

PROPERTIES OF *STREPTOCOCCUS THERMOPHILUS* FERMENTED MILK CONTAINING VARIABLE CONCENTRATIONS OF *BIFIDOBACTERIUM LONGUM* AND *LACTOBACILLUS ACIDOPHILUS*

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ABSTRACT

Sensory evaluation and analysis of pH, titratable acidity and microbial counts after 1, 7, 14 and 21 days of storage of five combinations of lyophilized *Bifidobacterium longum* and/or *Lactobacillus acidophilus* added to milk fermented with *Streptococcus thermophilus* were studied during storage at 4°C. The taste and acidity sensory attributes were significantly ($P < 0.05$) perceived as the storage time increased. Favourite combinations contained, initially, *Bif. longum* and *L. acidophilus* (10^8 and 10^7 cfu/mL, respectively) and *Bif. longum* only (10^8 cfu/mL). These combinations did not differ significantly among them and neither from the standard fermented milk. The lowest sensory scores and highest titratable acidity values were obtained for fermented milk added of *L. acidophilus* (10^8 cfu/mL initial concentration). During the 21 days of storage at 4°C the viable cell counts of: *Str. thermophilus* did not change. *Bif. longum* viable cells counts kept constant or reduced 1 logarithmic cycle and *L. acidophilus* reduced 1 or 2 logarithmic cycle. We did not observe inhibition of over-acidification caused by the presence of bifidobacteria or *L. acidophilus*.

Key words: *Bifidobacterium*, *Lactobacillus acidophilus*, fermented milk, sensory properties

INTRODUCTION

The use of *Lactobacillus acidophilus* and bifidobacteria, normal inhabitants of the human intestine, as dietary adjuncts, has been subject of interest of many researchers (3,4,9). According to Dave and Shah (3) yoghurt or yoghurt-like products have been used as the most popular vehicle for incorporation of probiotic organisms.

Saxelin *et al.* (18) reported that to maintain confidence in probiotic products it is important to demonstrate good survival of the bacteria in food products during their shelf life, associated to a pleasant taste and attractive texture, regardless of the "health message" of the product. The probiotic product must contain minimum values that vary from 10^5 to 10^7 cfu/g of probiotic bacteria at use-by date (4,6,15,16,19).

Properties such as growth rate; metabolic rate; proteolytic activity and flavour promotion of several probiotic strains are species specific and success in industrial applications may be obtained through careful management of these factors and tailored manufacturing processes (6,18).

Production of high-quality fermented milk products containing *Bifidobacterium* spp. and *L. acidophilus* is, according to Gomes and Malcata (6), a challenge to dairy plants owing to the sensitive character of the microorganisms. Additionally, Dave and Shah (3) reported that it is not feasible to ferment milk using only probiotics, due to the longer fermentation time required to reduce the pH of milk and to the objectionable taste produced by some probiotic bacterial strains. However, probiotic strains may be used with a support culture, like *Str. thermophilus* or another yoghurt culture (17,20).

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Lourens-Hattingh and Viljoen (9) reported that the probiotic culture may be added prior to fermentation, simultaneously with the conventional yogurt cultures or after fermentation to the product cooled to 4°C, before packaging. Saxelin *et al.* (18) did not observe a reduction in the number of probiotic bacteria in some combinations of probiotic and yoghurt cultures, concluding that it is important to select an optimal support culture to obtain good probiotic survival during the shelf life of the product.

Tamime *et al.* (21) reported that in Europe, United States and East Europe, fermented dairy products contained: (a) *Lac. acidophilus*, *Bifidobacterium* spp., *Lac. casei* sub-specie *casei* biovar *shirota*, *Lac. GG* strain, and/or combinations of these bacteria with common lactic starters; (b) bacteria isolated from human which are able to survive and to grow on the human intestine and to adhere to epithelial intestine cells; (c) more than 10⁶cfu/mL or gram of the product (20).

The use of bifidobacteria in cultured milks, according to Medina and Jordano (11), produces some of the following nutritional and technological advantages: i) mild sour taste; ii) limited after acidification; iii) less bitterness; iv) are beneficial as intestinal organisms.

