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Effects of added *Lactobacillus acidophilus* and *Bifidobacterium lactis* probiotics on the quality characteristics of goat ricotta and their survival under simulated gastrointestinal conditions

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ABSTRACT

This study evaluated the effects of incorporating the probiotics *Bifidobacterium animalis* subsp. *lactis* Bb-12 (*B. lactis*) or *Lactobacillus acidophilus* La-05 (*L. acidophilus*) into goat ricotta on the technological, physicochemical, physical and sensory parameters of this product during refrigerated storage, as well as the protective effects of the goat ricotta on the survival of the tested probiotics during exposure to simulated gastrointestinal conditions. Incorporating the tested probiotics did not affect the yield or syneresis of the obtained goat ricotta. The counts of *L. acidophilus* and *B. lactis* during the chosen storage period were approximately 6 log CFU/g. The ricotta samples containing a probiotic strain presented smaller and greater amounts of lactose and lactic acid, respectively, and exhibited greater hardness and lower brightness after storage compared with the samples lacking a probiotic. No differences were observed in the fatty acid profiles of the goat ricotta containing or not containing a probiotic. All of the ricotta samples were described as a soft cheese with a homogeneous texture; however, the goat ricotta cheeses containing *L. acidophilus* or *B. lactis* were described as having a more acidic flavor. At the end of a challenge using experimental human digestive conditions, the counts of each of the tested probiotic strains were approximately 6 log CFU/g if it had been incorporated into goat ricotta. These results demonstrated the feasibility of incorporating *L. acidophilus* or *B. lactis* into goat ricotta because these probiotics did not negatively affect the quality characteristics of this product and suggested that goat ricotta is an efficacious food matrix for maintaining the viability of these probiotics during storage and under the stressful conditions imposed by the human gastrointestinal tract.

1. Introduction

The consumption of goat dairy products has increased worldwide, with a consequent increase in the demand for goat milk in the major producing countries (Queiroga et al., 2013). The flavor and taste of goat dairy products are mainly due to the high content of short- and medium-chain fatty acids (such as caproic (C6:0), caprylic (C8:0) and capric (C10:0) fatty acids) in the fat contained in goat milk, which is a commonly cited obstacle to their widespread acceptance (Prandini, Sigolo, & Piva, 2011; Raynal-Ljutovac, Le Pape, Gaborit, & Barrucand, 2011). The lower content of "goat" fatty acids in the whey obtained during the production of goat cheese could increase the acceptance of goat whey-based products by consumers (Borba et al., 2013).

The interest in the development of new products using goat milk whey is also associated with the high nutritional value of this by-product. This use provides an environmental friendly destination for the whey generated during goat cheese manufacture, which is a large source of environmental pollution when improperly disposed (Silveira et al., 2014). Moreover, whey-based dairy products have been shown to be a suitable substrate for harboring, protecting and delivering probiotic bacteria (Castro et al., 2013).

Probiotics are viable microorganisms that are beneficial to the host when administered in appropriate quantities (FAO/WHO, 2002). Probiotics can protect human hosts from infections, primarily those that occur on the colonized mucosal surfaces of the gastrointestinal tract (Sanders, 2003; Vandenplas, Huys, & Daube, 2015). Researchers

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have reported dairy products (e.g., yogurt, beverages and cheese) to be suitable vehicles of probiotics that provide health benefits to the consumer (Boylston, Vinderola, Ghoddusi, & Reinheimer, 2004; Saarela, Lähteenmäki, Crittenden, Salminen, & Mattila-Sandholm, 2002). Cheeses are particularly interesting in this regard due to their solid consistency and high buffering capacity, which help maintain the viability of probiotics not only throughout the product shelf life but also during their passage through the gastrointestinal tract after consumption (Coman et al., 2012; Gregor, 2015). However, few studies have assessed the capacity of goat cheese to deliver probiotics either by monitoring the probiotic survival rate during the shelf-life period of the tested products or when such products are exposed to gastrointestinal conditions (Garcia, de Oliveira, Queiroga, Machado, & Souza, 2012; Oliveira, Garcia, Queiroga, & Souza, 2012; Oliveira et al., 2014).

In recent years, the growing public awareness of diet-related health issues has fueled the demand for foods with distinct health-promoting effects, such as food-related probiotics (Oliveira et al., 2014; Silveira et al., 2014). Probiotic products must have a microbial count of ≥6 log counting forming units per milliliters or gram until the end of their shelf-life period to produce their claimed benefits (Garcia et al., 2012; Roy, 2005). However, several factors can affect the viability of probiotic cells in dairy products during their storage, such as the nutrient content, acidity, pH, Aw and secreted inhibitory metabolites (e.g., organic acids and bacteriocins) (Cruz, Buriti, Souza, Faria, & Saad, 2009; Jankovic, Sybesma, Phothirath, Ananta, & Mercenier, 2010). Ricotta cheese is a soft cheese typically consumed in Italy and in Ibero-American countries (Borba et al., 2013). This product is defined as an unripened, creamy dairy product that is generally obtained via heat-induced coagulation and acid-precipitation of whey proteins from cow, sheep or goat milk (Buriti, Cardarelli, Filisetti, & Saad, 2007). The high moisture content, low salt content and initial pH above 6.0 (Davies, Bevis, & Delves-Broughton, 1997) make ricotta cheese a favorable environment for the survival of probiotic bacteria. Among the well-recognized probiotic bacteria, *Bifidobacterium animalis* subsp. *lactis* (*B. lactis*) and *Lactobacillus acidophilus* (*L. acidophilus*) have been widely used as active ingredients of functional dairy products (González-Sánchez, Azaola, Gutiérrez-López, & Hernández-Sánchez, 2010). When added to bovine or caprine fermented milks, yogurts, dairy beverages (Ranadheera, Evans, Adams, & Baines, 2013; Silveira et al., 2014; Vinderola & Reinheimer, 1999) or ewe cheese (Albenzio et al., 2013), *B. lactis* and *L. acidophilus* showed satisfactory viability and had no undesirable effects on the nutritional and sensory aspects of such products during their storage.

Considering these aspects, the aims of this study were as follows: 1) to manufacture goat ricotta cheese containing the well-known probiotic strains *B. lactis* (*B. lactis* Bb12) or *L. acidophilus* (*L. acidophilus* La-05) (Albenzio et al., 2013; Sanders & Huis in't Veld, 1999); 2) to assess the survival of each of the test probiotics when incorporated into the manufactured goat ricotta cheeses during their refrigerated storage; 3) to evaluate the effects of the added probiotic strain on the technological, physicochemical, physical and sensory parameters of the obtained cheeses during their refrigerated storage; and 4) to assess the protective effects of goat ricotta on the survival of the tested probiotics during exposure to simulated human gastrointestinal conditions.

2. Materials and methods

2.1. Raw materials

Goat whey was generated during the manufacture of coalho, a product typical of northeastern Brazil, which is a semi-hard cheese with a medium moisture content. This coalho cheese was produced using enzymatic coagulation according to a previously described procedure (Garcia et al., 2012). The goat milk used to manufacture the coalho cheese was obtained from Alpine breed goats and was pasteurized at 65 °C for 30 min.

2.2. Bacterial strains and growth conditions

Lyophilized cultures of *L. acidophilus* (La-05; Chr. Hansen SA, Valinhos, São Paulo) and *B. lactis* (Bb-12; Chr. Hansen SA, Valinhos, São Paulo) were grown for 24 h at 37 °C in de Man, Rogosa, and Sharpe (MRS) broth (Oxoid SpA, Milan, Italy) and in MRS + cysteine (0.05 g /100 mL, Sigma-Aldrich, Milan, Italy) (cMRS), respectively. After this period, the probiotic cultures were heated in a water bath at 65 °C for 30 min to induce heat adaptation, after which six successive heat treatments were performed (Minervini et al., 2012). The cell cultures (30 mL) were centrifuged (1200 ×*g* for 10 min) and the supernatants were discarded, and then 30 mL of sterile distilled water was added to each pellet obtained. The harvested cells were plated on selective growth medium to test their recovery after heat treatment. The counts of the *L. acidophilus* and *B. lactis* cells ranged from 8.5 and 8.0 log of counting forming units per milliliter (log cfu/mL).

2.3. Production of goat ricotta

Three different types of goat ricotta cheese were produced, as follows: R1 — goat ricotta without probiotic cells; R2 — goat ricotta containing *L. acidophilus* La-5 cells; and R3 — goat ricotta containing *B. lactis* Bb-12 cells. The cheeses were prepared using the manufacturing procedures shown in Flow Chart 1 (Fig. 1).

