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Effect of Storage on Physico-Chemical, Microbiological and Sensory Characteristics of Goat Milk Fermented by *Lactobacillus* Strains Isolated from Minas Artisanal Cheeses

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Abstract

Lactobacillus spp. are lactic acid bacteria which have important implications for the food industry due to their fermentation capacities. The aims of this research were to produce fermented goat milks with *Lactobacillus plantarum* B7 and *Lactobacillus rhamnosus* D1, isolated from Brazilian artisanal cheeses, and to evaluate their physico-chemical, microbiological and sensorial qualities during 30 days of storage at 7 °C. The goat milks, fermented by B7, D1, co-culture and a *Lactobacillus casei* Shirota control, possessed acceptable physico-chemical characteristics to meet fermented milk standards established by Brazilian legislation and maintain the viability of *Lactobacillus* spp. throughout the shelf life of the products. The products were microbiologically safe. D1 fermented goat milk gave higher consumer sensory quality acceptance and purchase intention ($p<0.05$) than other treatments, thus *Lactobacillus rhamnosus* D1 is recommended for fermented goat milk production.

Keywords: Lactic acid bacteria; Probiotics; Food quality; Milk and dairy products; Fermentation

1 Introduction

Lactic acid bacteria (LAB) are a group of Gram-positive, facultative anaerobic or microaerophilic, rod or cocci-shaped microorganisms. LAB may be naturally found in some foods such as animal products and vegetables, soil, water and mucosa of animals. The production of lactic acid by carbohydrate fermentation represents an important biochemical pathway of these bacteria (Gaenzle, 2015). Among the 13 genera of LAB, *Lactobacillus* spp. are the most commom and are generally recognized as safe by agencies such as Food and Agriculture Organization of the United Nations (FAO), Food and Drug Administration (FDA), Qualified Presumption of Safety (QPS) and European Food Safety Authority (EFSA) (Bermudez-Humaran et al., 2013). In addition, studies have shown that some *Lactobacillus* strains can be considered probiotics, that is, they may promote health benefits when consumed in adequate concentrations (Food and Ag-

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riculture Organization, 2002). For these reasons, *Lactobacillus* spp. are widely used in fermented foods (Ranadheera et al., 2016).

Goat milk is considered a functional food due to its health-beneficial properties, mainly the easier digestion and absorption of its components compared to the milk of other species (Slacanac et al., 2010). Because of its functional characteristics, goat milk is recommeded to individuals with malabsorption syndromes, neoplasms and allergies to cow milk (Yadav et al., 2016). Goat milk also has higher levels of macro and micronutrients than milks from other mammals (El-Hatmi et al., 2015). However, goat dairy products are rejected by many consumers because of their sensory characteristics. They have high concentrations of short and medium-chain fatty acids, such as butyric, caproic, caprylic, capric, lauric and myristic acids, which are responsible for the development of a "goaty" flavour (Slacanac et al., 2010).

In this regard, the fermentation of goat milk presents itself as a mechanism for reducing the perception of the "goaty" flavour. Some studies have shown that goat milk fermented by *Lactobacillus* strains had high acceptance by consumers (Ranadheera et al., 2016). This suggests that fermented goat milk may be an interesting option for the introduction of goat dairy products to consumers, while combining the health benefits provided by goat milk and probiotics. Based on this, the aims of this study were to produce fermented goat milks with *Lactobacillus plantarum* B7 and *Lactobacillus rhamnosus* D1, isolated from Brazilian artisanal cheeses, and to evaluate their physico-chemical, microbiological and sensorial qualities during 30 days of storage at 7 ^{o}C.

2 Materials and Methods

2.1 Lactic acid bacteria strains

The cultures of *L. plantarum* B7 and *L. rhamnosus* D1 were previously isolated from Minas artisanal cheeses produced in the Serra da Canastra region, Brazil. Both strains had shown probiotic properties in previous *in vitro* assays (Costa et al., 2013). *L. casei* Shirota (LC), used as control, was donated by the Departamento de Microbiologia at the Universidade Federal de Minas Gerais, Brazil.

2.2 Milk and inoculum preparation

8 % (w/v) sucrose was added to raw goat milk and the solution sterilized by autoclaving at 110 ^{o}C for 10 minutes (Autoclave CS, Generalmed, São Paulo, Brazil). Each strain was aerobically cultured in MRS broth (Difco, Detroit, Michigan, United States) at 37 ^{o}C from 24 to 48 hours to prepare the inocula. Then, 5 mL of the broths were transferred to glass bottles containing 200 mL of sterilized goat milk solution, followed by incubation at 37 ^{o}C until coagulation. The inocula of B7 and D1 reached a count of 6.1 x 10^{8} and 2.4 x 10^{8} cfu/mL, respectively. Next, the inocula were added to the sterilized goat milk, in the concentration of 2.5 % (v/v), to start the fermentation process. In the co-culture of B7 and D1, the concentration of each inoculum was 1.25 % (v/v) in the sterilized goat milk.

