

## Probiotic soft sheep's cheese: evaluation of probiotic survival and its influence on proteolysis and organoleptic characteristics

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### Abstract

In the present work, the survival of two commercial strains of probiotic bacteria: *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Lactobacillus acidophilus* LA-5, and their influence on the composition, proteolysis and sensory characteristics of soft sheep cheese were studied. Three different types of cheeses were made: control cheese (QT) without addition of probiotic bacteria, and two experimental cheeses with addition of BB-12 (QBb) and LA-5 (QLA). Gross composition, pH, counts of starter and probiotic bacteria and proteolysis were evaluated during ripening at 4, 15 and 30 days, and a descriptive sensory analysis was conducted at the end of ripening (30 d). The initial pH in all cheeses was  $5.15 \pm 0.02$  and was slightly increased at 30 days at a value of  $5.31 \pm 0.02$ ; pH values were similar in all cheeses. Gross composition was similar between control and probiotic cheeses; mean values of moisture and content of fat and protein (expressed in dry matter) were  $47.61 \pm 0.8$  (%w/w),  $53.38 \pm 1.52$  (%w/w) and  $42.87 \pm 1.15$  (%w/w) respectively. The counts of BB-12 and LA-5 in the curd were  $\sim 10^8$  CFU/g and were maintained at this level in the cheeses during all ripening time. The addition of BB-12 and LA-5 produced an increase in the levels of secondary proteolysis, being the effect more marked for LA-5. The descriptive sensory analysis of cheeses showed that the addition of LA-5 and BB-12 did not produce defects in flavor and taste but rather significantly improved ( $p < 0.05$ ) the elasticity, appearance of mass and mouthfeel of cheeses. Soft sheep cheese demonstrated to be a suitable carrier for these probiotic cultures, and the characteristics of these cheeses were slightly improved by the addition of probiotic bacteria.

### Keywords

Probiotic bacteria  
Soft sheep cheese  
Ripening  
Sensory characteristics

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### Introduction

In recent years, sheep milk and its derivatives have acquired a very important role, which is reflected in their increasing integration into the dairy market (Mc Cormick and Lynch, 2003). This trend could be attributed to their distinctive organoleptic characteristics, since they have delicate flavors, derived mainly from their composition and their high fat content (Ramos and Juárez, 2011). This fact, coupled with the growing demand for functional products that meet the nutritional consumer expectations, has promoted an important development of innovative products (Boylston *et al.*, 2004).

Cheese is one of the most efficient food matrices to keep the probiotic bacteria viable and enter them in the human diet (Vinderola *et al.*, 2003; Gomes da Cruz *et al.*, 2009; Bergamini *et al.*, 2010). In fact, ingestion of cheeses made with probiotic bacteria has been associated with a variety of health benefits such as improved immune response, serum cholesterol reduction, synthesis of vitamins, anti-cancer and

antibacterial activity (Gomes and Malcata, 1999; Medici *et al.*, 2004; Hatakka *et al.*, 2007; Ibrahim *et al.*, 2010; Modzelewska-Kapitulka *et al.*, 2010). Therefore, the addition of these strains to a fresh sheep cheese would represent an increase in value addition, which already has advantages inherent in its composition (Albenzio *et al.*, 2013a).

Nevertheless, it should be noted that, according to the amount, the addition of probiotic strains may influence (positively or negatively) cheese ripening through an increase in enzymatic processes that lead to changes in the sensory characteristics of the product, such as flavor and texture (Urala and Lähtenmäki, 2004; Buriti, da Rocha and Saad, 2005). In this sense, the results reported are different. Gomes da Cruz *et al.* (2009) found that the addition of 0.8% (w/v) of *Lb. acidophilus* in Minas type cheese, reduced consumer acceptance (compared to conventional commercial cheese), due to the low pH values and increased production of organic acids due to microbial metabolism, resulting in alterations in their appearance, aroma, taste and texture. Similarly,

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Grattepanche *et al.* (2008) reported that in Cheddar type cheese added with bifidobacteria, it significantly increased the production of acetic acid.