Samples of the three different goat ricotta cheeses were used for physicochemical, nutritional microbiological and sensory analyses. The ricotta cheese samples were analyzed immediately (day 1) and after 7 days of storage at 7 °C because it has been suggested that commercial version of this product must have a shelf-life of 7 days of storage under refrigeration (Brazil, M.S. Resolution RDC n° 12, 2001). Each day, three ricotta cheeses prepared from the same batch were unpacked. Samples (25 g) were aseptically collected from different parts of the cheeses for microbiological analysis. For the instrumental texture profile analysis, at least 0.5 cm of the rind was discarded, and the cheese samples were carefully collected along a line passing from the center to the exterior. The rest of the cheese was grated and immediately used for physicochemical, microbiological and sensory analyses. The study of the survival of each probiotic upon exposure to simulated gastrointestinal conditions was performed using goat ricotta samples that had been stored for 1 day.

2.4. Microbiological analysis and analysis of the viability of the probiotics during storage

For the microbiological analysis of the goat ricotta samples, counts of the total and thermotolerant coliforms (using the more probable number in *Escherichi coli* broth — Himedia, India; at 45 °C for 24 h), the mesophilic bacteria (using plate count agar — Himedia, India; at 35 °C for 24 h) and coagulase-positive *Staphylococcus* (using Baird Parker agar supplemented with 50 mL/L of egg yolk emulsion containing potassium tellurite (3.5%) — Himedia, India; at 35 °C for 24 h) were obtained and the presence of *Salmonella* spp. (using *Salmonella* differential agar — Himedia, India; at 35 °C for 24 h) and *Listeria monocytogenes* (using *Listeria* Agar Base containing selective supplement for *Listeria* II — Himedia, India; 30 °C for 24 h) was determined according to standard procedures described elsewhere (APHA, 2001).

The counts of *L. acidophilus* La-05 and *B. lactis* Bb-12 in the goat ricotta samples were determined using a viable-cell count procedure (Oliveira et al., 2014). For this procedure, at each pre-established time (1 and 7 days of storage), 25-g samples of cheese were homogenized in 225 mL of peptone water (1 mg/100 g) using a Bag Mixer 400 (Interscience Co., Saint Nom, France) and the homogenates were serially diluted (10^2–10^5) using the same diluent. Subsequently, a

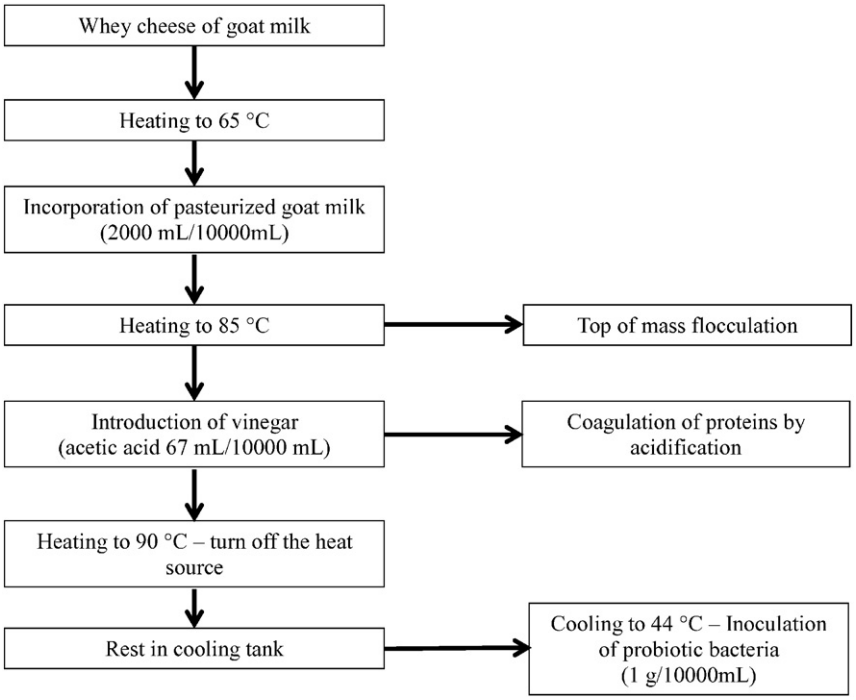

Fig. 1. Flowchart of the processes for manufacturing goat ricotta containing or not containing the probiotic bacterium *Lactobacillus acidophilus* La-05 or *Bifidobacterium lactis* Bb-12.

1-mL aliquot of each dilution was dispensed into MRS (Himedia, India) or MRS + cysteine agar (0.05 g/100 mL Sigma-Aldrich, Milan, Italy) (cMRS agar) (for counting *L. acidophilus* and *B. lactis*, respectively) using the pour-plate inoculation method and the plates were incubated for 3 days at 37 °C under anaerobic conditions (Anaerobic System Anaerogen, Oxoid). The counts were expressed as the log of the colony forming units per gram of cheese (log cfu/g).

2.5. Yield, syneresis, pH, Aw and proximate composition

The yield of each batch was expressed as the fresh weight of the goat ricotta obtained from each liter of whey used for production (g of cheese/L of whey) (Zeng, Soryal, Fekadu, Bah, & Popham, 2007). The level of syneresis (grams of whey per kilogram of cheese) was calculated as the weight of whey in grams released from each kilogram of ricotta in the package after different storage periods divided by the weight of the cheese in the same package in grams and multiplied by 100 (Borba et al., 2013). The proximate composition and the Aw value were determined according to the standard procedures (AOAC, 2005) for measuring the moisture (925.09), fat (2000.18), protein (939.02), lactose (923.09), extracted total solids (990.19) and ash (930.30) contents and the acidity (g/100 g of lactic acid) (920.124) and Aw (978.18) values. The pH values were measured using a digital potentiometer and the density (milligrams per deciliter — mg/dL) of the samples was measured at 15 °C using a thermo-lacto density meter.

2.6. Instrumental textural analysis

The textural properties of the ricotta samples were evaluated using a TA-XT2 Texture Analyzer TM (Stable Micro Systems, Godalming, England), using a two-bite compression of cylindrical samples (25-mm-diameter acrylic cylindrical probe (P25), a strain rate programmed to a speed of 1 mm/s and maximum penetration depth of 10 mm). The hardness, springiness, adhesiveness, cohesiveness, chewiness, gumminess were measured in three replicates of each sample (Garcia et al., 2012).

2.7. Color analysis

A CR-300 colorimeter® (Minolta Co., Osaka, Japan) was used for the instrumental color evaluation. The CIE Lab color scale (L*a*b*) was applied using D65 illumination (standard daylight) at a 10° angle. The L*, a* and b* parameters were determined according to the International Commission on Illumination (CIE, 1996). 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. The measurements were performed in triplicate using the inner section of the ricotta samples immediately after their unpacking (Sant'Ana et al., 2013).

2.8. Determination of the fatty acid profile

After extracting the total lipids (Folch, Less, & Stanley, 1957), followed by saponification and esterification (Hartman & Lago, 1986), the fatty acid profile of the ricotta samples was determined using a Varian 430-GC gas-chromatograph equipped with a flame ionization detector and a fused silica capillary column (Varian CP WAX 52 CB) with the dimensions of 60 m × 0.25 mm × 0.25-mm thick film. Helium was used as the carrier gas, at a flow rate of 1 mL/min. The oven temperature was initially 100 °C, which was increased at 2.5 °C/min to a final temperature of 240 °C, which was held for 20 min, for a total time of 76 min. The injector and detector temperatures were maintained at 250 °C and 260 °C, respectively. A 1.0-μL aliquot of the esterified extract was injected into a split/*splitless* type of injector at 250 °C, and chromatograms were recorded using Galaxie Chromatography Data System software. The fatty acids were identified by comparing the methyl ester retention times with those of the standards from a Supelco ME19-Kit (Fatty Acid Methyl Esters C6-C24). The fatty acid contents were quantified using area

normalization of the methyl ester peaks and were expressed as the percent (%) area.

2.9. Determination of the sugar profile

Ricotta samples (5 g) were precipitated by adding 20.0 g of 1.0 M perchloric acid and letting the preparation stand overnight at 4 °C. The supernatant (1 mL) was cleared by centrifugation (4000 rpm, 15 min, 4 °C), and this material was passed through a 0.45-mm membrane filter and subjected to HPLC analysis (Freitas, Pintado, Pintado, & Malcata, 1999). The sugar content was determined using a 1100 series Hewlett-Packard chromatograph equipped with a refractive index detector, operated at 50 °C, and a 300 × 7.8 mm CARBOsep CHO 682 column (Transgenomic, Glasgow, U.K.), operated at 80 °C. Distilled water was used as the mobile phase (flow rate of 0.4 mL/min). The HPLC sample peaks were identified by comparing their retention times with those of sugars standards (Sigma Aldrich®), namely lactose, galactose and glucose. Duplicate injections were performed, and the average peak areas were used for quantification.