2.3 Determination of fermentation kinetics

Initially, a study was conducted to determine the fermentation kinetics of B7, D1 and co-culture in goat milk. For this, the strains were inoculated in a liter of sterilized goat milk followed by incubation at 37 ^{o}C for 24 hours. Milk samples were collected at time 0 (immediately after inoculation of the strains) and every two hours until 24 hours of fermentation. The samples were analyzed for pH (Gehaka PG1800 digital pH meter, São Paulo, Brazil), titrable acidity (0.1N NaOH) and *Lactobacillus* counts on MRS agar (Difco, Detroit, Michigan, United States) (International Dairy Federation, 1988). Each assay was performed in triplicate.

2.4 Production of fermented goat milk

Goat milks, fermented by B7, D1, co-culture and LC, were produced for micobiological, physico-

chemical and sensory analyses. Each inoculum was added into erlenmeyers containing two liters of sterilized goat milk solution, with 8 % (w/v) sucrose. Then, the erlenmeyers were incubated at 37 ^{o}C and the fermentation time was determined by the results of previously performed fermentation kinetics. Soon after the end of the fermentation process, the erlenmeyers were stored in a BOD incubator (Eletrolab, São Paulo, Brazil), at 7 ^{o}C for 30 days, simulating the conditions of a domestic refrigerator. Each assay was also performed in triplicate.

2.5 Fermented goat milk analyses

Microbiological analyses

Fermented goat milks were analyzed after 0, 15 and 30 days of storage for the presence of *Salmonella* spp., the most probable number of coliforms at 30 ^{o}C and 45 ^{o}C, counts of coagulase-positive *Staphylococcus* and counts of molds and yeasts (Downes & Ito, 2001; Tournas et al., 2001). The count of *Lactobacillus* was also performed in order to evaluate the bacterial viability during storage (International Dairy Federation, 1988). The microbiological analyses were carried out in triplicate.

Physico-chemical analyses

Fermented goat milks at 0, 15 and 30 days of storage were submitted to the following physico-chemical analyses, according to Association of Official Analytical Chemists (2019): titratable acidity by titration with 0.1N NaOH (Official Methods of Analysis, 2019a); pH (Gehaka PG1800 digital pH meter, São Paulo, Brazil); and contents of fat by Roese-Gottlieb method (Official Methods of Analysis, 2019d), protein by micro-Kjeldahl method (Official Methods of Analysis, 2019c) solids and ash by gravimetric method (Official Methods of Analysis, 2019b). The free fatty acids concentration of the samples was also measured following the adapted methodology described by Deeth et al. (1975). The physico-chemical analyses were also performed in triplicate.

Sensory analysis

The evaluation of the sensory characteristics and purchase intention of fermented goat milks after 15 and 30 days of storage were also performed in three repetitions. Seventy non-trained panelists were served with approximately 30 mL of each sample, kept at 10 ^{o}C. Sensory evaluations were recorded on a 5-point hedonic scale, with 1 representing 'dislike very much' and 5 'like very much'. The purchase intention of each product was also determined by panelists, who had to mark "yes" or "no", if they would buy the product. Lastly, panelists were given the option to make general comments about the samples.
The consumer acceptability was determined by equation (1), proposed by Emediato et al. (2009). According to the authors, an acceptable outcome in sensory tests would be obtained when the sample acceptability index was equal to or greater than 70 %.

$$\text{Acceptability index} = \frac{\text{Average score}}{\text{Maximum value}} \times 100 \quad (1)$$

The sensory analyses were approved by the Ethics Committee on Research with Human Beings at the UFMG under protocol number: CAAE-48320015.1.0000.5149.

2.6 Statistical analysis

Data analyses were performed using GraphPad Prism 7.0 software (GraphPad software, San Diego, CA, USA). The means of physico-chemical parameters of fermented goat milks were submitted to Two-way ANOVA and compared using the Tukey test at a 5 % significance level. The same statistical test was used to compare *Lactobacillus* spp. counts during the storage period, after log-transformation of the data. The means of acceptance scores in sensory analyses were compared by the Tukey (between treatments) and t tests (between days of storage), at a significance level of 5 %. Finally, the purchase intention was analyzed by the Fisher test, at a significance level of 5 %.

