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Acceptability of prebiotic fiber-treated whey drink fermented with *Lactobacillus acidophilus*

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This work aimed to develop a fermented drink by diversifying the quantities of *Lactobacillus acidophilus* inoculum and prebiotic fiber in the form of inulin and using the total dry extract of whey and sucrose. After fermentation, the following measurements were made after 0, 7, 14, 21 and 28 days of storage at 6°C: titratable acidity (according to the Dornic method), pH, moisture content, fat content, ash content, protein content, lactose content and the probiotic cell count. After 28 days of storage, the viable *L. acidophilus* cell counts had decreased for all six treatments but were still above the minimum count of 7 log CFU/mL recommended by the Brazilian legislation. All samples presented satisfactory acceptability with the exception of treatment 6, in which the inulin was decanted, thereby altering the color and causing a decrease in acceptance.

Key words: Fermented milk, whey, probiotic microorganism, prebiotic, sensory evaluation.

INTRODUCTION

Fermented dairy products containing probiotic bacteria have received increasing attention in recent decades, including the expansion of the market for functional foods and research into the development of probiotic foods (Karimi et al., 2011).

Fermentation is the chemical transformation of organic substances into simpler compounds through the action of enzymes, complex organic catalysts produced by microorganisms such as molds, yeasts or bacteria (Jafarei and Ebrahimi, 2011). These bacteria produce lactic acid as a result of carbohydrate fermentation and are widely used in the production of fermented foods, from dairy products to fruit and vegetable products. The reasons for the widespread use of lactic acid bacteria

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(LAB) include increasing the shelf life; improving the safety, flavor, appearance and texture and enhancing the physiological and hygienic value due to the presence of viable cells and valuable LAB metabolites (Semjonovs et al., 2008; Kaboosi, 2011; Paraschiv et al., 2011).

The mechanisms of probiotics include the remodeling of microbial communities, the suppression of pathogens, immunomodulation via the up-regulation of anti-inflammatory factors, enhancement of immunity, effects on epithelial cell differentiation and proliferation, promotion of the intestinal barrier function (Preidis and Versalovic, 2009), reduction of serum cholesterol, vitamin synthesis, anti-carcinogenic activity and anti-bacterial activity (Belviso et al., 2009; Ibrahim et al., 2010; Lourens-Hattingh and Viljoen, 2001; Robinson and Samona, 1992; Songisepp et al., 2004; Arunachalam, 1999; Blanchette et al., 1995; Gomes and Malcata, 1999). The beneficial effects attributed to probiotic bacteria also include the alleviation of lactose-intolerance symptoms and constipation, a reduction in serum cholesterol, the prevention of drug-induced colitis and efficacy against a number of other conditions including ulcerative colitis, pouchitis, radiation colitis, atopic eczema and diarrhea (Fotiadis et al., 2008). Many studies have reported the effects of probiotics on gut function as well as visceral sensitivity (Thomas et al., 2012; Preidis et al., 2012), such as reductions in visceral nociceptive reflex responses in rodents and abdominal discomfort in humans (Tillisch et al., 2013).

Probiotics are defined as a dietary supplementation of beneficial bacteria, such as *Lactobacillus* spp. and *Bifidobacterium* spp. (Tannock, 1995; Fuller and Gibson, 1997; Macfarlane and Cummings, 1999; Rolfe, 2000; Sanders, 2000; Dunne, 2001; Ishibashi and Yamazaki, 2001; Marteau et al., 2001), which are consumed to antagonize pathogenic bacteria that can invade the human intestine and cause gastrointestinal diseases (Oliveira et al., 2011). The survival and permanence of these microorganisms in the intestinal tract depend on their ability to survive in the gastric medium and utilize the available nutrients (Barrangou et al., 2006).

Products obtained by LAB fermentation processes are therefore of special importance for functional foods such as probiotics (Semjonovs et al., 2008). In addition to dairy products, there are many other commercial probiotic products based on *L. acidophilus*. This bacterium is thought to produce healthy byproducts that protect the stomach, the gut and the reproductive area from harmful bacteria. *Lactobacillus acidophilus* is the best-known species of this Lactobacillus complex in the LAB group and is naturally present in the gastrointestinal tracts of humans and animals. In fermented food, the metabolic activity of this microorganism results in the production of flavor and aromas that produce the organoleptic properties of fermented foods and inhibit food spoilage (Parvaneh and Ebrahimi, 2011). Commercially, the most Important probiotic strains are lactic acid bacteria (Oliveira et al., 2011).