Hoier (7) reported that bifidobacteria produces, during fermentation, acetic and lactic acids at the ratio 3:2. So, excessive growth may yield products with vinegar-like taste and aroma. Also, Samona *et al.* (17) investigated the production of acetic acid and others organic acids during 21 days of storage at 4°C of fermented milks prepared with different combinations of two yogurt cultures and *Bif. adolescentis*, *Bif. bifidum* and *Bif. longum*. The acetic acid produced by bifidobacteria, which apparently has a inhibitory effect on *Lactobacillus delbrueckii* ssp *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*, may prevent post-production acidification, according to Samona *et al.* (17).

The objectives of this research were to study the influence of the *Bif. longum* and *L. acidophilus* concentrations, added in the lyophilized form to a *Str. thermophilus* fermented milk, on the taste and acidity sensorial attributes, pH, titratable acidity and microbial counts characteristics during 21 days of refrigerated storage.

MATERIALS AND METHODS

Test organisms

The following test organisms were used: freeze dried starter cultures of *Str. thermophilus* TA 559 (ST), *L. acidophilus* LAC4 (LAC) and *Bif. longum* BL (BL) from Ezal® - Texel, supplied by Rhodia Food (Paulínia, Brasil).

Culture media and reagents

The following culture media and reagents were used: (i) Triptone, (ii) bacto bile, (iii) bacto peptone, (iv) agar (Difco,

Detroit, Mi, USA); (v) sucrose, (vi) yeast extract, (vii) KH₂PO₄, (viii) sodium propionate (SIGMA, St. Louis, MO, USA); (ix) lithium chloride (Synth, Diadema, SP, Brasil); (x) Anaerobac (Probac, São Paulo, SP, Brasil); (xi) MRS (Man Rogosa Sharpe) agar (Oxoid, Basingstoke, Hampshire, En); (xii) Skim milk powder (Molico, Nestle, Araçatuba, SP, Brazil).

Starter and intermediate cultures preparation

Sterile reconstituted skim milk (11% ST, 115°C/10min) was inoculated with freeze-dried ST in laminar air flow cabinet, fermented to coagulation at 42°C and cooled. The pH of starters and intermediate cultures of ST was on the range of 4.4 - 4.7, after 4 hours of incubation at 42°C.

Fermented milks preparation

Twenty five liters of heat treated (90°C/5min) reconstituted (12% total solids) skim milk was inoculated with 2% intermediate culture, fermented to approximate pH 4.7 at 45°C and chilled to 38°C. This pH was reached around 2h45min of fermentation. The LAC and BL cultures were then added in quantities specified on Table 1, stirred and conditioned into sterilized glass bottles and stored at 4°C. This experiment was repeated twice for all 6 combinations (see Table 1).

Table 1. Probiotics Initial concentrations of the fermented milks^a.

| Combination | Microorganisms ^b | Initial Probiotics |
|-------------|-----------------------------|----------------------------------|
| | | Concentrations - IPC (cfu/mL) |
| C1 | ST | — |
| C2 | ST | — |
| | BL | 1x10 ⁸ |
| C3 | ST | — |
| | BL | 1x10 ⁷ |
| | LAC | 1x10 ⁸ |
| C4 | ST | — |
| | BL | 1x10 ⁸ |
| | LAC | 1x10 ⁸ |
| C5 | ST | — |
| | BL | 1x10 ⁸ |
| | LAC | 1x10 ⁷ |
| C6 | ST | — |
| | LAC | 1x10 ⁸ |

a: Determined by considering the probiotics counts of LAC and BL presented in the lyophilized powder. These counts were verified using microbiological methods described below. **b:** ST=*Streptococcus thermophilus*; BL=*Bifidobacterium longum*; LAC = *Lactobacillus acidophilus*

The reconstituted skim milk used at different steps of this research was prepared using skim milk powder (Molico, Nestle, Araçatuba, SP, Brazil) supplied by local markets.

Fermented milks evaluation

Fermented milks taste and acidity sensorial attributes evaluation, viable cell counts, pH and titratable acidity determinations were performed at 1, 7, 14 and 21 days of refrigerated storage at 4°C.

