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Effect of brine concentration on the ripening of an Argentinean sheep's milk cheese

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ABSTRACT

In the present work, it was studied the influence of brine concentration on the ripening of an Argentinean sheep cheese. The aim was to establish the conditions of the salting process for it to lead to a product with good quality and acceptability by consumers. Sheep cheeses were manufactured using a direct-vat-set culture of *Streptococcus thermophilus* as starter and chymosin as coagulant. The salting was carried out using brine with the following concentrations: 20, 15, 10 and 5% (w/v). The cheeses were vacuum packed on the fourth day and ripened for 60 days. In order to evaluate the influence of salt content in brine on cheese quality, there were analyzed gross composition, pH, microbial counts, proteolysis, sodium and calcium concentration, melting capacity and sensory characteristics. The increase of salt concentration in brine corresponded with a decrease in moisture as proteolysis advanced. This suggests some inhibition of proteolytic and peptidolytic enzymes involved in ripening due to these adverse environmental conditions. In the descriptive sensory analysis, differences were only found for bitter taste, which was significantly higher for the less salted cheeses. This was attributable and correlated with an increase in concentration of hybrophobic peptides due to an imbalance in proteolysis/peptidolysis during ripening. The melting capacity of the cheese was not affected by the level of salt used. Therefore, it is proposed that the salting process for an Argentinean sheep cheese should be with a 15% brine.

1. Introduction

Even though there are many world-famous sheep milk cheeses, such as Roquefort (France), Feta (Greece), Romano (Italy) or Manchego (Spain), in recent years, sheep milk and its derived products have gained great importance, as it is reflected in their growing presence in the market (Mc Cormick and Lynch, 2003). This can be attributed to organoleptic properties of these products, whose typical and delicate flavors are derived mainly from its high fat content and the composition of fatty acids (Ramos and Juarez, 2011). However, the biochemical changes that occur during the ripening period also have an important impact on sensory characteristics of cheeses and their acceptability by consumers. In connection with this, it is important to notice that the salt content in cheese has a direct influence on these processes, mainly due to its effect on water activity, microbial growth, protein hydration and enzyme activity in general (Møller et al., 2013; Rulikowska et al., 2013). Therefore, the salting process constitutes a major step in the development of a cheese, since the amount of salt (especially their level in the moisture) plays a critical role not only in the quality but also in conservation and safety (Guinee and Sutherland, 2011; Rulikowska et al., 2013). Thus, the salt level for each type of cheese must be regulated within a certain optimum range, which is defined according to the conditions under which this operation is performed. Salt levels below this range may lead to defects such as the development of undesirable microorganisms, uncontrolled enzyme activities or changes in the cortex (Melilli et al., 2005). Conversely, high salt concentrations may cause defects associated to an inhibition of primary starter or biochemical changes during ripening process, or to a direct effect on the gross composition (Guinee and Sutherland, 2011). In general, the range for salt content in the different varieties of cheeses ranges from 0.7 to 4%, while the salt-in-moisture is from 2 to 10% (Boylston, 2012).

Nowadays, there is a global trend towards the reduction of sodium in processed foods, because of its direct relationship with various diseases, especially hypertension (Appel et al., 2011). Several studies have been conducted about cheese salt reduction, the most common being the partial replacement of sodium chloride by potassium chloride (Sihufe et al., 2006; Grummer et al., 2013). However, based on the foregoing, reducing salt content of a cheese

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requires, in the first place, knowing the levels that are compatible with a product of an acceptable organoleptic quality (Ganesan et al., 2014).

In our country, despite the growing popularity of sheep cheeses in the last years, there is still no standardized technology that ensures uniformity and consistency in their quality (Candioti et al., 2009). In the present work, it has been studied the effect of brine concentration on the ripening process of a sheep cheese. The aim was to establish the salting conditions which result in a safe product with good organoleptic quality in accordance to the needs of small producers.

