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Development of probiotic cultures in a symbiotic soy beverage using different types of carbohydrate

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The objective of this study was to evaluate the fermentation of a soluble soy extract using lactic starters such as *Lactobacillus acidophilus* and *Streptococcus thermophilus* with *L. acidophilus, Bifidobacterium* spp. (SAB) and *Lactobacillus casei*, varying the formulations with respect to the type of carbohydrate used, such as sucrose and glucose. Twelve formulations were defined in order to verify the fermentation efficiency and possible differences in their behavior in the presence and absence of sugar supplementation. At the end of storage (28 days), the number of viable cells in the beverage fermented by *L. casei* varied between 10^{11} and 10^{12} viable cells/ml, demonstrating a high viability of the probiotic character. The formulations containing the SAB inoculums presented counts above 10^{11} viable cells/mL at the end of storage. For beverages fermented using *L. acidophilus*, the number of lactic acid bacteria fell to a basis of 10^{11} viable cells/ml during the 28 days of storage. As compared to the other formulations using different lactic starters, it could be seen that these showed a lower count after 28 days, but the probiotic characteristic remained viable, with significant differences between the formulations ($p<0.01$). Soy was shown to be an optimum medium for the development of the desired lactic cultures.

Key words: Fermentation, lactic cultures, storage, soluble soy, sugar supplementation.

INTRODUCTION

According to Codex Alimentarius (Food and Agriculture Organization of the United Nations, 2011), fermented milk is a milk product obtained by fermentation of milk which may have been manufactured from products obtained from milk with or without compositional modification by the action of suitable microorganisms and resulting in a reduction in pH with or without coagulation (iso-electric precipitation). The starter microorganisms must be viable, active and abundant in the product up to the date of minimum durability. If the product is heat treated after fermentation, the requirement for viable microorganisms does not apply.

Resolution GMC 47/97 of the Brazilian legislation, approved by Mercosul subgroup 3, also defined fermented milk as "a product to which other food substances may be added or not, obtained by a decrease in pH of milk or reconstituted milk to which other lactic products may be added or not, and submitted to lactic fermentation through the action of specific microorganisms" (Khurana and Kanawjia, 2007). According to Angelov et al. (2009), fermented milks represent a very important group amongst the functional

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foods.

The cultures used include various bacteria, amongst which are *Lactobacillus* spp., *Streptococcus* spp., *Propionibacterium* spp., *Leuconostoc* spp., *Bifidobacterium* spp., *Pediococcus* spp. and *Acetobacter* spp. Mathur and Singh (2005) defined fermented milk as a beverage produced using lactic starter cultures, such that the bacteria enter the intestine in large numbers and interact with the microbial flora.

According to Hauly et al. (2005), like milk, soybean extract is also adequate for the growth of lactic cultures, and the oligosaccharides (raffinose and stachyose), which are the major carbohydrates in soybeans (Omogbai et al., 2005), sucrose, amino acids and peptides present in the soybean stimulate microbial growth. Thus, fermented soybean beverages obtained by fermentation with probiotic microorganisms present sensory characteristics similar to those of traditional yoghurt. Soy beverages contain sucrose, stachyose, raffinose, glucose and fructose, providing probiotic bacteria with a broad choice of sugar substrates (Buckley et al., 2011). The addition of probiotic bacteria to a soy product provides the benefits associated with probiotics, including effects on the intestinal microbial flora and on the immune system, and also the diversification of soy foods with respect to flavor and texture (Buckley et al., 2011). However, Scalabrini et al. (1998) pointed out that the low fermentable carbohydrate content of soy limits the use of the water soluble soybean extract as a growth substrate for lactic cultures, which is necessary to add glucose, sucrose or even lactose to make the extract adequate for fermentation.

The main lactic bacteria, Gram-positive organisms belonging to the general category of lactic acid bacteria (Shehata, 2012), used to ferment the water soluble soybean extract include *Lactobacillus acidophilus*, *Lactobacillus bulgaricus, Bifidobacterium lactis* and *Streptococcus thermophilus*.

Probiotics are non-pathogenic microorganisms mostly of human origin which, when administered in adequate amounts, confer a health benefit on the host and are able to prevent or improve some diseases (Fric, 2007). They can be classified as homo or heterofermentative organisms (Axelsson, 2004). Probiotic lactobacilli are claimed to provide a number of health benefits, including antimicrobial effects against pathogens, anti-tumor effects, anti-cholesterol, immuno-modulation, anti-diabetic and the treatment of diarrhea and lactose intolerance (Nagpal et al., 2007, 2010, 2012). Fermenting soymilk with lactic acid bacteria considerably increases its health value (Božanić et al., 2011).

