el queso elaborado con leche PR presentó un porcentaje de humedad mayor en comparación a los quesos fabricados a partir de leche CR y PA. Los valores de humedad de los quesos se pueden relacionar con la cantidad de proteína del suero retenida en

el queso recién fabricado, que incrementaría la superficie efectiva de proteína susceptible de interaccionar con el agua. Recordemos que la pasteurización aplicada puede desnaturalizar hasta un 7% de la proteína del suero (Lawrence, 1991a), mientras que un TAP superior a 400 MPa a 25ºC puede producir la agregación de más del 50% de la proteína del suero (Felipe *et al.*, 1997).

TABLA 8. COMPOSICIÓN DE LOS QUESOS (DIA 1) ELABORADOS A PARTIR DE LECHE CRUDA, PASTEURIZADA Y TRATADA POR ALTAS PRESIONES[1]

	ES (%)		MG (%)		pH		NT (%)		S/H (%)	
CR	46,04^{A}	*2,75*	24,69	*2,05*	5,09	*0,09*	2,47	*0,23*	1,69	*0,41*
PA	45,14^{B}	*2,24*	24,31	*2,45*	5,05	*0,09*	2,63	*0,13*	1,61	*0,30*
PR	43,56^{C}	*2,72*	24,19	*2,59*	5,09	*0,06*	2,57	*0,25*	1,60	*0,34*

ES: extracto seco; **MG**: materia grasa; **NT**: nitrógeno total; **S/H**: sal en humedad.

[1] *Promedio de cuatro experimentos independientes analizados por duplicado (n=8).*
[A,B,C] *Diferentes superíndices indican diferencia significativa (P<0,05).*

En la Tabla 9 se puede observar los valores de composición de los tres tipos de quesos a los 15, 30 y 45 días de madurado. Globalmente, no se observaron diferencias en sal, materia grasa y nitrógeno total de los quesos.

TABLA 9. COMPOSICIÓN DE LOS QUESOS ELABORADOS A PARTIR DE LECHE CRUDA, PASTEURIZADA Y TRATADA POR ALTAS PRESIONES[1]

	ES (%)		MG (%)		pH		NT (%)		S/H (%)	
Día 15										
CR	55,53^{A}	*2,40*	31,13	*2,34*	4,78^{A}	*0,08*	3,13	*0,15*	2,71	*0,62*
PA	54,79^{A}	*2,67*	30,50	*1,95*	4,70^{B}	*0,07*	3,09	*0,13*	2,62	*0,59*
PR	53,02^{B}	*2,93*	29,44	*1,64*	4,66^{C}	*0,08*	3,05	*0,18*	2,55	*0,41*
Día 30										
CR	60,66^{A}	*2,69*	33,50^{A}	*3,62*	4,86^{B}	*0,06*	3,45^{B}	*0,10*	3,32	*0,61*
PA	60,26^{A}	*1,69*	33,06^{A}	*2,48*	4,94^{A}	*0,09*	3,58^{A}	*0,08*	3,22	*0,35*
PR	59,15^{B}	*0,41*	32,63^{B}	*1,89*	4,93^{A}	*0,13*	3,71^{A}	*0,10*	3,03	*0,08*
Día 45										
CR	61,86^{A}	*2,22*	33,81	*3,86*	4,90^{B}	*0,07*	3,88	*0,39*	3,35	*0,58*
PA	61,63^{A}	*1,67*	33,50	*3,46*	5,03^{A}	*0,12*	3,79	*0,13*	3,33	*0,36*
PR	60,33^{B}	*0,46*	32,50	*1,87*	4,98^{A}	*0,07*	3,71	*0,09*	3,14	*0,11*

ES: extracto seco; **MG**: materia grasa; **NT**: nitrógeno total; **S/H**: sal en humedad

[1] *Promedio de cuatro experimentos independientes analizados por duplicado (n=8).*
[A,B,C] *Diferentes superíndices indican diferencia significativa (P<0,05).*

En general, el pH descendió durante los primeros días de maduración debido a la acción del fermento sobre la lactosa residual transformándola en ácido láctico. A los 45 días de madurado los valores de pH fueron similares a los iniciales, debido a que los fenómenos proteolíticos originan compuestos nitrogenados que son los responsables del incremento de pH. Aunque no hubo diferencias en los valores de pH al inicio de la maduración, al día 30 y 45 los quesos de leche PR y PA presentaron un pH más elevado que los quesos de leche CR, lo que podríamos relacionar con el mayor nivel de proteolisis alcanzado por estos quesos respecto al de leche CR (ver sección 4.1.5).

Los valores de extracto seco en los tres tipos de queso aumentaron a lo largo del tiempo debido a la pérdida de agua por evaporación. Sin embargo, el queso elaborado a partir de leche PR presentó, en todos los períodos de maduración, un extracto seco significativamente menor que el de los quesos PA y CR.

4.1.4. Microbiología

La calidad microbiológica de las leches CR, PA y PR, así como la microbiota presente en los quesos elaborados a partir de las mismas y sus cambios a través de la maduración se describen en el artículo de la sección 3.2 (Buffa, Guamis, Royo y Trujillo, 2001b).

La leche CR mostró unos recuentos de microorganismos aerobios mesófilos totales de aproximadamente 8×10^5 ufc ml^{-1}, los cuales se redujeron alrededor de dos ciclos logarítmicos en las leches PA y PR. Tras los tratamientos de pasteurización o alta presión, la letalidad observada para los diferentes grupos microbianos estudiados (bacterias psicrótrofas, *Micrococcaceae* y enterococos) fue superior a las 3 unidades logarítmicas, llegándose a no detectar la presencia de lactobacilos y *Enterobacteriaceae* en las leches PA y PR. Estos valores evidencian que el TAP aplicado a la leche fue capaz de reducir la microbiota total de la leche de manera similar a la pasteurización.

Por lo que respecta a los quesos elaborados a partir de las leches CR, PA o PR, los recuentos de microorganismos aerobios totales en los tres tipos de quesos fueron del orden de $3{,}2 \times 10^9$ ufc g^{-1}, recuentos que concuerdan con los obtenidos en diferentes variedades de queso de cabra (Alonso-Calleja *et al.*, 2002).

Lactobacilos y lactococos fueron los microorganismos predominantes durante la maduración de todos los quesos. No se detectaron diferencias significativas en los recuentos de las bacterias del fermento (lactococos) de los quesos que, en concordancia con otros

estudios (McSweeney *et al.*, 1993; Beuvier *et al.*, 1997), disminuyeron 1-2 unidades logarítmicas durante la maduración.

El número de lactobacilos fue similar entre los quesos de leche PA y PR, pero menor (~3 unidades logarítmicas) a los encontrados en los quesos elaborados a partir de leche CR. En todos los casos, el número de lactobacilos se incrementó durante la maduración hasta llegar a valores de aproximadamente 10^8 ufc g^{-1} en los quesos elaborados con leche CR y de 10^6 ufc g^{-1} en los elaborados a partir de leche PA o PR.

