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Potentially probiotic ice cream from goat's milk: Characterization and cell viability during processing, storage and simulated gastrointestinal conditions

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ABSTRACT

In this work, the physicochemical characteristics, meltdown behavior and sensory properties of goat's milk ice cream produced with and without the probiotic bacteria *Bifidobacterium animalis* subsp. *lactis* BLC1 were analyzed. The ice cream with added *B. animalis* was further evaluated in regard to the probiotic viability during processing, frozen storage, and simulated gastrointestinal conditions. Results showed that the addition of *B. animalis* decreased the pH ($p < 0.05$), but it had no effect on physicochemical properties, including overrun and melting behavior of ice cream from goat's milk ($p > 0.05$). After 120 days of frozen storage, a survival rate of 84.7% was registered. With regard to cell viability during gastrointestinal conditions, the exposure to bile and pancreatin resulted in the decline of 3.82 log cycles in ice cream samples previously stored at −18 °C for 120 days. Overall, the goat's milk ice cream with *B. animalis* received good sensory scores and satisfactory probiotic viability (6–7 log CFU/g) was maintained throughout the 120 days of frozen storage. Therefore, this research shows that goat's milk ice cream is an adequate delivery vehicle for the probiotic bacteria *B. animalis*.

1. Introduction

Nowadays, probiotic dairy products constitute one of the most developed segments and represent a major branch of the functional foods industry (Saad, Delattre, Urdaci, Schmitter, & Bressollier, 2013). Studies have shown that ice cream is an excellent vehicle for probiotic bacteria when compared to fermented dairy products. The pH of ice cream is higher than regular fermented milk, and it constitutes an important advantage over other dairy products, since low pH may severely affect the survival of probiotic bacteria (Ranadheera, Evans, Adams, & Baines, 2012). On the other hand, the freezing and whipping processes involved in the ice cream production may lead to serious cell damage and consequent loss of probiotic viability (Abghari, Sheikh-Zeinoddin, & Soleimanian-Zad, 2011).

Products from goat's milk have become significantly important in many parts of the world (Haenlein, 2004). Despite its technological and market challenges (Bezerra, Souza, & Correia, 2012; Gomes et al., 2013; Yamazi, Moreira, Cavicchioli, Burin, & Nero, 2013), goat's milk has some advantages in comparison to cow's milk, including special nutritional characteristics such as easier digestion and the ability of improving the absorption of iron and copper (Barrionuevo, Alferez, Lopez-Aliaga, Sanz-Sampelayo, & Campos, 2002; Silanikove, Leitner, Merin, & Prosser, 2010). Nevertheless, few researches have focused on probiotic dairy products made with goat's milk and the traditional cow's milk derivatives still represent a larger portion of the probiotic market (Ranadheera, Evans, Adams, & Baines, 2013; Ranadheera et al., 2012).

Bifidobacterium strains are among the most common probiotic microorganisms used in food products (Baboota et al., 2013; Saad et al., 2013). The number of viable microorganisms at the time of consumption is extremely important in order to provide expected health benefits. Consequently, the probiotic survival during processing and storage should be monitored. Although the ideal number of viable probiotic microorganisms has not been universally

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established, levels between 10^6 CFU/g to 10^9 CFU/g are commonly accepted (Abadia-Garcia et al., 2013). In addition to the necessary probiotic survival in the final product, sensory characteristics are identified as a major factor in influencing the acceptance of functional foods (Urala & Lahteenmaki, 2007).

Therefore, this paper has the objective of comparing the physicochemical characteristics, meltdown behavior and sensory properties of caprine ice cream produced with and without the probiotic bacteria *Bifidobacterium animalis* subsp. *lactis* BLC1. In addition, the potentially probiotic ice cream was evaluated in regard to the viability of the probiotic strain after processing and during storage. In order to assess the ability of the probiotic cells to survive under acid and bile stress, *in vitro* tests that simulate harsh gastrointestinal conditions were conducted in ice cream samples after 30 (P30) and 120 (P120) days of frozen storage.

2. Material and methods

2.1. Probiotic bacteria

Freeze dried probiotic culture of *B. animalis* subsp. *lactis* BLC1 was obtained from Sacco (Campinas, SP, Brazil).

