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Sensory, microbiological and physicochemical screening of probiotic cultures for the development of non-fermented probiotic milk

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ABSTRACT

The aim of the present work was to screen probiotic cultures for the development of non-fermented probiotic milk that keeps its sensory, microbiological and physicochemical characteristics during storage. The study was conducted in three steps. Initially, five probiotic cultures (*Bifidobacterium animalis, B. longum, Lactobacillus acidophilus, L. casei, and L. rhamnosus*) were screened using sensory, microbiological and physicochemical techniques. Two cultures were selected for a more detailed evaluation based on their ability to maintain viability during storage without altering the sensory characteristics of the product: *L. acidophilus* and *B. animalis*. The milk with *L. acidophilus* showed a better sensory performance throughout storage. Finally, the shelf life of the probiotic milk obtained with L. *acidophilus* culture was estimated in 21.8 days from a sensory and microbiological point of view. *L. acidophilus* culture could be used by dairy companies as an innovative option to add health functionality to regular milk without any major change in its sensory characteristics.

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1. Introduction

Probiotics are defined as live microorganisms which, when administered in adequate amount, confer a health benefit to the host (FAO & WHO, 2006). Selection of a suitable base product for delivering probiotics is a key step of the development of probiotic foods (Ranadheera, Baines, & Adams, 2010). Dairy products are the most usual vehicles for delivering probiotics to consumers, being yogurt and fermented milk the most common products (Kumar, Vijayendra, & Reddy, 2015; Shori, 2015). These products are usually formulated with added-sugar and additives, which makes the development of non-fermented probiotic milk an interesting alternative to deliver probiotics to consumers using a daily consumed product with a strong healthy image as base carrier. The development of probiotic milk without any added ingredient is particularly interesting considering governmental and consumers' interest in reducing consumption of highly processed products (Moodie et al., 2013).

Once the base product has been defined, the other major step for the development of a probiotic product is the selection of the probiotic strain. Several species, belonging to the genera of *Lactobacillus*, *Bifidobacterium*, *Streptococcus* and *Lactococcus*, have been used as probiotics over the years (Kumar et al., 2015). Selection of probiotic strains has been based on their safety, functionality and the ability to survive during production and storage (FAO & WHO, 2006; Tripathi & Giri, 2014). Differences in the metabolism of probiotic strains should also be considered, as it markedly affects the characteristics of probiotic products (La Torre, Tamine, & Muir, 2003). The metabolism of probiotic bacteria, and particularly heterofermentation pathway, can result in the production of compounds that may negatively affect the sensory characteristics of the product, the so-called probiotic off-flavor (Bayarri, Carbonell, Barrios, & Costell, 2011).

Considering that previous research has shown that consumers are not willing to compromise the flavour of probiotic foods for potential health benefits (Tuorila & Cardello, 2002; Verbeke, 2006), the sensory characteristics of probiotic products should also be taken into account when selecting the most appropriate strain for a specific product (Cruz et al., 2010).

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In this context, the aim of the present work was to select a probiotic culture to develop a non-fermented probiotic milk that keeps its sensory, microbiological and physicochemical characteristics during storage.

2. Materials and methods

2.1. Cultures for non-fermented probiotic milk

Five commercial cultures of probiotics in the form of dried powder were considered to prepare the samples of non-fermented probiotic milk: *Bifidobacterium animalis* subsp. *lactis* BB-12® (BA) and *Lactobacillus acidophilus* LA-5® (LA) from Chr. Hansen (Denmark); *B. longum* BL-G301 (BL), *L. casei* LC-G11 (LC), and *L. rhamnosus* LR-G14 (LR) from Granolab (Brazil).

Probiotic liquid cultures were prepared by independently dispersing a weighted amount of each dried powder culture in 10 mL of pasteurized milk, in order to obtain a minimum bacterial concentration of 10^6–10^7 CFU/mL in the probiotic milks. The liquid cultures remained under refrigeration conditions for 30 min with agitation (20 times in 10 s) of a 50 mL Erlenmeyer every 5 min to allow the re-hydration and dispersion of the powder cultures (Muller, Stanton, Sybesma, Fitzgerald, & Ross, 2010).

2.2. Preparation of non-fermented probiotic milks

Pasteurized whole milk (75°C/15 s) was obtained from a local producer (Cooperativa Boa Nova, Valença, RJ, Brazil) and transported to Embrapa Food Technology (Rio de Janeiro, RJ, Brazil) in thermal boxes with ice, keeping the temperature at 6 ± 3 °C.