Aunque no se detectó la presencia de lactobacilos y *Enterobacteriaceae* en las leches PA y PR, estos microorganismos aparecieron en los correspondientes quesos. Este hecho se debe a la contaminación producida durante la fabricación del queso. Los recuentos de *Enterobacteriaceae* fueron mayores en los quesos de leche CR, aproximadamente 2×10^5 ufc g^{-1} y de 2-4 $\times 10^2$ ufc g^{-1} en los quesos elaborados con leche PA o PR. En todos los casos los recuentos disminuyeron hasta niveles no detectables al final de la maduración (45 días) en los quesos PA y CR, y en el día 30 en los quesos de leche PR.

Se ha sugerido que *Micrococcaceae* y enterococos pueden participar activamente en el madurado del queso, ya que poseen actividades proteolítica y lipolítica (Bhowmik, Riesterer, Boekel, Marth y Van, 1990; Menéndez, Centeno, Godínez y Rodríguez-Otero, 1998). Los recuentos de enterococos, al igual que los de *Micrococcaceae,* fueron mayores en los quesos de leche CR al inicio de la maduración, pero al final no se observaron diferencias significativa entre los quesos.

Globalmente, las diferencias entre los quesos al inicio de la maduración fueron los mayores recuentos de *Enterobacteriaceae, Micrococcaceae,* enterococos y lactobacilos en los quesos de leche CR. Sin embargo, estas diferencias desaparecieron a lo largo de la maduración, excepto los recuentos de lactobacilos que fueron ~3 unidades logarítmicas mayores en los quesos de leche CR durante los 45 días de madurado.

Por otra parte, los recuentos obtenidos en los grupos microbianos estudiados en los quesos elaborados a partir de leche PR fueron muy similares a los obtenidos en aquellos de leche PA. De acuerdo con estos resultados, es difícil que las diferencias observadas en la proteólisis de estos quesos en experiencias anteriores (Trujillo *et al.* 1999b) y en el presente trabajo (ver sección 4.1.5), puedan atribuirse exclusivamente a la microbiota residual de la leche tras los tratamientos de pasteurización o alta presión, ya que fueron muy similares. Sin embargo, se ha visto que otros factores además del número de BNF contribuyen al madurado del queso, como por ejemplo la especie y/o la cepa (Rehman *et al.*, 1999), por lo que sería interesante profundizar los estudios microbiológicos en esa dirección.

4.1.5. Proteólisis

En esta primera experiencia se evaluó el grado de proteólisis primaria (mediante electroforesis capilar de la fracción insoluble a pH 4,6) y secundaria (fraccionamiento de nitrógeno y perfil peptídico de la fracción soluble en agua por HPLC) como se describe en el artículo de la sección 3.3 (Trujillo, Buffa, Casals, Fernández y Guamis, 2002).

Proteólisis primaria

Globalmente, el perfil electroforético de la fracción insoluble a pH 4,6 fue similar en todos los quesos. La mayor diferencia cualitativa fue la aparición de un pico que correspondió a la η-LG incorporada en diferentes proporciones en la fracción insoluble a pH 4,6 debido a los tratamientos de pasteurización o de alta presión. Sin embargo, la η-LG se mostró resistente a la hidrólisis.

A lo largo de la maduración las áreas de los picos correspondientes a caseínas intactas ζ_{s1}-, ζ_{s2}- y η-CN disminuyeron, mientras que las de sus principales productos de degradación (ν-CN y ζ_{s1}-I) aumentaron. La ζ_{s1}-CN mostró una mayor degradación que la η-CN; tras 45 días de madurado los contenidos medios de ζ_{s1}- y η-CN disminuyeron un 76,6% y un 27,4%, respectivamente. La concentración de para-ρ-CN disminuyó durante la maduración en todos los quesos, pero lo hizo más rápidamente en los quesos elaborados con leche CR y PR.

A los 15 días de madurado, los quesos elaborados con leche PA presentaron menores valores de ζ_{s1}-CN intacta que los quesos fabricados con leche CR, mientras que los elaborados con leche PR mostraron valores intermedios. Sin embargo, al final de la maduración no se encontraron diferencias en los niveles de degradación de la ζ_{s1}-CN entre los quesos. También se pudo observar que al final de la maduración los quesos elaborados con leche PA presentaron mayor hidrólisis de las ζ_{s2}- y η-CN que los quesos de leche CR o PR.

La mayor hidrólisis de la ζ_{s1}-CN de los quesos de leche PA pudo deberse a una mayor retención de enzimas coagulantes en el queso (Gaya *et al.*, 1990; Centeno *et al.*, 1994; Gómez *et al.*, 1999). Por otro lado, la mayor degradación de las ζ_{s2}- y η-CN podría explicarse en base a la inactivación de los inhibidores de la plasmina y/o una activación del plasminógeno en plasmina tras la pasteurización (Richardson, 1983).

Los quesos elaborados con leche PR mostraron valores intermedios de degradación de la ζ_{s1}-CN (PA>PR>CR). Además, los quesos elaborados con leche PR y PA presentaron niveles similares de η-CN a los 15 días de madurado, pero menores que los encontrados en los de leche CR.

Son varios los factores que podrían controlar la proteólisis en los quesos fabricados con leche PR. Es importante resaltar que estos quesos tienen mayor humedad, y es conocido que la humedad del queso afecta de manera muy importante al madurado del queso (Lawrence *et al.*, 1987), acelerando este proceso. Por otra parte se ha descrito que los TAP (Ω500 MPa) no ocasionan cambios en la actividad de la plasmina (Trujillo, Ferragut, Gervilla, Capellas y Guamis, 1997; Scollard, Beresford, Murphy y Kelly, 2000a). Sin embargo, aunque la actividad de la plasmina no se ve afectada por estos tratamientos de alta presión, algunos autores han observado un incremento en los índices de proteólisis (nitrógeno soluble a pH 4,6, degradación de las η-, ζ_{s2}- y ζ_{s1}-CN) en la leche tras el TAP durante su conservación en refrigeración (García-Risco *et al.*, 1998; Scollard *et al.*, 2000b). Estos autores explican este incremento en la proteolisis de las leches PR a través de los cambios que provocan las altas presiones en las proteínas. Los TAP en la leche causan una disgregación de las caseínas en pequeñas partículas y una gran desnaturalización de las proteínas del suero, especialmente η4LG (Felipe *et al*., 1997; Law *et al*., 1998). Cuando la presión es liberada, las micelas de caseína de reagrupan, alterando su morfología y tamaño. Durante el proceso de reagrupación micelar, la η4LG desnaturalizada puede formar enlaces disulfuro con la ρ-CN o con la ζ_{s2}-CN o formar grandes agregados con otras moléculas de η4LG, las cuales pueden ser incluidas dentro de las micelas de caseína (Needs *et al.*, 2000), originando micelas de composición y/o estructura modificadas. Así, los cambios en las micelas de caseína provocados por la presión, que alterarían la matriz proteica del queso, podrían facilitar la acción de las enzimas proteolíticas, hecho que explicaría los niveles de degradación de la η4CN encontrados en los quesos de leche PR. Por otra parte, existe un mecanismo opuesto que puede controlar la proteólisis basado en el hecho que la η-LG desnaturalizada inhibe la acción de la plasmina y de la quimosina (Lo y Bastian, 1997).