2. Materials and methods

2.1. Cheese-making

Raw sheep milk, provided by the School of Agriculture, Farm and Livestock from Universidad Nacional del Litoral (EAGyG-UNL), was refrigerated and transported at 4 °C to the pilot plant of our Institute (Instituto de Lactología Industrial, UNL), where it was kept frozen at −20 °C until its use. Each cheese making day, 40 L of raw milk were unfrozen and pasteurized at 65 °C for 20 min, and then cooled to 39 °C (temperature of coagulation). A lyophilized commercial culture of *Streptococcus thermophilus* (ST-M5, Chr. Hansen, Inc., Denmark), previously resuspended in 100 mL of sterile milk, was added at a concentration of 10^6 CFU mL^{-1} of milk. After 10 min, it was added the chymosin produced by fermentation of *Aspergillus niger* var. awamori (Chy-Max, Inc. Chr Hansen, Denmark. 183 IMCU/mL) The amount of rennet was enough to obtain the proper firmness. For cutting the curd in 15–20 min. At this time, the curd was cut in the adequate grain size (approximately 25 mm). After 15 min, the mixture was stirred gently during 15 min to achieve proper moisture when left standing for about 10 min. Then, the whey was removed and the curd was placed into molds, and kept in a warm chamber (40 °C–3 h) until reaching pH 5.10 ± 0.05. Afterwards, the cheeses were placed in a conditioning chamber at 4 °C and 92% relative humidity (with air circulation at reduced speed to avoid excessive surface evaporation), where they were kept for 24 h. This stage has the purpose of regulating the cooling rate and allows the development of fermentation to compensate the rise of pH derived from the salts balance, which occurs during brining (Cuffia et al., 2011). Cheeses obtained, of approximately 4 kg, were divided into eight portions of 500 g each, labeled Q1, Q2, Q3 and Q4, which were salted by immersion in concentrated brines 20%, 15%, 10% and 5%, respectively, for a time equivalent to 1 h per kg cheese. After salting, the cheeses were placed in the same conditioning chamber (4 °C and 92% relative humidity), and on the fourth day they were packed under vacuum in shrink plastic bags, until completing their maturation. Four replicates of cheeses were made on successive cheese-making days.

2.2. Gross composition, pH, sodium and calcium analysis

At two months of ripening, cheeses were analyzed in order to determine: moisture (FIL-IDF, 1982), protein (FIL-IDF, 1993), pH (Bradley et al., 1993), and fat matter (Fil-IDF, 1997). At the same time, the concentration of calcium and sodium by atomic absorption flame (FAAS), and flame atomic emission (FAES) respectively, was determined by standard methods (AOAC, 1995).

For all experiences two replicates were made. Each one was analyzed in duplicate.

2.3. Microbiological analysis

Enumeration of total lactic acid bacteria was performed in cheeses at 3, 30 and 60 days of ripening by plating sample dilutions on skim milk agar (SMA) and counting plate colonies after 48 h of incubation at 37 °C according to American Public Health Association (APHA) standards (Frank et al., 1993). Coliforms were enumerated on Bile Red Violet Agar (BRVA) incubating the plates for 24 h at 37 °C, as stated in APHA (Christen et al., 1993).

The results were obtained from two replicates.

2.4. Proteolysis assessment

Proteolysis was assessed by the techniques described below:

2.4.1. Soluble nitrogen (SN)

Cheese samples were treated to obtain crude citrate extract and soluble fractions at pH 4.6, in TCA 12% and PTA 2.5%, according to Hynes et al. (2003). The crude cheese extract was obtained by adding 20 mL of sodium citrate 0.5 M to 10 g of cheese and grounding to homogeneity using a pestle. Deionized water was added to ~90 mL, and the pH was adjusted to 4.6. After centrifugation (3000 × *g*/15 min), the soluble fraction volume was adjusted to 100 mL. The TCA 12% and PTA 2.5% soluble fractions were obtained from 4.6 soluble fraction according to Gripon et al. (1975). The N content was determined in duplicate by the macro-Kjeldahl method according to the IDF method (FIL-IDF, 1993).

2.4.2. Electrophoresis

The insoluble residue at pH 4.6 was purified. In order to do that, samples were re-dissolved by adding 200 mL of distilled water and bringing the pH to 7 with stirring. After being kept about 10 min in these conditions, the insoluble residue was re-precipitated at pH 4.6, proceeding in the same manner as in the extraction. This operation was repeated twice. Finally, the insoluble residue was washed with distilled water twice (by suspension and centrifugation). Samples thus obtained were preserved in a freezer at −18 °C for subsequent electrophoretic analysis.

