

## Technological, physicochemical and sensory characteristics of a Brazilian semi-hard goat cheese (coalho) with added probiotic lactic acid bacteria

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**ABSTRACT:** Over the past few years, the use of probiotics, which are capable of exerting beneficial effects on the composition of intestinal microbiota, has increased. Cheeses have been suggested as a better carrier of probiotic bacteria than other fermented milk products. The effect of added cultures of probiotic lactic acid bacteria on the quality of a Brazilian goat semi-hard cheese (coalho) was assessed during 21 days of storage at 10 °C as follows: C1, *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* (standard cheese); C2, *Lactobacillus acidophilus* (LA-5); C3, *Lactobacillus paracasei* (*Lactobacillus casei*-01); C4, *Bifidobacterium lactis* (BB-12); and C5, *L. acidophilus*, *L. paracasei* and *B. lactis*. Differences in some physico-chemical, experimental texture and proteolysis parameters were found among the assessed cheeses. All of them presented high luminosity (L\*) with predominance of the yellow component (b\*). Numbers of lactic acid bacteria in the cheeses were greater than 10<sup>7</sup> cfu g<sup>-1</sup> during storage. Cheeses with the added probiotic strains alone and in co-culture were better accepted than cheeses without the probiotic strains. It is suggested that goat “coalho” cheese could be a potential carrier of probiotic lactic acid bacteria.

Keywords: dairy product, probiotic microorganisms, quality evaluation

### Introduction

In recent years, consumers have become more interested in the general quality of foods. Most consumers are concerned not only about safety and nutritional value but also about health benefits (Saarela et al., 2002). The Food and Agriculture Organization defines food-related probiotics as living organisms that, upon ingestion in certain numbers, exert health benefits to the consumer's health beyond inherent basic nutrition (FAO, 2001).

Probiotics are efficacious in treatment of gastrointestinal disorders, respiratory infections and allergic symptoms (Wohlgemuth et al., 2010) and also reduce blood cholesterol and improve immunity (Shah, 2007). The minimal population of the probiotic bacteria to ensure a favorable impact on health is 10<sup>7</sup> cfu g<sup>-1</sup> or mL<sup>-1</sup> of food at the time of consumption (De Vuyst, 2000). Furthermore, a probiotic strain must be amenable to food-grade production on an industrial scale, remain under good viability and functionality during storage and food consumption (Mätö et al., 2006), resist the bile salts and acidic conditions of the upper gastrointestinal tract (Cruz et al., 2009), adhere to the intestinal epithelium of hosts and possess antagonistic activity against bacterial pathogens (Lin et al., 2006).

Strains belonging to the genera *Lactobacillus* [*L. acidophilus* (LA-5), *L. paracasei* (*L. casei*-01)] and *Bifidobacterium* [*B. lactis* (BB 12), *B. longum* (1941)] have been evaluated for probiotic properties with satisfactory results (Buriti et al., 2005a; Buriti et al., 2005b; Buriti et al., 2007b; Ong and Shah, 2009; Phillips et al., 2006; Souza and Saad, 2009). These bacterial strains are normal constituents of the intestinal flora and present a long history of safe application in foods.

Considering the survival and viability of probiotic cultures, fermented dairy products, such as cheeses, yogurts and fermented milks, are promising food delivery systems for these cultures. Cheese has been suggested as a better carrier of probiotic bacteria than other fermented milk products due to its pH, higher content of fat and solid consistency, which offer greater protection to these microorganisms in the gastrointestinal tract (Ong et al., 2006).

“Coalho” cheese is a Brazilian product that has been produced for over 150 years in various states of the northeast region of Brazil. Coalho cheese is a semi-hard cheese with medium-to-high moisture produced from raw or pasteurized cow or goat milk. It is obtained after milk coagulation using rennet or proper coagulating enzymes, which are sometimes complemented with selected lactic acid bacteria. Coalho cheese is commonly marketed after seven days of storage at 10 °C (Brasil, 1997). This product has a high commercial value because its production technology is simple, the yield is high and consumers readily purchase it.

Probiotic bacteria, such as lactobacilli, bifidobacteria and enterococci, have been successfully incorporated into different cheese varieties, such as cheddar (McBrearty et al., 2001), Gouda (Gomes et al., 1998), Crescenza (Gobbetti et al., 1998), cream cheese (Buriti et al., 2007a) and Minas fresh cheese (Buriti et al., 2005b; Vinderola et al., 2000). The addition of selected lactic acid bacteria in simple and mixed cultures in cheeses can improve bacterial viability and increase the acidification, flavor development and sensory characteristics of the cheese during storage (Buriti et al., 2005a; Buriti et al., 2005b; Buriti et al., 2007c).

Currently, there is a lack of studies emphasizing the incorporation of probiotic cultures into coalho

goat cheese and the influence of probiotics on the quality parameters of the cheese during storage. Regarding these aspects, this study was performed with the main purpose of assessing the technological, physicochemical and sensory characteristics of coalho goat cheese supplemented with *Lactobacillus acidophilus* (LA-5), *Lactobacillus paracasei* (*L. casei*-01) and *Bifidobacterium lactis* (BB 12) in single and mixed culture during cold storage. A secondary aim was to assess the viability of the added probiotic strains in the cheeses during the evaluated storage periods.

## Materials and Methods

**Cultures** – The following freeze-dried commercial cultures used in the production of the cheeses were obtained in Valinhos, state of São Paulo, Brazil (22°58'14' S; 46°59'45' W): *Lactococcus lactis* subsp. *Lactis* and *Lactococcus lactis* subsp. *cremoris* (R704, batch 2937721) (non-probiotic cultures used as starter cultures in the standard method for the manufacture of coalho cheese); *Lactobacillus acidophilus* (LA-5, batch 2914230); *Lactobacillus paracasei* (*L. casei*-01, batch 2910976); and *Bifidobacterium lactis* (BB 12, batch 2280202). Five bacterial cultures (10 mg L<sup>-1</sup> of milk) were added during the production of the cheeses as follows: C1, *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*; C2, *L. acidophilus*; C3, *L. paracasei*; C4, *B. lactis*; and C5, *L. acidophilus*, *L. paracasei* and *B. lactis* (1:1:1 ratio).

**Manufacturing process of coalho goat cheeses** – Coalho cheeses were manufactured in 10-L vats from commercially pasteurized (65 °C per 15 min) goat milk heated to 45 ± 1 °C and followed by direct acidification with lactic acid (0.85 mL 100 mL<sup>-1</sup>) in an amount of 0.25 mL L<sup>-1</sup>. The cultures of lactic acid bacteria were added at a concentration of 10 mg L<sup>-1</sup> for direct vat inoculation. Calcium chloride (0.5 mL L<sup>-1</sup>) and a commercial coagulating agent (0.9 mL L<sup>-1</sup>) were also added to the vats. The vats were incubated at 36 °C until a firm curd was formed (approximately 40 min). The obtained gel was gently cut into cubes (1.5 to 2.0 cm), allowed to drain, placed in perforated rectangular containers (approximate capacity of 250 g), salted in brine (12 g L<sup>-1</sup> NaCl), maintained at 36 °C under pressure for 4 h and vacuum packaged. The cheese obtained after storage at 10 °C for 24 h was regarded as the final product. Each cheese was manufactured three times (replicates), and all analyses were performed in triplicate. The results of the analysis are presented as averages of the data collected for each replicate. For each replication, the cheeses were manufactured from the same milk batch.

**Analysis** – Cheeses from each treatment were used for physicochemical and technological analyses. The cheeses were analyzed both immediately (day 1) and after 7, 14 and 21 days of storage at 10 °C. Each day, three cheeses from the same batch and trial were unpacked. The samples (25 g) were collected aseptically from parts of the

cheeses for microbiological analysis. For the instrumental texture profile analysis, at least 0.5 cm of the rind of the cheeses was discarded, and the cheese samples were collected from the center to the outer part. The rest of the cheese was grated and immediately used for physicochemical, microbiological and sensory analyses.

**Yield and syneresis** – The yield of each batch is expressed as the fresh weight of the cheese obtained from each liter of milk used for its production (g of cheese L<sup>-1</sup> of milk). The syneresis (expulsion of whey from coagulum) was calculated as the weight of whey in grams released from each kilogram of cheese in the package after the various storage times, divided by the weight of cheese of the same package in grams and multiplied by 100 (Buriti et al., 2005b).