Proteólisis secundaria

Los valores de nitrógeno soluble en agua (NS) y de nitrógeno soluble en ácido tricloroacético al 12% (NSTCA) aumentaron durante la maduración en todos los quesos. Mientras que al inicio de la maduración no se encontraron diferencias significativas entre los valores de NS o NSTCA de los quesos, al cabo de 45 días aquellos elaborados a partir de leche PA y PR mostraron una concentración mayor, 15,4 y 10,2% respectivamente, que los de leche CR.

Los quesos fabricados con leches PA o CR presentaron concentraciones similares de aminoácidos libres totales, mientras que los quesos de leche PR exhibieron mayores ($P<0,05$)

valores durante la maduración (~15%). Estos resultados confirman las observaciones previas realizadas por Trujillo *et al.* (1999a,b) en queso de cabra fabricado con leche PA o PR.

En general, el perfil peptídico de la fracción soluble en agua, analizado por HPLC, de los quesos fue muy similar. La mayor diferencia cualitativa fue la aparición de un pico con tiempo de retención ~78 min, que correspondió a la η-LG nativa no desnaturalizada y presente en la fracción soluble en agua en los quesos elaborados a partir de leche CR, y en menor grado en la leche PA. En este estudio se pudo observar que, globalmente, los tratamientos alta presión aplicados a la leche no afectaron los niveles de péptidos hidrofílicos o hidrofóbicos de los quesos con relación a sus homólogos de leche CR. Sin embargo, los quesos de leche PA mostraron menor concentración ($P<0.05$) de péptidos hidrofílicos que los elaborados a partir de leche CR o PR al final de la maduración.

Según los datos bibliográficos disponibles, en general, los quesos elaborados con leche PA contienen mayor concentración de péptidos hidrofóbicos que los elaborados con leche CR, los cuales muestran mayores concentraciones de péptidos hidrofílicos (Lau, Barbano y Rasmussen, 1991; McSweeney *et al.*, 1993; Beuvier *et al.*, 1997). En lo que respecta a los quesos elaborados con leche PR, Trujillo *et al.* (1999a) observaron una mayor cantidad de péptidos, en la zona cromatográfica donde eluyen los péptidos hidrofóbicos, para los quesos de leche PA en comparación a los de leche PR. Sin embargo estos autores no realizaron un análisis cuantitativo.

En el presente trabajo, las concentraciones de péptidos hidrofóbicos de los quesos fabricados con leche PA fueron mayores que la de los quesos de leche CR y PR en todos los períodos de maduración evaluados, pero sólo se trató de una tendencia pues estas diferencias no resultaron significativas ($P>0,05$).

4.2. SEGUNDA EXPERIENCIA (PRODUCCIONES DE MAYOR FORMATO)

4.2.1. Composición de las leches y sueros

La composición de las leches utilizadas para la elaboración de las dos producciones de quesos de mayor formato y de los sueros de quesería se muestra en las Tablas 10 y 11, respectivamente.

Los tratamientos de pasteurización y de altas presiones no provocaron cambios en los valores de extracto seco, nitrógeno total, materia grasa y pH de las leches.

TABLA 10. COMPOSICIÓN DE LAS LECHES DE CABRA[1]

	ES (%)		MG (%)		pH		NT (%)	
Cruda	13,44	*0,18*	3,85	*0,75*	6,48	*0,01*	0,53	*0,02*
Pasteurizada	13,21	*0,29*	3,33	*0,67*	6,49	*0,01*	0,53	*0,03*
Presurizada	13,41	*0,15*	3,60	*0,46*	6,51	*0,01*	0,54	*0,01*

ES: extracto seco; **MG**: materia grasa; **NT**: nitrógeno total

[1] *Promedio de dos experimentos independientes analizados por duplicado (n=4).*

La composición de los sueros de quesería fue afectada por los tratamientos de la leche (Tabla 11). Los sueros provenientes de la leche PR tuvieron un porcentaje significativamente menor de nitrógeno total y materia grasa, y así menor extracto seco. Como se ha comentado anteriormente, la menor cantidad de nitrógeno en el suero de le leche PR se debe a la pérdida de solubilidad de las proteínas del suero, principalmente η-LG, que quedan retenidas en el queso.

TABLA 11: COMPOSICIÓN DE LOS SUEROS DE QUESERÍA[1]

	ES (%)		MG (%)		pH		NT (%)	
Crudo	$7,29^{A}$	*0,11*	$0,77^{A}$	*0,05*	$6,39^{A}$	*0,02*	$0,16^{A}$	*0,01*
Pasteurizado	$7,27^{A}$	*0,07*	$0,66^{A}$	*0,16*	$6,33^{B}$	*0,02*	$0,16^{A}$	*0,01*
Presurizado	$6,56^{B}$	*0,13*	$0,38^{B}$	*0,05*	$6,41^{A}$	*0,01*	$0,12^{B}$	*0,02*

ES: extracto seco; **MG**: materia grasa; **NT**: nitrógeno total

[1] *Promedio de dos experimentos independientes analizados por duplicado (n=4).*
[A,B] *Diferentes superíndices indican diferencia significativa (P<0.05).*

Las menores pérdidas de materia grasa pueden deberse a las diferencias en microestructura de los geles resultantes en la elaboración del queso. Después del tratamiento por alta presión de la leche, las proteínas y los glóbulos de grasa podrían formar una estructura más cerrada y compactada que en el caso de las leches PA o CR, lo que ocasionaría menores pérdidas de grasa en el suero.

4.2.2. Rendimiento quesero y composición

El rendimiento quesero obtenido en la elaboración de quesos de cabra con leche CR, PA y PR fue del 15,3, 15,9 y 15,7%, respectivamente, no observándose diferencias entre ellos. Las diferencias de rendimiento encontradas en la primera experiencia se debieron fundamentalmente a las diferencias de humedad de los quesos. Esto fue corroborado en la segunda experiencia, en los cuales al estandarizarse el extracto seco de los quesos no se observaron diferencias en los rendimientos queseros.

La composición de los quesos, y su evolución a través de la maduración, se muestra en la Tabla 12. No se observaron diferencias entre los valores de ES de los diferentes quesos en el primer día de madurado. Al inicio de la maduración tampoco hubo diferencias en los valores de nitrógeno total y materia grasa de los quesos, que representaron aproximadamente el 5,7 y 54,4% de los sólidos totales, respectivamente.