Prebiotic foods contain certain types of dietary fibers, that is, non-digestible carbohydrates with a molecular configuration that makes them resistant to enzyme action. Examples of efficient and commercially available prebiotics are fructooligosaccharides (FOS), inulin and galactooligosaccharides (Tuohy et al., 2003; Barrangou et al., 2003). Inulin is an oligomeric fructose-based carbohydrate that can be easily dispersed in water (Hoppert et al., 2013). The concept of synbiotics represents the combination of probiotics and prebiotics (Shukla et al., 2011; Rastall and Maitin, 2002; Tuohy et al., 2003; Holzapfel and Schillenger, 2002).

Considering the environmental impacts caused by the high chemical oxygen demand (COD) of whey, which liberates 50 KgO_2/ton permeate from 9 kg of whey produced for every kilogram of cheese manufactured, different uses for this waste material have been devised (Martin-Diana et al., 2006). In recent years, due to the need to minimize environmental pollution and the use of available nutrients to attenuate the demand, whey has become an eminent requirement (Ammar et al., 2011) because its proteins have a higher biological value than other proteins such as those of egg, soy and even milk caseins (Smithers, 2008).

Thus, the aim of the present study was to evaluate the fermentation of total dry whey extract and sucrose using a lactic starter, such as pure *L. acidophilus*, and varying the formulations with respect to the size of the inoculums and the amount of prebiotic such as inulin.

MATERIALS AND METHODS

Whey fermentation

This study was divided into three stages, with the first stage consisting of whey fermentation using varying amounts of inoculum (1 -2%) and prebiotic fiber (0, 2 and 4%). The primary purpose of this stage was to determine the best technology to ferment whey when reconstituted to 7% of the total solids and to obtain the highest viability of probiotic microorganisms. A concentrated freeze-dried probiotic starter for direct use was used, which was composed of *L. acidophilus* (*LA3*) donated by SACCO® (a dairy products company).

According to the specific experiment, the whey powder was reconstituted in water to approximately 7% of the total solids, and then sugar (5% w/v) was added with vigorous stirring, followed by the addition of different percentages of inulin. The inulin prebiotic consisted of Raftiline®GR (92% inulin and 8% glucose/-fructose/sucrose) and was donated by CLARIANT® (a company that represents ORAFTI®, Tienen-Belgium in Brazil). This mixture was heated to 85°C and maintained at this temperature for 20 min in a thermostatic bath. The mixture was then cooled to 37°C in an ice-water bath to

obtain the probiotic lactic culture under aseptic conditions. The product was then incubated at 37°C, and the fermentation time of the milk drink, as calculated from the inoculation, was used to obtain an acid value close to 60° Dornic. This temperature is within the optimum temperature range (37 to 40°C) for the growth of *L. acidophilus* (Ahmed et al., 2006). After fermentation was complete, the product was initially cooled to approximately 20°C, and the clots were broken by manual shaking for 30 s. Then, a final cooling step was performed in an ice-water bath, followed by the addition of fruit salad pulp (10% w/v) to enhance the flavor and mask the bitter taste of the whey. The beverage was filled into plastic cups and stored in a refrigerator at a temperature of approximately 6°C. In this stage, six samples were developed: T_1= Fermented drink with 1% inoculum and the addition of fruit salad pulp; T_2= Fermented drink with 1% inoculum and the addition of fruit salad pulp and 2% inulin; T_3= Fermented drink with 1% inoculum and the addition of fruit salad pulp and 4% inulin; T_4=Fermented drink with 2% inoculum and the addition of fruit salad pulp; T_5= Fermented drink with 2% inoculum and the addition of fruit salad pulp and 2% inulin; and T_6= Fermented drink with 2% inoculum and the addition of fruit salad pulp and 4% inulin.

Chemical, physicochemical and microbiological assessment

The second stage was related to the chemical, physicochemical and microbiological assessment. The following measurements were obtained: acidity as lactic acid (° Dornic), pH, moisture, fat, ash, protein, lactose (AOAC, 1997), and microbiological quality as required by law (Brazil, 2000). After fermentation, the physic-chemical analysis was carried out in triplicate. The pH values were determined using a digital potentiometer (DIGIMED) calibrated with pH 7.0 and 4.0 buffer solutions. The total acidity was determined by measuring the lactic acid content of 100 g of sample. In particular, 5-ml aliquots of the samples were titrated with 0.1 N NaOH in the presence of the indicator phenolphthalein, according to the technique described by Instituto Adolfo Lutz (2008).

Probiotic cell count and probiotic microorganism count

Microbiological analyses of the probiotic microorganisms under study were carried out in triplicate after 0, 7, 14, 21 and 28 days of storage, representing the third stage of the experiment. All samples were collected under aseptic conditions and were immediately taken to the laboratory.