2.10. Determination of the organic acid profile

Ricotta samples (5 g) were precipitated by adding 20.0 g of 1.0 M perchloric acid and letting the preparation stand overnight at 4 °C. The supernatant (1 mL) was cleared by centrifugation (4000 rpm, 15 min, 4 °C), and this material was passed through a 0.45-mm membrane filter and subjected to HPLC analysis (Freitas et al., 1999) to directly determine the organic acids content using an Agilent 1200 series HPLC instrument equipped with a refractive index (RI) detector (Agilent, Waldbronn, Germany) and operated at 50 °C. The other analytic conditions were as follows: an Aminex HPX-87H column (BioRad, Hercules, CA, USA); mobile phase, 0.003 M H_2SO_4; flow rate, 0.6 mL/min. The HPLC sample peaks were identified by comparing their retention times with those of organic acid standards (Sigma Aldrich®), namely acetic, formic and lactic. Duplicate injections were performed, and the average peak areas were used for quantification.

2.11. Sensory analysis

For the sensory analysis, the ricotta samples were characterized using the quantitative descriptive analysis (QDA) method (Stone & Sidel, 1993). The panel consisted of 10 panelists who were trained in pre-selection, the definitions of the descriptive terminology and descriptive analysis. The panelists participated in 10 training sessions (each session lasting one hour) to develop their descriptive terminology and become familiar with the reference materials. The attributes evaluated included the appearance (smooth, whitish and creamy), aroma (goat milk and butter), flavor (goat milk, butter, acidic and salty) and texture (soft and homogenous). An unstructured scale ranging from 0 (poor) to 9 (strong), which anchored the minimal and the maximal values, was used to assess the intensity of each described attribute (Borba et al., 2013). The analyses were performed in individual booths with controlled temperature and lighting, and the samples were served at the refrigeration temperature in disposable dishes coded with three random digits and accompanied by mineral water and crackers.

2.12. Effects of goat ricotta on the viability of probiotic bacteria exposed to simulated gastrointestinal conditions

Each probiotic strain was studied separately; for each strain, a set of three ricotta samples labeled S1, S2 and S3 were produced. S1 was a ricotta sample that was inoculated with the tested probiotic strain but was not exposed to the simulated gastrointestinal conditions, S2 was a ricotta sample that was not inoculated with the tested probiotic strain but was exposed to the simulated gastrointestinal conditions (and used to aseptically follow the pH adjustments during the sequential stages of the in vitro digestion); and S3 was a ricotta sample that was inoculated with the tested probiotic strain and was exposed to the simulated gastrointestinal conditions. Twenty-five gram samples containing each of the probiotic strains were prepared in sterile 50-mL flasks. The simulated gastrointestinal pathway used in this study, including the compounds utilized, their concentrations, the exposure period and the intensities of stirring at all steps (stirring was used to somewhat simulate peristaltic movements) is described in Table 1. The simulation process was continuous, so that the overall working volume increased (as happens during actual digestion) from that of the initial 25-g sample of ricotta (Madureira, Amorim, Gomes, Pintado, & Malcata, 2011). The number of viable cells referred to the volume at each stage so these values could be compared with the values for the R1 counterparts to compensate for the effect of dilution. All of the enzyme solutions were freshly prepared and were filter-sterilized using a 0.22-μm membrane filter (Millipore, Billerica, MA, USA) prior to use; after sterilization, all of the solutions were maintained in an ice bath during the entire period of simulation prior to their gradual addition (when appropriate). After exposure to each artificial digestion condition, a 1-mL aliquot of the system in each gastrointestinal compartment was aseptically collected and then was serially diluted using sterile peptone water [0.1 g/100 mL (Sigma, St. Louis MO, USA)]. A 1-mL aliquot of each dilution was dispensed into cMRS or MRS agar (Himedia, India) for counting *B. lactis* and *L. acidophilus* cells, respectively,

Table 1
The conditions used during each step of the simulated digestion and the obtained viable cell counts (n:3, mean values ± standard deviation, in log of cfu/g) for *L. acidophilus* La-05 (*L. acidophilus*) and *B. lactis* BB-12 (*B. lactis*) assayed in de Man, Rogosa and Sharpe (MRS) broth or into goat ricotta, after exposure to each digestion step.

Steps	Compartment	Conditions	Stirring (rpm)	Final pH	Time of exposure (min)	Viable cell counts (in log cfu/g)			
						L. acidophilus		*B. lactis*	
						MRS broth	Goat ricotta	MRS broth	Goat ricotta
1	Before simulation	–	–	–	–	6.36 (±0.2)^{A}	6.54 (±0.3)^{A}	6.49 (±0.1)^{A}	6.22 (±0.3)^{A}
2	Mouth	Saliva	200	6.9	2	6.34 (±0.1)^{A}	6.51 (±0.2)^{A}	6.01 (±0.3)^{A}	6.44 (±0.2)^{A}
3	Esophagus–stomach	Pepsin	130	5.5	10	6.14 (±0.3)^{A}	6.41 (±0.1)^{A}	5.73 (±0.2)^{A}	6.14 (±0.3)^{B}
4				4.6	10	5.94 (±0.2)^{A}	6.43 (±0.3)^{A}	5.43 (±0.2)^{A}	5.94 (±0.1)^{B}
5				3.8	10	6.13 (±0.2)^{A}	6.19 (±0.2)^{A}	5.44 (±0.3)^{A}	6.13 (±0.2)^{B}
6				2.8	20	6.08 (±0.3)^{A}	5.95 (±0.3)^{A}	5.32 (±0.2)^{A}	6.08 (±0.3)^{B}
7				2.3	20	6.06 (±0.1)^{A}	5.74 (±0.3)^{A}	4.60 (±0.3)^{A}	6.06 (±0.2)^{B}
8				2.0	20	6.38 (±0.2)^{A}	5.86 (±0.2)^{B}	3.50 (±0.1)^{A}	6.18 (±0.3)^{B}
9	Duodenum	Pancreatin + bile salts	45	5.0	30	6.58 (±0.2)^{A}	5.93 (±0.2)^{B}	≤2 (±0.0)^{A}	5.78 (±0.3)^{B}
10	Ileum		45	6.5	60	6.04 (±0.3)^{A}	6.01 (±0.3)^{A}	≤2 (±0.0)^{A}	6.27 (±0.1)^{B}

A–B: different superscript letters in the same row denote differences ($p \leq 0.05$) between the viable cell counts obtained for the same bacterial strain when assayed in MRS broth or into goat ricotta and exposed to the same step of the experimental digestion, according to the Tukey's test.

using the pour-plate inoculation method, followed by incubation for 3 days at 37 °C under anaerobic conditions (Anaerobic System Anaerogen, Oxoid). The counts were expressed as the log of the colony forming units per gram of ricotta (log cfu/g).

2.13. Statistical analysis

All analyses were conducted on three different occasions (repetitions) and the samples were assessed in triplicate. Initially, the data were assessed via descriptive analysis (means and standard deviation) to obtain the description order of the variables. Subsequently, inferential analyses were performed to determine significant differences ($p \leq 0.05$) between the results obtained from different treatments (ANOVA followed by post hoc Tukey test or student *t* test). Principal Component Analysis (PCA) was conducted due to its ability to provide accurate graphical representations (that best integrated all of the significant data) of objects or variables for studies of their proximity (Silva, Minim, Simiqueli, Gomide, & Minim, 2010). Fro this, the SigmaStat 3.1 software was used.

3. Results and discussion

3.1. Microbiological analysis of ricotta samples and viability of the probiotics during storage

At the chosen storage period (1 and 7 days), all of the goat ricotta samples had < 0.3 NMP/g of total and thermotolerant coliforms and lacked coagulase-positive *Staphylococcus*, *Salmonella* spp. and *L. monocytogenes*. These results indicated that the goat ricotta produced had a satisfactory microbiological quality as determined by current Brazilian legislation (Brazil, M.S. Resolution RDC n° 12, 2001). The counts of mesophilic bacteria in all of the ricotta samples were less than 6.0 log CFU/g at the end of the experimental storage period, which was in agreement with the European Union Directives (92/46 and 94/71 EU Directives) for mesophilic bacterial counts in goat cheeses.

The counts of *L. acidophilus* and *B. lactis* at 1 and 7 days of storage were approximately 6 log CFU/g in R2 (day 1: 6.01 ± 0.6 log cfu/g; day 7: 6.29 ± 0.9 log cfu/g) and R3 (day 1: 6.12 ± 0.4 log cfu/g; day 7: 6.31 ± 0.6 log cfu/g), although the initial counts in all of the ricotta samples were slightly higher (±0.3 log cfu/g) than those at the end of the storage period. Previous studies reported similar counts for the same probiotic strains tested in this study during the refrigerated storage of goat dairy products (e.g., dairy beverages and cheese) (Garcia et al., 2012; Oliveira et al., 2012; Silveira et al., 2014), as well as for *Lactobacillus* spp. and *Streptococcus thermophilus* in bovine fresh white cheese (Yerlikaya & Ozer, 2014). Maintaining viable counts of approximately 6 cfu/g during a 7-day storage period is noteworthy because this is the minimal count (6 cfu/g) of probiotics that must be present in food to provide their potential benefits to the host (Plessas, Bosnea, Alexopoulos, & Bezirtzoglou, 2012; Vandenplas et al., 2015).