Electrophoresis assessment was carried out by Urea-PAGE in a Mini-Protean II cube (BioRad Laboratories, California, USA) by Andrews (1983) method, with a concentration of acrylamide of 7.5%. Proteins were stained by Coomasie blue G-250.

2.4.3. RP-HPLC

The HPLC equipment consisted of a quaternary pump, an online degasser and UV–vis detector, all Series 200, purchased from PerkinElmer (PerkinElmer, Norwalk, CT, USA). An interface module connected to a computer was used for acquisition of chromatographic data with the software Turbochrom® (PerkinElmer). A 220 × 4.6 mm Aquapore OD-300C18, 5 μm – 300 A° analytical column was used (PerkinElmer). Water-soluble extracts of the cheeses were obtained by blending 5 g of cheese and 15 mL of distilled water with mortar and pestle, then warmed up to 40 °C and maintained for 1 h. The suspension was centrifuged (3000 × *g*/30 min), and filtered through fast flow filter paper. The filtered solution was adjusted to a final volume of 25 mL. Samples were filtered through 0.45 μm membranes (Millex, Millipore, São Paulo, Brazil), and 60 μL was injected into the HPLC chromatograph. Detection was performed at 214 nm, and column temperature was 40 °C. The gradient starting from 100% of solvent A (H_2O:trifluoroacetic acid (TFA) 1000:1.1, v/v) and 0% of solvent B (acetonitrile:H_2O:TFA 600:400:1, v/v), was generated 10 min after injection. The proportion of solvent B was increased by 1% min-1 (80 min), 20% min-1 (1 min), 0% min-1 (4 min), and then returned to starting conditions, which took 1 min. These last setting conditions were maintained for 10 min (Hynes et al., 2003). The different profiles were visually compared. In addition, total peak areas were calculated from each chromatogram and divided into 3 groups: peaks which elute between 0 and 30 min are mainly free amino acids and small hydrophilic peptides, peaks which elute between 30 and 70 min

Table 1
Gross composition, sodium and calcium concentration, and covered area from melting assay in cheeses salted with brines of 5 (Q4), 10 (Q3), 15 (Q2) and 20% (Q1) at 60 days of ripening. Values are means ± standard deviation ($n = 4$).

Cheese	Moisture (% w/w)	Fat matter (% w/w– DM)	Protein (% w/w–DM)	Sodium (mg/100 g of cheese)	Calcium (mg/100 g of cheese)	Covered area (cm^2)
Q_4	45.0 ± 0.7[a]	54.5 ± 1.6[a]	39.8 ± 1.5[a]	114.43 ± 1.56 [d]	1030.32 ± 70.77 [a]	54.8 ± 0.7[a]
Q_3	44.1 ± 0.5[a,b]	54.0 ± 1.2[a]	40.1 ± 1.2[a]	225.75 ± 2.81 [c]	1020.45 ± 210.89 [a]	53.0 ± 0.7[a]
Q_2	43.1 ± 0.6[b]	52.9 ± 1.4[a]	38.8 ± 0.9[a]	382.12 ± 2.35 [b]	1020.39 ± 70.43 [a]	52.0 ± 1.1[a]
Q_1	41.2 ± 0.5[c]	51.2 ± 1.7[a]	37.1 ± 15[a]	516.73 ± 3.47 [a]	1040.12 ± 140.87[a]	52.1 ± 1.9[a]

Values with different superscript letters within the same column are significantly different ($p < 0.05$).
DM: Dry matter.

are peptides with different levels of hydrophobicity, and finally, peaks which elute from 70 min to the end of the chromatogram are hydrophobic peptides.

2.5. Melting assay

Melting assays were performed using the Schreiber test (Muthukumarappan et al., 1999), modified by Mercanti et al. (2004). For that, cylinders of cheese samples (42 mm in diameter and 10 mm high, cut at least 1 cm from the edge) were taken from cheeses after 60 days of ripening. The cylinders were kept at 4 °C for 30 min in order to standardize initial temperature, and were then placed on a glass surface in a natural convection oven, at 130 °C for 15 min. After the treatment, it was evaluated the increase in the covered area produced by each cylinder. All melting assays were performed in triplicate.

2.6. Sensory analysis

Descriptive sensory analysis was performed at the end of the ripening (60 days). The four cheeses, identified by random numbers, were presented simultaneously to each evaluator. The panel was composed of eight participants trained in the subject, who, using unstructured scales anchored at the ends, evaluated in two separate sessions the following attributes: aroma, color, appearance of mass, elasticity, mouthfeel, cream flavor, salty taste, bitter taste, acid taste and residual flavor.