Los valores de ES de todos los quesos aumentaron a lo largo de la maduración (ℑ23,5%) debido a la evaporación del agua. Sin embargo, después de dos meses de madurado, los quesos elaborados a partir de leche PA mostraron el mayor valor de ES, sugiriendo que estos quesos tienen una mayor velocidad de evaporación de agua en relación con los quesos de leche CR o PR, tal como fue observado en las curvas de evaporación obtenidas por pesada de los quesos (ver sección 4.2.5).

La concentración de sal fue mayor en los quesos elaborados a partir de leche PA en comparación a los fabricados con leche CR o PR. Las observaciones realizadas en estos quesos a través del análisis de microestructura por microscopia láser confocal (ver sección 4.2.4) mostraron que la matriz de los quesos fabricados con leches PA tenía una estructura menos continua, presentando numerosos poros irregulares y una red más abierta. Contrariamente, los quesos de leche CR o PR exhibieron una matriz proteica más regular y cerrada. Cuando se aplican unas condiciones de salado estandarizadas (tiempo de salado, temperatura, concentración de sal, geometría del queso, etc.), la cantidad de sal absorbida depende principalmente de las propiedades intrínsecas del queso. Así, la presencia de poros

estrechos y de tamaños relativamente pequeños en la matriz del queso, presentará una oposición a la penetración de la salmuera disminuyendo la velocidad de difusión del NaCl (Guinee y Fox, 1993). De esta forma, la estructura más abierta presentada por los quesos de leche PA pudo facilitar la difusión de sal dentro de los quesos, lo que explicaría la mayor concentración de sal encontrada en éstos.

TABLA 12. COMPOSICIÓN DE LOS QUESOS ELABORADOS A PARTIR DE LECHE CRUDA, PASTEURIZADA Y TRATADA POR ALTAS PRESIONES[1]

	ES (%)		MG (%)		pH		NT (%)		S/H (%)	
Día 1										
CR	47,42	*0,28*	26,00	*0,75*	5,05^{A}	*0,03*	2,70	*0,05*	1,76^{B}	*0,52*
PA	47,76	*1,53*	25,50	*0,58*	4,99^{B}	*0,02*	2,69	*0,05*	2,25^{A}	*0,53*
PR	47,25	*0,31*	25,50	*0,26*	5,01^{B}	*0,06*	2,75	*0,12*	1,77^{B}	*0,41*
Día 30										
CR	63.08	*0.98*	35.38	*0.75*	4,80	*0,03*	3.53	*0.18*	5,00	*0,56*
PA	63.74	*0.92*	35.66	*0.71*	4,80	*0,01*	3.51	*0.19*	5,30	*0,68*
PR	62.69	*0.72*	35.15	*0.65*	4,79	*0,05*	3.67	*0.10*	5,00	*0,50*
Día 60										
CR	70.69^{B}	*0.10*	39.55^{B}	*0.64*	4,89^{A}	*0,02*	3.85	*0.17*	6,92^{B}	*0,41*
PA	71.97^{A}	*0.28*	40.90^{A}	*0.12*	4,84^{B}	*0,04*	3.96	*0.23*	7,51^{A}	*0,40*
PR	70.46^{B}	*0.46*	37.88^{C}	*0.75*	4,76^{C}	*0,02*	4.09	*0.08*	7,03^{B}	*0,54*

ES: extracto seco; **MG**: materia grasa; **NT**: nitrógeno total; **S/H**: sal en humedad

[1] *Promedio de 2 experimentos independientes analizados por duplicado (n=4).*
[A,B,C] *Diferentes superíndices indican diferencia significativa (P<0,05).*

4.2.3. Lipólisis y análisis sensorial

La lipólisis se evaluó mediante la cuantificación de ácidos grasos libres por cromatografía de gases, como se detalla en el trabajo incluido en la sección 3.4 (Buffa, Guamis, Pavia y Trujillo, 2001a).

La concentración de ácidos grasos libres aumentó significativamente a lo largo de la maduración de los quesos de leche CR y PR (de ~5 mg g^{-1} grasa a unos 8 mg g^{-1} grasa), mientras que en los quesos elaborados con leche PA aumentó hasta 6 mg g^{-1} grasa.

El perfil de ácidos grasos fue similar en todos los tipos de queso analizados, siendo el palmítico, oleico, mirístico, cáprico y esteárico los ácidos grasos individuales presentes en mayor proporción (~80% del total). Sin embargo, los quesos elaborados con leche CR o PR

mostraron mayores concentraciones en la mayoría de los ácidos grasos individuales, en relación con las encontradas en los quesos de leche PA.

Es conocido que la lipoproteín lipasa de la leche es una enzima relativamente termolábil (Driessen, 1989), pero sin embargo se ha observado que esta enzima muestra cierta actividad tras un tratamiento de alta presión de 500 MPa durante 15 min a temperatura ambiente (Trujillo *et al.*, 1999b). De esta manera, la lipólisis registrada en los quesos elaborados a partir de leche PR podría deberse principalmente a la actividad de la lipoproteín lipasa resistente al TAP, y también a la acción de las lipasas del fermento y de otras bacterias no pertenecientes al fermento.

El análisis sensorial realizado (ver anexo II) mostró que los catadores no percibieron diferencias significativas entre los quesos. Sin embargo, los jueces calificaron a aquellos elaborados con leche PR y CR como más aromáticos y con un sabor más intenso que los correspondientes de leche PA. Además, los quesos elaborados con leche CR y PR mostraron los mayores valores en los atributos amargo y picante, aunque estas diferencias se trataron sólo de tendencias.

4.2.4. Textura, microestructura y color

La textura de los quesos fue evaluada mediante ensayos de compresión uniaxial y de relajación al estrés; la microestructura se observó mediante técnicas de microscopía láser confocal, y el color mediante el uso de un colorímetro HunterLab, tal como se describe en la sección 3.5 (Buffa, Trujillo, Pavia y Guamis, 2001d).

Existen dos mecanismos opuestos que controlan la firmeza del queso. El primero consiste en la acción de las diferentes enzimas proteolíticas sobre matriz proteica, principalmente sobre la ζ_{s1}-CN, que da como resultado una disminución de la firmeza. El segundo es el efecto de pérdida de humedad, que al provocar una disminución de la hidratación de las proteínas conduce a una mayor interacción de las mismas provocando el aumento de la firmeza de la matriz proteica.

En los quesos analizados el efecto predominante fue la pérdida de humedad frente al de proteólisis, causando un incremento global de la firmeza a lo largo de la maduración. Los quesos elaborados a partir de leche PA fueron los más fracturables. Este hecho se podría explicar a través de las pequeñas diferencias encontradas en los valores de nitrógeno soluble y humedad, o como comentaremos a continuación, a diferencias en la microestructura de los quesos.

El valor de la deformación de los quesos en el punto de fractura disminuyó (P<0,05) a lo largo de la maduración. Según Creamer y Olson (1982) la pérdida de elementos estructurales elásticos por efecto de la proteólisis y la disminución de la cantidad de agua disponible en la red proteica, podría causar la disminución de la deformabilidad del queso.