Serial decimal dilutions were prepared by aseptically transferring 10 ml of sample into a sterile conical flask containing 90 mL of sterile 0.1% distilled peptone water. This solution was then homogenized, and dilutions were made.

Each sample was serially diluted from 10^{-1} to 10^{-15} in peptone water. One milliliter of each dilution was inoculated into triplicate plates containing MRS agar (De Man et al., 1960). The analyses were conducted each week over a 4-week storage period at 4°C. The probiotic bacteria (*L. acidophilus*) were counted in MRS agar, followed by 72-h incubation at 37°C under anaerobic conditions. Formulations with *L. acidophilus* were counted on MRS agar containing maltose using the spread plate method, followed by 72-h incubation at 37°C under aerobic conditions. Identification of the lactic acid bacteria was performed using the catalase test and Gram staining, according to the methods of Holt et al. (1994).

Sensory analysis

The sensory evaluation was performed in a single step and after the assessment of microbiological parameters to ensure the food safety of the volunteer participants. A nine-point hedonic scale was applied (Meilgaard et al., 1999) using an untrained panel of 50 teachers and students, between 19 and 50 years of age, who represented consumers at a higher education level from the Federal University of Technology-UTFPR. Statistical tests were performed using an analysis of variance (ANOVA) with test comparison by means of Tukey's test at 5% significance (Barbetta, 2002). The statistical analysis was performed using the software *Statistica 6.0*.

Ethical procedures

The volunteers provided free and informed consent according to standard procedures, and the Ethics Committee for Research with Human Beings of the Federal University of Santa Catarina approved the research project under approbation number 380/05.

RESULTS AND DISCUSSION

Acidity and pH parameters

Table 1 shows the evolution of the parameters of acidity and pH during fermentation for the six treatments. The values obtained during fermentation showed that as the lactic acid content increased, the pH value decreased, which is consistent with data obtained by previous researchers (Lourens-Hattingh and Viljoen, 2001). When the values obtained for these parameters were compared to those in Resolution nº 5 of November 13th, 2000 (Brasil, 2000) for the manufacturing of fermented milk, only treatment 1 presented values in agreement with this

Table 1. Values obtained for acidity and pH during fermentation.

*Treatment		Time (h)									
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	Titratable acidity (ºD)	13	19	39	45	51	56	58	60	-	-
	pH	6.30	4.97	4.75	4.50	4.31	4.29	4.22	4.10	-	-
2	Titratable acidity (ºD)	13	22	27	32	37	41	46	50	51	-
	pH	6.30	5.56	5.26	4.93	4.87	4.62	4.50	4.39	4.31	
3	Titratable acidity (ºD)	13	19	25	31	36	42	46	50	54	59
	pH	6.30	5.46	5.09	4.86	4.62	4.51	4.42	4.36	4.31	4.29
4	Titratable acidity (ºD)	13	16	23	26	30	33	39	46	48	50
	pH	6.30	5.92	5.59	5.36	5.12	5.00	4.88	4.75	4.71	4.50
5	Titratable acidity (ºD)	13	17	22	25	27	34	39	43	47	50
	pH	6.30	5.88	5.49	5.17	4.90	4.85	4.81	4.76	4.52	4.48
6	Titratable acidity (ºD)	13	16	21	26	28	33	37	41	46	48
	pH	6.30	5.83	5.47	5.15	4.98	4.87	4.65	4.51	4.47	4.41

*Data represent the evolution of the acidity and pH parameters during fermentation for the six treatments.

resolution at the end of the fermentation process (minimum of 60° Dornic). However, there is no quality standard for fermented beverages made with whey.

We found that the fermentation time was longer and the evolution of acidity (° Dornic) was slower as a function of the low multiplication rate of the probiotic cultures in relation to the traditional lactic bacteria, in accordance with the results of Lourens-Hattingh and Viljoen (2001). It was also observed that the time spent for fermentation was greater and the acidity evolution (° Dornic) was slower, due to the low rate of multiplication of probiotic cultures, in comparison to traditional lactic acid bacteria. These results are also consistent with those of Gomes and Malcata (1999).

In comparison to the values reported in Brasil (2000), the values for acidity and pH in Treatment 1 indicated that this formulation (with 1% starter and no inulin) is likely the most technologically and economically viable formulation for production on an industrial scale.

Physicochemical results

Table 2 shows the results obtained for the three replicates of the physicochemical analysis, considering acidity and pH.

It was observed that during storage, the pH values for all treatments decreased to below 4.5, a desirable value for preventing the growth of pathogenic contaminants (Micanel et al., 1997).