2.2. Production of goat's milk ice cream

Goat's milk ice cream was produced as described by Silva, Varela, and Correia (2010). The following ingredients were used to prepare the ice cream from goat's milk: dried goat's milk (Caprilat, Brazil), Emustab® emulsifying, Liga Neutra Extra® stabilizer, Algemix® guava flavouring, Selecta Cream® fat substitute (Duas Rodas, Brazil), corn syrup (Corn Products Brazil, Brazil) and commercial sugar.

Two experimental groups were defined: ice cream with added probiotics (PIC) and regular ice cream, without the addition of probiotics (RIC). Briefly, batches of 6.5 kg were prepared by mixing all the ingredients thoroughly followed by pasteurization at 70 °C for 30 min. The mixture was cooled and transferred to a refrigerated holding tank (Brasfrio, Brazil) where the mixes were aged at 4 °C for 20 h. After that, goat's milk previously incubated for 3 h at 37 °C with *B. animalis* subsp. *lactis* probiotic culture (10^9 CFU/g) was added to PIC batches. The RIC samples received the same quantity of goat's milk without probiotics. The aged mixes (PIC and RIC) received the guava flavor and the mixtures were frozen using an ice cream maker (PHB 80/100, Brasfrio, Brazil). The ice cream batches were drawn, packaged into 500 mL polyethylene containers, and stored in a freezer (Electrolux, Brazil) at −18 °C for 24 h to harden. Three batches of each experimental group (PIC and RIC) were prepared on different days and ice cream samples were collected for triplicate analysis.

2.3. Physicochemical analysis

Both experimental ice cream groups (PIC and RIC) were analyzed for their physicochemical characteristics. The pH, total solids, soluble solids, ash, fat and protein were determined according to AOAC (1998). Total sugars were determined by a modified 3.5-dinitrosalicylic acid (DNS) method (Miller, 1959).

2.4. Overrun

The overrun of the ice cream samples was calculated according to Akin, Akin, and Kirmaci (2007) using the expression: overrun = $[(W1 - W2)/W2] \times 100$, where W1 = weight of the mix and W2 = weight of the same volume of ice cream. Samples were analyzed after 1 week of storage.

2.5. Meltdown test

The meltdown test was conducted according to Muse and Hartel (2004). After 1 week of frozen storage, the ice cream samples (100 g) were placed on a wire screen (6 holes/cm) on the top of a funnel attached to a graduated cylinder. The samples were left to melt in controlled temperature chambers at 25 °C and the dripped volume was recorded after 5 min. The time (min) was plotted against the melted volume (%) and the slope of the curve was taken as the melting rate.

2.6. Sensory analyses

The hedonic sensory analyses were performed with 120 untrained 10 to 15 year-old panelists. The individuals were regular consumers of ice cream, not allergic to milk and willing to participate. Ice cream samples (PIC and RIC) were evaluated for overall appearance, aroma, consistency and overall flavor using a structured 9-point hedonic scale ranging from 1 (disliked it very much) to 9 (liked it very much) according to Meilgaard, Civille, and Carr (1999, chap. 12). Each sample (15 g) was coded by using a 3-digit random number and served in 50 mL disposable transparent plastic containers. The ice cream samples were sensory evaluated after 1 week of frozen storage.

2.7. Viable probiotic counts after processing and during storage

The viability of *B. animalis* was determined in the aged ice cream mix and also during the first 24 h after the production of the ice cream with added probiotics (0, 2, 4, 6, 8, 12, 16 and 24 h). In order to assess the probiotic viability during storage, samples of the ice cream were collected after 7, 30, 60, 90 and 120 days of frozen storage.

The *B. animalis* count was conducted according to Lapierre, Underland, and Cox (1992). Briefly, 25 g of sample was aseptically collected and diluted in 225 mL of 0.1 g/100 mL peptone water (Oxoid, UK). Serial dilutions were subsequently prepared with the same diluent. Populations of *B. animalis* were enumerated by the pour plating technique using 1 mL of each dilution in MRS-LP agar (Oxoid, UK) followed by anaerobic incubation (Anaerobic System Anaerogen, BBL, EUA) at 43 °C for 72 h. The results were expressed as log CFU/g and also as survival rate (%) according to Magarinos, Selaive, Costa, Flores, and Pizarro (2007).