On the other hand, even though there are a few studies about the effects on sheep cheeses manufactured with probiotic strains (Corbo *et al.*, 2001; Albenzio *et al.*, 2010); it has been reported that the inclusion of appropriate amounts of bifidobacteria and lactobacilli produces increased levels in proteolysis and lipolysis, which could improve the sensory characteristics (Santillo *et al.*, 2009). Additionally, in Argentina there have been reported interesting and promising results about the incorporation of commercial probiotic strains in sheep cheese (Cuffia *et al.*, 2012) and in the optimization of technology for its production thereof (Cuffia *et al.*, 2015). In this sense, the selection of probiotic strains and determination of their concentration to add are vital for the development of new probiotic cheeses with differential sensory characteristics. Accordingly, the aim of this study was to evaluate the survival of two probiotics strains, *Lactobacillus acidophilus* (LA-5) and *Bifidobacterium animalis* subsp. *lactis* (BB-12) and its influence on the composition, proteolysis and sensory characteristics of a fresh sheep cheese.

The aim of this work was to evaluate the survival of two commercial strains of probiotic bacteria: *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Lactobacillus acidophilus* LA-5, and their influence on the composition, proteolysis and sensory characteristics of soft sheep cheese.

## Materials and Methods

### Starter and probiotic cultures

A cheese starter culture consisting of *Streptococcus salivarius* subsp. *thermophilus* ST-M5 (Chr. Hansen, Inc., Denmark) and the probiotic cultures of *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Lactobacillus acidophilus* LA-5 (Chr. Hansen, Inc., Denmark) were used as cultures (freeze-dried) in cheesemaking experiments. Both commercial probiotic cultures from Chr. Hansen have probiotic functions which have been demonstrated in several works: Shioya *et al.* (2000), Sheu *et al.* (2002), Chourraqui *et al.* (2004) and Pitkälä *et al.* (2007) for *B. animalis* BB-12; and Shioya *et al.* (2000) and Sheu *et al.* (2002), for *Lb. acidophilus* LA-5.

### Cheesemaking

Raw sheep milk, provided by the School of Agriculture, Farm and Livestock from Litoral National University (EAGyG-UNL), was refrigerated and transported at 4°C to the pilot plant of our

Institute (Industrial Lactology Institute), where it was kept frozen at -20°C until its use. On each cheese making day, 40 L of raw milk were unfrozen and pasteurized at 65°C for 20 minutes, cooled to 39°C (temperature of coagulation) and divided into three vats. Starter and probiotic cultures, previously resuspended in 100 mL of sterile milk, were added at a concentration of 10<sup>7</sup> CFU mL<sup>-1</sup> of milk. Three batches of soft sheep cheeses were made comprising a control (QT) and two different probiotic cheeses added with *Bifidobacterium animalis* subsp. *lactis* BB-12 (QBb) and *Lactobacillus acidophilus* LA-5 (QLa), respectively. After 10 min, chymosin produced by fermentation of *Aspergillus niger* var. *wamori* (Chy-Max, Inc. Chr Hansen, Denmark. 183 IMCU/mL) was added in a suitable dose to obtain the proper firmness for cutting the curd. After 15-20 min., the curd was cut to the appropriate grain size (approximately 25 mm). After 15 min, the mixture was stirred gently to achieve proper moisture. Then, the whey was removed and the curd was placed into molds, and kept in a warm chamber (40°C - 3h) until reaching pH 5.10 ± 0.05. Afterwards, the cheeses were held in a conditioning chamber at 4°C and 92% relative humidity (with air circulation at reduced speed to avoid excessive surface evaporation), for 24 h. This stage has the purpose of regulating the cooling rate and allows the development of fermentation to compensate for the increase in pH derived from the salts balance, which occurs during brining (Cuffia *et al.*, 2011). Approximately 4 kg of cheese obtained, was divided into eight portions of 500 g and salted by immersion in brine at 15% p/w, as established in a previous work (Cuffia *et al.*, 2015). 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 maturation. Three replicates of cheeses were made on successive cheese-making days.