Sensory evaluation

The sensorial attributes evaluation was performed using the acceptability test methodology with 35 untrained panelists, using a 9-points balanced scale. Each panelist received 30g of the samples of the fermented milks described on Table 1 (C1 to C6). The samples were codified and presented to the panelist in randomized order as suggested Meilgaard *et al.* (12). The sensorial analysis sessions were carried out like described in Meilgaard *et al.* (12) and Macfie *et al.* (10).

The data were analyzed by ANOVA, using the Tukey test to evaluate differences among averages and the Statistical Analysis System (SAS 8.02 TS level 02M0). A P value < 0.05 was considered to be significant.

Microbiological analysis

Tubes containing 0.1% sterile peptone water and the samples of fermented milk were homogenized by Vortex shaking. After homogenization, the samples were serially diluted in 0.1% sterile peptone water, pour plated in ST agar (aerobiosis) at 30°C/48h according to Zacarchenco and Massaguer-Roig (25) for *Str. thermophilus*, in Bile-MRS agar (aerobiosis) at 37°C/72h according to Vinderola and Reinhermer (22) for *L. acidophilus* and in LP-MRS (lithium-propionate-MRS) agar (anaerobiosis) at 37°C/72h according to Vinderola and Reinhermer (22) and the modifications of Zacarchenco and Massaguer-Roig (26) for *Bif. longum*.

Determination of the acidity and pH

The evaluation of pH was performed with a pH meter Model PH320 (WTW®) and, the evaluation of the titratable acidity was performed according to AOAC (1), both in triplicate.

RESULTS

Data from measurements of pH, acidity, average of scores obtained at sensory evaluations and cell counts of fermented milks, performed at 1, 7, 14 and 21 days of storage of two experiments are presented on Tables 2 and 3.

Tamime *et al.* (21) reported that commercially produced bifidobacteria starters give more consistent activity than the in house production of liquid cultures which are subject to daily transfers. The experiments were realized by the authors using commercially produced BL and LAC starters.

Sensory evaluation of the taste attribute

The sensory differences among fermented milks added or not of LAC and BL (combinations) were significantly ($P < 0.05$) perceived as storage time increased.

At day 1 of refrigerated storage the fermented milks most preferred by the panelists were C1, C2 and C5 (Table 2) and did not differ ($P < 0.05$) from each other, in the experiment 1. These results are similar to those found after 21 days of storage. In the experiment 2, the favourites combinations were C1, C5 and C3 although C2 did not differ ($P < 0.05$) from these favourites. At day 1 of storage, in the experiment 1, C6 obtained the lowest sensory scores and differed ($P < 0.05$) from C1. In the experiment 2, C4 obtained the lowest sensory scores.

At 7 days of refrigerated storage sensory scores of the experiments 1 and 2 showed significant differences ($P < 0.05$) among favourites combinations (Table 2). In the first experiment the favourites combinations were C5, C1 and C2 and C3 was the fourth preferred. Similar results were obtained at 21 days of storage (Table 2). In the other hand, C2, C3 and C5 were the favourites combinations in experiment 2, while C1 was the fourth preferred. The most preferred combinations C5 and C2 kept their positions in the preference order at 14 and 21 days of storage (Table 2) although C1, C2, C3 and C5 did not differ significantly ($P < 0.05$).

It was noted at 7 days of storage that C4 obtained the lowest sensory scores and differed significantly ($P < 0.05$) from C5 in experiment 1. In experiment 2, in this same period of storage, C4 was the least preferred and differed significantly from C2.

At 14 days of refrigerated storage sensory scores of combinations C5 and C2, from experiments 1 and 2, were the most preferred combinations (Table 2). These combinations received similar sensory scores and did not differ significantly ($P < 0.05$) between each other. They did not differ significantly ($P < 0.05$) from C1, the standard fermented milk. The combination C4 differed significantly ($P < 0.05$) from C5 and C2 and received the lowest sensory scores. Until day 14th C4 and C6 did not differ from each other. This differentiation occurred after 14 days of storage.