## Gross composition and physicochemical analysis

– Titratable acidity was determined according to the appropriate standard method (AOAC, 2005) and expressed in terms of acidity in lactic acid (g 100 g<sup>-1</sup>). Water activity (A<sub>w</sub>) was determined at 25 °C. Moisture and dry matter contents were determined by drying 5 g of the samples at 105 °C until constant weight. The ash content was determined gravimetrically by heating the samples (3 g) at 550 °C until complete combustion. The protein content was estimated by measuring the N content by the Kjeldahl method and multiplying the value by a conversion factor (6.38) after drying the cheese samples (1 g). The fat content was determined by Gerber's method, and sodium chloride (NaCl) content was analyzed using the Mohr method. The analysis of lactose was performed by the Fehling reducing method. All analyses were performed in triplicate according to the standard methods suggested by AOAC (2005).

**Analysis of proteolysis and meltability** – The soluble nitrogen (SN) content in 1.2 g L<sup>-1</sup> of trichloroacetic acid (TCA) and the SN content at pH 4.6 in the cheese samples were determined using a micro-Kjeldahl method (AOAC, 2005). Proteolysis was evaluated with the proteolysis index (EPI) and depth of proteolysis index (DPI) using the following equations: EPI = (SN at pH 4.6) / TN (total nitrogen) × 100; and DPI = (SN in TCA) / TN × 100 (Andreatta et al., 2007). Meltability of the cheese samples was determined by the Schreiber test, as previously described by Koca and Metin (2004). The cheese samples (4-6 °C) were prepared using a glass borer and a sharp knife. The samples (36 mm × 7 mm) were placed on a Petri dish, which was then placed into an electrical oven preheated to 107 ± 1 °C for 5 min. The samples were removed from the oven and cooled for 30 min at room temperature. Sample expansion was measured using a scale with six lines (A - E) marked on a concentric set of circles. Schreiber meltability was expressed as the mean of six readings using an arbitrary scale (0-10 units) (Park et al., 1984).

**Analysis of instrumental texture profile** – The textural properties of the cheeses were evaluated using a two-bite compression of cylindrical samples (diameter of 5.0 cm and height of 2.0 cm) with a probe of 25.4 mm. The employed compression ratio was 50 % deformation from the initial height of the sample at a rate of 1 mm s<sup>-1</sup>. The compression distance was 10 mm, the contact force was 5.0 g, and the time between cycles was 5 s. After being cut, the cheese samples were stored in boxes containing ice prior to testing. The measured parameters consisted of hardness, springiness, adhesiveness, cohesiveness, chewiness and gumminess, which were obtained with the Texture Expert for Windows software version 1.20 (Stable Micro Systems).

**Color evaluation** – A CR-300 colorimeter was used for the instrumental color evaluation. The CIELab color scale (L\*a\*b\*) was used with a D<sup>65</sup> illuminant (standard daylight) at a 10° angle. The L\*, a\* and b\* parameters were determined according to the standards set by the International Commission on Illumination (CIE, 1986). Using reference plates, the apparatus was calibrated in the reflectance mode with specular reflection excluded. A 10-mm quartz cuvette was used for the readings. Measurements were performed in triplicate using the inner section of the cheeses immediately after unpacking.

**Counts of lactic acid bacteria** – Initially, a sample (25 g) of each cheese was blended with 225 mL of peptone water (1 g per L) in a Bag Mixer and submitted to serial dilutions (10<sup>1</sup> – 10<sup>-5</sup>) with the same diluent. A sample (1 mL) of each dilution was plated onto DeMan-Rogosa-Sharpe agar using the pour plate method and incubated for three days at 37 °C in an anaerobic atmosphere (Anaerobic System Anaerogen, Oxoid). The counts were expressed in colony forming units per gram of cheese (log cfu g<sup>-1</sup>) (FDA, 1992).

**Sensory evaluation** – Sensory evaluation was performed by three methods: the acceptance test, purchase intention and preference ranking test. These tests were performed using 50 non-trained panelists pre-selected according to interest with a habit of consuming coalho

cheese. All sensory evaluation assays were performed with the same panelists who worked in individual booths with controlled temperature and lighting conditions. Each panelist was served 20 g of each cheese on a small white plate coded with a random three-digit number, and the samples of the five types of cheese were served simultaneously using a blind method in a randomized sequence (assuring that each panelist was served with a specific order of samples) immediately after being taken out of the refrigerated storage. The panelists were asked to use low-salt crackers and water to cleanse their palates between the samples. The acceptability of appearance, color, flavor, taste, texture and general perception were evaluated on a 9-point unstructured hedonic scale ranging from 1 (dislike very much) to 9 (like very much). Purchase intention was evaluated using a 5-point unstructured hedonic scale ranging from 1 (certainly would not purchase) to 5 (certainly would purchase). For the preference ranking test, the panelists were asked to choose the most and least preferred sample based on their overall impressions.

**Statistical analysis** – The means of the results were evaluated using analysis of variance (ANOVA), and the Tukey test was used to compare differences ( $p < 0.05$ ) among the technological, physicochemical, microbiological and sensory evaluations. Statistical Analysis System (SAS, 1999) was used for the statistical analyses.

## Results and Discussion

The yields and synereses for the coalho goat cheeses with added probiotic lactic acid bacteria during storage at 10 °C (Table 1) ranged from 130.90 to 142.83 g L<sup>-1</sup>. Higher yields ( $p < 0.05$ ) were found for cheese C3 (*L. paracasei*) compared with cheeses C1 and C2.

The higher yield of C3 could be related to its lower acidity after one day of storage (Table 2). A similar result was observed with fresh white cheese supplemented with *L. paracasei* (Buriti et al., 2005b). Generally, cheeses produced through the direct addition of lactic acid to the milk (as in our study) resulted in higher yields and improved textures, and the products

Table 1 – Mean values (standard deviation) for yields of the final product and syneresis of coalho goat cheese with probiotic lactic acid bacteria during 21 days of storage at 10 °C.

Variables	Days of storage	Cheeses				
		C1	C2	C3	C4	C5
Yield <sup>1</sup>	1	133.60 (± 10.80) <sup>BC</sup>	130.90 (± 72.50) <sup>C</sup>	142.83 (± 33.80) <sup>A</sup>	136.27 (± 40.80) <sup>AC</sup>	139.84 (± 28.70) <sup>AB</sup>
	1	8.29 (± 0.47) <sup>BCc</sup>	18.50 (± 5.79) <sup>ab</sup>	10.01 (± 0.21) <sup>bb</sup>	2.45 (± 0.26) <sup>cc</sup>	2.27 (± 2.48) <sup>cb</sup>
	7	27.62 (± 1.89) <sup>Abc</sup>	42.87 (± 0.11) <sup>ab</sup>	43.24 (± 25.85) <sup>Ab</sup>	40.57 (± 6.56) <sup>Ab</sup>	51.08 (± 6.43) <sup>Aa</sup>
Syneresis <sup>2</sup>	14	49.25 (± 1.26) <sup>Aa</sup>	69.80 (± 30.31) <sup>Aa</sup>	68.91 (± 15.36) <sup>Aab</sup>	41.27 (± 12.58) <sup>Aab</sup>	60.47 (± 21.30) <sup>Aa</sup>
	21	34.78 (± 1.61) <sup>Bab</sup>	64.36 (± 10.17) <sup>ABa</sup>	103.08 (± 51.47) <sup>Aa</sup>	55.70 (± 15.47) <sup>Ba</sup>	77.19 (± 12.73) <sup>ABa</sup>

Results are averages of three replicates of cheeses manufactured at different times; the following abbreviations are used: C1, cheese treated with *Lactococcus lactis* subsp. *Lactis* and *Lactococcus lactis* subsp. *cremoris*; C2, cheese treated with *Lactobacillus acidophilus*; C3, cheese treated with *Lactobacillus paracasei*; C4, cheese treated with *Bifidobacterium lactis*; and C5, cheese treated with *L. acidophilus*, *L. paracasei* and *B. lactis*.<sup>1</sup> g of cheese L of milk<sup>-1</sup>; <sup>2</sup> g of whey kg of cheese<sup>-1</sup>; <sup>a-c</sup> For each trial, different superscript lowercase letters within a column denote differences ( $p < 0.05$ ) between values obtained for different days of storage according Tukey's test; <sup>A-C</sup> Different superscript capital letters within a row denote differences ( $p < 0.05$ ) between the values obtained for the different trials according to Tukey's test.

Table 2 – Mean values (standard deviation) for the gross composition and physicochemical characteristics of coalho goat cheese with probiotic lactic acid bacteria during 21 days of storage at 10 °C.