2.7. Statistics

The results were processed by analysis of variance (ANOVA) using Statgraphics Plus v3.0 (Statistical Graphics Corp.) software to determine the effect of the concentration of the brine on the studied variables. When significant differences were presented ($p < 0.05$), Duncan's test was applied to detect homogeneous groups of means, using the same software.

3. Results and discussion

3.1. Microbiological counts and gross composition of cheeses

The number of lactic acid bacteria was maintained above 10^7 cfu/g during all ripening process, while coliform counts was always <10 cfu/g.

The content of fat and protein did not differ ($p > 0.05$) between the cheeses salted with different concentrations of brine (Table 1). This result was expected since these parameters depend mainly on milk characteristics and the processing technology, which was the same for all cheeses.

On the contrary, the moisture content showed significant differences ($p < 0.05$), with lower values for cheeses as the concentration of the brine used was increased (Table 1). This observation is not surprising since it is known that salt uptake by cheese is accompanied by a simultaneous moisture loss during the process of salting by immersion in brine, due mainly to an osmotic effect, as salt (from brine) and moisture (from cheese) migrate in opposite directions during diffusion. Consequently, there is an inverse relationship between the levels of salt and moisture in cheese (Guinee and Sutherland, 2011). With regards to pH, all cheeses had a value in the order of 5.15 ± 0.02.

The levels of calcium and sodium in cheeses at the end of ripening are shown in Table 1. As expected, the amount of sodium increased concomitantly with the brine concentration, presenting significantly different values in the four cheeses evaluated. Nevertheless, the different levels of salt did not affect the concentration of calcium.

The differences in the levels of moisture without significant variations in the fat and protein content of the cheeses could be due to differences in the ash content, which was not measured in the present work. However, the sodium concentration in Q1 was ~4.5 times greater than in Q4, which is consistent with the hypothesis.

3.2. Proteolysis assessment

3.2.1. Soluble nitrogen

Mean values and standard deviations of the three fractions of soluble nitrogen (SN), expressed as percentage of total nitrogen, obtained for cheeses with different levels of salt are shown in Table 2. The percentage ratio of SN TCA and SN PTA, in relation to SN 4.6, is also shown.

The SN content in different fractions consists mainly of compounds of different size and molecular weight, which increases during ripening as a result of proteolysis mediated by the proteolytic agents present in the cheese. The SN 4.6 fraction is composed of proteins (except caseins), all peptides, amino acids and smaller sized N compounds. This is an index of primary proteolysis. Fraction of SN TCA includes medium sized to small peptides, free amino acids and smaller sized N compounds (such as amines/urea and ammonium). Finally, fraction of SN PTA includes free amino acids and smaller sized N compounds. These two last fractions are indicative of the level of secondary proteolysis (Ardö, 1999).

Nitrogen levels were similar at the beginning of the ripening in all cheeses, but were lower in cheeses after 30 and 60 days of ripening as brine concentration increased. In effect, significant differences ($p < 0.05$) were found for SN pH 4.6 and SN PTA in cheeses after 30 and 60 days of ripening. Taking as reference the results obtained at 60 days for Q4 (5% brine), the values of Q3, Q2 and Q1 cheeses were lower in the following percentages: 14, 22 and 35% for NS 4.6 and 6, 17 and 27% for the fraction of NS PTA, respectively. On the other hand, the ratios of the SN TCA / SN 4.6 and SN PTA/SN 4.6 were overall higher for cheeses with higher levels of salt (Table 2). These ratios are related to the peptidolytic activity of starter and non starter bacteria present in the cheese. So, these results suggest that the higher levels of SN in cheeses salted with less concentrated brine was mainly due to a higher release of high-sized peptides via

Table 2
Evolution of soluble nitrogen (SN) at pH 4.6, in trichloroacetic acid (TCA) and in phosphotungstic acid (PTA), expressed as percentage of total nitrogen (TN) and the porcentual ratio of these fractions, in cheeses salted with brines of 5 (Q4), 10 (Q3), 15 (Q2) and 20% (Q1) Values are means ± standard deviation ($n = 4$).