At 21 days of refrigerated storage sensory scores of the experiments 1 and 2 for the attribute taste showed good similarity (Table 2). The most favourite combinations were C5 and C2. C5 contained BL and LAC (IPC of 10^8 and 10^7 cfu/mL, respectively) and C2 contained BL only (IPC de 10^8 cfu/mL). However, C5 and C2 obtained similar sensory scores and did not differ significantly ($P < 0.05$). These favourite combinations did not differ significantly ($P < 0.05$) from C1, the standard fermented milk. Davidson *et al.* (4) found similar results in frozen yogurt. The combinations C3 and C6 obtained lowest sensory scores (Table 2). C3 contained BL and LAC (IPC of 10^7 and 10^8 cfu mL⁻¹, respectively) and C6 contained LAC only (IPC de 10^8 cfu/mL). C6 was significantly ($P < 0.05$) different from C5, C2 and C1, obtaining the lowest sensory scores.

Table 2. Values of pH, acidity and average of scores obtained at sensory evaluations of fermented milks added or not of the BL^x and LAC^x at days 1, 7, 14 and 21 of the storage for processings 1 and 2.

| Storage time (days) | Comb ^y . | pH | | T A ^x | | taste ^v | | acidity ^v | |
|---------------------|---------------------|--------|--------|------------------|--------|--------------------|-------------------|----------------------|-------------------|
| | | Proc.1 | Proc.2 | Proc.1 | Proc.2 | Proc.1 | Proc.2 | Proc.1 | Proc.2 |
| 1 | C1 | 4.52 | 4.53 | 106.4 | 106.1 | 6.9 ^a | 7.4 ^a | 7.2 ^a | 6.5 ^a |
| 1 | C2 | 4.45 | 4.57 | 109.3 | 109.3 | 6.3 ^{ab} | 5.7 ^{ab} | 6.7 ^a | 6.1 ^a |
| 1 | C3 | 4.65 | 4.58 | 102.6 | 104.0 | 5.5 ^b | 5.8 ^{ab} | 6.3 ^{ab} | 6.6 ^a |
| 1 | C4 | 4.63 | 4.5 | 94.9 | 109.6 | 5.9 ^{ab} | 5.5 ^b | 6.3 ^{ab} | 6.6 ^a |
| 1 | C5 | 4.54 | 4.48 | 112.1 | 102.2 | 6.1 ^{ab} | 6.6 ^{ab} | 6.3 ^{ab} | 5.7 ^a |
| 1 | C6 | 4.62 | 4.58 | 117.4 | 120.4 | 5.2 ^b | 5.6 ^{ab} | 5.5 ^b | 5.6 ^a |
| 7 | C1 | 4.32 | 4.2 | 108.03 | 114.43 | 6.7 ^{ab} | 6.3 ^{ab} | 6.2 ^a | 5.8 ^a |
| 7 | C2 | 4.26 | 4.25 | 114.70 | 118.78 | 6.5 ^{ab} | 6.8 ^a | 6.2 ^a | 5.6 ^a |
| 7 | C3 | 4.33 | 4.22 | 113.43 | 116.44 | 6.3 ^{ab} | 6.6 ^{ab} | 6.2 ^a | 6.5 ^a |
| 7 | C4 | 4.30 | 4.22 | 104.77 | 117.78 | 5.7 ^b | 5.8 ^b | 6.1 ^a | 5.7 ^a |
| 7 | C5 | 4.28 | 4.2 | 115.93 | 114.60 | 7.0 ^a | 6.4 ^{ab} | 6.7 ^a | 6.0 ^a |
| 7 | C6 | 4.38 | 4.35 | 125.77 | 135.12 | 6.3 ^{ab} | 5.8 ^b | 5.9 ^a | 6.0 ^a |
| 14 | C1 | 4.26 | 4.35 | 114.90 | 118.77 | 6.5 ^{ab} | 6.5 ^{ab} | 6.3 ^a | 6.4 ^a |
| 14 | C2 | 4.21 | 4.22 | 117.43 | 119.10 | 6.9 ^a | 7.4 ^a | 6.2 ^a | 5.7 ^a |
| 14 | C3 | 4.29 | 4.32 | 116.60 | 117.17 | 5.7 ^{ab} | 5.6 ^{ab} | 5.8 ^a | 5.5 ^a |
| 14 | C4 | 4.24 | 4.24 | 110.33 | 116.2 | 5.6 ^b | 5.5 ^b | 5.7 ^a | 5.0 ^a |
| 14 | C5 | 4.22 | 4.12 | 123.10 | 122.13 | 7.0 ^a | 6.7 ^a | 6.2 ^a | 5.9 ^a |
| 14 | C6 | 4.35 | 4.28 | 135.07 | 140.38 | 6.2 ^{ab} | 5.0 ^{ab} | 5.9 ^a | 5.6 ^a |
| 21 | C1 | 4.25 | 4.18 | 107.60 | 130.13 | 6.7 ^a | 6.1 ^{ab} | 6.3 ^a | 5.8 ^a |
| 21 | C2 | 4.16 | 4.14 | 118.60 | 137.34 | 6.4 ^{ab} | 6.5 ^a | 6.1 ^a | 5.9 ^a |
| 21 | C3 | 4.24 | 4.17 | 114.43 | 127.89 | 4.8 ^{cd} | 5.0 ^b | 5.2 ^{ab} | 5.5 ^{ab} |
| 21 | C4 | 4.20 | 4.16 | 108.77 | 130.77 | 5.6 ^{bc} | 5.9 ^{ab} | 5.5 ^{ab} | 5.8 ^a |
| 21 | C5 | 4.11 | 4.16 | 119.43 | 126.61 | 6.8 ^a | 6.1 ^{ab} | 6.2 ^a | 5.8 ^a |
| 21 | C6 | 4.22 | 4.28 | 123.67 | 153.82 | 4.5 ^d | 3.8 ^c | 4.6 ^b | 4.6 ^b |