3 Results and Discussion

3.1 Fermentation kinetics profiles

The changes of pH, titratable acidity and *Lactobacillus* count during goat milk fermentation by B7, D1 and co-culture are shown in Figures 1, 2 and 3. The curves are representative of the fermentation process because they illustrate the bacterial growth in milk and acid production by the strains over time (decreasing pH and increasing titratable acidity). The pH values between treatments did not show significant differences in the first ten hours of fermentation. However, after 12 hours, goat milk fermented by B7 had a significantly lower pH than the others. Statistical differences between the three treatments were verified after 18 hours of fermentation and the mean of the pH values were found to be 4.18 (B7), 4.80 (D1) and 4.53 (co-culture) at the end of the fermentation process. The titratable acidity curves presented the same trend: the same lactic acid concentrations in the goat milks fermented by B7, D1 and co-culture up to ten hours of fermentation and differences between them after 20 hours. The lactic acid concentrations in the goat milks fermented by B7, D1 and co-culture were, respectively, 0.90, 0.61 and 0.77 g/100 g at 24 hours.

Minervini et al. (2009) observed a more pronounced reduction in pH during fermentation of goat milk by *L. plantarum*. They used 1 % (v/v) of inoculum with 10^7 cfu/g. After eight hours of incubation at 30 ^{o}C, the pH value was approximately 4.60. Despite differences in inoculum and incubation conditions, this result highlights the variation in the fermentative pattern that may exist between different strains. Salva et al. (2011) also showed that co-cultures of *L. rhamnosus* and *Streptococcus thermophilus* with different ratios caused a faster pH decrease in goat milk, when incubated at 42 ^{o}C.

B7 showed higher growth in the inoculm preparation stage, according to the highest cell concentration at the beginning of the fermentation (time 0). The differences between B7 and D1 and co-culture counts remained throughout the whole process, resulting in a higher fermentation rate for the strain B7, observed in the pH and titratable acidity curves. On the other hand, among the three treatments, D1 showed the lowest initial counts. This profile resulted in the lowest pronounced increase of acidity and decrease of pH in goat milk during fermentation. In the study of (Zalan et al., 2010), *L. rhamnosus* also produced less organic acid than other bacteria when used to ferment skim milk. Among the *Lactobacillus* strains used for fermentation of different substrates - such as milk and MRS broth - for 18 hours, the products with *L. rhamnosus* showed the lowest titratable acidity. In addition, Gaudreau et al. (2005) suggest that the growth and consequently the acid production of *L. rhamnosus* in cow milk are slower than that of different bacteria.

The curves of pH, titratable acidity and bacterial counts in the goat milk fermented by co-culture were intermediate to the B7 and D1 curves, that is, while B7 and D1 showed the highest and the lowest values, respectively, the co-culture results were within the range of the values found for the isolate cultures. Ranadheera et al. (2016) also observed the same behavior comparing the fermentation of goat milk by co-culture and pure cultures of *Lactobacillus*. The fermented milk standards established by the Brazilian legislation are titratable acidity ranging from 0.6 to 2 g/100 g and *Lactobacillus* count higher than 10^6 cfu/g (Brasil, 2007). In addition, studies have shown that the final pH to maintain the viability of *Lactobacillus* spp. throughout the shelf life of the product should be from 4.5 to 5.0 (Lee & Salminen, 1995). Based on this information and the fermentation kinetics' profiles, it was possible to determine the fermentation time for each treatment as 16, 24 and 20 hours, respectively for B7, D1 and co-culture. At these times, it is possible to associate approximate values of pH (4.89 $\pm$ 0.02, 4.80 $\pm$ 0.24 and 4.83 $\pm$ 0.19, respectively), titratable acidity (0.55 $\pm$ 0.01, 0.61 $\pm$ 0.10 and 0.61 $\pm$ 0.02 g/100 g) and bacterial counts (9.05 $\pm$ 0.13, 8.63 $\pm$ 0.05 and 8.62 $\pm$ 0.32 log cfu/g) to meet the requirements of Brazilian legislation.