		Cheeses			
		Q4	Q3	Q2	Q1
SN 4.6/TN (%)	0 days	4.7 ± 1.2^{a}	4.4 ± 1.1^{a}	$4.9. \pm 1.3^{a}$	4.7 ± 1.1^{a}
	30 days	9.1 ± 0.3^{a}	6.8 ± 0.2^{b}	6.0 ± 0.2^{c}	5.0 ± 0.4^{d}
	60 days	14.8 ± 0.4^{a}	12.2 ± 0.4^{b}	11.6 ± 0.3^{b}	9.7 ± 0.2^{c}
SN TCA/TN (%)	0 days	2.5 ± 0.2^{a}	2.5 ± 0.1^{a}	2.5 ± 0.1^{a}	2.6 ± 0.3^{a}
	30 days	2.9 ± 0.1^{a}	2.7 ± 0.1^{a}	2.7 ± 0.1^{a}	2.8 ± 0.1^{a}
	60 days	4.0 ± 0.5^{a}	3.7 ± 0.4^{a}	3.5 ± 0.2^{a}	3.5 ± 0.2^{a}
SN PTA/TN	0 days	0.8 ± 0.1^{a}	0.9 ± 0.2^{a}	0.8 ± 0.1^{a}	0.8 ± 0.2^{a}
	30 days	1.1 ± 0.1^{a}	0.9 ± 0.1^{b}	0.9 ± 0.1^{b}	0.8 ± 0.1^{c}
	60 days	1.5 ± 0.1^{a}	1.4 ± 0.1^{b}	1.3 ± 0.1^{c}	1.1 ± 0.1^{d}
SN TCA/SN 4.6 (%)	0 days	54.0 ± 2.0^{a}	56.9 ± 2.8^{a}	51.8 ± 3.5^{a}	55.3 ± 2.3^{a}
	30 days	31.5 ± 1.0^{d}	40.4 ± 1.7^{c}	45.7 ± 3.2^{b}	55.5 ± 5.2^{a}
	60 days	27.0 ± 3.5^{b}	30.7 ± 2.7^{b}	29.8 ± 1.4^{b}	35.9 ± 2.2^{a}
SN PTA/SN 4.6 (%)	0 days	17.4 ± 0.7^{a}	19.6 ± 0.8^{a}	17.1 ± 0.8^{a}	17.4 ± 0.5^{a}
	30 days	12.0 ± 0.3^{c}	13.8 ± 0.8^{b}	$15.0 \pm 0.9^{a,b}$	15.4 ± 1.3^{a}
	60 days	10.3 ± 0.3^{b}	11.5 ± 0.4^{a}	10.7 ± 0.4^{b}	11.6 ± 0.3^{a}

Values with different superscript letters in the same row are significantly different ($p < 0.05$).

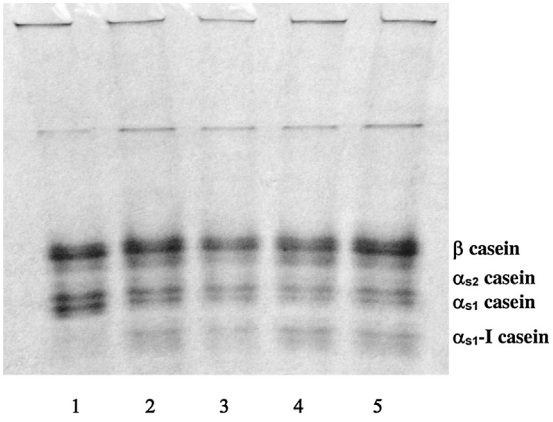

Fig. 1. Urea-PAGE electrophoretic profiles of cheeses with different levels of salt after 60 days of ripening. 1: unsalted cheese before ripening; 2, 3, 4 and 5: cheeses salted with brines of 5 (Q4), 10 (Q3), 15 (Q2) and 20% (Q1), respectively.

proteases, while the release of medium and small-sized peptides via peptidases had a minor influence.