El queso elaborado con leche CR mostró la mayor deformabilidad al inicio de la maduración. En varios trabajos se destaca la importancia del pH en la textura del queso (Creamer y Olson, 1982; Lawrence *et al.*, 1987; Rohm, Lederer y Ginzinger, 1992). Creamer y Olson (1982) y Rohm *et al.* (1992) observaron que quesos con mayor pH presentaban una mayor deformabilidad. A mayor pH las moléculas de caseína adquieren carga negativa, lo que conduce a un incremento en el grado de solvatación de las mismas. De esta forma, la mayor deformabilidad observada en el queso de leche CR pudo deberse a su mayor pH.

El test de relajación al esfuerzo mostró que los quesos elaborados a partir de leche PR tuvieron un comportamiento más elástico (P<0,05), aunque todos los quesos fueron perdiendo esas características a lo largo de la maduración, como cabía esperar.

Mediante microscopía láser confocal se pudo observar que los quesos elaborados a partir de leche PR presentaron una matriz proteica regular y cerrada, con glóbulos grasos pequeños y uniformes en tamaño y forma, mientras que los quesos de leche PA mostraron una matriz más abierta y con numerosas cavidades irregulares. A lo largo de la maduración la estructura de la matriz proteica se fue compactando, suavizando las diferencias observadas entre los quesos. De esta manera, la matriz proteica del queso de leche PR, cerrada y uniforme, podría explicar el comportamiento más elástico observado en el test de relajación al esfuerzo.

Mediante el análisis instrumental del color se pudo apreciar una mayor luminosidad (P<0,05) en los quesos elaborados a partir de leche CR y PR, en relación a aquellos de leche PA. Esto puede ser atribuido a que los quesos de leche PR y CR presentan una estructura mucho más cerrada y uniforme, lo cual facilitaría la reflexión de la luz en su superficie. Sin embargo, el análisis sensorial del color por parte de un panel de catadores (Anexo II) no encontró diferencias entre los quesos. Los jueces tampoco percibieron diferencias significativas en los parámetros de dureza, fracturabilidad y elasticidad de los quesos de leche CR, PA y PR al día 60 de maduración, resultados que coinciden, en líneas generales, con aquellos obtenidos mediante análisis instrumental.

4.2.5. Grados de interacción del agua en el queso

Como se ha descrito en la introducción de este trabajo, los TAP causan numerosas modificaciones en la leche que afectan a la manufactura y madurado del queso. Varios autores han observado que los quesos elaborados con leche PR tienen mayor humedad, debido fundamentalmente a que en la cuajada se incorporan proteínas del suero que presentan gran capacidad de retención de agua (Drake *et al.*, 1997; Trujillo *et al.*, 1999b).

Con el objetivo de cuantificar los diferentes tipos de agua contenidos en el queso y su evolución durante la maduración, se estudió el proceso de desecación mediante pesada y los grados de interacción del agua del queso por termogravimetría, tal como se describe en el trabajo incluido en la sección 3.6.

En estas producciones de queso se observó que la concentración, grado de interacción y evolución del agua a lo largo de la maduración se vió afectada según el tipo de leche de partida utilizada en la elaboración del queso.

El agua retenida con menos energía a la matriz del queso (W_1) y la más fuertemente ligada (W_2) disminuyeron durante el madurado en todos los quesos, aunque, como era de esperar, W_1 lo hizo en mayor proporción, pasando de representar un 31-32% del peso del queso al inicio de la maduración a constituir un 15-17% al final.

Al principio de la maduración, W_1 representó el 60% del agua del sistema, siendo al final de la maduración aproximadamente la mitad. Los mayores descensos en W_1 fueron registrados en los quesos de leche PA en comparación a los elaborados con leche CR o PR, que mostraron un comportamiento similar. Este resultado puede explicarse por las diferencias estructurales de la matriz del queso comentadas anteriormente. El gradiente de concentración de agua existente entre el queso y la atmósfera de la cámara hizo que el queso perdiese humedad. Este movimiento de agua a través del queso se ve facilitado por una estructura abierta y porosa (Pierre, Michel, Le Graët y Berrier, 1999), tal como aquella observada en los quesos de leche PA. Sin embargo, al final de la maduración, etapa en la que el gradiente de humedad entre el queso y el ambiente es mínima, la pérdida de humedad se ralentiza considerablemente. Además, al final de la maduración la microestructura que presentan los quesos es más compacta, lo que dificulta el movimiento del agua hacia la superficie. Estos dos factores serían los responsables de la desaceleración del proceso de desecación del queso.

En cuanto a W_2, al inicio de la maduración no se observaron diferencias entre los diferentes quesos, pero a los 30 días los quesos elaborados a partir de leche PA mostraron los mayores porcentajes (20,2%) en relación con los de leche CR o PR (~17,6%). Estas diferencias no tuvieron significancia estadística al final de la maduración, encontrándose valores muy semejantes entre todos los quesos.

Según De Angelis-Curtis *et al.* (1999; 2000) la proteólisis, y en menor medida, la lipólisis, provocan una liberación de agua fuertemente ligada. En los quesos de leche PA estos dos procesos se ven retardados, en relación con los quesos de leche CR o PR, lo que explicaría sus elevados valores en W_2. Debido a que la transformación de W_2 en W_1 está retardada en los quesos de leche PA, una vez eliminada parte de W_1 ésta se restituye más lentamente que en los otros tipos de queso, dando como resultado un menor valor de W_1.

4.2.6. Ácidos orgánicos

La concentración de ácidos orgánicos presentes en los quesos elaborados a partir de leche CR, PA o PR fueron analizados mediante HPLC, como se detalla en el trabajo incluido en la sección 3.7 (Buffa, Guamis, Saldo y Trujillo, 2003a).

Los tratamientos de pasteurización o de alta presión no produjeron diferencias en la concentración de ácidos orgánicos totales de los quesos al inicio de la maduración (aproximadamente ~420 mg kg^{-1} ES). Estos valores fueron aumentando con el tiempo hasta alcanzar ~600 mg kg^{-1} ES en los quesos de leche CR y PR al final de la maduración, concentración significativamente mayor que la obtenida en los de leche PA (~540 mg kg^{-1} ES).

La concentración de ácidos orgánicos individuales varió según el tipo de queso. Así, los quesos elaborados a partir de leche PR mostraron la mayor concentración de lactato, mientras que los de leche PA presentaron elevadas concentraciones de málico y pirúvico y las más bajas de butírico. Los quesos fabricados con leche CR, que tuvieron el mayor recuento de lactobacilos, presentaron valores elevados de propiónico y acético y bajas concentraciones de pirúvico y cítrico.

La concentración de ácido láctico al final de la maduración fue mayor en los quesos de leche PR, mientras que en los de leche CR y PA fue similar. Los menores valores de ácido láctico podrían explicarse, en el caso de los quesos elaborados con leche CR, basándose en la gran heterogeneidad de la microbiota presente en este tipo de leche que metabolizaría la lactosa o el lactato para producir otros compuestos (propionato, acetato, etanol, CO_2, etc.), mientras que la mayor concentración de sal ralentizaría la degradación de la lactosa en los quesos de leche PA.