Variables	Days of storage	Cheeses				
		C1	C2	C3	C4	C5
Moisture (g 100g <sup>-1</sup> )	1	57.57 (± 0.41) <sup>Ca</sup>	56.62 (± 0.51) <sup>Ca</sup>	57.64 (± 1.10) <sup>BCa</sup>	59.18 (± 0.73) <sup>ABa</sup>	60.27 (± 0.31) <sup>Aa</sup>
	7	57.09 (± 0.15) <sup>Ca</sup>	57.48 (± 0.52) <sup>Ca</sup>	58.15 (± 0.88) <sup>BCa</sup>	58.83 (± 0.73) <sup>Ba</sup>	60.41 (± 0.05) <sup>Aa</sup>
	14	54.97 (± 0.12) <sup>Cb</sup>	56.72 (± 1.09) <sup>Ba</sup>	54.39 (± 0.99) <sup>Cb</sup>	59.46 (± 0.32) <sup>Aa</sup>	59.29 (± 0.30) <sup>Ab</sup>
	21	54.20 (± 0.11) <sup>Cb</sup>	56.06 (± 0.89) <sup>Ba</sup>	55.23 (± 0.09) <sup>Cb</sup>	56.76 (± 0.82) <sup>Bb</sup>	59.97 (± 0.14) <sup>Aa</sup>
Dry matter (g 100 g <sup>-1</sup> )	1	42.85 (± 0.17) <sup>Ac</sup>	43.47 (± 0.37) <sup>Aa</sup>	41.29 (± 0.36) <sup>Bb</sup>	40.39 (± 0.19) <sup>Bb</sup>	40.25 (± 0.94) <sup>Bb</sup>
	7	42.91 (± 0.15) <sup>Ac</sup>	42.52 (± 0.52) <sup>Aa</sup>	41.85 (± 0.88) <sup>ABb</sup>	41.18 (± 0.73) <sup>Bb</sup>	39.56 (± 0.08) <sup>Cb</sup>
	14	45.50 (± 0.12) <sup>Ab</sup>	43.28 (± 1.09) <sup>Ba</sup>	45.61 (± 0.99) <sup>Aa</sup>	43.23 (± 0.11) <sup>Ba</sup>	40.71 (± 0.30) <sup>Cb</sup>
	21	45.80 (± 0.11) <sup>ABa</sup>	43.94 (± 0.89) <sup>CDa</sup>	44.78 (± 0.09) <sup>BCa</sup>	43.24 (± 0.82) <sup>Da</sup>	46.86 (± 0.31) <sup>Aa</sup>
Ashes (g 100 g <sup>-1</sup> )	1	4.03 (± 0.04) <sup>Ba</sup>	4.25 (± 0.07) <sup>Aa</sup>	4.02 (± 0.11) <sup>Ba</sup>	4.37 (± 0.14) <sup>ABc</sup>	4.24 (± 0.05) <sup>Aa</sup>
	7	4.13 (± 0.15) <sup>Aa</sup>	4.25 (± 0.04) <sup>Aa</sup>	4.16 (± 0.19) <sup>Aa</sup>	4.32 (± 0.03) <sup>Ac</sup>	4.23 (± 0.06) <sup>Aa</sup>
	14	4.13 (± 0.09) <sup>Ba</sup>	4.25 (± 0.04) <sup>Ba</sup>	4.20 (± 0.06) <sup>Ba</sup>	4.56 (± 0.09) <sup>ABb</sup>	4.25 (± 0.06) <sup>Ba</sup>
	21	4.22 (± 0.20) <sup>Ba</sup>	4.10 (± 0.10) <sup>Bb</sup>	4.09 (± 0.11) <sup>Ba</sup>	4.70 (± 0.11) <sup>Aa</sup>	4.20 (± 0.02) <sup>Ba</sup>
Total protein (g 100 g <sup>-1</sup> )	1	21.82 (± 0.10) <sup>Ac</sup>	20.01 (± 0.16) <sup>Bc</sup>	20.32 (± 0.14) <sup>Bb</sup>	19.27 (± 0.32) <sup>Cb</sup>	18.42 (± 0.03) <sup>Bb</sup>
	7	22.73 (± 0.16) <sup>Ab</sup>	21.66 (± 0.12) <sup>ABa</sup>	19.98 (± 0.96) <sup>CDb</sup>	21.12 (± 0.43) <sup>Ba</sup>	19.44 (± 0.26) <sup>Da</sup>
	14	23.32 (± 0.37) <sup>Aa</sup>	20.69 (± 0.19) <sup>Cb</sup>	21.86 (± 0.60) <sup>Ba</sup>	20.96 (± 0.54) <sup>BCa</sup>	19.37 (± 0.64) <sup>Da</sup>
	21	21.59 (± 0.24) <sup>Ac</sup>	21.27 (± 0.33) <sup>Aa</sup>	20.87 (± 0.47) <sup>ABb</sup>	21.70 (± 0.74) <sup>Aa</sup>	19.10 (± 0.16) <sup>BAB</sup>
Fat matter (g 100 g <sup>-1</sup> )	1	17.00 (± 0.00) <sup>Ab</sup>	16.50 (± 0.00) <sup>BAb</sup>	17.25 (± 0.29) <sup>Ab</sup>	15.13 (± 0.25) <sup>Cc</sup>	16.13 (± 0.25) <sup>Ba</sup>
	7	18.63 (± 0.48) <sup>Aa</sup>	16.00 (± 0.58) <sup>Bb</sup>	18.63 (± 1.03) <sup>Aa</sup>	17.50 (± 0.00) <sup>Aa</sup>	16.13 (± 0.48) <sup>Ba</sup>
	14	18.83 (± 0.41) <sup>Aa</sup>	17.50 (± 0.41) <sup>Ba</sup>	19.13 (± 0.25) <sup>Aa</sup>	15.75 (± 0.29) <sup>Cbc</sup>	16.17 (± 0.26) <sup>Ca</sup>
	21	19.00 (± 0.45) <sup>Aa</sup>	16.75 (± 0.61) <sup>BAb</sup>	19.25 (± 0.65) <sup>Aa</sup>	16.63 (± 0.75) <sup>BAb</sup>	16.38 (± 0.25) <sup>Ba</sup>
Lactose (g 100 g <sup>-1</sup> )	1	1.54 (± 0.04) <sup>Ca</sup>	1.90 (± 0.02) <sup>Ba</sup>	2.08 (± 0.02) <sup>Aa</sup>	1.54 (± 0.04) <sup>Cc</sup>	2.09 (± 0.07) <sup>Aa</sup>
	7	1.07 (± 0.00) <sup>Bb</sup>	1.93 (± 0.02) <sup>Bb</sup>	1.56 (± 0.05) <sup>Cab</sup>	2.04 (± 0.07) <sup>Aa</sup>	1.85 (± 0.02) <sup>Bb</sup>
	14	0.99 (± 0.02) <sup>Cc</sup>	1.68 (± 0.02) <sup>BCc</sup>	1.32 (± 0.34) <sup>CDb</sup>	2.12 (± 0.02) <sup>Aa</sup>	1.76 (± 0.10) <sup>ABbc</sup>
	21	0.97 (± 0.02) <sup>Cc</sup>	2.03 (± 0.04) <sup>Aa</sup>	1.16 (± 0.37) <sup>BCb</sup>	1.94 (± 0.01) <sup>Ab</sup>	1.65 (± 0.02) <sup>Ac</sup>
NaCl (g 100 g <sup>-1</sup> )	1	0.61 (± 0.04) <sup>Bb</sup>	0.62 (± 0.06) <sup>Ba</sup>	0.58 (± 0.04) <sup>Bb</sup>	0.77 (± 0.05) <sup>Aa</sup>	0.55 (± 0.00) <sup>Bb</sup>
	7	0.67 (± 0.05) <sup>ABb</sup>	0.59 (± 0.04) <sup>Ba</sup>	0.70 (± 0.03) <sup>Aa</sup>	0.65 (± 0.02) <sup>ABb</sup>	0.63 (± 0.03) <sup>ABa</sup>
	14	0.66 (± 0.02) <sup>ABAb</sup>	0.61 (± 0.03) <sup>BCa</sup>	0.59 (± 0.05) <sup>Cb</sup>	0.69 (± 0.01) <sup>Ab</sup>	0.60 (± 0.02) <sup>Ca</sup>
	21	0.69 (± 0.01) <sup>Aa</sup>	0.53 (± 0.04) <sup>Ba</sup>	0.56 (± 0.01) <sup>Bb</sup>	0.65 (± 0.05) <sup>Ab</sup>	0.64 (± 0.03) <sup>Aa</sup>
pH	1	6.52 (± 0.16) <sup>Da</sup>	7.03 (± 0.02) <sup>BCab</sup>	7.16 (± 0.00) <sup>ABa</sup>	7.26 (± 0.01) <sup>ABb</sup>	6.93 (± 0.06) <sup>Ca</sup>
	7	6.31 (± 0.30) <sup>Ba</sup>	7.12 (± 0.09) <sup>ABb</sup>	7.06 (± 0.02) <sup>ABb</sup>	7.16 (± 0.08) <sup>Ab</sup>	6.98 (± 0.01) <sup>Aa</sup>
	14	6.01 (± 0.09) <sup>Da</sup>	7.25 (± 0.02) <sup>Aa</sup>	6.79 (± 0.05) <sup>Cb</sup>	7.16 (± 0.04) <sup>Ab</sup>	6.91 (± 0.05) <sup>Ba</sup>
	21	6.22 (± 0.41) <sup>Ca</sup>	6.88 (± 0.25) <sup>ABb</sup>	6.35 (± 0.25) <sup>Cc</sup>	7.38 (± 0.11) <sup>Aa</sup>	6.53 (± 0.16) <sup>BCb</sup>
Titratable acidity (g 100 g <sup>-1</sup> )	1	0.53 (± 0.00) <sup>Bc</sup>	0.71 (± 0.00) <sup>Aa</sup>	0.18 (± 0.00) <sup>Dd</sup>	0.53 (± 0.00) <sup>Ba</sup>	0.36 (± 0.00) <sup>Cb</sup>
	7	1.06 (± 0.00) <sup>Ab</sup>	0.53 (± 0.00) <sup>Cb</sup>	0.71 (± 0.00) <sup>Bc</sup>	0.44 (± 0.10) <sup>CDa</sup>	0.36 (± 0.00) <sup>Bb</sup>
	14	1.06 (± 0.00) <sup>Ab</sup>	0.53 (± 0.00) <sup>Bb</sup>	1.06 (± 0.00) <sup>Ab</sup>	0.35 (± 0.00) <sup>Ca</sup>	0.53 (± 0.00) <sup>Ba</sup>
	21	1.23 (± 0.00) <sup>Ba</sup>	0.35 (± 0.00) <sup>Dc</sup>	1.24 (± 0.00) <sup>Aa</sup>	0.35 (± 0.00) <sup>Da</sup>	0.53 (± 0.00) <sup>Ca</sup>
Aw	1	0.971 (± 0.00) <sup>Aa</sup>	0.967 (± 0.00) <sup>BAb</sup>	0.970 (± 0.00) <sup>Ab</sup>	0.966 (± 0.00) <sup>Bb</sup>	0.962 (± 0.00) <sup>Cc</sup>
	7	0.966 (± 0.00) <sup>Cb</sup>	0.969 (± 0.00) <sup>Ba</sup>	0.972 (± 0.00) <sup>ABb</sup>	0.963 (± 0.00) <sup>Bb</sup>	0.971 (± 0.00) <sup>ABb</sup>
	14	0.967 (± 0.00) <sup>Cb</sup>	0.966 (± 0.00) <sup>CDb</sup>	0.963 (± 0.00) <sup>Dc</sup>	0.973 (± 0.00) <sup>Ba</sup>	0.978 (± 0.00) <sup>Aa</sup>
	21	0.966 (± 0.00) <sup>Bb</sup>	0.962 (± 0.00) <sup>Bc</sup>	0.978 (± 0.01) <sup>Aa</sup>	0.975 (± 0.01) <sup>Aa</sup>	0.976 (± 0.00) <sup>Aa</sup>