### Survival of bacteria in cheeses

Viability of starter and probiotic bacteria was assessed during production (in inoculated milk and acidified curd), and in cheeses during ripening at 4, 15 and 30 days according to Vinderola *et al.* (2003). Starter bacteria was enumerated on M17 agar (Biokar Diagnostics, Beauvais, France) after incubation under microaerobic condition at 37°C for 48 h. The counts of *Bifidobacterium animalis* subsp. *lactis* were determined on MRS agar with addition of 0.1% (w/v) cysteine (Biopac - Buenos Aires, Argentina) (MRSc), after incubation under anaerobic conditions (AnaeroPack® - Anaero,

Mitsubishi Gas Chemical CO., INC., Tokyo, Japan) at 37°C for 48 h. *Lactobacillus acidophilus* was enumerated on MRS agar, with addition of 0.1% (w/w) sorbitol, after incubation under anaerobic conditions (AnaeroPack®-Anaero, Mitsubishi Gas Chemical CO., INC., Tokyo, Japan) at 43°C for 72 h.

#### *Gross composition and pH*

Cheese samples were grated and analyzed in duplicate for fat matter by Gerber van Gulik method (FIL - IDF N° 152 A 1997), moisture by oven-drying at 102°C (AOAC 926.08, 1990), and total protein by Kjeldahl method (AOAC 920.123, 1990) using a Digestion System 6 (1007 Digester, Tekator, Switzerland) and BÜCHI Distillation Unit B-324 (Sweden). The pH of cheese was measured according to Bradley *et al.* (1993), with a pH meter (Orion Research Incorporated, United States) introduced in slurry prepared by blending a mix 1:1 of grated cheese in H<sub>2</sub>O. Cheese composition and pH were analyzed at day 4, 15 and at the end of ripening (30 days).

#### *Soluble nitrogen (SN)*

Cheese samples were treated to obtain crude citrate extract and soluble fractions at pH 4.6 (SN 4.6), in TCA 12% (SN TCA) and PTA 2.5% (SN PTA), according to Hynes *et al.* (2003). The crude cheese extract was obtained by adding 20 mL of 0,5 M sodium citrate 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 x 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 (IDF, 1993).

#### *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 for about 10 min under 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.

Electrophoretic 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 Coomassie blue G-250.

#### *Sensory analysis*

Descriptive sensory analysis was performed at the end of the ripening (30 days). The three 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: odor, color, appearance of mass, elasticity, mouthfeel, cream flavor, salty taste, bitter taste, acid taste and residual flavor.

#### *Statistical analysis*

The results were processed by analysis of variance (ANOVA) using Statgraphics Plus v3.0 software (Statistical Graphics Corp.) to determine the effect of the addition of probiotic bacteria on gross composition, pH, proteolysis and sensory characteristics of cheeses. When significant differences were presented ( $p < 0.05$ ), Duncan's test was applied to detect homogeneous groups of means, using the same software.

## **Results**

#### *Cheese composition during ripening*

Values of pH were similar in control and probiotic cheeses at each sampling day (Table 1). Nevertheless, in all cheeses, pH increased significantly along the ripening. On the other hand the levels of moisture, fat matter and total protein were also similar between control and probiotic cheeses, which demonstrate that the cheese making was reproducible. Furthermore, the values of moisture were established according to the Argentinean legislation (Código Alimentario Argentino).

#### *Survival of bacteria during manufacture and repining of soft sheep cheeses*

Microbiological counts of starter bacteria increased approximately one log order in the curd regarding its concentration in milk and were kept in a range of 8 log<sub>10</sub> throughout ripening time (Table 2). In addition, starter population was similar in control and experimental cheeses, demonstrating that the addition of probiotic bacteria in experimental cheeses did not affect starter viability.