As mentioned by Gomes and Malcata (1999), the probiotic species of Bifidobacterium and Lactobacillus, particularly *L. acidophilus*, in addition to the benefits they provide in terms of nutrition and health, have the advantage of promoting reduced acidification (° Dornic) during storage, as confirmed in the six treatments evaluated in this study. This reduced acidification is important because it helps maintain the level of viable probiotic bacteria in drinks (Dave and Shah, 1997).

As shown in Table 2, at the end of twenty-eight days of storage, the values for acidity (° Dornic) and pH of the six treatments were close to the minimum values required by Resolution No. 5, November 13th, 2000 (Brasil, 2000) for fermented milks (that is, 60° Dornic).

The pH values obtained in this study at the beginning of the storage period were similar to those obtained in the formulation of acidophilus milk (pH 4.68) by Zacarchenco and Massaguer-Roig (2004), although the values for acidity were lower than those reported by these authors (77° Dornic).

This difference is likely related to our use of whey, whereas the aforementioned study used cow's milk, which may have contributed to better fermentation, thereby increasing the acidity of the drink.

Considering the values obtained for pH, moisture, fat, total carbohydrates, lactose, ash and total solids (Table 3), a significant difference was observed between the treatments (p-value <0.05). Only the protein content (p-value> 0.05) showed no significant difference among the six treatments, which supports the finding of Klaver et al. (1993) that probiotic bacteria grow slowly in milk and have low proteolytic activity.

The varied percentages of inoculum and prebiotic used in each of the six treatments could have been the cause of the different values obtained for pH. However, these values were similar (pH 4.68) when compared to those of the acidophilus milk produced in the study of Zacarchenco and Massaguer-Roig (2004).

As expected, there was a significant difference (p-value < 0.05) in the values for moisture content between

Table 2. Values obtained for acidity and pH during storage.

Sample		Storage period (days)				
		0	**7**	**14**	**21**	**28**
1	Acidity (ºD)	60	70	73	77	79
	pH*	4.10 ± 0.02	3.98 ± 0.02	3.96 ± 0.01	3.81 ± 0.02	3.75 ± 0.02
2	Acidity (ºD)	51	56	58	63	66
	pH*	4.31 ± 0.01	3.87 ± 0.02	3.81 ± 0.01	3.67 ± 0.01	3.62 ± 0.01
3	Acidity(ºD)	59	61	64	69	71
	pH*	4.29 ± 0.01	3.97 ± 0.01	3.88 ± 0.01	3.73 ± 0.02	3.70 ± 0.01
4	Acidity (ºD)	48	50	52	55	56
	pH*	4.50 ± 0.02	4.15 ± 0.01	4.09 ± 0.02	4.00 ± 0.01	3.88 ± 0.01
5	Acidity (ºD)	50	52	54	55	57
	pH*	4.48 ± 0.02	4.08 ± 0.01	3.93 ± 0.01	3.88 ± 0.02	3.84 ± 0.02
6	Acidity (ºD)	48	50	54	57	57
	pH*	4.41 ± 0.02	4.19 ± 0.02	3.97 ± 0.02	3.82 ± 0.02	3.80 ± 0.01

*Data represent the means ± S.D of three replicates. T1 = fermented drink with 1% starter and fruit saladflavor; T2 = fermented drink with 1% starter, fruit salad flavor, and supplementation with 2% inulin; T3 = fermented drink with 1% starter, fruit salad flavor and the addition of 4% inulin; T4 = fermented drink with 2% starter and fruit salad flavor; T5 = fermented drink with 2% starter, fruit salad flavor and supplementation with 2% inulin; T6 = fermented drink with 2% starter, fruit salad flavor and the addition of 4% inulin.

Table 3. Values for the physical and chemical determinations of the flavored fermented beverages obtained from the various treatments.