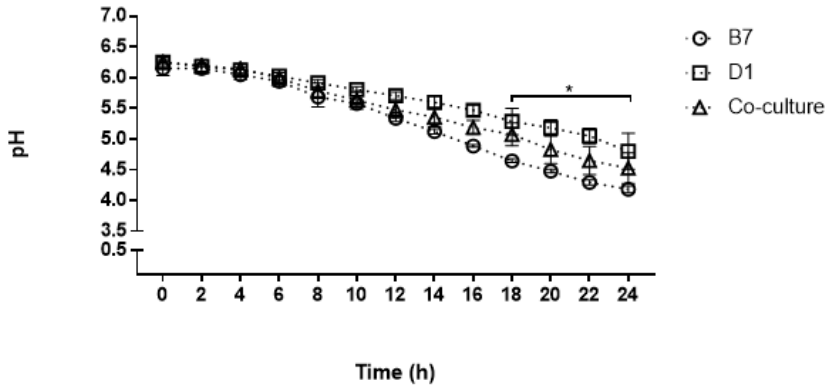

Figure 1: Means and standard deviations of pH during goat milk fermentation by *Lactobacillus* plantarum B7, *L. rhamnosus* D1 and co-culture for 24 hours. Data are plotted as means ± SD. The results shown are the average of triplicate experiments. *($p < 0.05$) Two-way ANOVA, followed by Tukey's test.

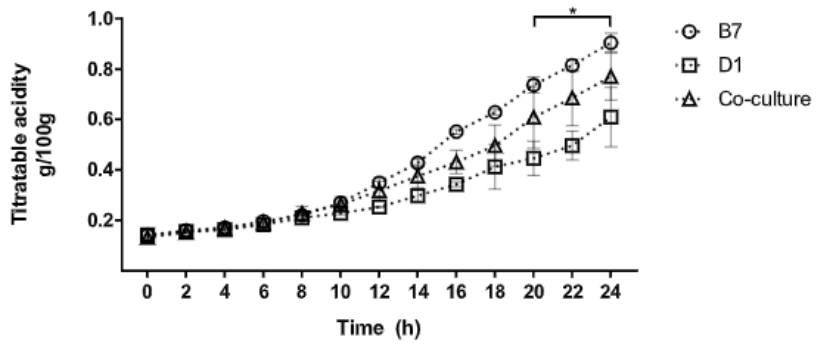

Figure 2: Means and standard deviations of titratable acidity during goat milk fermentation by *Lactobacillus plantarum* B7, *L. rhamnosus* D1 and co-culture for 24 hours. Data are plotted as means ± SD. The results shown are the average of triplicate experiments. *($p < 0.05$) Two-way ANOVA, followed by Tukey's test.

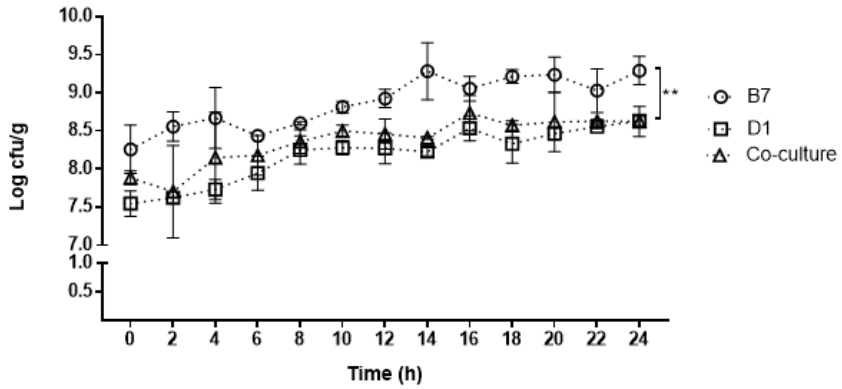

Figure 3: Means and standard deviations of *Lactobacillus* count during goat milk fermentation by *Lactobacillus plantarum* B7, *L. rhamnosus* D1 and co-culture for 24 hours. Data are plotted as means ± SD. The results shown are the average of triplicate experiments. *($p < 0.05$) Two-way ANOVA, followed by Tukey's test.

3.2 Physico-chemical parameters of fermented goat milk during storage

Titratable acidity

Only the sample D1 on 0 day of storage had titratable acidity outside the range established by the Brazilian legislation (from 0.6 to 2.0 g/100 g). However, it can be seen that the results of this parameter were adequate in the subsequent analyses (Brasil, 2007).

During the storage period, an increase in the acidity of the fermented milks with B7 and D1 was observed, while the milks inoculated with co-culture and LC showed a decrease in acidity from 15 to 30 days. The increase in acid concentrations during refrigeration is an expected phenomenon. Lactic acid bacteria can maintain their metabolism even at temperatures ranging from 0 to 5 ^{o}C. The decrease in the titratable acidity of fermented milks stored under refrigeration was also observed by Amani et al. (2017). Bacterial cultures with high proteolytic activity may cause a decrease of acidity in products under these conditions, due to the production of alkaline compounds (Lim et al., 2019). As noted in Table 1, this event may not directly influence pH changes since the behavior of this parameter is determined by other acidic components besides lactic acid, such as fatty acids.