#### *Proteolysis assessment*

The nitrogen content in the different soluble

Table 1. pH and gross composition of control (QT) and probiotic (QBb and QLa) soft sheep cheeses during ripening

	Moisture (%w/w)			Fat Matter (%w/w DM)			Protein (%w/w DM)			pH		
	4 days	15 days	30 days	4 days	15 days	30 days	4 days	15 days	30 days	4 days	15 days	30 days
QT	49.03±0.15	48.06±0.45	46.70±0.30	54.81±1.68	53.41±1.59	54.16±1.26	43.95±0.84	42.82±1.17	41.22±0.58	5.16±0.04	5.23±0.02	5.32±0.04
QBb	48.60±1.13	47.70±0.80	46.46±0.57	53.53±2.35	53.49±2.19	52.75±0.98	44.11±1.15	42.64±0.35	43.53±0.97	5.17±0.03	5.24±0.02	5.31±0.04
QLa	48.56±0.25	47.13±0.15	46.23±0.15	53.27±0.89	51.51±0.47	53.44±0.91	43.29±0.50	42.05±0.91	42.22±0.73	5.13±0.04	5.23±0.01	5.29±0.02

Values are means of three replicates analyzed in duplicate.

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

DM. Dry Matter

Table 2. Viable counts ( $\text{Log}_{10}$  CFU/g) of primary starter and probiotic bacteria during cheesemaking (milk and curd) and ripening of control and probiotic soft sheep cheeses.

Cheese	Bacteria	Cheese-making		Cheeses during ripening		
		Milk	Curd	4 days	15 days	30 days
QT	Starter	7.56±0.12	8.67±0.14	8.50±0.09	8.48±0.16	8.33±0.16
QBb	Starter	7.50±0.15	8.58±0.21	8.40±0.30	8.28±0.30	8.16±0.14
	<i>Bifidobacterium bifidum</i>	7.30±0.23	8.57±0.46	8.36±0.44	8.22±0.26	7.99±0.17
QLa	Starter	7.34±0.18	8.45±0.08	8.39±0.09	8.26±0.11	8.19±0.10
	<i>Lactobacillus acidophilus</i>	7.37±0.31	8.57±0.22	8.45±0.23	8.27±0.35	8.15±0.13

Values are means of three replicates evaluated in duplicate

fractions (SN), and the value of Degree of Ripening for the control (QT) and probiotic (QBb and QLa) soft sheep cheeses are shown in Table 3. The values of each fraction increased during ripening as a result of the proteolytic process. The levels of the fractions of SN pH 4.6 and SN TCA, and the values of the Degree of Ripening, were similar in all cheeses at each ripening time. On the contrary, the levels of the fraction of SN PTA showed significant ( $p < 0.05$ ) differences between QT, QBb and QLa, at each sampling time. The results of this fraction were in the following order QT < QBb < QLa.

Urea-PAGE electrophoretic profiles of pH 4.6 insoluble fraction for control and probiotic cheeses are presented in Figure 1. All cheeses exhibited a very similar profile, which is in agreement with the similar levels in the SN 4.6 fraction.

#### Sensory characteristics

Results of descriptive sensory analysis of the cheeses after 30 days of ripening are shown in Figure 2.

The addition of the probiotic bacteria BB-

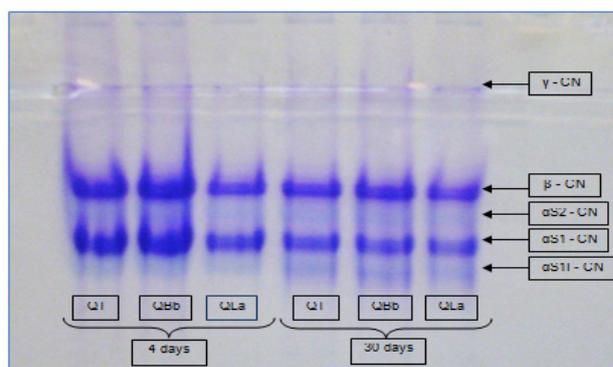


Figure 1. Urea-PAGE electrophoretic profiles for the control (QT) and probiotic (QBb and QLa) Argentinean soft sheep cheeses during ripening (4 and 30 days).