x: BL, LAC and T. A. means, respectively, *Bif. longum*, *L. acidophilus* and titratable acidity. **y:** see Table 1. **v:** different letters indicate significant differences ($P < 0.05$) among combinations of the fermented milks.

According to Davidson *et al.* (4) other authors also have reported that the activity of yogurt starter and probiotic bacteria caused specific changes in the products affecting sensory characteristics and that fermented products cultured with bifidobacteria were different from the yogurts produced with traditional starters.

Baig and Prasad (2) studied the effect of adding *Bif. bifidum* as a supplementary starter culture on the biochemical, microbiological and organoleptic characteristics of yogurt. These authors reported that supplementation with *Bif. bifidum* reduced ($P < 0.05$) the flavour score slightly concluding that the reduction in the flavour score was related to acetic acid production by *Bifidobacterium*. Also the acetic acid production by different *Bifidobacterium* strains was evaluated by Ostlie *et al.* (14).

The differences in the preference order of C3 and C1 can be related to others organic acids produced by the BL and/or LAC,

instead of lactic acid, in the fermented milk C3. The production of larger quantities of acetic acid from 7th day to 14th of storage, for example, could cause sensory differences that panelist could perceive as suggested by Gomes and Malcata (6).

The results presented by Samona *et al.* (17) showed an increase on the acetic acid concentration at different shelf life periods at 4°C of yogurts that contained *Bif. adolescentis*, *Bif. bifidum* and *Bif. longum*. The increase in the acetic acid concentration varied according to probiotic strain and source used of yogurt starter. Some yogurts reached, after 21 days of storage, the double of the acetic acid concentration measured at the day of yogurt processing. Our results regarding sensory preferences, probably, could be explained by the microorganisms behaviors similar to that described by Samona *et al.* (16). The reduction in the sensory scores of C3 observed after 7 days, in experiment 1, and after 14 days of storage, in

experiment 2, could be related to the acetic acid production described by Samona *et al.* (17).

Sensory evaluation of the acidity attribute

After 1 day of storage, in the experiment 1, the most preferred fermented milks was C1 (Table 2). The fermented milk C6 obtained the lowest sensory scores, in experiments 1 and 2, and differed significantly ($P<0.05$) from C1 and C2. In the experiment 2, the most preferred fermented milks were C3, C4 and C1.

At days 14, 7 and 1 of storage the fermented milks obtained acidity sensory scores that did not differ significantly ($P<0.05$) (Table 2).