Parameter	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	F Test
pH	4.100 ± 0.0000^{f}	4.307 ± 0.0058^{d}	4.290 ± 0.0000^{e}	4.510 ± 0.0100^{a}	4.477 ± 0.0058^{b}	4.413 ± 0.0058^{c}	P<0.0001
Moisture content (%)	77.300 ± 0.2500^{a}	75.500 ± 0.3606^{b}	73.800 ± 0.2000^{c}	77.600 ± 0.2646^{a}	75.650 ± 0.1323^{b}	73.600 ± 0.2646^{c}	P<0.0001
Proteín (%)	0.890 ± 0.0100^{a}	0.800 ± 0.0700^{a}	0.820 ± 0.0436^{a}	0.850 ± 0.0265^{a}	0.840 ± 0.0700^{a}	0.830 ± 0.0721^{a}	P=0.4830
Fat (%)	0.300 ± 0.0000^{a}	0.200 ± 0.0000^{b}	0.200 ± 0.0000^{b}	0.300 ± 0.0000^{a}	0.200 ± 0.0000^{b}	0.200 ± 0.0000^{b}	P<0.0001
Carbohydrates (%)	21.210 ± 0.0000^{f}	22.940 ± 0.0000^{c}	24.680 ± 0.0000^{b}	21.020 ± 0.0000^{f}	22.840 ± 0.0000^{d}	24.830 ± 0.0000^{a}	P<0.0001
Lactose (%)	5.340 ± 0.0265^{a}	5.340 ± 0.0624^{a}	5.250 ± 0.0265^{ab}	5.150 ± 0.0500^{b}	4.800 ± 0.0458^{c}	5.150 ± 0.0300^{b}	P<0.0001
Fixed min. res. (%)	0.300 ± 0.0361^{b}	0.460 ± 0.0173^{a}	0.500 ± 0.0529^{a}	0.230 ± 0.0656^{b}	0.470 ± 0.0458^{a}	0.540 ± 0.0557^{a}	P<0.0001
Total solids content (%)	22.700 ± 0.0436^{c}	24.400 ± 0.2646^{b}	26.200 ± 0.1732^{a}	22.400 ± 0.1000^{c}	24.350 ± 0.0458^{b}	26.400 ± 0.0173^{a}	P<0.0001

*Data represent the means ± S.D of three replicates. *A p-value<0.01 indicates a significant difference between the formulations. Means sharing the same letter within a column are not significantly different at the 5% significance level.

treatments with different amounts of prebiotic fiber, which followed the changes in the percentage of total solids between the beverages. The fat content only showed a significant difference in those treatments with no added prebiotic fiber. When compared to the parameters of Normative Instruction n° 51 (Brasil, 2002), the fermented beverage in the present study was classified as skimmed because it showed a maximum total milk fat content below 0.5%. As expected, the carbohydrate contents showed no significant difference (p-value < 0.05) between the treatments with no added prebiotic fiber (treatments 1 and 4).

The lactose content was determined post-fermentation to verify whether variation in the size of the initial inoculum influenced the consumption of lactose by the probiotic microorganisms. According to Fuller (1999), probiotics are characterized by their ability to decrease the residual lactose level in the final product. This concept was confirmed in our study, as an increase in the amount of probiotic starter inoculated generally led to an increase in the consumption of lactose by the micro-organism inoculated.

Measurement of the fixed mineral residue and total dry extract levels demonstrated that the values obtained were only significantly different (p-value < 0.05) when the percent of added inulin (prebiotic fiber) was increased, as this modification altered the total solids content of the treatments.

Table 4. Results obtained in the analyses of the microbiological quality of the fermented beverage samples.

Microorganism	*Acceptance criteria	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6
Coliforms at 35°C (MPN/mL)	10^2	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Coliforms at 45°C (MPN/mL)	10^1	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Yeasts and molds (CFU/mL)	2×10^2	<10	$2x10^1$	<10	<10	<10	<10

*According to Brazilian Legislation, Resolution nº5 of November 13th, 2000.

Table 5. Acceptability of the six samples of fermented whey beverage.

Sample	*Average scores for the attributes			
	Color	Flavor	Aroma	Consistency
(T_1)	7.54 ± 1.46^a	7.34 ± 1.56^a	7.44 ± 1.51^a	6.9 ± 1.73^a
(T_2)	6.86 ± 1.60^a	6.76 ± 1.72^a	6.68 ± 1.65^b	6.68 ± 1.68^a
(T_3)	6.96 ± 1.52^a	7.04 ± 1.61^a	7.08 ± 1.35^a	7 ± 1.61^a
(T_4)	7.54 ± 1.47^a	7.16 ± 1.62^a	7.32 ± 1.22^a	7 ± 1.47^a
(T_5)	6.9 ± 1.46^a	7.04 ± 1.58^a	7.14 ± 1.37^a	7.08 ± 1.54^a
(T_6)	5.84 ± 2.31^b	6.9 ± 1.85^a	7.02 ± 1.57^a	6.9 ± 1.66^a
**HSD	0.72	0.76	0.60	0.60

*Average scores of the 50 consumers. Hedonic score: (9) *Like extremely*; (8) *Like very much*; (7) *Like moderately*; (6) *Like slightly*; (5) *Neither like nor dislike*; (4) *Dislike slightly*; (3) *Dislike moderately*; (2) *Dislike very much*; (1) *Dislike extremely*.** HSD: *Tukey´s* significant difference at the 5% level.[a, b, c, d] Scores (average and standard deviation) followed by the same letter (same column) do not differ from each other. T1 = fermented drink with 1% starter and fruit salad flavor; T2 = fermented drink with 1% starter, fruit salad flavor, and supplementation with 2% inulin; T3 = fermented drink with 1% starter, fruit salad flavor and the addition of 4% inulin; T4 = fermented drink with 2% starter and fruit salad flavor; T5 = fermented drink with 2% starter, fruit salad flavor and supplementation with 2% inulin; T6 = fermented drink with 2% starter, fruit salad flavor and the addition of 4% inulin.