Thus, the use of more concentrated brine during salting of Argentinean sheep cheese led to a less marked proteolysis during the ripening period. These results can be attributed to a more restrictive effect exerted by lower levels of moisture and higher salt levels, achieved in cheese salted with more concentrated brines, on the activity of proteolytic enzymes present in the product, such as indigenous milk proteases or microbial peptidases (Candioti et al., 2001). Indeed, the salt can inhibit the growth of microorganisms, mainly due to lower water activity produced in the cheese which, in turn, influences the microbial enzyme activity (Pérez Elortondo et al., 1999; Guinee and Sutherland, 2011). At the same time, the salt has an effect on other proteolytic agents in the cheese matrix, mainly on the residual coagulant activity (Candioti et al., 2010). Our results are in agreement with those obtained by other researchers on the study of the influence of the salt level on Cheddar cheese proteolysis (Rulikowska et al., 2013).

3.2.2. Electrophoresis

Typical electrophoretic pattern for sodium caseinate (data not shown) was used to identify the different bands of caseins in electrophoretic profiles of cheeses at 60 days of ripening (Fig. 1). The α_{s1}-I peptide was observed in all cheeses, regardless of salt concentration. As it is known, this peptide is the result of the main biochemical transformation performed by the coagulant on α_{s1} casein, in bovine (Hynes et al., 2003) and sheep milk (Irigoyen et al., 2002). As in bovine milk, it has been shown in solutions of sheep α_{s1} casein, that α_{s1}-I peptide is a product of the Phe_{23}-Phe_{24} union breakdown by action of chymosin (Trujillo et al., 2000). Similar intensity for α_{s1}-I peptide, and also for α_{s1} casein, suggests that different salt concentrations had no significant influence on the residual coagulant activity in the cheeses. In addition, β casein band was similar for all cheeses, which indicates that the hidrolysis of this protein, mainly mediated by plasmin,was unaffected by the salt level. In the type of cheese studied in the present work, in which no cooking step is included during cheesemaking, chymosin is one of the most important enzymes which affect the proteolysis during ripening, while the influence of plasmin activity is lower (Vélez et al., 2015).

3.2.3. Peptide profiles

The RP-HPLC profiles of the curd and 60-d-old cheeses are shown in Fig. 2. Very low quantity of peaks was observed in the RP-HPLC profiles from the samples of curd before ripening (0 days), which increased significantly in the samples at the end of ripening (60 days) due to the evolution of proteolysis. Quantitative data of profiles are shown in Table 3; these results showed numerical differences but they were not significant. Total peak areas of cheese salted with more concentrated brine was the lowest, which indicates a decrease in proteolysis that is in agreement with the results of the soluble fractions of nitrogen. On the other hand, a higher proportion of peak areas in the last region of chromatograms, in which hydrophobic peptides elute, was found for the less salted cheeses. It is important to note that high levels of these peptides have been associated with the occurrence of the defect of bitterness in cheese. On the contrary, cheeses with more levels of salt had higher proportion of peak areas in the first and second region of chromatogram.

As already mentioned, these results can be attributed to an inhibition of proteolytic and peptidolytic enzymes involved in the ripening at higher salt concentrations. Salt has influence on the majority of the enzymes involved in the ripening of cheese; the activity of hydrophilic enzymes, such as proteases and peptidases, decreases with the reduction in water activity with the concomitant increase of the level of salt (Guinee and Sutherland, 2011). On the other hand, a low level of salt can allow the development of undesirable microorganisms, and/or lead to an uncontrolled enzyme

