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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] × 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