Algunos autores han observado en quesos de pasta cocida elaborados a partir de leche PA menores concentraciones de ácidos acético y propiónico (de 5 a 10 veces), en relación con aquellos elaborados con leche CR, resultado que fue atribuido a la drástica reducción en el número de propionibacterias debido al tratamiento de pasteurización (Bouton y Grappin, 1995;

Beuvier *et al.*, 1997). Así, los tratamientos de pasteurización y de alta presión, los cuales reducen de manera similar las BNF, podrían causar una disminución en las concentraciones de estos ácidos orgánicos.

Los menores valores de citrato encontrados en los quesos de leche CR pueden atribuirse a los elevados recuentos de BNF, ya que éstas pueden utilizar el citrato como substrato metabólico (Peterson y Marshall, 1990; Bouzas, Kantt, Bodyfelt y Torres, 1993; McSweeney y Sousa, 2000).

Finalmente, los bajos valores de ácido butírico encontrados en los quesos de leche PA pueden deberse a la inactivación de la LPL endógena de la leche por efecto del tratamiento térmico.

4.2.7. Proteólisis

En esta experiencia la proteolisis fue evaluada mediante el fraccionamiento de nitrógeno, la caracterización de la fracción insoluble a pH 4,6 por electroforesis capilar y la determinación del perfil de aminoácidos libres por cromatografía líquida, tal como se recoge en la sección 3.8 (Buffa, Guamis y Trujillo, 2003).

Proteolisis primaria

De manera análoga a lo observado en las producciones de quesos de pequeño formato, el perfil electroforético de la fracción insoluble a pH 4,6 fue similar en todos los quesos, siendo la mayor diferencia cualitativa la aparición de un pico que correspondió a la η-LG en los quesos de leche PR y PA. Esta proteína se mostró resistente a la hidrólisis durante el período de maduración estudiado.

Las áreas de los picos correspondientes a caseínas intactas (ζ_{s1}-, ζ_{s2}- y η-CN) disminuyeron en todos los quesos durante la maduración, mientras que las correspondientes a sus productos de degradación (principalmente ν-CN y ζ_{s1}-I) aumentaron. La ζ_{s1}-CN fue degradada en mayor proporción que la η-CN, sin embargo, la degradación de estas proteínas fue más importante en las producciones de quesos de pequeño formato. La ζ_{s1}-CN se degradó ~80% en los quesos de pequeño formato, mientras que en los de mayor formato lo hizo en un 50%. En lo que respecta a la degradación de la η-CN, ésta fue de ~30% y 13% en los quesos de pequeño y gran formato, respectivamente. Estos resultados pueden ser explicados

basándonos en los valores de humedad y sal que presentaron los quesos, ya que estos parámetros influencian en gran medida el madurado del queso (Lawrence *et al.*, 1987). En este contexto se ha de recordar que los quesos de pequeño formato conservaron aproximadamente un 10% más de humedad debido al hecho que fueron encerados al día 18 de maduración. De la misma manera, aunque inicialmente los quesos elaborados en las dos experiencias tuvieron similar concentración de sal, al final de la maduración los quesos de mayor formato presentaron mayores niveles de sal (más del doble) con respecto a los de pequeño formato, debido al fenómeno de desecación.

Los quesos elaborados con leche PR mostraron la mayor velocidad de degradación de la ζ_{s1}-CN (PR>PA=CR), hecho que concuerda con la presencia de una mayor cantidad de su principal producto de degradación ζ_{s1}-I. En las producciones de quesos de pequeño formato la degradación de la ζ_{s1}-CN fue más intensa en el queso elaborado con leche PA, mientras que el queso de leche CR fue el menos proteolizado, mostrando una degradación intermedia el producido a partir de leche PR.

En esta segunda experiencia, tanto la η-CN como la ζ_{s2}-CN fueron hidrolizadas en mayor porcentaje en los quesos de leche PR. El orden de degradación de la η-CN fue PR>PA=CR, mientras que en las producciones de pequeño formato este orden fue PA>PR=CR. Estos últimos resultados, como anteriormente se indicó, podrían ser explicados por la activación del plasminógeno en plasmina y/o la inactivación de los inhibidores de la plasmina por efecto de la pasteurización. Sin embargo, la menor degradación de η-CN observada en la segunda experiencia en los quesos de leche PA, respecto a los PR, podría deberse a una mayor concentración de sal en los primeros. En este contexto, Delacroix-Buchet y Trossat (1991) estudiaron el efecto de la concentración de sal en la proteolisis del queso Gruyère, encontrando que la actividad de la plasmina sobre la η-CN se incrementaba hasta una concentración de sal en humedad de ℑ2%, para posteriormente declinar regularmente a medida que aumenta la concentración de sal.

Las diferencias en proteólisis primaria encontradas entre los quesos elaborados con leche PR podrían ser atribuidas, como hemos comentado en la sección 4.1.5, a los cambios que sufre la leche tras el TAP. Cuando la leche es sometida a las altas presiones ocurre una fragmentación micelar y una gran desnaturalización de proteínas del suero. Una vez liberada la presión, las micelas de caseína, probablemente modificadas en estructura, se reagruparían rápidamente, pudiendo incrementar su susceptibilidad a la acción proteolítica de diferentes enzimas.

Proteólisis secundaria

Las concentraciones de NS y de NSTCA aumentaron (P<0,05) durante la maduración de los quesos CR, PA y PR. Sin embargo, no se encontraron diferencias significativas entre los valores de NS o NSTCA de los quesos en ninguna etapa del madurado.

Los aminogramas obtenidos mediante cromatografía líquida de intercambio iónico mostraron que la pasteurización de la leche parece enlentecer la liberación de aminoácidos en el queso, mientras que los quesos de leche CR y PR, aunque mostraron valores similares en aminoácidos libres totales, difirieron en la concentración de algunos de ellos. Los quesos de leche CR exhibieron los mayores niveles de prolina y los menores de serina, tirosina, arginina y AABA. Los quesos de leche PR mostraron los mayores niveles de arginina.

La concentración de aminoácidos individuales en el queso depende de las rutas metabólicas seguidas durante la degradación de los péptidos, así como de las interconversiones o degradaciones de los diferentes aminoácidos (Polo, Ramos y Sánchez, 1985). Al igual que lo observado con los ácidos orgánicos, los tratamientos de pasteurización y de alta presión afectaron el metabolismo de los aminoácidos, debido presumiblemente a los drásticos cambios observados en la microbiota de la leche de partida. Es posible también que los cambios provocados por los TAP en la leche de quesería alteren de alguna manera la susceptibilidad de las proteínas a la acción de las enzimas proteolíticas. De esta forma, la mayor proteolisis primaria observada en los quesos de leche PR, que incrementaría el número de posibles substratos para el ataque enzimático, facilitaría la liberación de aminoácidos en el queso.