Results are averages of three replicates of cheeses manufactured at different times; the following abbreviations are used: C1, cheese treated with *Lactococcus lactis* subsp. *Lactis* and *Lactococcus lactis* subsp. *cremoris*; C2, cheese treated with *Lactobacillus acidophilus*; C3, cheese treated with *Lactobacillus paracasei*; C4, cheese treated with *Bifidobacterium lactis*; and C5, cheese treated with *L. acidophilus*, *L. paracasei* and *B. lactis*.<sup>a-c</sup> For each trial, different superscript lowercase letters within a column denote differences ( $p < 0.05$ ) between values obtained for different days of storage according to Tukey's test; <sup>A-C</sup> Different superscript capital letters within a row denote differences ( $p < 0.05$ ) between the values obtained for the different trials according to Tukey's test.

are more durable due to the delayed acidification (Ong et al., 2006). However, the use of this procedure could result in cheeses with high pH and moisture levels, which are more susceptible to spoilage by contaminating microorganisms (Cunha et al., 2006).

All the cheeses presented increased ( $p < 0.05$ ) syneresis after 21 days of storage (Table 1). The increased expulsion of the whey in cheeses C1, C3 and C4 during

storage could be the cause of the decreased moisture of these cheeses after 21 days of storage ( $p < 0.05$ ) (Table 2). Buriti et al. (2005b) and Souza and Saad, (2009) also found increased syneresis in bovine fresh white cheese supplemented with *L. paracasei* and *L. acidophilus*. The syneresis rate is directly related to the acidity and, therefore, inversely related to pH (Souza and Saad, 2009). As a consequence of the progressive increase in the hydro-

gen ion concentration during acidification, the repulsive forces decrease and casein micelles begin to aggregate, followed by whey expulsion and establishment of the increased syneresis. However, in our study, this relation was found only for cheeses C3 and C5. Cheese C3 presented the highest acidity in lactic acid ( $1.24 \text{ g } 100 \text{ g}^{-1}$ ) after 21 days of storage (Table 2), explaining the loss of whey ( $103.08 \text{ g kg}^{-1}$ ) during the same storage period.

Differences in gross composition and physico-chemical characteristics of coalho goat cheese supplemented with lactic acid bacteria during 21 days of cold storage were observed among the types of bacteria and throughout the time of storage ( $p < 0.05$ ) (Table 2). The moisture content of cheeses C1, C3 and C4 decreased during storage time, concomitant with an increase in dry matter, both of which could be related to an increase in the percentage of protein.

Lower values for lactose were observed in cheeses C1 ( $1.55$  and  $1.09 \text{ g } 100 \text{ g}^{-1}$  at 1 and 21 days, respectively), C3 ( $2.08$  and  $1.16 \text{ g } 100 \text{ g}^{-1}$ , respectively) and C5 ( $2.09$  and  $1.65 \text{ g } 100 \text{ g}^{-1}$ , respectively), suggesting a higher consumption of lactose by the starter culture and probiotic strains during storage, which may have been accompanied by greater lactic acid production (Table 2).

Proteolysis is one of the major biochemical events for the development of proper flavor in cheeses during storage, mainly due to the production of peptides and free amino acids (Sousa et al., 2001). The mean values for the proteolysis parameters of coalho goat cheeses supplemented with different lactic acid bacteria during 21 days of cold storage are listed in Table 3. Storage time exhibited no influence ( $p > 0.05$ ) on the extent of the proteolysis index - EPI (primary proteolysis), with the exception of cheese C5, in which EPI decreased ( $p < 0.05$ ) after seven days of storage. This finding could be related to the processing of the cheeses, where the type and the amount of commercial coagulating agent was the same for all the produced cheeses, and therefore, different proteolytic enzymes did not act on the casein in the cheeses, producing different high-molecular-weight peptides.

Our findings are consistent with Gardiner et al. (1998) who did not observe differences in the proteolysis parameters between cheddar cheeses with and without *L. paracasei* during refrigerated storage. Similar results were also noted in semi-hard cheeses supplemented with *L. acidophilus* and *L. paracasei* (Bergamini et al., 2006). This same feature for primary proteolysis was also found in previous studies of cheddar cheese supplemented with *Bifidobacterium bifidum* and *L. paracasei* (Dinakar and Mistry, 1994; Gardiner et al., 1998), Gouda cheeses with *Bifidobacterium* spp. and *L. acidophilus* (Gomes et al., 1995) and goat cheeses with *B. lactis* and *L. acidophilus* (Gomes and Malcata, 1998).

Higher average values for the depth of the proteolysis index - DPI (secondary proteolysis) were found in cheese C5 (*L. acidophilus*, *L. paracasei* and *B. lactis*) at all storage times, suggesting that the combined use of the assayed bacteria could enhance the proteolysis of cheeses and provide an increased release of peptides (medium and small) and free amino acids. Only in the C1, C2 and C4 cheeses did increased secondary proteolysis occur over the assessed storage periods. The increased levels of secondary proteolysis in cheeses with *L. acidophilus* (LA-5) and *B. lactis* (BB-12) suggest that these strains present a more active peptidolytic system than the assayed strain of *L. paracasei* (*L. casei*-05). Similar results were also observed by Bergamini et al. (2009) who reported that *L. paracasei* exerted no influence on secondary proteolysis in semi-hard cheese after 60 days of storage. Enzymes are more likely to influence proteolysis in cheeses than the type of lactic acid bacteria used during processing (Buriti et al., 2005a; Gomes and Malcata, 1998). In fact, changes in proteolysis are mainly catalyzed by residual chymosin and, to a lesser extent, by other proteases present in the curd, such as plasmin, or proteases from the cell envelopes of the starter culture (Sousa et al., 2001); this was not observed in the present study.