2.8. In vitro gastrointestinal tolerance assay

In order to infer about the possible effect of frozen storage on the cell viability under simulated gastrointestinal conditions, two experimental groups were investigated: ice cream samples after 30 (P30) and 120 (P120) days of frozen storage at −18 °C. The tolerance of *B. animalis* to *in vitro* simulated gastric and enteric conditions was performed according to the method described by Buriti, Castro, and Saad (2010), with modifications. Initially, the samples (25 g) were homogenized in 225 mL of 0.5 g/100 mL NaCl solution. For the gastric phase simulation, the pH of aliquots (10 mL) was adjusted to 2.1–2.6 with 0.5 mL of HCl (0.5 mol equi/L) and 0.3 mL of pepsin solution (3 g/L, porcine stomach mucosa P6887, Sigma-Aldrich, MO, USA). Flasks were incubated at 37 °C for 2 h with agitation of approximately 150 rpm (Water Bath Dubnoff MA-095, Marconi, SP, Brazil).

In order to simulate enteric conditions, the pH of samples was increased to 4.9–5.4 using an alkaline solution (150 mL of 1 mol equi/L NaOH solution, 14 g of $PO_4H_2Na \cdot 2H_2O$ and distilled water up to 1 L). Bovine bile (B3883, Sigma-Aldrich, MO, USA) and pancreatin (P3292, Sigma-Aldrich, MO, USA) were added to reach a concentration of 10 g/L and of 1 g/L, respectively. Samples were incubated again at 37 °C for 2 h under agitation. After 4 h, the pH

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was increased to 7.5–7.7 using the same alkaline solution, bile and pancreatin concentrations were added (10 g/L and 1 g/L, respectively), and samples were incubated again at 37 °C for 2 h under agitation, achieving 6 h of assay. The enumeration of *B. animalis* was performed in aliquots collected after 2 h, 4 h and 6 h as described in item 2.7.

2.9. Statistical analysis

Three batches were prepared for each sample and all analyses were carried out in triplicate ($N = 9$). Results were expressed as mean ± standard deviation. Statistical analysis of the average values obtained from ice cream samples were calculated by Analysis of Variance (ANOVA) and the Tukey's test ($p < 0.05$) using Satistica 7.0 software (StatSoft, Inc., USA). The equations that describe the melting behavior were obtained by regression analysis.

3. Results and discussion

3.1. Physicochemical characterization

According to Table 1, the addition of the bifidobacteria led to lower pH ($p < 0.05$), but it had no effect on the other physicochemical properties of caprine ice cream samples. Similar findings are reported by Abghari et al. (2011) and Alamprese, Foschino, Rossi, Pompei, and Savani (2005). The pH and total solids content are similar to the results of probiotic caprine ice cream previously shown by Ranadheera, Evans, Adams, and Baines (2013).

3.2. Overrun and meltdown tests

The overrun expresses the expansion of ice cream resultant from the air incorporation into the product. It is a technical parameter and may vary according to different elaboration procedures and ingredients (Karaca, Guven, Yasar, Kaya, & Kahyaoglu, 2009). Our results for RIC and PIC groups (Table 1) are respectively identical and lower than those obtained for non-fermented ice cream prepared with *Lactobacillus* strains (Abghari et al., 2011), but higher than the probiotic ice cream from goat's milk prepared with bifidobacteria (Ranadheera et al., 2013) and *Lactobacillus rhamnosus* GG (Alamprese et al., 2005). Some reports have shown that the probiotic strain does not affect the overrun (Abghari et al., 2011; Alamprese et al., 2005), but Akalin and Erisir (2008) found that higher overrun may decrease the viability of *B. animalis* Bb-12.

Besides the overrun, the meltdown characteristics are important quality parameters of ice cream (Erkaya, Dagdemir, & Sengul, 2012). Fig. 1 shows the evolution of drained ice cream with time. The curve presents a sigmoidal shape, which was used to obtain the regression equations that correlate the drained volume (y, mL/100 mL) and time (x, min) for RIC ($y = 2.88x - 6.94$, $R^2 = 0.974$) and PIC ($y = 2.76x - 11.91$, $R^2 = 0.968$) ice cream formulations.