At 21 days of storage sensory scores for the attribute acidity were similar to those of the attribute taste (Table 2). The fermented milks C2, C5 and C1 obtained the highest scores and did not

differ from each other. Similarly to the attribute taste, C6 obtained the lowest sensory scores and differed from C2, C5 and C1 in the two experiments.

The fermented milk C6 was the least preferred by the sensory panelists when evaluating the attributes taste and acidity. C6 showed the highest titratable acidity.

Measurements of pH and titratable acidity of the fermented milks

The fermented milk C6 that contained LAC and ST, showed the highest titratable acidity during the storage time in all the measurements performed, in the two experiments. However the C6 fermented milk did not show the lowest pH which probably is due to differences in the ionization constant, amount and relative proportions of the organic acids produced. According to Shahani, Vakil and Kilara (20) *L. acidophilus* produces lactic acid, acetic acid, benzoic acid, hydrogen peroxide and bacteriocins and microcins. The authors of the current research did not realized separated determination of acetic acid. In the fermented milk C6, ST produced the lactic acid during fermentation and storage and LAC produced additionally to lactic acid other acids like acetic acid, during the storage period.

During the 21 days of the storage period, generally, the fermented milks showed increasing titratable acidity values and decreasing pH values. Only at experiment 1, the titratable acidity values were higher at 14 days of storage then at 21 days.

Viable cell counts of the fermented milks

All the fermented milks showed constant viable cell counts of ST during the 21 days of storage, in the two experiments (Table 3). Oliveira *et al.* (13) reported similar results for counts of *Str. thermophilus* in fermented lactic beverages containing probiotic bacteria. However, Vinderola, Mocchiutti and Reinheimer (24) reported that lactic bacteria were inhibited by the probiotic bacteria and vice-versa. These authors stressed the existence of lactic bacteria strains which were not inhibited and the necessity of a careful restriction of the results obtained to the strains studied.

Table 3. Values of viable cells counts of the fermented milks added or not of BL^x and LAC^x at days 1, 7, 14 and 21 of the storage for processings 1 and 2.