12 and LA5 in soft sheep cheeses did not modify organoleptic characteristics associated with the odor, colour, cream flavor, residual flavor, salty, bitter and acid taste. However, the scores for the appearance of mass, elasticity and mouthfeel were significantly higher ( $p < 0.05$ ) in both cheeses inoculated with probiotic bacteria: QBb and QLa, in comparison to control cheese (QT).

Table 3. Evolution of soluble nitrogen at pH 4.6 (SN 4.6), in trichloroacetic acid (SN TCA) and in phosphotungstic acid (SN PTA), expressed as g of nitrogen 100 g<sup>-1</sup> of cheese, and degree of ripening (SN 4.6 / TN), for the control (QT) and probiotic (QBb and QLa) soft sheep cheeses during ripening.

		Cheeses		
		QT	QBb	QLa
SN 4.6	4 day	0.20 ± 0.01	0.20 ± 0.01	0.18 ± 0.02
	15 day	0.28 ± 0.02	0.26 ± 0.01	0.25 ± 0.01
	30 day	0.36 ± 0.01	0.36 ± 0.02	0.36 ± 0.02
SN TCA	4 day	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
	15 day	0.11 ± 0.01	0.11 ± 0.01	0.10 ± 0.01
	30 day	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.01
SN PTA	4 day	0.02 ± 0.01 <sup>a</sup>	0.03 ± 0.01 <sup>b</sup>	0.04 ± 0.01 <sup>c</sup>
	15 day	0.02 ± 0.01 <sup>a</sup>	0.03 ± 0.01 <sup>b</sup>	0.04 ± 0.01 <sup>c</sup>
	30 day	0.02 ± 0.01 <sup>a</sup>	0.03 ± 0.01 <sup>b</sup>	0.04 ± 0.01 <sup>c</sup>
Degree of Ripening (SN 4.6 / TN)	4 day	4.50 ± 0.30	4.60 ± 0.30	4.70 ± 0.40
	15 day	6.30 ± 0.30	6.00 ± 0.20	6.50 ± 0.20
	30 day	8.20 ± 0.10	8.40 ± 0.40	8.30 ± 0.40

Values are expressed as means of three replicates evaluated in duplicate.

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

The letters have not been included in the cases when significant differences were not found ( $p < 0.05$ ).

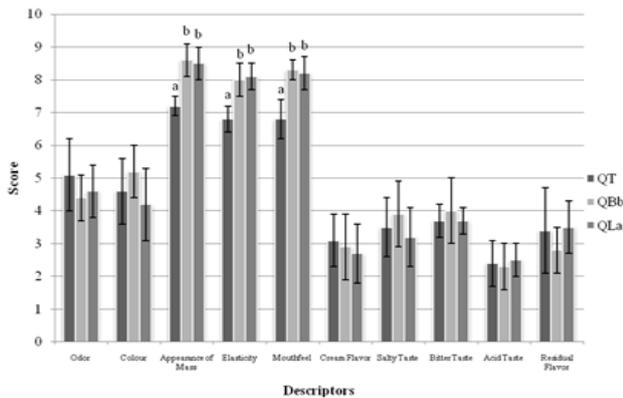


Figure 2. Sensory descriptors analyzed for the control (QT) and probiotic (QLa and QBb) Argentinean soft sheep cheeses at the end of ripening (30 days). Values are expressed as means of three replicates evaluated in duplicate. Values with different letters for each descriptor are significantly different ( $p < 0.05$ ).