3.2. Yield, syneresis, pH, Aw and proximate composition

Incorporating *L. acidophilus* (R2) or *B. lactis* (R3) in goat ricotta did not affect the yield of the obtained samples. The yields of the goat ricotta cheeses that did or did not contain the tested probiotics were similar ($p > 0.05$), ranging from 4.26 g/100 g to 4.51 g/100 g (Table 2). A previous study reported a higher yield (13–14%) for goat semi-hard cheese containing *Lactobacillus paracasei* compared with the same cheese containing *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and/or the cheese containing *L. acidophilus* La-05 (Oliveira et al., 2012). However, these investigators used a starter culture (containing *L. paracasei*) to manufacture cheese that was not supplemented with *L. acidophilus* and thus the observed yield was most likely due to specific characteristics of the product that were primarily related to the technological qualities of the starter culture used. It is well known that the curds obtained via acid precipitation of goat milk (as applied in our study) are fragile, resulting in a low yield of the cheeses obtained using it (Raynal-Ljutovac et al., 2011).

Table 2
Yield, syneresis and physicochemical parameters (n:3, mean values ± standard deviation) of goat ricotta cheese not containing or containing *L. acidophilus* La-05 or *B. lactis* Bb-12, after 1 and 7 days of refrigerated storage.

Parameters	Days of storage	Cheeses		
		R1	R2	R3
Yield[1]	1	4.40 (±0.20)^{A}	4.26 (±0.39)^{A}	4.51 (±0.28)^{A}
Syneresis[2]	1	1.79 (±0.10)^{Ca}	2.13 (±0.02)^{Aa}	2.27 (±0.01)^{Bb}
	7	2.70 (±0.02)^{Bc}	2.77 (±0.04)^{Cc}	3.12 (±0.04)^{Ac}
Moisture (g/100 g)	1	70.23 (±0.36)^{Aa}	68.39 (±0.41)^{Ba}	70.39 (±0.07)^{Aa}
	7	68.55 (±0.42)^{Bab}	66.51 (±0.08)^{Cb}	68.93 (±0.14)^{Bb}
Dry matter (g/100 g)	1	29.77 (±0.36)^{Cb}	33.49 (±0.08)^{Aa}	31.07 (±0.14)^{Ba}
	7	31.45 (±0.42)^{Aab}	31.61 (±0.41)^{Ab}	29.61 (±0.07)^{Bb}
Ashes (g/100 g)	1	1.16 (±0.03)^{Aa}	1.18 (±0.03)^{Aa}	1.22 (±0.06)^{Aa}
	7	1.04 (±0.07)^{Ab}	1.03 (±0.02)^{Ab}	1.06 (±0.01)^{Ab}
Total protein (g/100 g)	1	9.49 (±0.39)^{Aa}	8.68 (±0.12)^{Aa}	8.65 (±0.46)^{Aa}
	7	7.14 (±0.19)^{Ab}	7.27 (±0.24)^{Ab}	7.35 (±0.20)^{Ab}
Fat (g/100 g)	1	16.00 (±1.41)^{Aa}	15.00 (±1.56)^{Aa}	17.00 (±1.29)^{Aa}
	7	18.00 (±1.52)^{Aa}	17.50 (±1.61)^{Aa}	16.00 (±1.73)^{Aa}
Lactose (g/100 g)	1	3.35 (±0.06)^{Aa}	2.37 (±0.07)^{Ba}	3.08 (±0.10)^{Ca}
	7	2.18 (±0.01)^{bA}	2.19 (±0.01)^{Ab}	2.14 (±0.02)^{Bb}
pH	1	6.96 (±0.10)^{Ba}	6.93 (±0.12)^{Ba}	6.98 (±0.10)^{Aa}
	7	6.97 (±0.10)^{Aa}	6.70 (±0.10)^{Bb}	6.69 (±0.11)^{Bb}
Titratable acidity (g/100 g)	1	0.25 (±0.01)^{Aa}	0.23 (±0.01)^{Aa}	0.22 (±0.03)^{Aa}
	7	0.26 (±0.01)^{Aa}	0.26 (±0.02)^{Ab}	0.25 (±0.01)^{Ab}
Aw	1	0.99 (±0.00)^{Aa}	0.99 (±0.00)^{Aa}	0.99 (±0.00)^{Aa}
	7	0.99 (±0.00)^{Aa}	0.99 (±0.00)^{Aa}	0.99 (±0.00)^{Aa}

R1: goat ricotta lacking probiotic bacteria.
R2: goat ricotta containing *L. acidophillus* La-05.
R3: goat ricotta containing *B. lactis* Bb-12.
A–C: different superscript capital letters in the same row denote differences ($p \leq 0.05$) between different treatments, according to the Tukey's test.
a–c: different superscript lowercase letters in the same column denote differences ($p \leq 0.05$) in the same treatment during storage, according to the Tukey's test.

After 7 days of storage, the syneresis value of all the goat ricotta samples (R1, R2 and R3) had increased, most likely due to the observed decrease in the moisture content that occurred during storage (Table 2). Similar results were reported for fresh white bovine cheese (Minas Frescal) containing *L. paracasei* (Buriti, Rocha, Assis, & Saad, 2005). The moisture content of all of the ricotta samples was greater than 55 g/100 g whereas the Aw value was approximately 0.99 (Table 2). These findings showed that incorporating the tested probiotic strains did not change the characteristics of goat ricotta that are required by the Brazilian legislation (Brazil, M.S. Resolution RDC n° 12, 2001), such as a high moisture content. Similarly, a previous study reported that the production of fresh white cheese containing different co-cultures comprising probiotic strains of *Lactobacillus* spp. and *S. thermophilus* did not affect negatively the cheese characteristics (Yerlikaya & Ozer, 2014).

Some investigators have suggested that the syneresis rate is directly related to the acidity level and therefore is inversely related to the pH value (Souza & Saad 2009); however, in our study, these predicted relationships were valid only for ricotta containing the probiotic strains (R2 and R3). The pH value of R2 and R3 decreased during refrigerated storage, and the acidity level of these samples increased, but not that of R1 (control without probiotics) (Table 2). The increase in the acidity of R2 and R3 was most likely due to the increase in the contents of organic acids caused by the metabolism of the probiotics added to these cheeses (Salminen, Von Wright, & Ouwehand, 2004). The decrease in the pH values and consequent increase in the acidity level during refrigerated storage have been reported in goat-milk beverages containing *B. lactis* (BLC1) (Silveira et al., 2014) and in Minas fresh cheese containing *L. acidophilus* (La-05) (Buriti, Da Rocha, & Saad, 2005).

The ash content (fixed mineral residue) of all of the ricotta samples decreased during 7 days of storage ($p \leq 0.05$) (Table 2). Despite the differences among the treatments, the decrease in ash content may be related to the loss of minerals that occurred when whey was released (syneresis) during storage (Borba et al., 2013; Sant'Ana et al., 2013).

The protein content of goat ricotta containing or not containing a probiotic decreased ($p \leq 0.05$) during 7 days of storage (Table 2). The decrease in the values for this parameter can be partially explained by the well-documented protein degradation that occurs during the storage of fresh cheeses, which affects the rheological characteristics of these products, primarily their texture and flavor (Sánchez-Macías et al., 2011).

The fat contents of the goat ricotta samples studied ranged from 15 to 17 g/100 g, with no differences ($p > 0.05$) among the different samples after 1 or 7 days of storage. Some researchers have stated that the high rate of heating used to manufacture ricotta could be related to the high retention of fat in this product (Pintado, da Silva, & Malcata, 1996). Ricotta samples without an added probiotic had a higher content ($p \leq 0.05$) of lactose compared with that of samples containing *L. acidophilus* La-05 or *B. lactis* Bb-12 (R2 and R3, respectively). However, the lactose content decreased during the storage period (Table 2) in all of the ricotta samples. The lower lactose content of goat ricotta cheeses containing *L. acidophilus* or *B. lactis* could be attributed to the sugar- metabolism profile of the added probiotics because *L. acidophilus* and *B. lactis* are typical lactose-fermenting microorganisms (Oliveira et al., 2012).