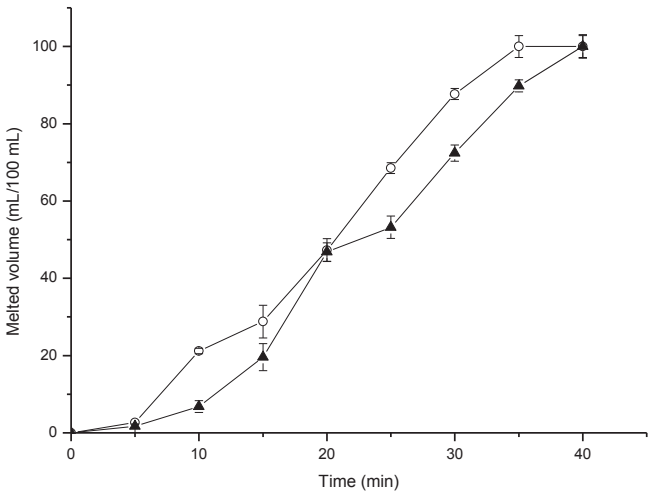

Fig. 1. Melting behavior of regular goat's milk ice cream without the addition of probiotics (RIC,○) and goat's milk ice cream with added *B. animalis* subsp. *lactis* (PIC,▲). The error bars represent the standard deviation ($n = 9$).

Results show similar melting rates ($p > 0.05$) for PIC and RIC samples. Several parameters influence the melting behavior, including the overrun (Sofjan & Hartel, 2004), the fat content and use of fat replacers (Granger, Legerb, Bareyb, Langerdorffb, & Cansell, 2005; Karaca et al., 2009), as well as the type of milk (Correia, Magalhaes, Pedrini, Cruz, & Clementino, 2008; Pandya & Ghodke, 2007). Both PIC and RIC samples were prepared by using the same type of milk and they have similar chemical composition and overrun values (Table 1). Therefore, the addition of *B. animalis* had no effect on the melting behavior of goat's milk ice cream, which corroborates with previous reports (Abghari et al., 2011; Favaro-Trindade, Balieiro, Dias, Sanino, & Boschini, 2007).

3.3. Sensory evaluation

In general, the sensory scores for both experimental groups were positive, ranging from "liked it regularly" and "liked it very much" (Table 2). With regard to overall appearance, aroma and overall flavor, the samples with or without probiotics had similar results ($p > 0.05$). Concerning the consistency, the RIC group reached a higher score ($p < 0.05$).

No unpleasant or strange taste in the ice cream with bifidobacteria (PIC) associated to the presence of probiotic or the use of goat's milk was reported, which is probably due to the inclusion of guava flavoring. Similar sensory impressions were reported by Favaro-Trindade, Bernardi, Barbosa, Balieiro, and Almeida (2009) and Turgut and Cakmakci (2009) when investigating probiotic ice cream.

3.4. Viable probiotic counts after processing and during storage

Initially, the population of *B. animalis* subsp. *lactis* was monitored during the first 24 h after processing, which coincides with the

Table 1
Physicochemical characterization of regular goat's milk ice cream without the addition of probiotics (RIC) and goat's milk ice cream with added *B. animalis* subsp. *lactis* (PIC).

	RIC	PIC
pH	6.62 ± 0.02[a]	6.45 ± 0.07[b]
Total solids, g/100 g	38.1 ± 0.3	35.6 ± 0.5
Soluble solids, Brix	36.2 ± 1.8	36.2 ± 1.8
Ash, g/100 g	0.7 ± 0.1	0.7 ± 0.1
Total sugars, g/100 g	13.5 ± 1.2	14.5 ± 0.6
Fat, g/100 g	2.0 ± 0.0	2.0 ± 0.1
Protein, g/100 g	3.1 ± 0.1	3.4 ± 0.1
Overrun, g/100 g	48.0 ± 1.0	48.0 ± 0.9

Results are presented as means ± standard deviation (n = 9).
a–b: Means in the same line followed by different letters are significantly different by HSD Tukey's test ($p < 0.05$).

Table 2
Sensory evaluation of regular goat's milk ice cream without the addition of probiotics (RIC) and goat's milk ice cream with added *B. animalis* subsp. *lactis* (PIC).