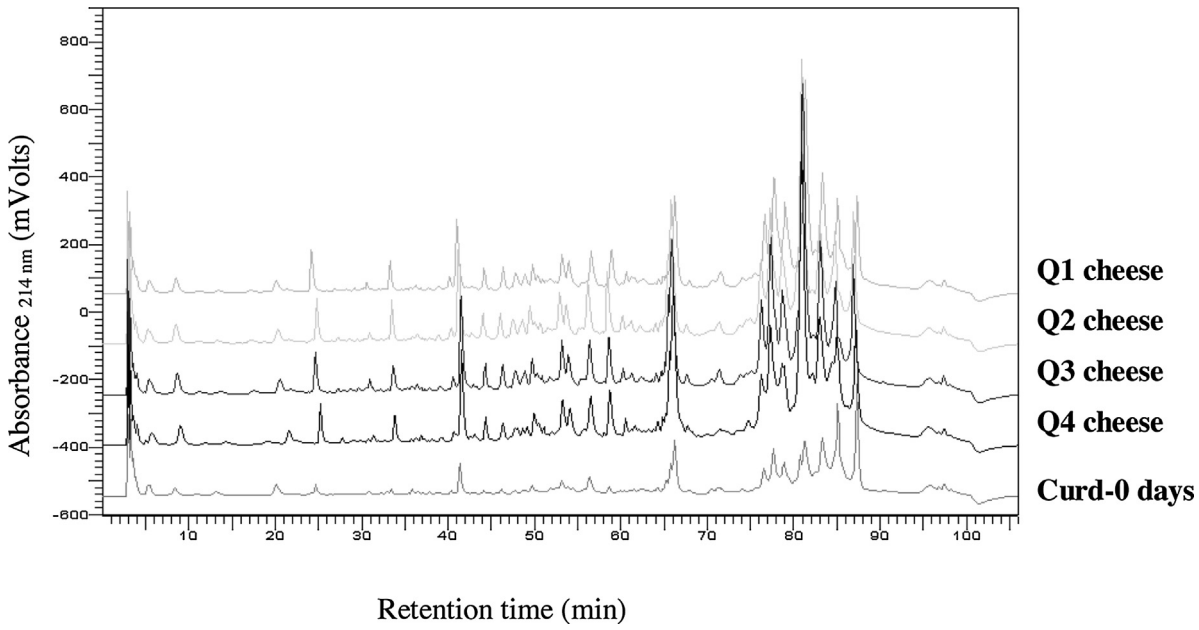

Fig. 2. Peptide profiles of curd before ripening ($t = 0$ days) and cheeses salted with brines of 5 (Q4), 10 (Q3), 15 (Q2) and 20% (Q1) at 60 days of ripening.

Table 3
Levels of peptides in different regions of RP-HPLC profiles of water-soluble fraction of cheeses salted with brines of 5 (Q4), 10 (Q3), 15 (Q2) and 20% (Q1). Values are means ± standard deviation ($n = 4$).

		Q4	Q3	Q2	Q1
Total area (x10^6)[a]		386 ± 58	415 ± 50	376 ± 24	281 ± 109
Percentual peak area (%)[b]	0–30 min region	5.1 ± 1.0	4.9 ± 0.7	5.8 ± 1.4	7.3 ± 2.1
	30–70 min region	31.5 ± 0.9	30.8 ± 0.3	32.9 ± 3.0	36.4 ± 2.3
	70–106 min region	63.4 ± 1.9	64.3 ± 1.1	61.3 ± 1.6	56.3 ± 4.4

[a] Area in arbitrary units expressed in dry matter.
[b] Percentual ratio of areas (arbitrary units) for each particular region in relation to total area.

activity, which can generate defects in cheese such as bitterness (Guinee and Sutherland, 2011).

3.3. Melting assay

The melting capacity of a cheese is the degree of spreading achieved after being heated. It is one of the functional properties of greater influence on the quality and acceptability of a cheese when it is used as an ingredient in hot culinary preparations (Kindstedt et al., 2004). In this work, the area covered by cylinders of cheese after heat treatment in melting assays was similar for all cheeses (Table 1). So, despite the differences in the degree of proteolysis, the melting capacity of the cheese was not affected by the different salt content. This result is consistent with those of previous studies, in which it was demonstrated that different levels of proteolysis due to different residual coagulant activity did not affect the texture or the melting of soft cheeses (Candioti et al., 2010; Bértola et al., 2011). Indeed, these properties have been more associated with variations in the balance between ionic and colloidal calcium, than with the proteolysis. In this sense, it has been suggested that the demineralization of the micelle continues during the cheese ripening and promotes the breakdown of the network and the softening of the mass of cheese (Sheehan and Guinee, 2004; O'Mahony et al., 2005). Therefore, it is not surprising that the melting capacity was similar for all cheeses because those salty conditions did not significantly affect neither the pH nor calcium content of cheese.

3.4. Sensory analysis

The results of descriptive sensory analysis of the cheeses after 60 days of ripening are shown in Fig. 3.