All the evaluated cheeses, when submitted to proper temperatures, did not melt but reduced in diameter. Cheeses tend to melt and deform when exposed to heat at pH values below 5.7 (Sousa et al., 2001). The

Table 3 – Mean values (standard deviation) for proteolysis parameters of coalho goat cheese with probiotic lactic acid bacteria during 21 days of storage at  $10^\circ \text{C}$ .

Variables	Days of storage	Cheeses				
		C1	C2	C3	C4	C5
EPI	1	10.54 ( $\pm 2.04$ ) <sup>Ab</sup>	14.50 ( $\pm 3.65$ ) <sup>Ab</sup>	11.44 ( $\pm 4.75$ ) <sup>Ab</sup>	12.72 ( $\pm 3.44$ ) <sup>Ab</sup>	12.32 ( $\pm 3.05$ ) <sup>Ab</sup>
	7	13.28 ( $\pm 3.82$ ) <sup>Ab</sup>	12.39 ( $\pm 2.54$ ) <sup>Ab</sup>	11.83 ( $\pm 3.12$ ) <sup>Ab</sup>	12.97 ( $\pm 1.85$ ) <sup>Ab</sup>	10.16 ( $\pm 1.14$ ) <sup>Bb</sup>
	14	13.27 ( $\pm 0.38$ ) <sup>ABa</sup>	11.81 ( $\pm 2.03$ ) <sup>Ba</sup>	12.44 ( $\pm 1.82$ ) <sup>ABa</sup>	11.65 ( $\pm 2.64$ ) <sup>Ba</sup>	15.37 ( $\pm 1.65$ ) <sup>Aa</sup>
	21	14.41 ( $\pm 0.54$ ) <sup>Aa</sup>	12.56 ( $\pm 2.40$ ) <sup>Aa</sup>	13.66 ( $\pm 3.20$ ) <sup>Aa</sup>	11.59 ( $\pm 2.77$ ) <sup>Aa</sup>	14.12 ( $\pm 3.89$ ) <sup>Ab</sup>
DPI	1	9.34 ( $\pm 0.38$ ) <sup>Dc</sup>	9.64 ( $\pm 0.39$ ) <sup>CDb</sup>	10.29 ( $\pm 0.55$ ) <sup>BCa</sup>	10.52 ( $\pm 0.39$ ) <sup>Bb</sup>	16.25 ( $\pm 0.03$ ) <sup>Aa</sup>
	7	8.87 ( $\pm 0.04$ ) <sup>Dbc</sup>	8.89 ( $\pm 0.49$ ) <sup>DBa</sup>	9.83 ( $\pm 0.71$ ) <sup>BDa</sup>	10.07 ( $\pm 0.50$ ) <sup>ABb</sup>	16.89 ( $\pm 0.14$ ) <sup>Aa</sup>
	14	8.70 ( $\pm 0.04$ ) <sup>Cb</sup>	15.16 ( $\pm 0.79$ ) <sup>Ba</sup>	9.73 ( $\pm 0.48$ ) <sup>Ca</sup>	9.66 ( $\pm 0.43$ ) <sup>Cb</sup>	16.38 ( $\pm 0.54$ ) <sup>Aa</sup>
	21	14.60 ( $\pm 0.44$ ) <sup>Ca</sup>	14.42 ( $\pm 0.15$ ) <sup>Ca</sup>	9.82 ( $\pm 0.22$ ) <sup>Da</sup>	15.42 ( $\pm 0.48$ ) <sup>Ba</sup>	16.59 ( $\pm 0.11$ ) <sup>Aa</sup>

Results are averages of three replicates of cheeses manufactured at different times; the following abbreviations are used: EPI, extent proteolysis index; DPI, depth of proteolysis index; C1, cheese treated with *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*; C2, cheese treated with *Lactobacillus acidophilus*; C3, cheese treated with *Lactobacillus paracasei*; C4, cheese treated with *Bifidobacterium lactis*; and C5, cheese treated with *L. acidophilus*, *L. paracasei* and *B. lactis*.<sup>a-c</sup> For each trial, different superscript lowercase letters within a column denote differences ( $p < 0.05$ ) between values obtained for different days of storage according to Tukey's test; <sup>A-C</sup> Different superscript capital letters within a row denote differences ( $p < 0.05$ ) between the values obtained for the different trials according to Tukey's test.

pH values (ranging from 6.22 to 7.26) measured in the cheeses may justify the decreased diameter observed in all the tested samples (Table 2). The mean values for the textural parameters of the coalho goat cheeses supplemented with different lactic acid bacteria during 21 days of storage at 10 °C are shown in Table 4. After 21 days of storage, an increase ( $p < 0.05$ ) in hardness was observed for cheeses C1, C3 and C5. Cheese C1 presented the lowest values ( $p < 0.05$ ) for springiness and cohesiveness at 1, 7 and 21 days of storage. The gumminess increased in cheeses C3 and C5 during storage, while chewiness increased during storage only in cheese C5.

The increase in syneresis and decrease in moisture content during storage may have been related to the increase in the hardness of the cheeses (Bhaskaracharya and Shah, 2001). Buriti et al. (2005a) and Souza and Saad (2009) reported that Minas frescal cheese with *L. acidophilus* became harder during storage. The rate and level of acidification have a major impact on cheese texture due to demineralization of the casein micelles (Buriti et al., 2005a). In the present study, cheeses C1, C3 and C5 presented greater acidification during storage, which could have contributed to the increased hardness. Among the texture parameters analyzed in the present study, hardness was the most important, as cheeses with

brittle texture are normally rejected by consumers (Souza and Saad, 2009).

The  $L^*$  value decreased ( $p < 0.05$ ) in the all assessed cheeses during storage (Table 5). Cheeses C2, C3 and C4 showed an increase in the  $a^*$  value during storage, and most of the cheeses also presented an increase in the  $b^*$  value during storage. The  $L^*$  parameter indicates lightness and the capacity of the object to reflect or transmit light based on a scale ranging from 0 to 100. Therefore, higher lightness values result in clearer objects. The assessed samples had high luminosity ( $L^*$ ) with a predominance of the yellow component ( $b^*$ ) rather than the green component ( $a^*$ ), suggesting that the white yellowness contributed the most to the color characteristics of the cheeses. Rohm and Jaros (1996) evaluated changes in the color of Emmental cheese during storage and noted a decrease in  $L^*$  and increases in  $a^*$  and  $b^*$ .

No differences ( $p > 0.05$ ) were found for the bacterial counts between the types of cheese over the period of storage (Figure 1). The lactic acid bacteria counts in cheeses C2, C3, C4 and C5 were always higher than  $6.5 \log \text{cfu g}^{-1}$  after 1 day of storage, while after 21 days, these values were higher than  $7 \log \text{cfu g}^{-1}$  of cheese. Buriti et al. (2007c) noted decreases in the counts of lactic acid bacteria in Minas frescal cheese with a mixed culture of

Table 4 – Mean values (standard deviation) for textural parameters of coalho goat cheese with probiotic lactic acid bacteria during 21 days of storage at 10 °C.