	Overall appearance	Aroma	Consistency	Overall flavor
RIC	7.7 ± 1.3	7.8 ± 0.9	8.1 ± 1.0	7.6 ± 1.9
PIC	7.1 ± 1.6	7.1 ± 1.7	7.2 ± 1.5	7.5 ± 1.7

Results are presented as means ± standard deviation (n = 9).

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hardening of the product (Fig. 2). The viable count in the mix was 8.1 log CFU/g which is similar to the population determined for the ice cream sample at $t = 0$ (8.2 log CFU/g). The observed survival rate of 98.8% reveals that the formulation, whipping and air incorporation did not affect the survival of *B. animalis*. The same tendency was observed for the survival of *Lactobacillus delbrueckii* in ice cream with different fat levels (Leandro, Araújo, Conceição, Moraes, & Carvalho, 2013) and in fermented ice cream with addition of *Bifidobacterium longum* and *Bifidobacterium lactis* (Favaro-Trindade et al., 2009). Previous reports show that oxygen toxicity caused by the incorporation of air during the ice cream production may seriously affect the growth of anaerobic bifidobacteria (Ferraz et al., 2012; Homayouni, Azizi, Ehsani, Yarmand, & Razavi, 2008), but this was not observed in the present study. Similarly, Ranadheera et al. (2013) showed no oxygen effect on *B. lactis* survival in low overrun (26.17%–33.83%) ice cream from goat's milk. In our case, the overrun was only 48%, which is low compared to other studies (Abghari et al., 2011). Thus, we hypothesize that noticeable oxygen effects may only be observed in higher overrun levels.

The population of *B. animalis* decreased during the first 24 h after processing (Fig. 2). After the first 2 h of frozen storage a substantial reduction of 1.08 log cycles was observed which is slightly superior to the results reported by Hekmat and McMahon (1992). According to Jay (2000, chap. 16), the microbial destruction is higher at the beginning of the freezing process and tends to decrease as the freezing progresses. His observation is coherent with our findings, which show a severe decline in *B. animalis* population, during the first 8 h and stabilization of the bacterial counts after this. Data allow inferring that the higher impact on probiotic survival was caused by the freezing process itself, which may be a result of the actual freezing of the cells, resulting in microbial death (Turgut and Cakmakci, 2009). Severe cell injuries caused by the mechanical stress involved in freezing and osmotic impacts caused by the temperature decrease also have to be considered (Magarinos et al., 2007; Ordonez, Jeon, & Roberts, 2000). Despite the detected viability loss, during the first 24 h the probiotic counts were kept near 7 CFU/g, which means a high survival rate of 84.3%. This result is similar to what Turgut and Cakmakci (2009) observed in ice cream produced with different cream levels, but contrary to Ranadheera et al. (2013), who obtained higher *B. animalis* survival in caprine ice cream packaged in different materials.

The viability of *B. animalis* fluctuated along the 120 days of frozen storage (Fig. 3). By comparing Figs. 2 and 3, it can be observed that the higher viability loss was observed during the first 24 h of frozen storage. Considering the initial ($t = 0$) and final ($t = 120$) probiotic counts, the viability decreased 1.26 log cycles, which means a survival rate of 84.7%. Salem, Fathi, & Awad (2005) found similar numbers after 12 weeks of probiotic ice cream storage, reporting a decrease of 1.68 log cycles in the *Bifidobacterium bifidum* population.

The *B. animalis* was able to maintain satisfactory viability ($\geq$6.5 log CFU/g) throughout the frozen storage. Overall, the possible cryoprotector properties of the ice cream mixture, which contains casein, sucrose and lactose might play a role in preserving the viability of probiotic cells during frozen storage (Magarinos et al., 2007). The observed survival rate and probiotic count is much higher than the survival of *B. lactis* in ice cream produced with and without inulin (Akin et al., 2007). With regard to probiotic viability, our results for the goat's milk ice cream are similar to previous literature reports of ice cream from cow's milk. Therefore, no clear influence of goat's milk on probiotic viability was observed in this study.