| Storage time (days) | Comb ^y . | ST | | LA | | BL | |
|---------------------|---------------------|----------------------|---------------------|----------------------|----------------------|----------------------|----------------------|
| | | Proc.1 | Proc.2 | Proc.1 | Proc.2 | Proc.1 | Proc.2 |
| 1 | C1 | 2.0x10 ⁹ | 1.9x10 ⁹ | - ^w | - ^w | - ^w | - ^w |
| 1 | C2 | 2.1 x10 ⁹ | 3.0x10 ⁹ | - ^r | - ^r | 1.0 x10 ⁸ | 3.7 x10 ⁷ |
| 1 | C3 | 2.0 x10 ⁹ | 2.0x10 ⁹ | 3.5 x10 ⁷ | 5.3 x10 ⁷ | 2.0 x10 ⁷ | 4.4x10 ⁷ |
| 1 | C4 | 2.0x10 ⁹ | 2.1x10 ⁹ | 2.8 x10 ⁷ | 4.3x10 ⁷ | 8.5 x10 ⁷ | 3.510 ⁷ |
| 1 | C5 | 2.0 x10 ⁹ | 1.8x10 ⁹ | 1.7 x10 ⁷ | 2.4x10 ⁷ | 7.4 x10 ⁷ | 2.7x10 ⁷ |
| 1 | C6 | 2.3 x10 ⁹ | 2.2x10 ⁹ | 3.4 x10 ⁷ | 5.4x10 ⁸ | - ^s | - ^s |
| 7 | C1 | 2.2 x10 ⁹ | 2.3x10 ⁹ | - ^w | - ^w | - ^w | - ^w |
| 7 | C2 | 2.2 x10 ⁹ | 2.1x10 ⁹ | - ^r | - ^r | 1.9 x10 ⁷ | 3.0 x10 ⁷ |
| 7 | C3 | 2.2x10 ⁹ | 2.6x10 ⁹ | 2.9 x10 ⁷ | 3.1 x10 ⁷ | 9.1 x10 ⁶ | 4.5x10 ⁷ |
| 7 | C4 | 2.3 x10 ⁹ | 1.9x10 ⁹ | 2.7 x10 ⁷ | 2.0x10 ⁷ | 2.4 x10 ⁷ | 3.610 ⁷ |
| 7 | C5 | 2.1 x10 ⁹ | 1.9x10 ⁹ | 1.8 x10 ⁷ | 2.0x10 ⁷ | 2.2 x10 ⁷ | 4.6x10 ⁷ |
| 7 | C6 | 2.3 x10 ⁹ | 2.8x10 ⁹ | 2.9 x10 ⁷ | 5.9x10 ⁷ | - ^s | - ^s |
| 14 | C1 | 2.4 x10 ⁹ | 2.6x10 ⁹ | - ^w | - ^w | - ^w | - ^w |
| 14 | C2 | 2.6 x10 ⁹ | 2.4x10 ⁹ | - ^r | - ^r | 4.0 x10 ⁷ | 4.9 x10 ⁷ |
| 14 | C3 | 2.0x10 ⁹ | 2.6x10 ⁹ | 6.7 x10 ⁶ | 1.2 x10 ⁷ | 2.1 x10 ⁷ | 4.4x10 ⁷ |
| 14 | C4 | 1.9 x10 ⁹ | 2.0x10 ⁹ | 9.0 x10 ⁶ | 1.5x10 ⁷ | 2.5 x10 ⁷ | 3.0x10 ⁷ |
| 14 | C5 | 2.3 x10 ⁹ | 2.0x10 ⁹ | 6.0 x10 ⁵ | 6.0x10 ⁵ | 9.0 x10 ⁶ | 4.6x10 ⁷ |
| 14 | C6 | 2.4 x10 ⁹ | 1.5x10 ⁹ | 1.1 x10 ⁷ | 1.8x10 ⁷ | - ^s | - ^s |
| 21 | C1 | 2.1 x10 ⁹ | 3.1x10 ⁹ | - ^w | - ^w | - ^w | - ^w |
| 21 | C2 | 2.0 x10 ⁹ | 1.6x10 ⁹ | - ^r | - ^r | 2.0 x10 ⁷ | 2.7 x10 ⁶ |
| 21 | C3 | 2.0x10 ⁹ | 2.2x10 ⁹ | 4.2 x10 ⁶ | 5.1 x10 ⁶ | 1.6 x10 ⁷ | 5.0x10 ⁶ |
| 21 | C4 | 2.0 x10 ⁹ | 1.4x10 ⁹ | 5.0 x10 ⁶ | 4.0x10 ⁶ | 1.3 x10 ⁷ | 2.8x10 ⁶ |
| 21 | C5 | 2.1 x10 ⁹ | 2.5x10 ⁹ | 3.3 x10 ⁵ | 3.7x10 ⁵ | 1.4 x10 ⁷ | 4.4x10 ⁶ |
| 21 | C6 | 2.1 x10 ⁹ | 1.8x10 ⁹ | 9.0 x10 ⁶ | 7.5x10 ⁶ | - ^s | - ^s |

x: BL, LAC and ST means, respectively, *Bif. longum*, *L. acidophilus* and *Str. thermophilus*. **y:** see Table 1. **w:** - Fermented milk without addition of *Bif. longum* and *L. acidophilus*; **r** and **s:** Fermented milk without addition of *L. acidophilus* and of *Bif. longum*, respectively.

The viable cell counts of ST in our study showed that the inhibition of ST by the BL and LAC did not occur.

The viable cell counts of BL were kept around 10^7 and 10^6 cfu/mL until the 21th of storage at 4°C. The addition to the ST fermented milk of 10^7 BL cfu/mL instead of 10^8 represents an economic advantage since less lyophilized material is necessary. The addition of 10^7 BL cfu/mL to ST fermented milk results in viable cell counts of BL in the final product, until 21 days of storage at 4°C, which are in agreement to recommended values for health benefits (21).

The difference between BL counts of the experiments 1 and 2 could be related to the sensibility to oxygen of the BL. During the stirring of the fermented milk of the experiment 2 (Table 3) more quantities of oxygen could be added. The sensibility to oxygen of bifidobacteria was related by researchers like Vinderola, Bailo and Reinheimer (23).