Approximate values of titratable acidity were found in other studies on physico-chemical parameters of fermented goat milk under refrigeration. Salva et al. (2011) found results ranging from 0.60 to 0.95 g/100 g in products stored at 4 ^{o}C for 15 days, varying according to the storage time and the concentration and proportion of inoculum added. Using *L. rhamnosus* as a starter culture, dos Santos et al. (2017) found titratable acidity of 0.52, 0.69 and 0.87 g/100 g in products with 1, 14 and 28 days of refrigeration, respectively.

pH

The lower pH values were observed in goat milks fermented by B7, coinciding with the higher concentration of lactic acid obtained in the titratable acidity analysis. There was no significant difference (p>0.05) between them and the pH of the products with co-culture and LC, while D1 fermented milks had significantly (p<0.05) higher pH than the others treatments, independently of the storage period. Regardless of the starter culture, there was a tendency to decrease pH throughout the refrigeration. As discussed, post-acidification is an expected process, but if

Table 1: Results of physico-chemical analyses of goat milk fermented by *Lactobacillus plantarum* B7, *L. rhamnosus* D1, co-culture and *L. casei* Shirota (LC) during 30 days of storage at 7 ^{o}C

Parameter	Storage days	*Lactobacillus* strain			
		B7	D1	Co-culture	LC
Titratable acidity	0	$0.68^{Bac} \pm 0.10$	$0.49^{Bb} \pm 0.07$	$0.65^{Ca} \pm 0.12$	$0.78^{Bc} \pm 0.11$
(g/100 g)	15	$0.92^{Aa} \pm 0.02$	$0.73^{Ab} \pm 0.04$	$0.90^{Aa} \pm 0.09$	$0.91^{Aa} \pm 0.03$
	30	$0.94^{Aa} \pm 0.14$	$0.73^{Ab} \pm 0.04$	$0.80^{Bb} \pm 0.09$	$0.76^{Bb} \pm 0.03$
	0	$4.53^{Abc} \pm 0.30$	$5.20^{Aa} \pm 0.19$	$4.72^{Ab} \pm 0.29$	$4.36^{Ac} \pm 0.20$
pH	15	$3.99^{Bb} \pm 0.09$	$4.42^{Ba} \pm 0.27$	$4.14^{Bb} \pm 0.07$	$4.08^{Bb} \pm 0.13$
	30	$3.93^{Bb} \pm 0.10$	$4.21^{Ca} \pm 0.18$	$4.02^{Bb} \pm 0.10$	$4.01^{Bb} \pm 0.13$
Free fatty acids	0	$1.2^{Ba} \pm 0.12$	$1.09^{Ba} \pm 0.11$	$1.2^{Ba} \pm 0.21$	$1.09^{Ba} \pm 0.20$
(μ equiv./mL)	15	$1.09^{Ba} \pm 0.10$	$1.07^{Ba} \pm 0.18$	$1.07^{Ba} \pm 0.10$	$1.07^{Ba} \pm 0.16$
	30	$1.55^{Aa} \pm 0.24$	$1.41^{Aa} \pm 0.22$	$1.59^{Aa} \pm 0.28$	$1.8^{Aa} \pm 1.05$
Fat	0	$3.04^{Aa} \pm 0.14$	$3.34^{Ab} \pm 0.20$	$3.23^{Aab} \pm 0.14$	$3.32^{Ab} \pm 0.36$
(g/100 g)	15	$3.01^{Aa} \pm 0.27$	$3.25^{Aa} \pm 0.31$	$3.13^{ABa} \pm 0.11$	$3.03^{Ba} \pm 0.11$
	30	$2.9^{Aa} \pm 0.23$	$3.07^{Ba} \pm 0.15$	$3.04^{Ba} \pm 0.05$	$3.00^{Ba} \pm 0.09$
Protein	0	$2.73^{Aa} \pm 0.16$	$2.85^{Aa} \pm 0.10$	$2.83^{Aa} \pm 0.04$	$2.78^{Aa} \pm 0.13$
(g/100 g)	15	$2.64^{Aa} \pm 0.14$	$2.60^{Ba} \pm 0.11$	$2.69^{Ba} \pm 0.10$	$2.64^{Ba} \pm 0.17$
	30	$2.50^{Ba} \pm 0.17$	$2.57^{Ba} \pm 0.09$	$2.55^{Ca} \pm 0.06$	$2.55^{Ba} \pm 0.11$
Total solids	0	$17.93^{Aa} \pm 0.45$	$17.88^{ABa} \pm 0.19$	$18.05^{Aa} \pm 0.35$	$18.02^{Aa} \pm 0.41$
(g/100 g)	15	$18.17^{Aa} \pm 0.77$	$18.31^{Aa} \pm 0.63$	$18.30^{Aa} \pm 0.32$	$18.22^{Aa} \pm 0.56$
	30	$17.05^{Ba} \pm 0.20$	$17.39^{Ba} \pm 0.36$	$17.29^{Ba} \pm 0.21$	$17.33^{Ba} \pm 0.28$
Ash	0	$0.69^{Ba} \pm 0.02$	$0.70^{Ba} \pm 0.02$	$0.68^{Ba} \pm 0.03$	$0.68^{Ba} \pm 0.02$
(g/100 g)	15	$0.73^{Aa} \pm 0.03$	$0.73^{Aa} \pm 0.03$	$0.72^{Aa} \pm 0.03$	$0.72^{Aa} \pm 0.02$
	30	$0.73^{Aa} \pm 0.03$	$0.72^{ABa} \pm 0.03$	$0.71^{Aa} \pm 0.03$	$0.71^{ABa} \pm 0.04$