3.5. In vitro gastrointestinal tolerance assay

The viability of probiotic bacteria is highly affected by the harsh conditions found in the gastrointestinal tract (Leandro et al., 2013). The *B. animalis* subsp. *lactis* BLC1 survival to *in vitro* simulated gastrointestinal conditions is shown in Fig. 4. Samples submitted to frozen storage for 30 and 120 days were investigated. The first 2 h of the assay mimic the gastric phase, when the ice cream samples were exposed to simulated gastric fluid consisting of HCl and pepsin in pH around 2.0. After this phase, the population of *B. animalis* was reduced in 1.24 log cycles and 1.00 log cycles for P30 and P120 samples, respectively. No significant difference ($p > 0.05$) was observed between the final probiotic population in P30 and P120 ice cream samples.

Our results show higher probiotic survival than what was observed for *B. animalis* subsp. *lactis* BB-12 inoculated in goat's milk ice cream and submitted to similar gastric conditions (Ranadheera et al., 2012). The *B. animalis* survival is also superior to the survival of *Lactobacillus acidophilus* in refrigerated and frozen mousses, which experienced a reduction of more than 3 log cycles after 30 min of simulated gastric conditions (Buriti et al., 2010).

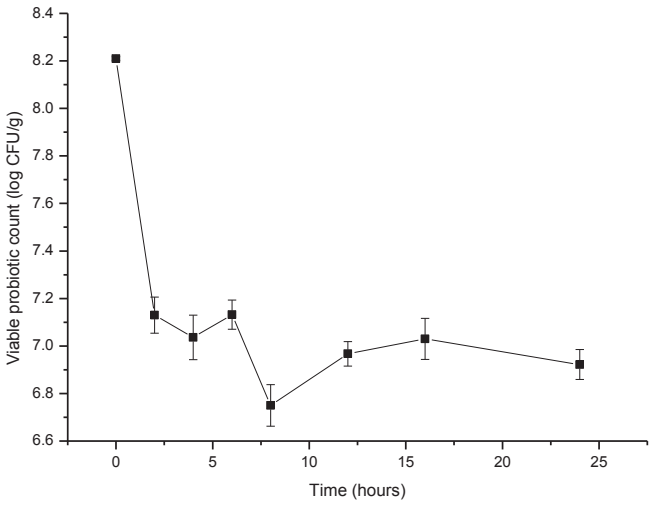

Fig. 2. Viable counts (log CFU/g) of *B. animalis* subsp. *lactis* in goat's milk ice cream during the first 24 h of frozen storage at -18 °C. The error bars represent the standard deviation ($n = 9$).

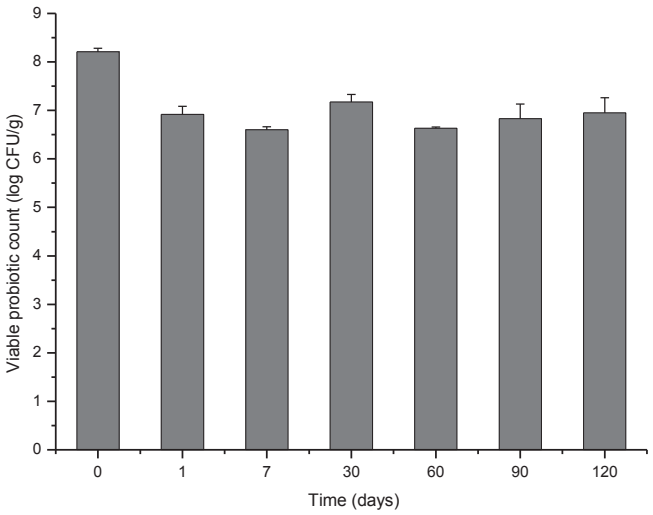

Fig. 3. Viable counts (log CFU/g) of *B. animalis* subsp. *lactis* in goat's milk ice cream during 120 days of frozen storage (−18 °C). The error bars represent the standard deviation ($n = 9$).