## Discussion

Increase of pH along the ripening could be due to the great mineral content (mainly calcium) of ovine milk, which provides high buffer capacity. In effect, since most of  $\text{Ca}^{2+}$  is associated with the colloidal

fraction (roughly 75-80% of total) (Park *et al.*, 2007; Ramos and Juarez, 2011), its release from the micelles, owing to ionic equilibrium during ripening, can raise the pH.

Viability of strains is consistent with the fact that starters provide the most significant contribution to the microbial biomass in young curd, typically attaining densities of  $10^8$  CFU g<sup>-1</sup> within one day of manufacture (Beresford and Williams, 2004). This is a very important aspect, since one of the prerequisites of probiotics strains, is that they must be compatible with cheese starter cultures, and must not affect their growth (Tamime *et al.*, 2005). Similar to the case of the starter, viable cell counts of probiotic bacteria in the curd were one log order higher than the levels in the milk, and the counts remained during ripening at concentration  $>10^8$  CFU g<sup>-1</sup> in cheeses (Table 2). These results show that soft sheep cheeses can be an efficient vehicle for probiotic bacteria until they reach the consumer. It is important to remark that in previous works conducted by our institute, we showed that Argentine cow cheeses, both semihard and soft, are good carriers for different probiotic cultures due to the high probiotic viability demonstrated during

all ripening time (Vinderola *et al.*, 2003; Bergamini *et al.*, 2009; Burns *et al.*, 2012). In general, cheeses show great advantages over other fermented dairy products like yogurt as a vector for incorporating probiotic bacteria to diet because they have a higher pH and buffer capacity, higher consistency and more fat content (Ong and Shah, 2009). These characteristics would be able to offer more protection to probiotic bacteria during storage and transit in the gastrointestinal tract (Gardiner *et al.*, 1998).

The nitrogen content in the soluble fraction at pH 4.6 represents the primary proteolysis, and it is produced from the breakdown of intact caseins (especially  $\alpha$ s1 and  $\beta$  caseins) mostly by non-microbial proteases. In soft cheeses, in particular, residual rennet is the main non-microbial agent which participates in proteolysis during ripening (Delacroix-Buchet and Fournier, 1992; Vélez *et al.*, 2015).

On the other hand, the nitrogen content in the SN TCA and SN PTA represents the secondary proteolysis, which is mainly produced by the activity of microbial proteolytic enzymes from starter and adjunct cultures and also from NSLAB (non-starter lactic acid bacteria) (Fox *et al.*, 1996). These enzymes hydrolyze large and medium peptides leading to the production of smaller peptides and free amino acids. The compounds contained in SN TCA are medium-sized to small peptides, amino acids and smaller nitrogen compounds, such as amines, urea and ammonium, whereas SN PTA fraction contains very small peptides, amino acids and smaller N compounds except dibasic amino acids and ammonia (Ardö, 1999). The addition of both probiotic strains produced a significant impact on the secondary proteolysis but only on the levels of the SN PTA, being this effect higher for *Lb. acidophilus*. Thus, our results show that these strains, despite not affecting the primary proteolysis, have peptidolytic enzymes which led to an increase in the production of small peptides and amino acids. Similar to our results, Gardiner *et al.* (1998) and McBrearty *et al.* (2001) did not detect any influence of probiotic cultures of lactobacilli and bifidobacteria on primary proteolysis of Cheddar cheese, evaluated by means of the peptide profiles obtained by size exclusion HPLC of water-soluble peptides. In addition, other authors also found some differences in the secondary proteolysis between cheeses with and without probiotic bacteria. Corbo *et al.* (2001) verified that peptide profiles obtained by reversed-phase fast protein liquid chromatography of the soluble fraction of probiotic Canestrato Pugliese cheeses (sheep cheeses) containing bifidobacteria were more complex than

those of control cheese. Likewise, Ong *et al.* (2006) showed an increase of the fraction of SN PTA after 4 months of ripening in Cheddar cheese with probiotic strains of *Lb. acidophilus*, *Lb. casei*, *Lb. paracasei*, and bifidobacteria.