3.3. Instrumental textural profile

The instrumental textural profile of the different goat ricotta samples characterized these products as soft in texture, non-elastic, easily deformable, cohesive and having a fragile structure. After 7 days of storage, an increased ($p \leq 0.05$) hardness was observed in all of the samples; however, the goat ricotta containing one of the tested probiotics had higher values ($p \leq 0.05$) for this parameter compared with that of the ricotta lacking a probiotic (Table 3). The increase in hardness that occurred during storage can be attributed to the increased degree of crosslinking among the proteins resulting in the formation of three-dimensional networks, which would be the consequence of the greater level of syneresis of these sample causing compression of the cheese structure and consequently placing the proteins in closer proximity (Lobato-Calleros et al., 2007; Oliveira et al., 2012). The difference ($p \leq 0.05$) in the hardness of the goat ricotta samples containing a probiotic compared with that of the control samples could be related to changes in the cheeses promoted by bacterial metabolism. A previous study reported that Minas frescal cheese containing *L. acidophilus* became harder during storage, which was proposed to be related to the increase in acidity (Buriti, Da Rocha, & Saad, 2005). This finding is interesting because the increased acidity of R2 and R3 observed after 7 days of storage compared with that observed in R1 could have contributed to the increased hardness of these samples, as well as to the greater levels of chewiness and gumminess (hardness derivative parameters) ($p \leq 0.05$). Another important factor related to the greater chewiness and gumminess of goat ricotta containing probiotics is the ability of lactobacilli and bifidobacteria to produce exopolysaccharides (EPSs). These EPSs can improve the texture and viscosity of dairy products because they modify their structures (Salazar et al., 2009).

3.4. Instrumental color profile

Studies have shown that incorporating probiotics into cheeses affected the color changes that occurred during their storage (Garcia et al., 2012; Rohm & Jaros, 1996). The brightness (L^* value) of the goat ricotta containing or not containing *L. acidophilus* La-05 or *B. lactis* Bb-12 significantly differed ($p \leq 0.05$). Lower values for brightness (L^*) and higher values ($p \leq 0.05$) for green color (a^* value) were found for R2 and R3 compared with those for R1 (Table 3). The differences between the ricotta containing either of the tested probiotics and the control ricotta might be associated with their ability to synthesize certain nutrients, particularly the B vitamins (such as, riboflavin — B12 vitamin), which contribute to the production of green pigments in food (Gomes & Malcata, 1999; Salminen et al., 2004). The yellow color (b^* values) had increased ($p \leq 0.05$) in R2 and R3 and had decreased ($p \leq 0.05$) in R1 by the end of experimental storage period. Few studies have assessing the color changes that occur in goat ricotta, a making it difficult to thoroughly discuss the data obtained in this study; nevertheless, yellowing during the

Table 3
Mean values (n:3, ± standard deviation) for textural parameters of goat ricotta cheese not containing or containing *L. acidophilus* La-05 or *B. lactis* Bb-12, after 1 and 7 days of refrigerated storage.

Parameters	Days of storage	Cheeses		
		R1	R2	R3
Textural parameters				
Hardness	1	427.92 (±1.19)^{Aa}	277.05 (±1.69)^{Cb}	370.56 (±0.69)^{Bb}
	7	438.18 (±1.39)^{Ca}	493.65 (±2.13)^{Aa}	467.85 (±1.25)^{Ba}
Adhesiveness	1	−68.37 (±9.26)^{Aa}	−30.73 (±4.28)^{Aa}	−57.68 (±12.13)^{ab}
	7	−41.21 (±3.64)^{Ab}	−30.27 (±6.52)^{Aa}	−29.61 (±5.23)^{Ab}
Springiness	1	0.81 (±0.06)^{Aa}	0.81 (±0.03)^{Aa}	0.78 (±0.01)^{Aa}
	7	0.80 (±0.07)^{Aa}	0.76 (±0.05)^{Aa}	0.80 (±0.01)^{Aa}
Cohesiveness	1	0.37 (±0.03)^{Aa}	0.29 (±0.04)^{Aa}	0.30 (±0.08)^{Aa}
	7	0.43 (±0.03)^{Ab}	0.40 (±0.03)^{Ab}	0.42 (±0.04)^{Ab}
Gumminess	1	160.49 (±19.03)^{Aa}	111.60 (±15.24)^{Bb}	113.06 (±2.53)^{Bb}
	7	142.71 (±16.43)^{Cb}	154.95 (±29.62) ^{Ba}	214.19 (±5.15)^{Aa}
Chewiness	1	131.09 (±1.25)^{Aa}	90.15 (±9.72)^{Ba}	88.69 (±2.93)^{Ba}
	7	135.42 (±4.15)^{Aa}	114.88 (±3.87)^{Cb}	122.92 (±2.38)^{Bb}
Color parameters				
L	1	90.86 (±0.13)^{Ab}	69.37 (±0.25)^{Ba}	57.12 (±0.10)^{Ca}
	7	93.33 (±0.10)^{Aa}	55.67 (±0.10)^{Bb}	52.37 (±0.17)^{Bb}
A	1	−2.90 (±0.08)^{Cb}	−1.52 (±0.02)^{Ba}	−1.43 (±0.04)^{Ab}
	7	−2.12 (±0.03)^{Ba}	−1.76 (±0.01)^{Cb}	−1.31 (±0.02)^{Aa}
B	1	7.73 (±0.01)^{Aa}	5.47 (±0.02)^{Ba}	4.28 (±0.02)^{Ca}
	7	5.86 (±0.06)^{Ab}	5.76 (±0.04)^{Bb}	4.43 (±0.01)^{Cb}

R1: goat ricotta lacking probiotic bacteria.
R2: goat ricotta containing *L. acidophilus* La-05.
R3: goat ricotta containing *B. lactis* Bb-12.
A–C: different superscript capital letters in the same row denote differences ($p \leq 0.05$) between different treatments, according to the Tukey's test.
a–c: different superscript lowercase letters in the same column denote differences ($p \leq 0.05$) in the same treatment during storage, according to the Tukey's test.

storage of cheeses made using goat milk (ricotta and Ibores cheese) without probiotics was not detected in previous studies (Delgado, González-Crespo, Cava, & Ramírez, 2012; Pizzillo, Claps, Cifuni, Fedele, & Rubino, 2005), suggesting that there is relationship between the detected color changes and the presence of the tested probiotic strains.

3.5. Fatty acid profile

The fatty acid profiles of all of goat ricotta samples included higher levels of long- and medium chain fatty acids than short-chain fatty acids, as shown by the large amounts of myristic (C14:0), palmitic (C16:0), stearic (C18:0) acids and monounsaturated oleic acid (C18:1n9c) (Table 4). Similar fatty acid profile was previously reported for creamy ricotta made with a mixture of whey and cow's milk and goat's milk (Borba et al., 2013), fresh goat cheese (Galiou et al., 2015) and low-ripened goat cheese (Poveda & Cabezas, 2006). The presence of higher amounts of long- and medium chain fatty acids and lower levels of short-chain fatty acids, particularly caproic, caprylic and capric acids, in obtained ricotta cheeses is interesting because the residual goat flavor and taste in goat dairy products is mainly attributed to the high content of short- and medium-chain fatty acids (Prandini et al., 2011; Raynal-Ljutovac et al., 2011).

The fatty acid profiles of the goat ricotta samples containing or not containing one of the tested probiotics showed the presence of essential fatty acids, such as oleic (C18:1n9c) acid, which have beneficial effects on health, particularly those related to protection against cardiovascular chronic diseases. Some studies have associated differences in fatty acid profile and essential fatty acids production in cheeses with the metabolism (mostly lipolysis activity) of autochthonous, starter or added probiotic cultures (Lavasani & Ehsani, 2012; Medina, Oliszewski, Abeijón Mukdsi, Van Nieuwenhove, & González, 2011; Taboada, Van Nieuwenhove, Alzogaray, & Medina, 2015). However, the influence of probiotic strains on fatty acid profile of cheeses has been observed in ripened rather than in fresh cheeses (such as ricotta cheese) (Lavasani & Ehsani, 2012; Taboada et al., 2015).

Table 4
Fatty acids (n:3, mean values, ± standard deviation) in goat ricotta cheese not containing or containing *L. acidophilus* La-05 or *B. lactis* Bb-12, after 1 and 7 days of refrigerated storage.