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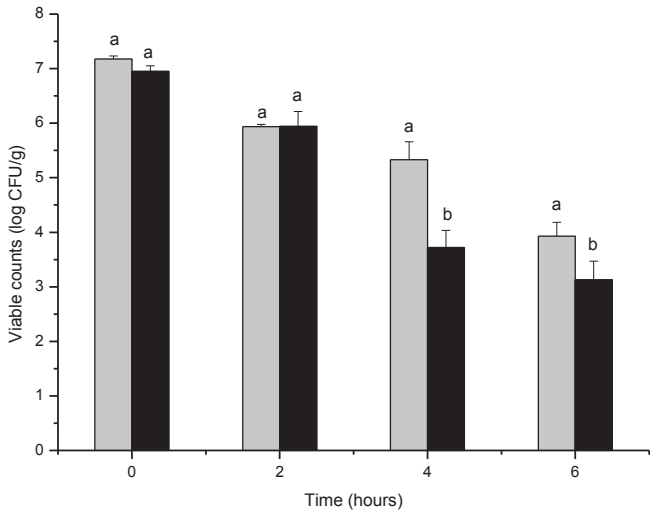

Fig. 4. Viable counts (log CFU/g) of *B. animalis* subsp. *lactis* in goat's milk ice cream during exposure to simulated gastric (2 h) and enteric (4 h and 6 h) conditions. Samples were analyzed after 30 days (▒) and 120 days (■) of frozen storage at −18 °C. The error bars represent the standard deviation ($n = 9$). a, b: Different superscript letters mean significant differences between trials for the same sampling period of the *in vitro* gastrointestinal tolerance assay ($p < 0.05$).

In order to simulate enteric conditions, bovine bile and pancreatin were added to ice cream samples with adjusted pH. It is known that bile acids are able to hamper the development and survival of probiotic bacteria (Bustos, Raya, Bru, Valdez, & Taranto, 2011). The *B. lactis* population suffered a remarkable decrease from 0 to 6 h of assay for the two frozen storage periods evaluated (30 d and 120 d). Moreover, the reduction was more evident after 4 h and 6 h of assay, which coincides with the enteric phase. For these 2 stages (4 h and 6 h), it was also observed that the storage time influenced the probiotic viability and the P120 samples reached lower bacterial counts when compared to P30 ($p < 0.05$).

The higher loss of viability was detected for the samples stored for 120 days after 6 h (decrease of 3.82 log cycles). Ding and Shah (2007) investigated the bile tolerance of several probiotic bacteria, including bifidobacteria strains, using two types of bile salts (oxgall and taurocholic acid). The *B. lactis* strains were especially susceptible to the selected bile salts and a decrease of more than 4 log cycles was reported.

This considerable reduction is also close to what was observed for *L. acidophilus* La-5 submitted to similar assay conditions (Bedani, Rossi, & Saad, 2013). On the other hand, the authors reported a remarkably higher resistance of *B. animalis* Bb-12, which maintained counts above 7 log CFU/g after 6 h of exposure to gastrointestinal conditions. Even though many other factors should be considered, in general, Gram-positive bacteria like bifidobacteria seem to be less resistant to the deleterious effects of bile. One of the possible mechanisms by which bile affects probiotic cells would be through membrane-damaging effects and/or disrupting the cell stability (Begley, Gahan, & Hill, 2005).

Despite this fact, bile tolerance is a strain-specific characteristic and therefore, it cannot be generalized. In addition, Begley et al. (2005) remind that the concentration of bile acid varies from one person to the other and, in general, experimental assays are not able to simulate the exact bile composition found in humans.

4. Conclusion

The present study showed that the addition of the probiotic *B. animalis* influenced the pH, but it had no effect on physicochemical properties, including overrun and melting behavior of ice cream made from goat's milk. The ice cream with added probiotics received good sensory scores and no flavors associated to goat's milk or the addition of probiotics was recorded. Results also show that most of the reduction of the organisms' viability occurred during the first 24 h of frozen storage. Despite this fact, satisfactory viability of *B. animalis* (6–7 log CFU/g) was maintained throughout the 120 days of frozen storage. With regard to probiotic viability during gastrointestinal conditions, the higher loss of viability was detected during the enteric phases, when the exposure to bile and pancreatin resulted in the decline of approximately 4 log cycles in ice cream samples previously stored for 120 days at −18 °C. Overall, this research shows the technological potential and adequacy of using goat milk to produce potentially probiotic ice cream with satisfactory physicochemical, sensory and cell viability results.

Acknowledgments

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