$^{a-c}$Means within rows with distinct superscripts differ significantly ($p < 0.05$); $^{A-C}$Means within columns with distinct superscripts differ significantly ($p < 0.05$).

exacerbated, it can lead to undesirable changes in the products, such as bacterial death, syneresis and rejection by consumers (Coggins et al., 2010). In another study, fermented goat milks showed pH oscillating from 3.83 to 4.60 during 28 days of storage (dos Santos et al., 2017).

Free fatty acids

A significant difference (p<0.05) between the concentration of free fatty acids of the treatments was not observed. However, significant increases (p<0.05) in the content of these substances were observed in the products analyzed after 30 days of refrigeration.

Lipolysis may occur in dairy products from the activity of enzymes, such as lipases, produced by psychrotrophic or natural lipases of milk. However, sterilization of milk immediately after milking is capable of denaturing endogenous lipases and eliminating the producing bacteria, although it has no effect on enzymes that have previously been produced by psychrotrophs. In this way, it was concluded that the lipolytic activity of B7, D1, co-culture and LC in the fermented goat milks presented the same intensity during the storage.

Fat

In general, practically no significant differences (p>0.05) in fat content were observed between the treatments. However, goat milks fermented by D1, co-culture and LC showed a decrease in the fat percent over the storage period. The development of lipolytic microorganisms in products is attributed as the main cause of this reduction. As observed in the analysis of free

fatty acids, all strains studied were able to cause lipolysis which may have resulted in this decrease.
Most of the similar studies showed products with higher fat contents, reaching a concentration of 5.37 g/100 g (Ranadheera et al., 2016; Salva et al., 2011). These differences may occur due to variations in the composition of the raw material, since fat is the component most subjected to oscillations in milk. In addition, this study used fresh milk, without addition of cream but with addition of 8 % (w/v) sucrose, which exerted a dilutive effect on the other dairy components.

Protein

No sample, regardless of the treatment or refrigeration period, presented the minimum value of 2.9 g/100 g established by the Brazilian legislation. The same regulation provides that fermented milks may have lower fat and protein concentrations than those recommended where other substances are added, such as sucrose (Brasil, 2007).
No significant differences ($p>0.05$) were observed between the treatments as to the protein content but during the storage period there were significant decreases ($p<0.05$). Ahmed and Razig (2017) reported the influence of the proteolytic activity of cultures and the casein hydrolysis process on the decrease of protein concentration in yogurts during storage. In other studies on fermented goat milk, the protein contents were higher and ranged from 3.51 to 5.39 g/100 g (Ranadheera et al., 2016; Salva et al., 2011). These differences may occur due to the quality of the raw material, as well as the addition of protein components to milk, such as milk powder and whey (Martin-Diana et al., 2003).

Solids

As observed in the analysis of fat and protein contents, the solids concentration also showed a significant decrease ($p<0.05$) during the 30 days of refrigeration. Reports in the scientific literature indicated variations in solids contents of fermented goat milk, ranging from 11.5 to 18.9 g/100 g (Martin-Diana et al., 2003; Ranadheera et al., 2016). As already discussed, these differences are due to the composition of raw milk used and the addition of solid components during production.