Microbological quality of the fermented beverage samples

Table 4 shows the results obtained in the analysis of the quality of the flavored fermented beverages. It was found that the counts obtained for coliforms at 35 and 45°C (MPN/mL) and those for yeasts and molds (CFU/mL) were below the maximum acceptable numbers established by Resolution n° 5 of November 13th, 2000 (Brasil, 2000) for fermented milk. This result assured that the beverages were microbiologically safe for consumption.

Acceptance of the fermented beverage

Table 5 demonstrates the results for acceptability of the six samples of fermented beverages. The analysis of variance (ANOVA) showed there was a significant difference ($p<0.05$) between treatments regarding the attributes of color and aroma, whereas there was no significant difference ($p>0.05$) for the attributes of flavor and consistency. It was observed that sample T_1 (Fermented drink with 1% inoculum plus added fruit salad pulp) and sample T_4 (fermented drink with 2% starter and fruit salad flavor) presented greater acceptability, as they were both classified in the category “Like moderately”, and this finding indicates that these two beverages presented good acceptability. In addition, sample T_6 (fermented drink with 2% starter, fruit salad flavor and the addition of 4% inulin) was scored between the categories “Neither like nor dislike” and “Like slightly”, which suggests that improvement could be made in relation to the attribute of color.

Probiotic bacterial cell count

Table 6 shows the results obtained for the probiotic bacterial counts for the six treatments during the storage period. Significant differences in probiotic bacterial counts were observed between the treatments (p value <0.05) throughout the storage period (Table 6). Thamer and Penna (2005) developed a fermented beverage with different amounts of whey and sugar fructooligosaccharides and assessed the growth of the probiotics and their physicochemical characteristics. The highest counts for the probiotic microorganisms corresponded to the treatment that showed low acidity and high levels of solids, as in treatments 4, 5 and 6 of this study, which showed the greatest initial growth of *L. acidophilus* due to the low level of acidity.

Various studies on the survival of probiotic

Table 6. *L. acidophilus* counts during storage at 6°C.

Storage period (days)	T1 (Log cfu/mL)	T2 (Log cfu/mL)	T3 (Log cfu/mL)	T4 (Log cfu/mL)	T5 (Log cfu/mL)	T6 (Log cfu/mL)	Test F
0	10.340 ± 0.0400^{c}	10.507 ± 0.0586^{b}	10.160 ± 0.0458^{d}	10.650 ± 0.0458^{a}	10.627 ± 0.0252^{a}	10.700 ± 0.0173^{a}	P<0.0001
7	10.287 ± 0.0473^{c}	10.453 ± 0.0208^{b}	10.083 ± 0.0513^{d}	10.637 ± 0.0416^{a}	10.587 ± 0.0153^{a}	10.623 ± 0.0252^{a}	P<0.0001
14	9.357 ± 0.0981^{b}	10.010 ± 0.6165^{ab}	9.833 ± 0.2082^{ab}	10.353 ± 0.1680^{a}	9.767 ± 0.0751^{ab}	9.713 ± 0.0981^{ab}	P=0.0204
21	8.900 ± 0.0700^{b}	8.877 ± 0.0850^{b}	9.543 ± 0.0513^{a}	8.947 ± 0.0153^{b}	8.763 ± 0.1026^{bc}	8.600 ± 0.0361^{c}	P<0.0001
28	7.360 ± 0.1473^{b}	7.477 ± 0.0503^{b}	8.493 ± 0.1102^{a}	7.330 ± 0.0985^{b}	8.267 ± 0.2779^{a}	7.367 ± 0.1069^{b}	P<0.0001

Data represent the means ± S.D of three replicates. Values followed by same superscript are not significantly different (p> 0.05).