Fatty acids	Cheeses		
	R1	R2	R3
Short chain			
Caproic (C6:0)	0.31 (±0.12)^{A}	nd	0.75 (±0.33)^{A}
Caprylic (C8:0)	0.80 (±0.11)^{A}	0.69 (±0.10)^{A}	0.65 (±0.16)^{A}
Pelargonic (C9:0)	0.03 (±0.01)^{A}	0.02 (±0.01)^{A}	0.04 (±0.01)^{A}
Capric (C10:0)	6.53 (±1.30)^{A}	4.93 (±0.50)^{A}	5.20 (±1.02)^{A}
Undecanoic (C11:0)	0.27 (±0.02)^{A}	0.05 (±0.01)^{B}	0.05 (±0.01)^{B}
Medium chain			
Lauric (C12:0)	3.64 (±1.35)^{A}	2.81 (±0.15)^{B}	2.95 (±0.13)^{B}
Myristic (C14:0)	10.71 (±1.60)^{A}	9.13 (±1.51)^{A}	9.55 (±1.10)^{A}
Myristoleic (C14:1)	0.25 (±0.03)^{B}	0.40 (±0.02)^{A}	0.42 (±0.02)^{A}
Pentadecanoic (C15:0)	1.04 (±0.03)^{A}	1.08 (±0.01)^{A}	1.14 (±0.10)^{A}
Palmitic (C16:0)	27.32 (±2.01)^{A}	25.48 (±1.80)^{A}	26.70 (±1.20)^{A}
Palmitoleic (C16:1)	0.88 (±0.02)^{A}	0.87 (±0.02)^{A}	0.92 (±0.02)^{A}
Long chain			
Heptadecanoic (C17:0)	0.65 (±0.06)^{A}	0.67 (±0.04)^{A}	0.71 (±0.04)^{A}
Cis-10-heptadecanoic (C17:1)	0.30 (±0.03)^{A}	0.36 (±0.03)^{A}	0.37 (±0.03)^{A}
Stearic (C18:0)	10.42 (±1.02)^{A}	9.08 (±1.13)^{C}	9.63 (±1.09)^{B}
Oleic (C18:1 n9cis)	30.92 (±4.50)^{A}	26.49 (±4.37)^{A}	27.92 (±3.32)^{A}
Vaccenic (C18:1 n11cis)	1.07 (±0.05)^{B}	1.41 (±0.05)^{A}	1.49 (±0.04)^{A}
Linoleic (C18:2 n6cis)	1.74 (±0.06)^{B}	1.85 (±0.04)^{B}	1.97 (±0.05)^{A}
Nonadecanoic (C19:0)	0.14 (±0.01)^{A}	0.16 (±0.01)^{A}	0.15 (±0.01)^{A}
Alpha-linolenic (C18:3 n3)	0.55 (±0.10)^{A}	0.74 (±0.11)^{A}	0.81 (±0.10^{A}
Eicosanoic (C20:0)	0.34 (±0.07)^{A}	0.16 (±0.05)^{C}	0.30 (±0.09)^{B}
Cis-9-eicosenoic (C20:1 n9)	0.06 (±0.03)^{A}	0.10 (±0.02)^{A}	0.16 (±0.05)^{A}
Heneicosanoic (C21:0)	nd	0.29 (±0.01)^{A}	0.30 (±0.01)^{A}
Docosanoic (C22:0)	0.74 (±0.05)^{B}	0.63 (±0.03)^{A}	0.10 (±0.03)^{C}
Erucic (C22:1n9)	nd	0.22 (±0.02)^{B}	0.40 (±0.02)^{A}

R1: goat ricotta lacking probiotic bacteria.
R2: goat ricotta containing *L. acidophilus* La-05.
R3: goat ricotta containing *B. lactis* Bb-12.
A–C: different superscript letters in the same row denote differences ($p \leq 0.05$) among the different treatments, according to the Tukey's test.
nd: not detected.

3.6. Sugar profile and organic acid profiles

The sugar present in the highest concentration in all of the goat ricotta samples was lactose (Table 5). Ricotta samples lacking a probiotic had a higher level ($p \leq 0.05$) of lactose compared with samples containing *L. acidophilus* La-05 or *B. lactis* Bb-12 (R2 and R3, respectively) after 7 days of storage. Lower levels of glucose ($p \leq 0.05$) were found in goat ricotta cheeses containing a probiotic (R2 and R3) compared with ricotta lacking a probiotic (R1) after both 1 and 7 days of storage. The lower lactose and glucose content of the ricotta containing a probiotic is likely to be associated with the conversion of these sugars into organic acids by the incorporated probiotic bacteria (Garde, Ávila, Gaya, Arias, & Nuñez, 2012). *L. acidophilus* and *B. lactis* are typical glucose/lactose-fermenting microorganisms that use these monosaccharides as substrates for lactic fermentation (Oliveira et al., 2012). The use of glucose and lactose by lactic-acid bacteria (LAB) could also be involved in their EPS production because galactose was found to be a constituent of the EPSs produced by mesophilic and thermophilic LAB (Salminen et al., 2004) and the level of galactose increased in goat ricotta cheeses containing each of the tested probiotic strains during storage.

Organic acids are important flavor compounds of dairy products (typically in aged cheeses). These acids are formed in cheeses as result of bacterial metabolism and/or the degradation of milk proteins, fats, lactose and citrate during their manufacture and/or storage (Seçkin & Esmer, 2011). Acetic acid was the most abundant organic acid in all of the goat ricotta samples, which was most likely the consequence of using vinegar for the production of curds during the ricotta manufacturing process (Table 5). A significant decrease in the level of acetic acid was observed in all of the ricotta samples after 7 days of storage, whereas the level of lactic acid increased in R2 and R3 during this period ($p \leq 0.05$). These

Table 5
Sugars and organic acids (n:3, mean values, ± standard deviation) in goat ricotta cheese not containing or containing *L. acidophilus* La-05 or *B. lactis* Bb-12, after 1 and 7 days of refrigerated storage.

Sugars	Days of storage	Cheeses		
		R1	R2	R3
Lactose	1	142.82 (±4.28)^{Cb}	154.96 (±1.10)^{Aa}	168.92 (±4.31)^{Ba}
	7	145.20 (±2.50)^{Ca}	138.25 (±4.50)^{Bb}	135.50 (±6.55)^{Ab}
Galactose	1	3.50 (±0.70)^{Cb}	3.15 (±0.63)^{Bb}	4.33 (±0.87)^{Ab}
	7	4.00 (±0.54)^{Ca}	4.80 (±0.96)^{Ba}	5.21 (±0.68)^{Aa}
Glucose	1	6.20 (±0.99)^{Aa}	3.52 (±0.56)^{Ca}	4.87 (±0.78)^{Ba}
	7	6.19 (±0.93)^{Aa}	3.49 (±0.60)^{Ba}	4.11 (±0.70)^{Ba}
Organic acids				
Lactic	1	0.7 (±0.01)^{Bb}	1.90 (±0.04)^{Ab}	1.60 (±0.02)^{Ba}
	7	0.6 (±0.05)^{Aa}	2.20 (±0.03)^{Ba}	1.80 (±0.01)^{Ca}
Formic	1	0.14 (±0.03)^{Bb}	0.17 (±0.03)^{Ab}	0.16 (±0.03)^{Aba}
	7	0.23 (±0.05)^{Aa}	0.25 (±0.04)^{Aa}	0.22 (±0.04)^{Ba}
Acetic	1	1.08 (±0.11)^{Ba}	0.92 (±0.06)^{Ca}	1.12 (±0.11)^{Aa}
	7	0.87 (±0.09)^{Bb}	0.79 (±0.05)^{Cb}	1.04 (±0.10)^{Ab}

R1: goat ricotta lacking probiotic bacteria.
R2: goat ricotta containing *L. acidophilus* La-05.
R3: goat ricotta containing *B. lactis* Bb-12.
A–C: different superscript capital letters in the same row denote differences ($p \leq 0.05$) between different treatments, according to the Tukey's test.
a–c: different superscript lowercase letters in the same column denote differences ($p \leq 0.05$) in the same treatment during storage, according to the Tukey's test.

results were somewhat expected because lactic acid is the main product of sugar fermentation by the probiotics that were incorporated into the goat ricotta cheeses. Moreover, *Bifidobacterium* can produce acetic acid from glucose or lactose via an unusual pathway (Gomes & Malcata, 1999; Hughes & Hoover, 1991), which could explain the higher content of acetic acid ($p \leq 0.05$) in R3 compared with that in R2 after 7 days of storage.

3.7. Quantitative descriptive analysis

All of the goat ricotta samples (R1, R2 and R3) were considered cheeses with a soft and homogeneous texture (Table 6). In general, no differences in the evaluated sensory attributes ($p \geq 0.05$) were observed among the samples containing or not containing each of the tested probiotics, with exception of acidity. Earlier studies reported similar results for bovine cheddar cheese (Gardiner, Ross, Collins, Fitzgerald, & Stanton, 1998; Stanton et al., 1998) containing probiotic *Lactobacillus* strains. After 7 days of refrigerated storage, R2 and R3 received higher scores for acidity than did R1. The higher acidity of these cheeses perceived by the panelists was most likely the result of the acid production of the incorporated probiotics via lactose fermentation, which decreased the pH values. The creamy color also increased only in R2 and R3 during the experimental storage period, which was consistent with the yellowing observed in these samples during this period (as detected using color instrumental analysis).

It is noteworthy that the "goat milk flavor" and "goat milk aroma" scores were not changed by storing any of the ricotta samples. Thus, using goat whey to manufacturing the ricotta and maintaining the goat milk content at a minimal level might have contributed to the acceptance of this product because goat whey has a low content of short- and medium-chain fatty acids, which are negatively related to the desired sensory aspects of goat dairy products (Raynal-Ljutovac et al., 2011).