Ash

Although the statistical analysis has shown differences in ash content over the storage period, numerically the variation observed between the means is practically inexpressive. In other studies, the ash content ranged from 0.75 to 1.39 g/100 g (Martin-Diana et al., 2003; Ranadheera et al., 2016; Salva et al., 2011). The addition of compounds to milk is the main cause of variation of ash concentration in fermented milks. The ash content in the fermented goat milks produced did not reach higher values since the addition of sucrose exerted a dilutive effect. On the other hand, Martin-Diana et al. (2003) added whey protein concentrate, which has a high mineral content, to the products.

3.3 Microbiological parameters of fermented goat milk during storage

There was no detection of potentially pathogenic microorganisms in the fermented goat milks analyzed, irrespective of the storage period. Cisse et al. (2019) highlighted that fermented goat milks produced under uncontrolled hygienic conditions may have an undesirable microbial population. On the other hand, the enumeration of *Lactobacillus* in the products indicated that these bacteria remained viable throughout the storage period, as shown in Figure 4. While B7, D1 and LC showed constant counts during refrigeration, the co-culture counts showed a significant decrease ($p<0.05$), from days 0 to 15, and from days 0 to 30. However, B7, D1, co-culture and LC counts were greater than 108 cfu/g in fermented goat milks during the 30 days. Likewise, Moreno-Montoro et al. (2018) observed that the counts of *L. plantarum* C4 were maintained above 108 cfu/mL in fermented goat milk stored for six weeks. *Lactobacillus* count in the analyzed products met the requirements established by the Brazilian legislation for inspection of fermented milks and products containing pro-

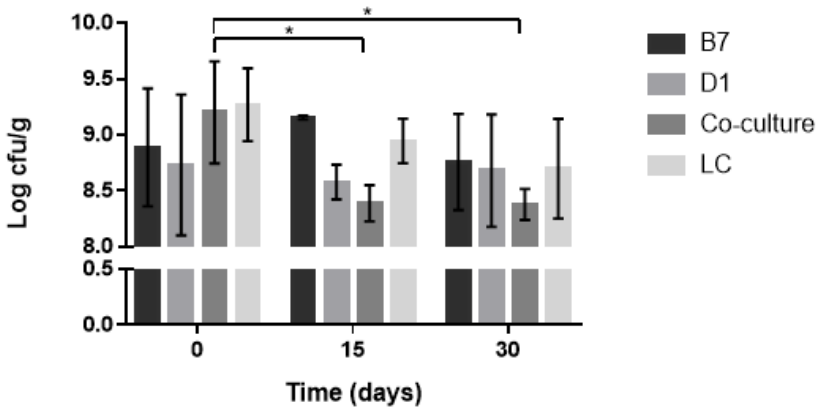

Figure 4: Enumeration of *Lactobacillus plantarum* B7, *L. rhamnosus* D1, co-culture and *L. casei* Shirota (LC) in fermented goat milk during 30 days of storage at 7 ^{o}C. Data are plotted as means ± SD. The results shown are the average of triplicate experiments. *($p < 0.05$) Two-way ANOVA, followed by Tukey's test.

biotics (Brasil, 2007; Ranadheera et al., 2016). The strains analyzed in this research presented a better stability over the storage period compared to other bacteria described in previous studies, in which the lactic acid bacteria count in fermented goat milks showed a reduction of up to 1 log during the storage (Martin-Diana et al., 2003; Ranadheera et al., 2016). On the other hand, dos Santos et al. (2017) observed that *L. rhamnosus* counts remained equal during the first seven days of storage of fermented product made from goat milk (7.38 log cfu/g) and presented a considerable increase until day 28 (7.78 log cfu/g).

3.4 Sensory analyses of fermented goat milk during storage

The overall acceptability, the purchase intention and the acceptability indexes of fermented goat milks attributed by the panelists are shown in Table 2. It can be noticed that the goat milks fermented by D1 excelled over the others in the three evaluated items, both after 15 and 30 days of storage. On the other hand, the goat milks fermented by co-culture tended to present the worst results of sensorial attributes.

The scores attributed to goat milks fermented by B7, D1 and LC were significantly higher ($p<0.05$) than the product inoculated with co-culture after 15 days of refrigeration. At 30 days of storage, there was a considerable decrease ($p<0.05$) in the acceptability of B7 and LC products, while D1 maintained the same score. Although the goat milks fermented by co-culture did not show any variation ($p>0.05$) in acceptability between the analyzed periods, it remained with a lower score ($p<0.05$) than fermented milk D1 at the end of the storage period.