5. CONCLUSIONES

1) Los tratamientos de pasteurización y de alta presión no provocan cambios en la composición general de las leches utilizadas para la elaboración del queso. Sin embargo, se observa una disminución en la cantidad de NNC en las leches PR respecto a las demás, fenómeno atribuido a la gran desnaturalización de las proteínas del suero por acción de la presión.
2) El tratamiento de pasteurización no produce cambios importantes en la aptitud a la coagulación enzimática, aunque muestra mayores tiempos de floculación en comparación con la leche CR. La leche PR exhibe tiempos más largos de floculación que las leches PA y CR, pero una mayor velocidad de agregación y firmeza del gel. Globalmente, el TAP mejora las propiedades de coagulación de la leche de cabra, ya que disminuye el tiempo de endurecimiento óptimo para el corte del gel.
3) Los quesos elaborados a partir de leche PR presentan mayor rendimiento quesero, debido a la incorporación de las proteínas del suero (especialmente η-LG) en la cuajada, ocasionando mayor retención de agua. Sin embargo, estas diferencias no son observadas cuando se estandariza el contenido de humedad de los quesos de partida.
4) Respecto a la composición general de los quesos, los tratamientos de pasteurización y alta presión dan lugar a un mayor contenido de humedad, especialmente en el queso de leche PR, no observándose otras diferencias de composición el día 1. Durante la maduración, los quesos de leche PR presentan contenidos de humedad superiores respecto a los quesos de leche CR y PA.
5) La aplicación de los tratamientos de pasteurización y de alta presión en leche con una microbiota inicial media de 8 x 10^5 ufc ml^{41}, disminuyen aproximadamente 2 ciclos logarítmicos los recuentos de bacterias mesófilas totales, 3 ciclos logarítmicos los

recuentos de psicrótrofos, *Micrococcaceae* y enterococos, no detectándose la presencia de lactobacilos y *Enterobactereaceae*. Así, el TAP aplicado es capaz de disminuir la microbiota de la leche de manera similar al tratamiento de pasteurización.

6) Los recuentos obtenidos en los grupos microbianos estudiados son muy similares en los quesos elaborados a partir de leche PA y PR. Sin embargo, los quesos elaborados con leche CR muestran unos recuentos mayores (aproximadamente 3 logaritmos) en lactobacilos, respecto a los otros tratamientos.
7) Desde el punto de vista cualitativo, el estudio de la proteolisis primaria de los quesos no muestra diferencias en el perfil electroforético de las fracciones insolubles a pH 4,6, excepto en la presencia de un pico correspondiente a la η-LG incorporada en los tratamiento de pasteurización y de alta presión, siendo considerablemente mayor en este último tratamiento.
8) Desde el punto de vista cuantitativo, el estudio de la proteolisis primaria da a conocer diferencias respecto a los tratamientos aplicados a la leche así como al contenido inicial de humedad en los quesos estudiados. Así, la ζ_{s1}-CN es degradada en los quesos con diferente contenido de humedad en el orden PA>PR>CR, y PR>PA=CR para los quesos de igual extracto seco de partida. En lo que respecta a la η-CN, este orden es PA>PR=CR en los quesos con diferente contenido de humedad, y PR>PA=CR para los quesos de igual extracto seco de partida. Los resultados observados podrían ser explicados bien en base a un predominio en el contenido de humedad o bien al de los efectos del tratamiento tecnológico sobre los constituyentes de la leche (enzimas nativos, p.e. plasmina, micelas de caseína, proteínas del suero, etc.).
9) Respecto a la proteolisis secundaria únicamente se observan mayores contenidos en NS y NSTCA a los 45 días de madurado en los quesos de leche PA y PR de la primera experiencia (quesos con diferente contenido de humedad de partida). Por otra parte, la concentración de aminoácidos libres totales es mayor en los quesos de leche PR>PA>CR en todos los períodos de maduración evaluados en los quesos de la primera experiencia. Sin embargo, en aquellos elaborados con igual contenido de humedad, el tratamiento de pasteurización ralentiza la liberación de aminoácidos libres totales en el queso, mientras que los quesos de leche CR y PR, aunque muestran valores similares en aminoácidos totales, difieren en la concentración de algunos de ellos. Los quesos de leche CR exhiben los mayores niveles de prolina y los más bajos de serina, tirosina, arginina y GABA, mientras que los quesos elaborados con leche PR muestran la mayor concentración de arginina.
10) El perfil peptídico de la fracción soluble en agua de los quesos es muy similar, siendo la mayor diferencia cualitativa un pico que corresponde a la η-LG no desnaturalizada en

los quesos de leche CR y en menor proporción en los de leche PA. En general, el tratamiento de alta presión no afecta la concentración de péptidos hidrofílicos ni hidrofóbicos de los quesos con relación a los de leche CR, mientras que los quesos elaborados con leche PA exhiben niveles más bajos de péptidos hidrofílicos al final de la maduración.

11) El estudio de la lipólisis muestra que el perfil de ácidos grasos libres es semejante en los quesos elaborados con igual contenido de humedad de partida. El tratamiento de pasteurización afecta negativamente la liberación de ácidos grasos durante el madurado, mientras que la concentración de ácidos grasos libres en los quesos de leche PR y CR es similar. Estos resultados pueden explicarse en base a la inactivación de la LPL de la leche por el tratamiento térmico, y a la relativa resistencia de esta enzima a la presión.
12) Los tratamientos de pasteurización y de alta presión de la leche provocan cambios en la estructura de los quesos. La observación a través del microscopio láser confocal muestra una matriz más regular, cerrada y con una distribución homogénea de los espacios libres y de la grasa en los quesos de leche PR, que se asemeja a la de los quesos de leche CR, mientras que los quesos elaborados con leche PA presentan una matriz más abierta e irregular. Estas diferencias entre los quesos se suavizan durante la maduración.
13) Los cambios ocurridos en la estructura de los quesos se reflejan también en las propiedades mecánicas. Los quesos elaborados con leche CR y PR son más firmes y menos fracturables que los de leche PA. Además los quesos de leche PR muestran el comportamiento más elástico, diferencias que, al igual que lo ocurrido en la microestructura, se hacen menos notables hacia el final de la maduración.
14) Aunque no se observan diferencias significativas en la evaluación sensorial de los quesos, cabe destacar las tendencias observadas en el perfil descriptivo realizado por el panel; los quesos de leche PR y CR son descritos como los más aromáticos y con mayor intensidad de sabor, respecto a los de leche PA. Asimismo, el queso de leche PR fue el más apreciado en su valoración global.
15) Las curvas de desecación y el grado de interacción del agua con la matriz de los quesos con similar valor de humedad inicial a lo largo de la maduración, se ve afectada por los tratamientos aplicados a la leche. El agua retenida con menos energía a la matriz proteica y la más fuertemente ligada exhiben un comportamiento muy similar en los quesos de leche PR y CR durante la maduración, hecho atribuible a las similitudes de su matriz y características bioquímicas.