After the characteristics of stored R1, R2 and R3 had been studied, PCA was used to assess the overall effect of incorporating each of the tested probiotics into goat ricotta based on the principal components that defined the ricotta samples stored for 7 days. The parameters of goat ricotta that contributed most to PC1 were the lactose content, perceived acidity, brightness, lactic acid content, homogenous texture and goat milk flavor (Fig. 2A). PC1, which explained 39.57% of the variance among the samples, clearly separated the perceived acidity, lactic acid content, homogeneous texture and brightness from the lactose content and goat milk flavor. Most of the variability related to the effects of the probiotics incorporated into goat ricotta could be explained by these variables. PC2, which explained 32.84% of the variance among the samples, was defined by the hardness, smooth appearance, whitish color, butter aroma and goat milk aroma. The variability not explained by PC1 was explained by these variables. In the case of PC2, the hardness and butter aroma together with a whitish color and smooth appearance were found to be the most important variables that separated the ricotta containing each of the added probiotics (R2 and R3) from the ricotta that did not contain a probiotic (R1) (Fig. 2B).

Previous studies also reported a whitish color, perceived acidity and goat milk aroma as important characteristics of goat fresh cheese (Sant'Ana et al., 2013). Similar to the findings of our study, a butter aroma and smooth appearance better described creamy ricotta manufactured using a mixture of goat and cow whey (Borba et al., 2013). PC1 also explained the separation of the goat ricotta containing *L. acidophilus* (R2) from that containing *B. lactis* (R3) and ricotta without an added probiotic (R1) (Fig. 2B), most likely due to the higher acidity perceived by the sensory panelists after this sample (R2) had been stored for 7 days. PC2 explained the separation of either goat ricotta containing *L. acidophilus* La-05 or *B. lactis* Bb-12 (Fig. 2B) from the goat ricotta not containing a probiotic due to its lower level of hardness (Table 3) and smoother appearance (Table 6).

The angle between the vectors that represented the variables showed the correlation among the variables. Vectors with angle of 90° indicated that the variables were not correlated, whereas angles smaller or greater than 90° suggested a positive or negative correlation between the variables, respectively (Silva et al., 2010). For the goat ricotta samples studied, perceived acidity was positively correlated with the lactic acid and lactose content ($r = 0.89$), whereas a negative correlation was observed for chewiness ($r = -0.81$) and brightness ($r = -0.89$). There was also a negative correlation between gumminess and a smooth appearance ($r = -0.74$) of all of the ricotta samples, as demonstrated by the inverse relationship between the values obtained for these parameters (Tables 3 and 6).

3.8. Viability of probiotic bacteria exposed to simulated gastrointestinal conditions

The counts of viable *L. acidophilus* La-05 and *B. lactis* Bb-12 in MRS broth and in goat ricotta cheeses after these samples were exposed to the simulated gastrointestinal conditions were monitored (Table 1). *B. lactis* Bb-12 maintained higher ($p \leq 0.05$) viable counts when incorporated into goat ricotta compared with the counts observed when this strain was assayed in MRS broth at each successive step comprising the simulated digestive process. At the beginning of the in vitro digestive process (time zero, before exposure to the experimental mouth conditions), the counts of both of the probiotic strains incorporated into either MRS or goat ricotta were approximately 6 log CFU/g (±0.5). The counts of the samples collected at the end of the experimental digestive process (after the 10th digestive step) were approximately 6.5 log CFU/g and 6.0 log CFU/g for *L. acidophilus* La-05 when incorporated into MRS and into goat ricotta, respectively, and ≤ 2 CFU/g and approximately 6.3 log

Table 6
Parameters of sensory descriptive quantitative analysis (n:3, mean values, ± standard deviation) in goat ricotta cheese not containing or containing *L. acidophilus* La-05 or *B. lactis* Bb-12, after 1 and 7 days of storage.

Attributes*	Days of storage	Cheeses		
		R1	R2	R3
Smooth appearance	1	4.01 (±1.60)^{Aa}	3.27 (±1.19)^{Aa}	3.35 (±1.59)^{Aa}
	7	5.26 (±1.73)^{Aa}	4.23 (±1.27)^{Aa}	3.68 (±1.34)^{Aa}
Whitish color	1	7.02 (±1.58)^{Aa}	7.26 (±1.29)^{Aa}	7.16 (±1.80)^{Aa}
	7	7.55 (±0.68)^{Aa}	7.14 (±0.90)^{Aa}	7.41 (±0.96)^{Aa}
Creamy color	1	1.24 (±0.50)^{Aa}	0.85 (±0.34)^{Cb}	0.97 (±0.31)^{Bb}
	7	1.46 (±0.58)^{Ca}	1.78 (±0.53)^{Aa}	1.54 (±0.48)^{Ba}
Syneresis	1	3.33 (±1.00)^{Aa}	2.29 (±0.92)^{Aa}	2.79 (±0.99)^{Aa}
	7	3.36 (±1.34)^{Aa}	1.56 (±0.62)^{Aa}	2.47 (±1.88)^{Aa}
Goat milk aroma	1	2.06 (±0.82)^{Aa}	2.73 (±0.87)^{Aa}	3.46 (±1.99)^{Aa}
	7	3.16 (±1.17)^{Aa}	2.27 (±0.91)^{Aa}	2.94 (±1.21)^{Aa}
Butter aroma	1	1.84 (±0.74)^{Aa}	2.86 (±0.97)^{Aa}	3.14 (±1.40)^{Aa}
	7	2.24 (±0.90)^{Aa}	2.96 (±1.07)^{Aa}	3.28 (±1.00)^{Aa}
Goat milk flavor	1	2.90 (±1.16)^{Aa}	2.97 (±1.19)^{Aa}	3.54 (±1.69)^{Aa}
	7	2.95 (±1.09)^{Aa}	3.14 (±1.26)^{Aa}	4.29 (±2.03)^{Aa}
Butter flavor	1	2.51 (±1.00)^{Aa}	2.43 (±0.97)^{Aa}	2.38 (±0.84)^{Aa}
	7	2.60 (±1.04)^{Aa}	2.79 (±0.98)^{Aa}	2.92 (±0.82)^{Aa}
Acidity flavor	1	1.46 (±0.58)^{Aa}	0.83 (±0.38)^{Bb}	0.69 (±0.28)^{Bb}
	7	0.96 (±0.38)^{Ab}	1.89 (±0.61)^{Ba}	1.39 (±0.44)^{Ba}
Salty flavor	1	1.28 (±0.15)^{Aa}	1.17 (±0.47)^{Aa}	1.27 (±0.31)^{Aa}
	7	0.97 (±0.35)^{Aa}	1.47 (±0.60)^{Aa}	1.49 (±0.35)^{Aa}
Soft texture	1	6.68 (±1.95)^{Aa}	7.90 (±1.36)^{Aa}	7.57 (±1.99)^{Aa}
	7	6.80 (±1.63)^{Aa}	7.18 (±2.16)^{Aa}	7.34 (±1.97)^{Aa}
Homogeneous texture	1	4.65 (±1.40)^{Aa}	5.58 (±2.06)^{Aa}	5.72 (±2.68)^{Aa}
	7	5.78 (±1.86)^{Aa}	6.25 (±2.30)^{Aa}	6.25 (±2.01)^{Aa}

R1: goat ricotta lacking probiotic bacteria.
R2: goat ricotta containing *L. acidophilus* La-05.
R3: goat ricotta containing *B. lactis* Bb-12.
A–C: different superscript capital letters in the same row denote differences ($p \leq 0.05$) between different treatments.
a–c: different superscript lowercase letters in the same column denote differences ($p \leq 0.05$) in the same treatment during storage.
* Intensity of each attribute was assessed using an unstructured scale ranging from 0 (poor) to 9 (strong), which anchored the minimal and the maximal values.