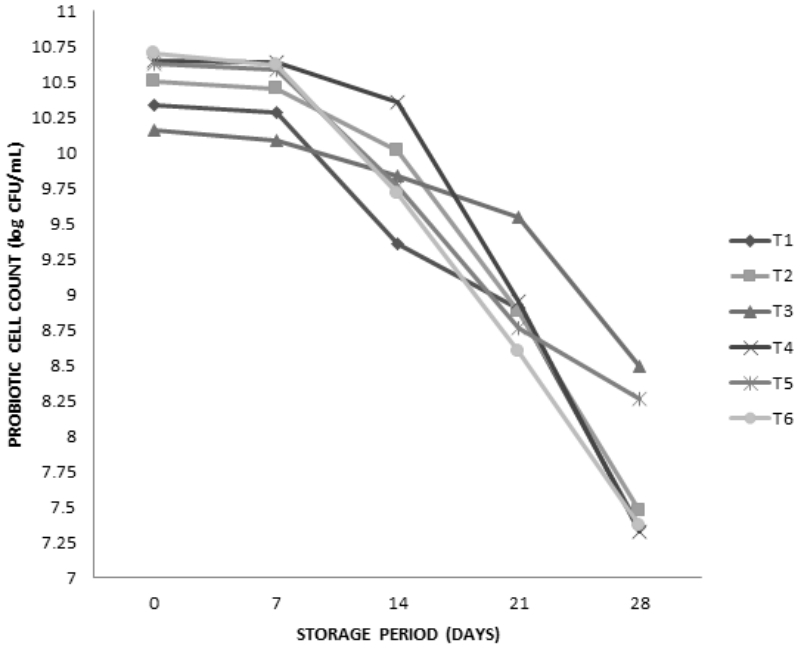
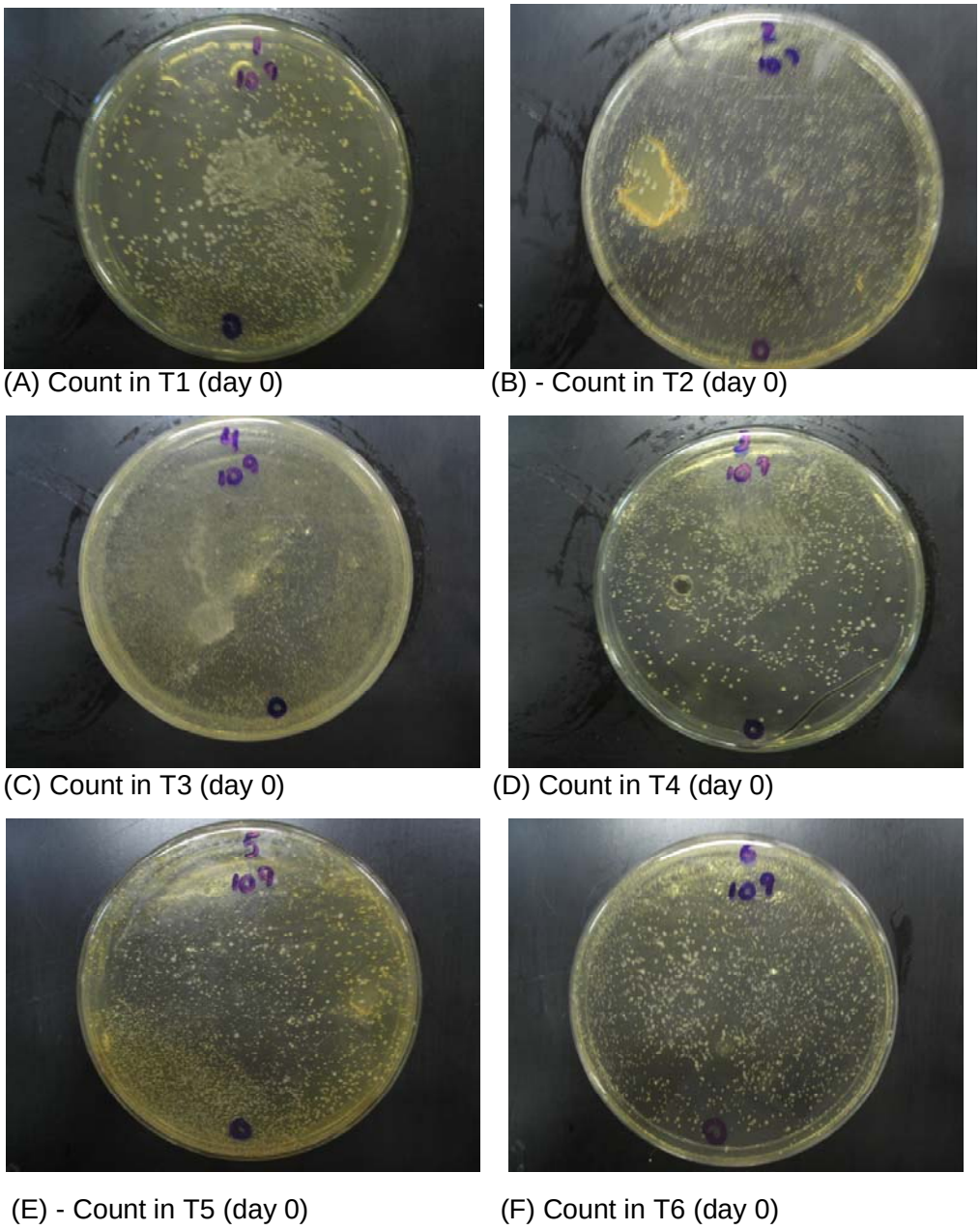