Sensory descriptors related to the cheese texture, such as appearance of mass, elasticity and mouthfeel, were not affected by different levels of salt used. On the contrary, the scores for some descriptors related to flavor showed numeric differences. In this sense, Q3 and Q4 cheeses received higher scores values for bitter taste and residual flavor, while Q1 and Q2 cheeses had greater scores for salty taste, and only Q1 had more acid taste. Besides these differences, a significant ($p < 0.05$) one was the bitter taste. These results correlate with those obtained in the evaluation of the proteolysis, because the bitterness defect is commonly associated with the presence of hydrophobic peptides (Molina et al., 1999; Guinee and Sutherland, 2011). A higher concentration of hydrophobic peptides in a cheese may result from an imbalance in the normal proteolytic process in which the bitter peptides are formed and then degraded to non-bitter compounds. Thus, any factor that affects the activity of some of the agents involved in normal proteolytic process during ripening of a particular cheese, such as a deficiency in their salt content, can lead to a defect of bitterness (Banks, 2011). In the same way, bitterness increased with NaCl reduction in Cheddar cheeses, which was attributed to a higher degradation of β-casein due to a greater activity of plasmin (Rulikowska et al., 2013). On the other hand, the salty taste was intensified in cheeses Q1 and Q2 in which the highest concen-

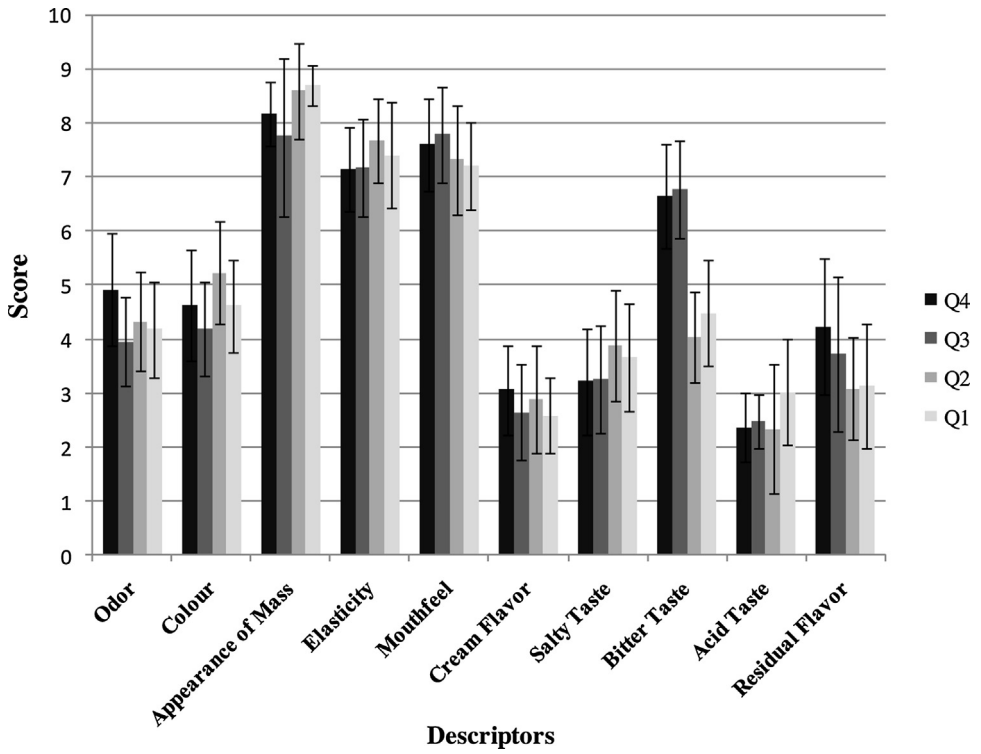

Fig. 3. Sensory descriptors analyzed of cheeses salted with brines of 5 (Q4), 10 (Q3), 15 (Q2) and 20% (Q1) at 60 days of ripening. Values are mean ± standard deviation.

tration of brine was used, but the differences were not significant ($p > 0.05$).

4. Conclusion

The salting of Argentinean cheeses made from sheep milk with brines between 5 and 20% produced differences in the level of proteolysis, being this process more pronounced as the level of salt used was lower. Furthermore, the use of the more dilute brines (5–10%) led to an increase in the bitterness of these cheeses due to an imbalance in proteolysis; the slight increase in the levels of hydrophobic peptides in these cheeses could be correlated with these results. Despite the differences in proteolysis, the cheese melting capacity was not affected by the concentration of the brine used. On the other hand, the cheese salted with the most concentrated brine (20%) showed too low moisture levels for this type of cheese. Taking into account the obtained results, a brine of 15% is proposed for the making of a sheep cheese of good quality.

Conflict of interest

None.

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