The viable cell counts of LAC for fermented milk C5 from 14 days of storage were on the range of 10^5 cfu/mL which represents a 2 logarithmic cycles reduction of LAC, since for the production of C5 were added 10^7 cfu/mL of LAC and 10^8 cfu/mL of BL. Gregurek (5) reported that counts and the stability of bifidobacteria starter cultures used were higher than those of *L. acidophilus*. Vinderola *et al.* (24) reported that *L. acidophilus* was the sole species inhibited by the others tested (*Bifidobacterium*, *Streptococcus* and *Lb.casei*). These authors also reported that *L. acidophilus* is more sensible than bifidobacteria to media like yogurts and acidified milks when considering ranges of the pH from 3.5 to 4.5. Oliveira *et al.* (13) also reported a 2 logarithmic cycles reduction of the *L. acidophilus* populations after 28 days of storage at 4°C in the presence of ST.

In the fermented milks C3, C4 and C6 the viable cell counts of LAC were kept above 1×10^6 cfu/mL until the end of 21 days of storage

DISCUSSION

The sensory effects related to the presence of *Bif. longum* and *L. acidophilus* in the fermented milks were significantly ($P < 0.05$) perceived while storage time increased. The favourite combinations contained, initially, *Bif. longum* and *L. acidophilus* (10^8 and 10^7 cfu/mL, respectively, C5) and *Bif. longum* only (10^8 cfu/mL, C2). These combinations did not differ significantly among them and neither from the standard fermented milk (C1). This result represents that it is possible to improve the acceptability of fermented products containing probiotics. The low acceptability of fermented milks containing probiotics were reported by many authors (2,8,16,20).

The lowest sensory scores and highest titratable acidity values was for *L. acidophilus* combination (10^8 cfu/mL initial concentration, C6). The sensory scores of C6 were significantly different ($P < 0.05$) from C5, C2 and C1 at 21 days of storage. The

fermented milk C6 showed the highest acidity during the storage time in all the measurements performed. However it was not observed inhibition of over-acidification by bifidobacteria or *L. acidophilus*.

All the fermented milks showed constant viable cell counts of ST during the 21 days of storage. The viable cell counts of BL were kept constant or reduced 1 logarithmic cycle and viable cell counts of LAC were reduced 1 or 2 logarithmic cycle. However, viable cell counts of LAC and BL kept the minimum values that the probiotic product must contain, which vary from 10^5 to 10^7 cfu/g of probiotic bacteria, at use-by date like reported by many authors (4,6,15,16,19).

RESUMO

Propriedades de leites fermentados por *Streptococcus thermophilus* contendo concentrações variáveis de *Bifidobacterium longum* e *Lactobacillus acidophilus*

Foram estudadas as características sensoriais, de pH e acidez de cinco combinações de leites fermentados por *Streptococcus thermophilus*, adicionados de *Bifidobacterium longum* e/ou *Lactobacillus acidophilus* liofilizados. Os efeitos sobre as características sensoriais tornaram-se significantes ($p < 0,05$) com o aumento do tempo de estocagem. As combinações favoritas continham, inicialmente, *Bif. longum* e *L. acidophilus* (10^8 e 10^7 ufc/mL, respectivamente) e *Bif. longum* apenas (10^8 ufc/mL). Estas combinações não diferiram significativamente do leite fermentado padrão, nem entre si. As notas mais baixas e os maiores valores de acidez titulável foram apresentados pelo leite fermentado por *Streptococcus thermophilus* contendo apenas *L. acidophilus* (10^8 ufc/mL, concentração inicial). Durante 21 dias de estocagem a 4°C, as contagens de células viáveis de *Str. thermophilus* não mudaram, as de *Bif. longum* mantiveram-se constantes ou reduziram em um ciclo logarítmico e, as de *L. acidophilus* reduziram de 1 a 2 ciclos logarítmicos. Não foi observada inibição da pós-acidificação resultante da presença de bifidobactéria ou *L. acidophilus*.

Palavras-chave: *Bifidobacterium*, *Lactobacillus acidophilus*, leite fermentado, análise sensorial

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