Figure 1. Total viable count of *Lactobacillus acidophilus* during the storage period. T1 = fermented drink with 1% starter and fruit salad flavor; T2 = fermented drink with 1% starter, fruit salad flavor, and supplementation with 2% inulin; T3 = fermented drink with 1% starter, fruit salad flavor and the addition of 4% inulin; T4 = fermented drink with 2% starter and fruit salad flavor; T5 = fermented drink with 2% starter, fruit salad flavor and supplementation with 2% inulin; T6 = fermented drink with 2% starter, fruit salad flavor and the addition of 4% inulin.

microorganisms (Gomes and Malcata, 1999) have generally agreed that products with high acidity (e.g., yoghurt) generate an increased loss of viability compared to probiotic products with low acidity (° Dornic). This concept also explains the high counts of probiotic microorganisms observed in this study throughout the 28 days of storage.

Of the two variables studied (inoculum and prebiotic fiber), a larger amount of inoculum resulted in significantly increased growth of the probiotic microorganism (p-value <0.05). Considering that autolysis reduces the number of probiotic bacteria (Kang et al., 1998; Koch et al., 2008) and that there is a reduction in autolysis in the presence of prebiotics (Saran et al., 2012), the efficient growth of the probiotic bacteria in this study can also be explained by the addition of inulin.

According to Collado and Sanz (2006), Mattila-Sandholm (2002), and Ouwehand et al. (1999), survival of the probiotic bacteria in a food product is fundamental, and sufficiently populations (typically greater than 7 log CFU/mL or g) are of physiological importance to the consumer. This value was achieved in all six treatments in this study. Figure 1 shows that the total lactic bacteria count required for fermented milk (7 log CFU/g),



(A) Count in T1 (day 0) (B) - Count in T2 (day 0)

(C) Count in T3 (day 0) (D) Count in T4 (day 0)

(E) - Count in T5 (day 0) (F) Count in T6 (day 0)

Figure 2. Photos showing the *L. acidophilus* count for the six treatments under study on the first day of storage.

according to the Brazilian Legislation (Brasil, 2000), was reached in all six treatments.

The acidophilus milk produced by Zacarchenco and Massaguer-Roig (2004) showed an initial L. acidophilus count of 8.869 log CFU / mL, a value which exceeded that in all six treatments of the present study on the first day of storage. On day 21 of storage, the probiotic count in the acidophilus milk was 8.322 log CFU/mL, which exceeded that observed in all six treatments in this study.

As expected, after 28 days of storage, the *L. acidophilus* count in the six treatments had decreased but remained above the minimum count of 7 log CFU/ mL. Thus, according to Jelen and Lutz (1998), this beverage could be classified as a probiotic food.

Illustration of probiotic microorganism growth

Figure 2 (A, B, C, D, E, and F) shows the growth of *L. acidophilus* in the six treatments on the first day of storage (day 0). The total probiotic counts in the six formulations ranged from 10.160 to 10.700 log CFU/mL

on the first day and from 7.330 to 8.493 log CFU/mL after 28 days of storage. These levels met the requirements described in the literature as well as those in the Brazilian legislation for fermented milk (Brasil, 2000), which recommends that all microorganisms producing lactic fermentation must be present and viable in the product at a level of 7 log CFU/mL.

Conclusions

With reference to the effects on the lactic acid bacteria population due to the amount of inulin and the inoculum used in various experiments, only changes in the inoculum led to a significant increase (p-value < 0.05) in the *L. acidophilus* population.

All treatments evaluated in this study showed good acceptability for the attributes of color, flavor, aroma and consistency, with the exception of T_6 (fermented drink with 2% starter, fruit salad flavor and the addition of 4% inulin), which presented low acceptability for the attribute of color. Thus, improvements should be made for this formulation to enhance the color via the addition of a larger amount of fruit pulp or a natural color additive.

Although all six treatments presented optimal microbiological, sensory and physicochemical results, the fermented beverage obtained from treatment one presented the best requisites for potential production on an industrial scale, considering the acidity and pH standards proposed by the Brazilian legislation.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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