Variables	Days of storage	Cheeses				
		C1	C2	C3	C4	C5
Hardness	1	1254.42 ( $\pm$ 182.45) <sup>ab</sup>	1240.48 ( $\pm$ 334.07) <sup>ab</sup>	1293.77 ( $\pm$ 470.90) <sup>ab</sup>	1163.23 ( $\pm$ 430.98) <sup>ab</sup>	1012.84 ( $\pm$ 213.49) <sup>ab</sup>
	7	2042.54 ( $\pm$ 508.49) <sup>ab</sup>	1891.10 ( $\pm$ 820.35) <sup>ab</sup>	1795.68 ( $\pm$ 282.18) <sup>ab</sup>	1762.84 ( $\pm$ 911.12) <sup>ab</sup>	1349.64 ( $\pm$ 357.70) <sup>ab</sup>
	14	2027.19 ( $\pm$ 574.70) <sup>ab</sup>	1424.30 ( $\pm$ 232.26) <sup>ab</sup>	1911.82 ( $\pm$ 324.65) <sup>ab</sup>	1709.28 ( $\pm$ 127.61) <sup>ab</sup>	1654.55 ( $\pm$ 339.96) <sup>ab</sup>
	21	2490.71 ( $\pm$ 566.32) <sup>ab</sup>	1814.84 ( $\pm$ 745.69) <sup>ab</sup>	2582.29 ( $\pm$ 598.43) <sup>ab</sup>	1880.77 ( $\pm$ 691.88) <sup>ab</sup>	2011.70 ( $\pm$ 435.50) <sup>ab</sup>
Adhesiveness	1	-0.60 ( $\pm$ 0.45) <sup>ab</sup>	-0.84 ( $\pm$ 0.37) <sup>ab</sup>	-1.05 ( $\pm$ 0.93) <sup>ab</sup>	-0.70 ( $\pm$ 0.53) <sup>ab</sup>	-1.11 ( $\pm$ 0.41) <sup>ab</sup>
	7	-3.26 ( $\pm$ 3.03) <sup>ab</sup>	-1.66 ( $\pm$ 1.32) <sup>ab</sup>	-1.40 ( $\pm$ 0.50) <sup>ab</sup>	-0.98 ( $\pm$ 0.35) <sup>ab</sup>	-0.59 ( $\pm$ 0.44) <sup>ab</sup>
	14	-1.40 ( $\pm$ 0.55) <sup>ab</sup>	-0.745 ( $\pm$ 0.68) <sup>ab</sup>	-1.36 ( $\pm$ 0.71) <sup>ab</sup>	-0.66 ( $\pm$ 0.18) <sup>ab</sup>	-0.99 ( $\pm$ 0.65) <sup>ab</sup>
	21	-5.90 ( $\pm$ 5.02) <sup>ab</sup>	-1.16 ( $\pm$ 0.21) <sup>ab</sup>	-6.57 ( $\pm$ 8.33) <sup>ab</sup>	-1.14 ( $\pm$ 0.84) <sup>ab</sup>	-1.57 ( $\pm$ 1.20) <sup>ab</sup>
Springiness	1	0.89 ( $\pm$ 0.02) <sup>ba</sup>	0.93 ( $\pm$ 0.00) <sup>ab</sup>	0.93 ( $\pm$ 0.01) <sup>ab</sup>	0.93 ( $\pm$ 0.01) <sup>ab</sup>	0.93 ( $\pm$ 0.01) <sup>ab</sup>
	7	0.78 ( $\pm$ 0.13) <sup>ba</sup>	0.92 ( $\pm$ 0.02) <sup>ab</sup>	0.91 ( $\pm$ 0.03) <sup>ab</sup>	0.92 ( $\pm$ 0.01) <sup>ab</sup>	0.91 ( $\pm$ 0.03) <sup>ab</sup>
	14	0.85 ( $\pm$ 0.11) <sup>ab</sup>	0.91 ( $\pm$ 0.01) <sup>ab</sup>	0.90 ( $\pm$ 0.03) <sup>ab</sup>	0.91 ( $\pm$ 0.02) <sup>ab</sup>	0.88 ( $\pm$ 0.05) <sup>ab</sup>
	21	0.73 ( $\pm$ 0.14) <sup>ba</sup>	0.92 ( $\pm$ 0.02) <sup>ab</sup>	0.83 ( $\pm$ 0.10) <sup>ab</sup>	0.92 ( $\pm$ 0.01) <sup>ab</sup>	0.88 ( $\pm$ 0.05) <sup>ab</sup>
Cohesiveness	1	0.75 ( $\pm$ 0.04) <sup>ba</sup>	0.82 ( $\pm$ 0.01) <sup>ab</sup>	0.81 ( $\pm$ 0.00) <sup>ab</sup>	0.81 ( $\pm$ 0.01) <sup>ab</sup>	0.81 ( $\pm$ 0.01) <sup>ab</sup>
	7	0.55 ( $\pm$ 0.22) <sup>ba</sup>	0.81 ( $\pm$ 0.02) <sup>ab</sup>	0.79 ( $\pm$ 0.03) <sup>ab</sup>	0.81 ( $\pm$ 0.02) <sup>ab</sup>	0.79 ( $\pm$ 0.07) <sup>ab</sup>
	14	0.65 ( $\pm$ 0.26) <sup>ab</sup>	0.80 ( $\pm$ 0.03) <sup>ab</sup>	0.76 ( $\pm$ 0.07) <sup>ab</sup>	0.77 ( $\pm$ 0.03) <sup>ab</sup>	0.76 ( $\pm$ 0.08) <sup>ab</sup>
	21	0.44 ( $\pm$ 0.26) <sup>ba</sup>	0.80 ( $\pm$ 0.04) <sup>ab</sup>	0.65 ( $\pm$ 0.15) <sup>ab</sup>	0.80 ( $\pm$ 0.02) <sup>ab</sup>	0.72 ( $\pm$ 0.11) <sup>ab</sup>
Gumminess	1	720.83 ( $\pm$ 506.26) <sup>ab</sup>	1018.20 ( $\pm$ 261.32) <sup>ab</sup>	1051.94 ( $\pm$ 378.80) <sup>ab</sup>	942.27 ( $\pm$ 343.82) <sup>ab</sup>	819.26 ( $\pm$ 165.18) <sup>ab</sup>
	7	1253.44 ( $\pm$ 676.67) <sup>ab</sup>	1548.93 ( $\pm$ 712.23) <sup>ab</sup>	1428.75 ( $\pm$ 260.27) <sup>ab</sup>	1429.05 ( $\pm$ 764.93) <sup>ab</sup>	1063.61 ( $\pm$ 268.72) <sup>ab</sup>
	14	1100.12 ( $\pm$ 288.14) <sup>ab</sup>	1241.69 ( $\pm$ 306.54) <sup>ab</sup>	1311.86 ( $\pm$ 98.61) <sup>ab</sup>	1329.53 ( $\pm$ 128.81) <sup>ab</sup>	1223.35 ( $\pm$ 202.96) <sup>ab</sup>
	21	1163.32 ( $\pm$ 816.79) <sup>ab</sup>	1477.72 ( $\pm$ 664.08) <sup>ab</sup>	1621.85 ( $\pm$ 275.55) <sup>ab</sup>	1504.69 ( $\pm$ 577.28) <sup>ab</sup>	1425.34 ( $\pm$ 166.06) <sup>ab</sup>
Chewiness	1	861.38 ( $\pm$ 195.70) <sup>ab</sup>	948.90 ( $\pm$ 243.74) <sup>ab</sup>	980.02 ( $\pm$ 358.79) <sup>ab</sup>	873.20 ( $\pm$ 309.91) <sup>ab</sup>	759.76 ( $\pm$ 162.12) <sup>ab</sup>
	7	1043.67 ( $\pm$ 692.88) <sup>ab</sup>	1428.00 ( $\pm$ 673.82) <sup>ab</sup>	1307.96 ( $\pm$ 262.91) <sup>ab</sup>	1321.47 ( $\pm$ 716.90) <sup>ab</sup>	972.687 ( $\pm$ 248.37) <sup>ab</sup>
	14	962.87 ( $\pm$ 344.72) <sup>ab</sup>	1131.96 ( $\pm$ 272.07) <sup>ab</sup>	1176.82 ( $\pm$ 51.10) <sup>ab</sup>	1216.86 ( $\pm$ 136.59) <sup>ab</sup>	1072.38 ( $\pm$ 128.77) <sup>ab</sup>
	21	943.70 ( $\pm$ 798.04) <sup>ab</sup>	1362.80 ( $\pm$ 628.28) <sup>ab</sup>	1357.18 ( $\pm$ 340.23) <sup>ab</sup>	1388.27 ( $\pm$ 538.22) <sup>ab</sup>	1258.26 ( $\pm$ 147.22) <sup>ab</sup>

Results are averages of three replicates of cheeses manufactured at different times; the following abbreviations are used: C1, cheese treated with *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*; C2, cheese treated with *Lactobacillus acidophilus*; C3, cheese treated with *Lactobacillus paracasei*; C4, cheese treated with *Bifidobacterium lactis*; and C5, cheese treated with *L. acidophilus*, *L. paracasei* and *B. lactis*. <sup>a-b</sup> For each trial, different superscript lowercase letters within a column denote differences ( $p < 0.05$ ) between values obtained for different days of storage according to Tukey's test; <sup>A-B</sup> Different superscript capital letters within a row denote differences ( $p < 0.05$ ) between the values obtained for the different trials according to Tukey's test.

Table 5 – Mean values (standard deviation) for the color evaluation parameters for coalho goat cheese with probiotic lactic acid bacteria during 21 days of storage at 10 °C.