16) El tratamiento de pasteurización ralentizó el desarrollo de los ácidos orgánicos durante la maduración de los quesos con similares valores de humedad de partida. Los quesos elaborados a partir de leche PR o PA, los cuales poseen similares características microbiológicas, se diferencian de los quesos de leche CR en las concentraciones de los ácidos propiónico, acético, pirúvico y cítrico, hecho que puede atribuirse a la acción de la microbiota autóctona de la leche CR durante el madurado.
17) El tratamiento de alta presión hidrostática (500 MPa, 15 min, 20ºC) se muestra efectivo para su aplicación en la producción de quesos de cabra madurado. Este tratamiento incrementa la seguridad alimentaria del producto, mostrando características similares al queso elaborado con leche CR.

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7. ANEXO I

TABLA I: MÉTODOS DE ANÁLISIS QUÍMICO		
CONSTITUYENTE	MÉTODO	REFERENCIA
Sólidos totales	Desecación a 102±2ºC	Leche: IDF 21B (1987) Queso: IDF 4A (1982)
Grasa	Butirométrico	Leche y suero: IDF 105 (1981) Queso: ISO 3433-33 (1975)
Sal	Conductimétrico	Autoanalizador de cloruros Corning 926
pH	Potenciométrico	
Nitrógeno total	Valoración Kjeldahl	Leche, suero y queso: IDF 20B (1993)
Nitrógeno soluble a pH 4,6	Fraccionamiento de nitrógeno a pH 4,6	Leche: Rowland (1938) Queso: Kuchroo y Fox (1982)
Nitrógeno no proteico	Precipitación con ácido tricloroacético al 12%	Leche: IDF 20B (1993) Queso: Kuchroo y Fox (1982)
Aminoácidos libres	Espectrofotométrico con cadmio-ninhidrina	Folkertsma y Fox (1992)
Caseínas	Análisis por electroforesis capilar de la fracción insoluble en agua	Recio y Olieman (1996)
Péptidos	Análisis por HPLC de la fracción soluble en agua	González del Llano *et al.* (1995)
Ácidos grasos libres	Cromatografía de gases	Ha y Lindsay (1990)
Ácidos orgánicos	Análisis por HPLC de la fracción soluble en ácido fosfórico 0,1 N	Lues *et al.* (1998)
Aminoácidos libres	Cromatografía de intercambio iónico con derivatisación post column con ninhidrina	Spackman *et al.* (1958)
Agua	Análisis termogravimétrico	De Angelis Curtis *et al.* (1999)
IDF: International Dairy Federation ISO: International Organization of Standarization		

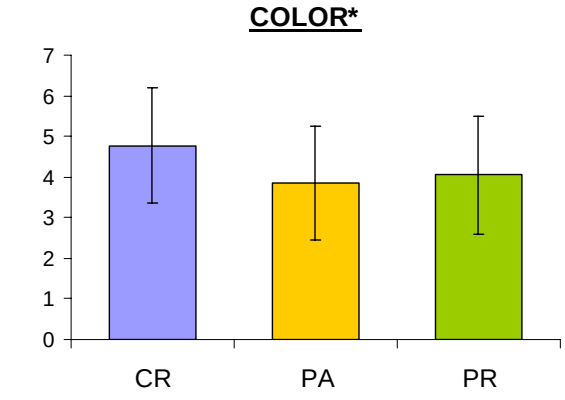
8. ANEXO II

Análisis sensorial de los quesos elaborados a partir de leche cruda, pasteurizada y tratada por altas presiones

El análisis sensorial de los quesos fue realizado al segundo mes de maduración por un panel de 10 miembros familiarizados con queso de cabra, como se detalla en el trabajo incluido en la sección 3.4. Se realizó un análisis descriptivo para la calificación de los atributos sensoriales distribuidos en diferentes grupos: color, textura, e intensidad de aroma y sabor, en una escala estructurada comprendida entre 1 (poco intenso) y 8 (muy intenso).

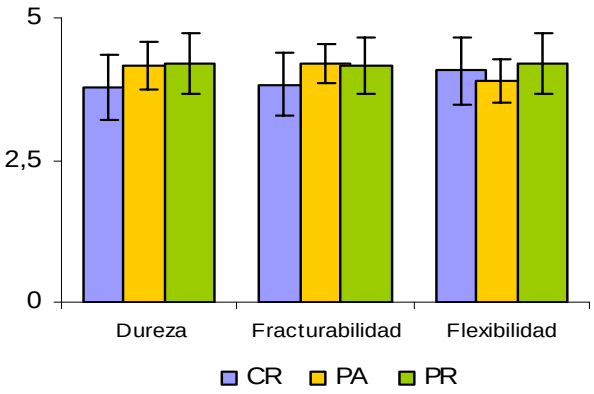
Las muestras, cortadas en forma de cuña de aproximadamente 80 mm de largo por 5 mm de espesor, se colocaron en platos blancos tapados con papel aluminio a temperatura ambiente, y se codificaron con números de tres dígitos. Los resultados fueron analizados mediante análisis de la varianza ($P<0,05$).

Los gráficos siguientes muestran los resultados obtenidos en el análisis sensorial de las muestras. Los catadores no observaron diferencias significativas entre los quesos para ninguno de los atributos sensoriales, aunque los quesos elaborados a partir de leche PR mostraron los mayores valores de intensidad de aroma y sabor. Además el panel otorgó a los quesos de leche PR la mayor valoración global, aunque estas diferencias tampoco fueron significativas.

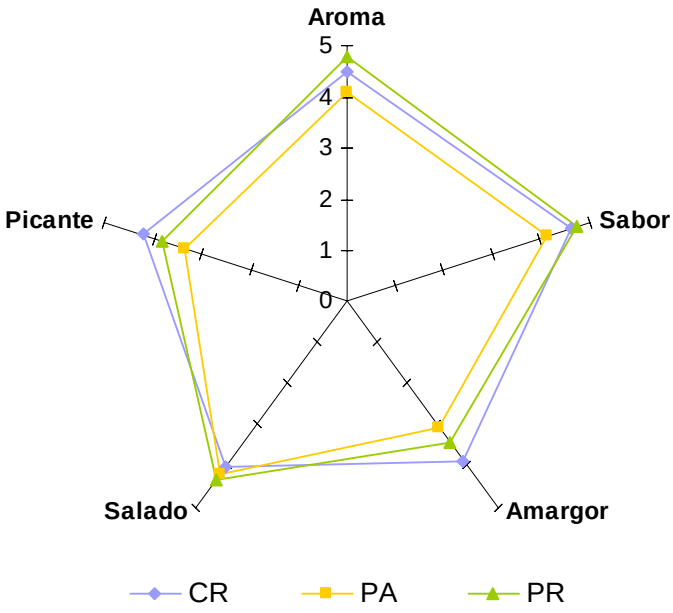


*Color (1-blanco; 8-amarillo)

TEXTURA



SABOR Y AROMA



VALORACIÓN GLOBAL