Pasteurized milk was placed in a sterilized glass container and independently inoculated with the probiotic liquid culture in the ratio 1000:1. Using aseptic techniques, every probiotic milk batch was agitated for 30s and transferred to sanitized glass bottles of 1 L with polypropylene screw caps. The probiotic milk bottles were stored under refrigeration (5 °C ± 2 °C).

2.3. Experiment design

The study was conducted in three steps. Initially, five probiotic cultures (BA, LA, BL, LC, and LR) were screened for the development of non-fermented probiotic milk, based on their ability to maintain the viability during storage without altering the sensory characteristics of the product. Samples were produced and stored at 5 °C for 0, 5 and 10 days. After each storage time, the sensory, microbiological and physicochemical characteristics of the samples were evaluated.

In the second step, two of the cultures screened in step 1 were selected for a more detailed evaluation of the sensory, microbiological and physicochemical characteristics of non-fermented probiotic milk: LA and BA. Batches were produced at 7, 14 and 19 days to obtain samples with different storage times. Besides, a pasteurized milk sample with 1 day of storage was considered as control; therefore, seven samples were used in this step of the study.

Finally, the third step estimated the shelf life of the non-fermented probiotic milk obtained using the selected culture screened in the second step (LA). A reversed experimental design was used (Giménez, Ares, & Ares, 2012). Probiotic milk samples were produced at different times, so that all products with different storage times were evaluated on the same day. Samples were kept at 5 °C for 0, 8, 11, 14, 20 and 25 days of storage.

2.4. Evaluation of non-fermented probiotic milk

2.4.1. Microbiological and physicochemical analyses

The enumerations of probiotic viable cells were performed by inoculating appropriate decimal dilutions of homogenized samples into MRS medium, using the pour plate technique, followed by incubation at 37 °C for 72 h. Petri dishes of *Bifidobacterium* strains were incubated in aerobic jars, while Petri dishes of *Lactobacillus* strains were incubated in aerobic conditions (IDF, 1995).

The pH of the probiotic milk samples was determined with a pH meter Micronal B-375 (Micronal, São Paulo, Brazil) equipped with a penetration electrode model (Marshall, 1993). The acidity index (°Dornic) was obtained by titration (AOAC., 2003). All analyses were performed in triplicate. These analyzes were performed in conjunction with the sensory analyzes described below.

2.4.2. Sensory evaluation

In all sensory tests, assessors were recruited among workers from Embrapa Food Technology (Rio de Janeiro, RJ, Brazil) according to their consumption of fresh milk (at least once a week). Testing took place in a sensory laboratory in standard sensory booths that were designed in accordance with ISO 8589 (ISO, 2007). Still mineral water was used for rinsing between samples. Different sensory methodologies were used in each of the three steps of the study. Methodologies were selected considering to the specific aim of each step and the number of samples involved.

2.4.2.1. Polarized sensory positioning (PSP). The sensory characteristics of the probiotic milk samples produced with the five probiotic cultures (BA, LA, BL, LC, and LR) were evaluated using Polarized Sensory Positioning (PSP), a reference-based methodology (Teillet, 2014). Three samples were selected as poles to reflect the main sensory characteristics of fresh and altered milk. One of the poles (R1) corresponded to the pasteurized milk at the first day of storage, aiming at representing the sensory characteristics of the fresh milk. The other two poles represented the sensory characteristics of altered milks. The probiotic milk stored for 13 days prepared with two different probiotic cultures: LR (R2) and LA (R3) were used. Poles were selected considering results from pilot testing with a semi-trained panel.

Thirty untrained assessors (37% female, ages ranging between 18 and 74 years) participated in the study. The assessors evaluated six samples, which comprised the five probiotic milks plus the control sample (fresh pasteurized whole milk, CT) at 5 and 10 days of storage. Assessors received 50 mL of each one of the three poles (R1, R2 and R3) at 8 ± 2 °C, and approximately 30 mL of the six samples (CT, LR, BL, LC, LA and BA), coded with three-digit random numbers. They were asked to try the poles and each of the samples and to quantify the overall difference between the coded samples and the three different poles using 10 cm line scales ranging from 'exactly the same' to 'completely different'.

2.4.2.2. Sorting. The sensory characteristics of the seven samples described in step 2 were evaluated using a sorting task. In this methodology, assessors have to group samples according to their similarities and differences (Lawless, Sheng, & Knoops, 1995).

Eighty untrained assessors (70% female, 18–74 years old) received 30 mL of each of the seven samples in coded plastic cups at 8 ± 2 °C. They were asked to look, smell and taste and then group the samples according to the similarities and differences in terms of the sensory characteristics into a minimum of two groups and a maximum of six groups. After grouping, assessors were asked to describe each of the groups using a maximum of four words.

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