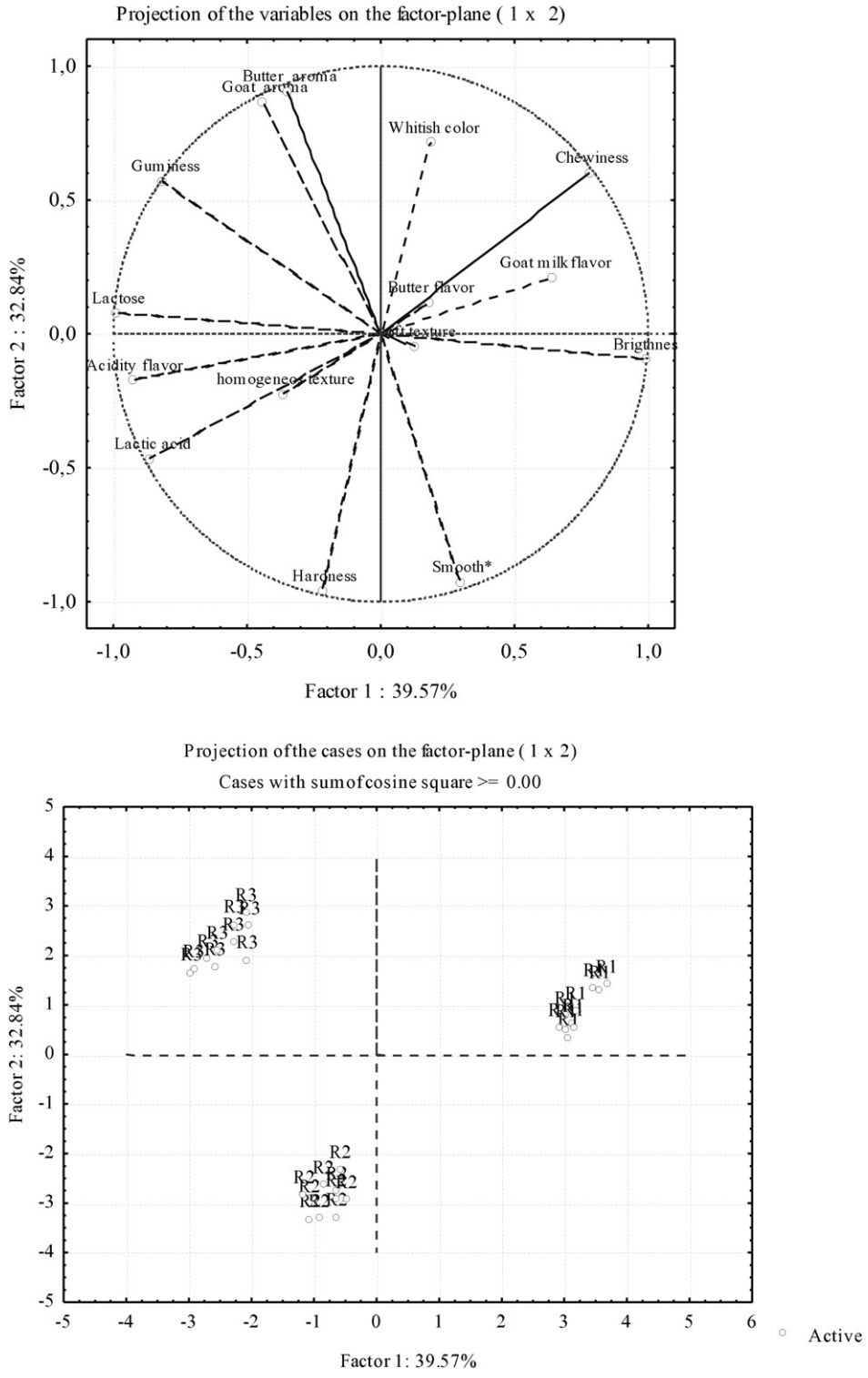

Fig. 2. (A) Principal Component Analysis (PCA) graph of the physicochemical and sensory aspects of the goat ricotta samples; (B) distribution of the ricotta samples according to the PCA. R1: goat ricotta lacking probiotic bacteria; R2: goat ricotta containing *L. acidophilus* La-05; R3: goat ricotta containing *B. lactis* Bb-12.

cfu/g for *B. lactis* Bb-12 when incorporated into MRS and into goat ricotta, respectively.

The counts of viable *L. acidophilus* La-05 that had been assayed in MRS or incorporated into goat ricotta were approximately 6 log CFU/g during exposure to each of the steps of the simulated digestion conditions, with no differences ($p > 0.05$) found between the counts at the end of the experimental digestive process (after the 10th digestive step) and before exposure to the experimental mouth conditions. After the 6th digestive step (experimental esophagus-stomach condition), the counts of *L. acidophilus* La-05 that was incorporated into goat ricotta had slightly decreased (± 0.3 log CFU/g). However, the counts of *L. acidophilus* La-05 obtained at further experimental digestive steps (duodenum and ileum) were not different ($p > 0.05$) from the counts obtained in the earliest digestive steps.

After exposure to the 1st step of the esophagus-stomach experimental condition, a decrease of > 1.5 log cfu/g was observed in the counts of

B. lactis Bb-12 assayed in MRS broth. At the end of the esophagus-stomach experimental step (8th digestive step), the counts of *B. lactis* Bb-12 were approximately 3.0 log cfu/g, whereas after exposure to the duodenum and ileum experimental conditions, the counts were ≤ 2 log CFU/g. In contrast, the counts of *B. lactis* Bb-12 incorporated into goat ricotta and exposed to the steps comprising the esophagus-stomach and duodenum experimental conditions were approximately 6 log cfu/g, and no reductions in the counts were observed until the last experimental digestive step (10th).

The ability to tolerate digestive stresses is one of the most important characteristics of probiotics that can be successfully incorporated into foods. The survival of the well-known probiotic strains (such as those utilized in the present study) during exposure to simulated gastrointestinal conditions was expected. However, in the present study, after the 3rd experimental digestive step (comprising the esophagus–stomach conditions), the counts of *B. lactis* Bb-12 were lower than the minimum required (6 log cfu/g) in foods at the moment of intake to ensure a favorable effect on the health of the consumer (Talwalkar, Miller, Kailasapathy, & Nguyen, 2004). However, this behavior was observed only in *B. lactis* incorporated into MRS broth as opposed in goat ricotta. This is an interesting result because researchers have reported a decrease in the counts of *B. lactis* at the earliest experimental digestive steps when this bacterium was incorporated into goat semi-hard cheese (Oliveira et al., 2014) and whey cheese (Madureira et al., 2011) using the same experimental digestive model. In this study, goat ricotta exhibited protective effects on *B. lactis* Bb-12 because throughout the successive experimental digestive steps, the viable counts were approximately 6 log cfu/g, whereas the counts of the same bacterium incorporated into MRS both and exposed to the experimental digestive conditions reached values as low as ≤2 log cfu/g. The protective effects were particularly obvious when the strain was exposed to the highest level of acidity (pH 2.0–4.6) under the esophagus–stomach conditions (4th–8th digestion steps) and to bile salts under the duodenum (9th digestive step) experimental condition. The buffering capacity of the goat ricotta matrix most likely resulted in an environment favorable to the viability of *B. lactis* cells. Moreover, the greater fat content and the more solid consistency of goat ricotta might also promoted (protect) the survival of *B. lactis* during exposure to the stomach and intestinal conditions (Cruz et al., 2009). When the *B. lactis* cells incorporated into ricotta samples were later exposed to bile salts, they were able to tolerate the inhibitory effects of these compounds, which can dissolve bacterial membranes.

The *L. acidophilus* La-05 cells was less affected by the stressful conditions imposed during the experimental digestion than were *B. lactis* Bb-12 cells because no differences ($p > 0.05$) were observed between the counts of viable *L. acidophilus* La-05 cells incorporated into MRS broth or goat ricotta and exposed to the simulated gastrointestinal conditions. An earlier study also found a smaller decrease in the counts of *L. acidophilus* and a greater decrease in the counts of *B. lactis* that had been incorporated into MRS broth at the end of an artificial digestion challenge (Oliveira et al., 2014). Upon initial consideration, the lack of difference between the viability of *L. acidophilus* La-05 assayed in either MRS broth or goat ricotta could be considered unimportant. However, these results are interesting because when this probiotic strain was incorporated into a goat semi-hard cheese, the survival rate decreased within 72 min of exposure to gastric juice (pH of 2.3; 6th digestion step; esophagus–stomach condition) (Oliveira et al., 2014). These findings might be attributed to the more favorable environment for bacterial survival provided by goat ricotta due to its higher Aw compared with that of goat semi-hard cheese because it has been proposed that the ability of bacteria to tolerate low pH conditions was directly affected by the Aw of the their environment (Cruz et al., 2009).

4. Conclusions

This study demonstrated that incorporating a well-known probiotic strain, either *L. acidophilus* La-05 or *B. lactis* Bb-12, into goat ricotta did not affect the yield, syneresis rate or physicochemical characteristics of the product, with the exception of the acidity level and the pH value, which resulted in a more acidic flavor, most likely associated with the concentration of lactic acid in these cheeses. Incorporating either of the tested probiotics into goat ricotta affected specific physical characteristics of the cheeses, as demonstrated by the increased yellowish color and hardness level. The results of bacterial viability study revealed that goat ricotta is a good matrix for delivering probiotic *L. acidophilus* La-05 or *B. lactis* Bb-12 cells in counts sufficient to provide health benefits to the consumer. Moreover, when incorporated into goat ricotta, both of the tested probiotics were able to tolerate the stressful conditions imposed by the experimental digestive process, although goat ricotta exhibited a stronger protective effect on *B. lactis* Bb-12. Overall, these results showed the feasibility of incorporating *L. acidophilus* La-05 and *B. lactis* Bb-12 into goat ricotta because these probiotics did not negatively affect the general quality characteristics of this product and suggested that goat ricotta is an efficacious food matrix for maintaining the viability of these probiotics during storage and under the stressful conditions imposed by the human gastrointestinal tract.

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