Color	Days of storage	Cheeses				
		C1	C2	C3	C4	C5
L	1	93.50 (± 0.30) <sup>Aa</sup>	92.63 (± 0.30) <sup>Ba</sup>	93.38 (± 0.16) <sup>Aa</sup>	92.67 (± 0.15) <sup>Ba</sup>	92.50 (± 0.25) <sup>Ba</sup>
	7	92.81 (± 0.13) <sup>ABb</sup>	92.23 (± 0.15) <sup>Ca</sup>	93.30 (± 0.33) <sup>Aab</sup>	92.70 (± 0.30) <sup>BCa</sup>	92.51 (± 0.21) <sup>BCa</sup>
	14	92.51 (± 0.27) <sup>ABb</sup>	92.52 (± 0.19) <sup>ABa</sup>	92.84 (± 0.21) <sup>Abc</sup>	92.60 (± 0.18) <sup>ABa</sup>	92.42 (± 0.33) <sup>Ba</sup>
	21	91.36 (± 0.23) <sup>Bc</sup>	91.48 (± 0.21) <sup>Bb</sup>	92.73 (± 0.35) <sup>Ac</sup>	91.71 (± 0.80) <sup>Bb</sup>	91.69 (± 0.20) <sup>Bb</sup>
a	1	-3.18 (± 0.08) <sup>Ba</sup>	-3.09 (± 0.00) <sup>ABb</sup>	-3.04 (± 0.05) <sup>Abc</sup>	-3.14 (± 0.04) <sup>Abc</sup>	-3.08 (± 0.02) <sup>ABa</sup>
	7	-3.12 (± 0.17) <sup>ABa</sup>	-3.17 (± 0.28) <sup>Bb</sup>	-3.09 (± 0.21) <sup>ABc</sup>	-2.78 (± 0.04) <sup>Ab</sup>	-3.09 (± 0.12) <sup>ABa</sup>
	14	-2.88 (± 0.21) <sup>Ba</sup>	-2.86 (± 0.08) <sup>ABab</sup>	-2.81 (± 0.06) <sup>ABb</sup>	-2.65 (± 0.03) <sup>Aa</sup>	-3.04 (± 0.02) <sup>Ba</sup>
	21	-2.88 (± 0.23) <sup>Ba</sup>	-2.68 (± 0.14) <sup>Ba</sup>	-2.34 (± 0.17) <sup>Aa</sup>	-2.74 (± 0.06) <sup>Bb</sup>	-3.18 (± 0.14) <sup>Ba</sup>
b	1	9.17 (± 0.82) <sup>Ac</sup>	8.87 (± 0.02) <sup>Ac</sup>	9.37 (± 0.05) <sup>Aa</sup>	9.03 (± 0.04) <sup>Ac</sup>	9.49 (± 0.16) <sup>Ac</sup>
	7	9.46 (± 0.37) <sup>Ac</sup>	9.60 (± 0.11) <sup>Abc</sup>	9.61 (± 0.50) <sup>Aa</sup>	9.20 (± 0.05) <sup>Abc</sup>	9.70 (± 0.40) <sup>Abc</sup>
	14	10.31 (± 0.24) <sup>Ab</sup>	10.32 (± 0.71) <sup>Ab</sup>	9.70 (± 0.33) <sup>ABa</sup>	9.58 (± 0.20) <sup>Bb</sup>	10.33 (± 0.07) <sup>Ab</sup>
	21	11.16 (± 0.32) <sup>Aa</sup>	10.70 (± 0.47) <sup>ABa</sup>	9.76 (± 0.14) <sup>Ca</sup>	10.48 (± 0.24) <sup>Ba</sup>	11.09 (± 0.33) <sup>Aa</sup>

Results are averages of three replicates of cheeses manufactured at different times; the following abbreviations are used: C1, cheese treated with *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*; C2, cheese treated with *Lactobacillus acidophilus*; C3, cheese treated with *Lactobacillus paracasei*; C4, cheese treated with *Bifidobacterium lactis*; and C5, cheese treated with *L. acidophilus*, *L. paracasei* and *B. lactis*.<sup>a-c</sup> For each trial, different superscript lowercase letters within in column denote differences ( $p < 0.05$ ) between values obtained for different days of storage according to Tukey's test; <sup>A-C</sup> Different superscript capital letters within a row denote differences ( $p < 0.05$ ) between the values obtained for the different trials according to Tukey's test.

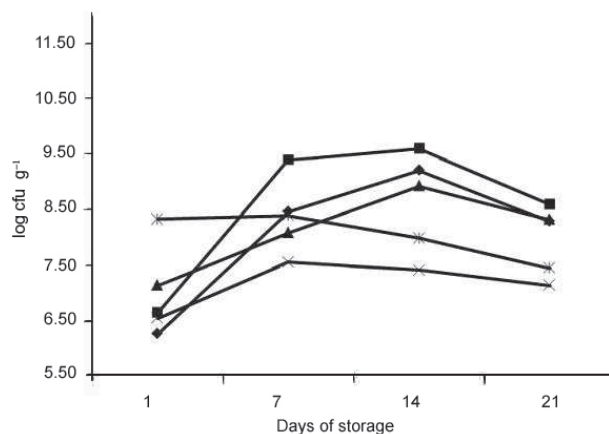


Figure 1 – Counts of lactic acid bacteria in coalho goat cheese with probiotic lactic acid bacteria during 21 days of storage at 10 °C. C1 (—●—): control cheese. C2 (—■—): cheese with *L. acidophilus*. C3 (—▲—): cheese with *L. paracasei*. C4 (—×—): cheese with *B. lactis*. C5 (—\*—): cheese with *L. acidophilus*, *L. paracasei* and *B. lactis*. Results are average of three replicates of cheeses manufactured at different times.

*L. lactis* subsp. *Lactis* and *L. lactis* subsp. *Cremoris* after 21 days of refrigerated storage. However, other authors have reported increases in the populations of lactic acid bacteria (e.g., *L. paracasei* and *L. acidophilus*) in Minas frescal cheese during storage (Buriti et al., 2005b; Souza and Saad 2009). Similarly, Ryhänen et al. (2001) found counts of *L. acidophilus* over 6.0 log cfu g<sup>-1</sup> in Festivo cheese during 16 weeks of storage. Yilmaztekin et al. (2004) reported counts of *L. acidophilus* La-5 in white-brined cheese approximately 6.0 log cfu g<sup>-1</sup> after 90 days of storage. Vinderola et al. (2000) tested the addition of *L. acidophilus* in mixed culture with different bifidobac-

teria (*Bifidobacterium* sp., *B. bifidum*, and *B. longum*) to Argentinian fresco cheese and observed counts of *L. acidophilus* above 6.0 log cfu g<sup>-1</sup> after 60 days of storage.

In this study, all cheeses presented counts of lactic acid bacteria higher than 10<sup>7</sup> cfu g<sup>-1</sup> (7.0 log cfu g<sup>-1</sup>) after seven days of cold storage, which is considered the minimum count of probiotic bacteria required at the moment of intake to assure a favorable impact on consumer health (De Vuyst, 2000; Talwalkar et al., 2004). Some characteristics of low-ripened white cheeses, such as the coalho cheese, suggest that these matrices are suitable carriers of probiotic lactic acid bacteria: i) temperatures exceeding 45 °C applied in the processing inhibit the survival of contaminating bacteria, and the matrices provide an appropriate physical structure for the protection of the probiotic cultures during the storage; ii) they contain high amounts of the proteins, lipids, vitamins and minerals needed for the survival and multiplication of probiotic cultures; and iii) they are slightly acidic, which enables the growth of lactic acid bacteria and inhibits microorganisms that commonly cause spoilage and food-borne diseases (Buriti et al., 2005a; Buriti et al., 2005b).

The lowest ( $p < 0.05$ ) values for appearance, flavor, taste, texture and general perception were found for cheese C1 (standard) at all evaluated storage periods (Table 6). No differences ( $p > 0.05$ ) in appearance, color or flavor were found between cheeses C2, C3, C4 and C5 in the evaluated storage period. Cheese C5 had higher values ( $p < 0.05$ ) than C3 for general perception after 21 days of storage. A decrease ( $p < 0.05$ ) in the scores for general perception for C3 was observed over the storage time.

When asked to report about the intent to purchase, the panelists reported differences between cheese C1 and the other cheeses at all storage periods. The responses fell between "possibly would not purchase" and "maybe would purchase/maybe would not purchase".

Table 6 – Mean (standard deviation) sensory scores<sup>1</sup> for coalho goat cheese with probiotic lactic acid bacteria treatments after storage at 10 °C.