According to the observations made by the panelists, the goat milks fermented by co-culture with 15 days of storage had a "goaty" flavour, being a factor that may have contributed to the lower acceptance. Lipolysis could explain such perception by the panelists. However, there was no significant difference ($p>0.05$) between the concentration of free fatty acids in the products. In addition, the texture was another point highlighted by the panelists. Reports indicated that the product containing co-culture showed greater fluidity. However, as the evaluation was done by untrained panelists, the comparative standards used by them were commercial fermented milks, which are added with additives that positively affect texture perception. Martin-Diana et al. (2003) also observed lower acceptance scores of

Table 2: Overall acceptability, purchase intention and acceptability index of goat milk fermented by *Lactobacillus plantarum* B7, *L. rhamnosus* D1, co-culture and *L. casei* Shirota (LC) after storage at 7 ^{o}C for 15 and 30 days

Parameter	Storage days	*Lactobacillus* strain			
		B7	D1	Co-culture	LC
Overall acceptability	15	$3.28A^{Ab} \pm 1.09$	$3.54^{Aa} \pm 1.08$	$3.14^{Ab} \pm 1.08$	$3.54^{Aa} \pm 1.12$
	30	$2.91^{Bb} \pm 1.23$	$3.53^{Aa} \pm 1.06$	$2.99^{Ab} \pm 1.14$	$3.13^{Bb} \pm 1.14$
Purchase intention (%)	15	49.05^{Ab}	60.00^{Aa}	45.71^{Ab}	58.10^{Ab}
	30	39.52^{Bb}	60.66^{Aa}	38.10^{Ab}	44.29^{Bb}
Acceptability index (%)	15	65.60	70.80	62.80	70.80
	30	58.20	70.60	59.80	62.60

$^{a-c}$Means within rows with distinct superscripts differ significantly ($p < 0.05$); $^{A-C}$Means within columns with distinct superscripts differ significantly ($p < 0.05$).

fermented goat milk when they had lower viscosity and firmness values. Similarly, Ranadheera et al. (2016) observed that the texture is one of the main determinants of the differences observed during the evaluation of yogurts made from goat milk.

At 30 days of storage, the panelists indicated changes in the texture and flavor of goat milks fermented by B7 and LC. Descriptions of flavors that refer to whey and cheese may have contributed to the reduction of acceptance of these products. Whey flavor may be indicative of the occurrence of syneresis throughout storage. Ranadheera et al. (2016) showed that the period of higher syneresis coincided with the lower scores of the products. In addition, the cheese flavor suggests the occurrence of proteolysis in these products, since this is one of the main biochemical reactions that occur during cheese ripening. Proteolysis can be triggered by several factors, such as bacterial enzyme activity, including those produced by lactic acid bacteria (Salva et al., 2011). The reduction in the acceptability of both products with 30 days of storage coincides with the period of higher concentration of free fatty acids. Although this factor did not cause an apparent effect on the acceptance of goat milks fermented by D1, it may have influenced the results of the other treatments.

The goat milks fermented by D1 was also superior to the other products regarding the purchase intention throughout the whole storage period ($p<0.05$). In addition, these products were able to maintain the same standard during refrigeration, since the purchase intention was statistically similar ($p>0.05$) in the two evaluated periods. On the other hand, a significant decrease ($p<0.05$) in purchase intention of products B7 and LC was observed from days 15 to 30 of storage. Garcia and Travassos (2012) observed values varying from 30.95 % to 42.86 % for the purchase intention of fermented goat milk. Alves et al. (2009) observed a purchase intention of approximately 61 % for frozen yogurt made from goat milk added probiotics.

Corroborating with the results already presented, the acceptability index of products inoculated with D1 and LC exceeded 70 %, after 15 days of storage, which are considered satisfactory values. At 30 days of refrigeration, only the D1 fermented milks continued to present an acceptability index in this range, proving their sensory superiority compared to the other treatments.

4 Conclusions

According to Brazilian legislation and scientific reference studies, goat milk fermented by B7, D1, co-culture and LC satisfied microbiological and physico-chemical standards during storage at 7 ^{o}C for 30 days. In addition, the samples were free of pathogenic microorganisms throughout the period and maintained the lactic bacteria counts at a desirable concentration ($>10^8$ cfu/g).

In the sensory analyses, goat milk fermented by D1 showed greater overall acceptability and purchase intention (p>0.05) than the other treatments. Therefore, it is suggested that *L. rhamnosus* D1 is the most promising microorganism to be used as a starter culture for fermentation of goat milk.

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