In previous works, we found that different strains of probiotic bacteria produced a different impact on secondary proteolysis of probiotic semihard cheeses, made with cow milk. In particular, *Lb. acidophilus* produced the higher effect with an increase in the levels of free amino acids and the fraction of SN PTA, while *Lb. paracasei* only influenced on the levels of a few amino acids and *Bb. lactis* did not produce any change in the proteolytic process (Bergamini *et al.*, 2009). The differences found in the proteolytic activity of probiotic bacteria can be attributed to the heterogeneity in their peptidolytic potential, and actual activity of the enzymes in the cheese matrix, which are largely strain-dependent (Corbo *et al.*, 2001; Ong *et al.*, 2007; Gómez *et al.*, 2010; Albenzio *et al.*, 2013b).

The Urea-PAGE results suggested that, in general,  $\alpha$ s1 casein underwent a limited hydrolysis, mainly by the residual rennet, releasing the typical peptide  $\alpha$ s1-I casein, which was more noticeable at 30 days of ripening (Irigoyen *et al.*, 2002).

Sensory analysis is very useful to know the organoleptic properties of the cheese through the senses. It is fundamental to determine the acceptability of the product by the consumer, this constituting a very important parameter for marketing the product (Carpenter *et al.*, 2000). Appearance and mouthfeel attributes are important drivers of consumer liking, as they can affect choice behavior (Wadhvani and McMahon, 2012). These results demonstrate that both commercial probiotic cultures did not produce defects of flavor, and could even improve organoleptic characteristics of soft sheep cheeses, apart from providing probiotic characteristics based on the properties of these strains.

The addition of probiotic cultures should not result in lower acceptance of the food compared with a similar conventional product (Cruz *et al.*, 2010). Some authors have reported the effect of the addition of probiotic cultures on the sensory characteristics of different types of cheeses. In most of these works it is highlighted that the influence is dependent on the strain (Albenzio *et al.*, 2013a). While some strains produce an improvement in the sensory properties (Minervini *et al.*, 2012), other strains do not produce changes or produce defects in the product (Tungjaroenchai *et al.*, 2001; Sarantinopoulos *et al.*, 2002). Dinakar and Mistry (1994) reported that bifidobacteria added to Cheddar cheese did not affect

the taste, texture or appearance through 24 weeks of storage. In particular bifidobacteria added cheeses had higher concentrations of acetic and lactic compared to control ones but the sensory characteristics were unchanged (Gobbetti *et al.*, 1998; Ong *et al.*, 2007). Buriti, da Rocha and Saad (2005) observed that apart from increasing proteolysis (as in our results), the addition of *Lb. acidophilus* to Minas fresh cheese had no influence on flavor and preference compared with a control cheese. In addition, the incorporation of the *Lb. acidophilus*, when producing the previously mentioned fresh cheese, conferred sensory stability during storage of the product for up to 14 d, particularly in cheese produced with the addition of the probiotic in co-culture with *S. thermophilus* (Souza *et al.*, 2008).

## Conclusion

*Bifidobacterium animalis* subsp. *lactis* BB-12 and *Lactobacillus acidophilus* LA-5 showed suitable properties for their use as adjunct probiotic cultures in soft sheep cheeses, in which they achieve the dual role of being secondary starters and probiotic cultures. During ripening, they remained viable in the cheese matrix, showing high counts, always in the order of  $8 \text{ Log}_{10} \text{ CFU g}^{-1}$ . They neither affected the overall composition nor did they produce over-acidification. Moreover, BB-12 and LA-5 slightly increased the level of secondary proteolysis and improved some organoleptic characteristics of cheeses. Soft sheep cheese is a good matrix for use as carrier of commercial probiotic strains studied in the present work.

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