Variables	Days of storage	Cheeses				
		C1	C2	C3	C4	C5
Appearance	1	6.66 (± 2.12) <sup>Ba</sup>	7.83 (± 1.28) <sup>Aa</sup>	7.71 (± 1.40) <sup>Aa</sup>	7.65 (± 1.43) <sup>Aa</sup>	7.86 (± 1.60) <sup>Aa</sup>
	7	6.18 (± .98) <sup>Ba</sup>	7.27 (± 1.56) <sup>Ab</sup>	7.42 (± 1.56) <sup>Aa</sup>	7.62 (± 1.27) <sup>Aa</sup>	7.72 (± 1.15) <sup>Aa</sup>
	14	5.93 (± 2.12) <sup>Ba</sup>	7.58 (± 1.27) <sup>Ab</sup>	7.56 (± 1.36) <sup>Aa</sup>	7.71 (± 1.25) <sup>Aa</sup>	7.77 (± 1.08) <sup>Aa</sup>
	21	5.93 (± 2.07) <sup>Ba</sup>	7.75 (± 1.30) <sup>Ab</sup>	7.32 (± 1.41) <sup>Aa</sup>	7.47 (± 1.47) <sup>Aa</sup>	7.84 (± 1.22) <sup>Aa</sup>
Color	1	7.54 (± 1.61) <sup>Aa</sup>	7.80 (± 1.34) <sup>Aa</sup>	7.91 (± 1.18) <sup>Aa</sup>	7.84 (± 1.21) <sup>Aa</sup>	8.06 (± 0.87) <sup>Aa</sup>
	7	7.27 (± 1.50) <sup>Ba</sup>	7.61 (± 1.17) <sup>ABa</sup>	7.70 (± 1.23) <sup>ABa</sup>	7.79 (± 1.15) <sup>Aa</sup>	7.79 (± 1.08) <sup>Aa</sup>
	14	7.24 (± 1.59) <sup>Ba</sup>	7.80 (± 1.20) <sup>Aa</sup>	7.75 (± 1.28) <sup>Aa</sup>	7.91 (± 1.10) <sup>Aa</sup>	7.84 (± 1.14) <sup>Aa</sup>
	21	7.11 (± 1.53) <sup>Ba</sup>	7.85 (± 1.27) <sup>Aa</sup>	7.61 (± 1.32) <sup>ABa</sup>	7.68 (± 1.36) <sup>Aa</sup>	7.75 (± 1.34) <sup>Aa</sup>
Flavor	1	5.88 (± 2.23) <sup>Ba</sup>	6.72 (± 1.77) <sup>Aa</sup>	6.77 (± 1.69) <sup>Aa</sup>	6.84 (± 1.63) <sup>Aa</sup>	7.22 (± 1.64) <sup>Aa</sup>
	7	5.69 (± 2.32) <sup>Ba</sup>	6.51 (± 1.77) <sup>Aa</sup>	6.49 (± 1.84) <sup>Aa</sup>	6.50 (± 1.67) <sup>Aa</sup>	6.86 (± 1.61) <sup>Aa</sup>
	14	5.96 (± 2.08) <sup>Ba</sup>	6.61 (± 1.54) <sup>ABa</sup>	6.62 (± 1.69) <sup>ABa</sup>	6.93 (± 1.58) <sup>Aa</sup>	6.93 (± 1.64) <sup>Aa</sup>
	21	6.18 (± 1.94) <sup>Ba</sup>	6.89 (± 1.69) <sup>Aa</sup>	6.49 (± 1.53) <sup>ABa</sup>	6.63 (± 1.66) <sup>ABa</sup>	6.93 (± 1.65) <sup>Aa</sup>
Taste	1	5.26 (± 2.49) <sup>Ba</sup>	7.05 (± 1.85) <sup>Aa</sup>	6.84 (± 1.93) <sup>Aa</sup>	7.01 (± 1.68) <sup>Aa</sup>	7.28 (± 1.75) <sup>Aa</sup>
	7	4.93 (± 2.56) <sup>Ba</sup>	6.54 (± 1.93) <sup>Aa</sup>	6.48 (± 1.89) <sup>Ab</sup>	6.42 (± 1.92) <sup>Aa</sup>	7.10 (± 1.61) <sup>Aa</sup>
	14	4.74 (± 2.39) <sup>Ba</sup>	6.79 (± 1.75) <sup>Aa</sup>	6.55 (± 1.93) <sup>Ab</sup>	7.02 (± 1.69) <sup>Aa</sup>	6.92 (± 1.63) <sup>Aa</sup>
	21	5.27 (± 2.57) <sup>Ca</sup>	7.06 (± 1.87) <sup>Aa</sup>	5.96 (± 2.15) <sup>BCb</sup>	6.73 (± 2.04) <sup>ABa</sup>	6.95 (± 1.84) <sup>Aa</sup>
Texture	1	5.81 (± 2.31) <sup>Ba</sup>	7.36 (± 1.64) <sup>Aa</sup>	6.66 (± 2.07) <sup>Aa</sup>	7.07 (± 1.79) <sup>Aa</sup>	7.36 (± 1.65) <sup>Aa</sup>
	7	5.35 (± 2.32) <sup>Ca</sup>	6.59 (± 2.01) <sup>Bb</sup>	6.80 (± 1.82) <sup>ABa</sup>	6.99 (± 1.76) <sup>ABa</sup>	7.38 (± 1.30) <sup>Aa</sup>
	14	5.15 (± 2.09) <sup>Ba</sup>	7.03 (± 1.54) <sup>Ab</sup>	6.87 (± 1.73) <sup>Aa</sup>	7.48 (± 1.48) <sup>Aa</sup>	7.28 (± 1.39) <sup>Aa</sup>
	21	5.38 (± 2.53) <sup>Ca</sup>	7.51 (± 1.50) <sup>Aa</sup>	6.60 (± 1.93) <sup>Ba</sup>	7.02 (± 1.93) <sup>ABa</sup>	7.44 (± 1.55) <sup>Aa</sup>
General perception	1	5.50 (± 2.30) <sup>Ba</sup>	7.23 (± 1.59) <sup>Aa</sup>	6.97 (± 1.55) <sup>Aa</sup>	7.06 (± 1.43) <sup>Aa</sup>	7.55 (± 1.29) <sup>Aa</sup>
	7	5.32 (± 2.22) <sup>Ca</sup>	6.76 (± 1.64) <sup>ABa</sup>	6.66 (± 1.71) <sup>ABa</sup>	6.74 (± 1.78) <sup>ABa</sup>	7.41 (± 1.31) <sup>Aa</sup>
	14	5.06 (± 2.16) <sup>Ba</sup>	7.06 (± 1.32) <sup>Aa</sup>	6.78 (± 1.74) <sup>Ab</sup>	7.27 (± 1.45) <sup>Aa</sup>	7.17 (± 1.39) <sup>Aa</sup>
	21	5.43 (± 2.31) <sup>Ca</sup>	7.22 (± 1.47) <sup>Aa</sup>	6.25 (± 1.86) <sup>Bb</sup>	6.79 (± 1.78) <sup>ABa</sup>	7.22 (± 1.54) <sup>Aa</sup>

<sup>1</sup>Scored according to a hedonic scale ranging from 1 (dislike very much) to 9 (like very much).

Results are averages of three replicates of cheeses manufactured at different times; the following abbreviations are used: C1, cheese treated with *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*; C2, cheese treated with *Lactobacillus acidophilus*; C3, cheese treated with *Lactobacillus paracasei*; C4, cheese treated with *Bifidobacterium lactis*; and C5, cheese treated with *L. acidophilus*, *L. paracasei* and *B. lactis*.<sup>a-b</sup> For each trial, different superscript lowercase letters within in column denote differences ( $p < 0.05$ ) between values obtained for different days of storage according to Tukey's test; <sup>A-C</sup> Different superscript capital letters within a row denote differences ( $p < 0.05$ ) between the values obtained for the different trials according to Tukey's test.

The intent to purchase was similar for cheeses C3 and C5 during the evaluated storage periods, where the hedonic term was between "maybe would purchase/maybe would not purchase" and "purchase". There was an increase in purchase intention for cheeses C2 and C4 during the assessed storage period, where the hedonic term was "possibly purchase".

In the preference ranking test, cheese C5 was the most preferred, and cheese C1 was the least preferred ( $p < 0.05$ ) after 21 days of storage. Cheeses C2, C3 and C4 were similarly ( $p > 0.05$ ) classified as being moderately desirable. Under certain conditions, *L. lactis* (which was present in the starter culture added to cheese C1) rapidly ferments available carbon sources into lactic acid and converts sugars into other organic compounds, such as formate, acetate and ethanol, negatively influencing the sensory properties of unripened cheeses, such as the coalho cheese (Even et al., 1999). Moreover, the different profile of secondary proteolysis (depth of proteolysis index) found in cheeses manufactured with some strains of probiotic lactic acid bacteria could cause a satisfactory impact on the sensory characteristics of these cheeses, particularly in their flavor, when compared with cheeses

manufactured without the addition of these strains. Although cheese C1 had also presented increased secondary proteolysis, the higher acidity developed in this cheese over the storage period could have overcome the possible positive effects of secondary proteolysis on its sensory characteristics, resulting in a decrease in the sensory acceptance of the product.

## Conclusions

The addition of a standard starter culture of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* in coalho goat cheese resulted in a more acidic product. The addition of the assayed strains of probiotic lactic acid bacteria in co-culture resulted in higher proteolysis rates and depths. Coalho goat cheese may be a good carrier for the delivery of probiotic lactic acid bacteria because the cheeses presented viable counts of the added bacteria during 21 days of cold storage at levels higher than those recommended for health benefits. Coalho goat cheeses with the added probiotic lactic acid strains alone and in co-culture were better accepted in the sensory evaluation than cheeses without the probiotic strains.



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