The use of probiotic cultures prevents a variety of health disorders due to their protective effect against pathogens, including coliforms (Sakhare and Narasimha, 2003; Coeuret et al., 2004), *Staphylococcus aureus* (Sakhare and Narasimha, 2003; Coeuret et al., 2004; Ameshima et al., 1998), *Listeria monocytogenes* (Rrantes et al., 2004; Coeuret et al., 2004), *Salmonella* spp. (Coeuret et al., 2004), *Candida* spp., *Zygosaccharomyces bailii*e and *Penicillium* spp. (Schwenninger et al, 2005). Probiotics are living, health-promoting microorganisms that are incorporated into various kinds of food (Kaboosi, 2011; Paraschiv et al., 2011).

Prebiotics are defined as non-digestible components that are metabolized by specific health-promoting bacteria such as *Lactobacillus* spp. and Bifidobacterium spp. (Gibson et al., 2010; Ringo et al., 2010). The use of probiotic strains together with prebiotic substances provides a combined effect named "symbiotic" (Cummings, 2009; Rastall and Maitin, 2002).

Thus, the objective of the present study was to evaluate the fermentation of a soluble soy extract using lactic starters such as pure *L. acidophilus, S. thermophilus with L. acidophilus* and *Bifidobacterium* spp. with another lactic starter culture of pure *Lactobacillus casei*, varying the formulations with respect to the types of carbohydrate used, such as sucrose and glucose.

MATERIALS AND METHODS

The formulations with water soluble soybean extract were reconstituted in 10% water. After the homogenization process, 15% of guava fruit pulp, which masked the beany flavor of the soluble soy extract, and 2% of a prebiotic substance such as inulin, were added to each formulation. Different carbohydrate concentrations (100% sucrose, 100% glucose, 50% glucose, 50% sucrose and 0% sugar) were used to evaluate the fermentation of the soluble soy extract using lactic starters donated by SACCO® (a dairy products company), such as pure *L. acidophilus (LA3)*, *S. thermophilus* with *L. acidophilus* and *Bifidobacterium* spp. (SAB 440) with another lactic starter culture of pure *L. casei* (BGP 93). Twelve formulations were defined in order to verify the fermentation efficiency and possible differences in their behavior in the presence and absence of sugar supplementation. After homogenization, 15% sugar was added, varying the type of sugar according to the formulation defined. They were then homogenized again, packed into individual volumetric flasks and submitted to heat treatment. The heat treatment adopted was 121°C for 15 min, applied using an autoclave, thus promoting sterilization and producing a contaminant-free medium suitable for cultivation of the starter culture in an adequate way.

After heat treatment, the samples were cooled to T^a = 37°C before inoculation with the starter culture. Equal amounts of culture were inoculated into both samples, and the inoculated samples were incubated at a constant temperature rigorously controlled at 39°C. This temperature is within the optimum temperature range for the growth of *L. acidophilus* (37 to 40°C) (Ahmed et al., 2006).

The pH value and titratable acidity were monitored at approximately two hourly intervals for a maximum of 14 h, giving rise to the graphs shown in Figures 1, 2 and 3. Fermentation was considered complete when the pH reached a value close or equal to 4.5 according to Wang et al. (1994), where it stabilized. At the end of the fermentation process, the lactic acid bacterial counts were monitored during 28 days of storage. 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, by titrating 5 ml aliquots of the samples with 0.1 N NaOH in the presence of the indicator phenolphthalein, according to the technique described by

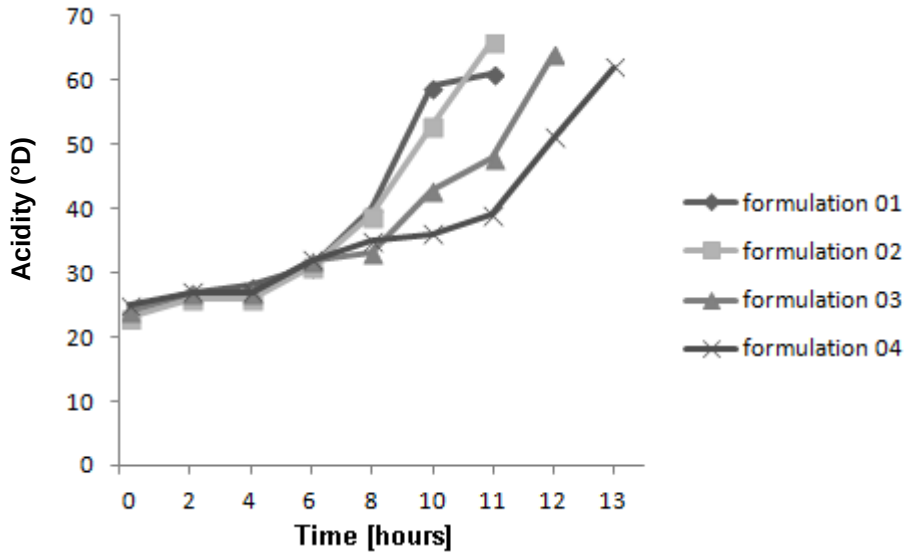

Figure 1. Fermentation time of the formulations with *L. casei*.

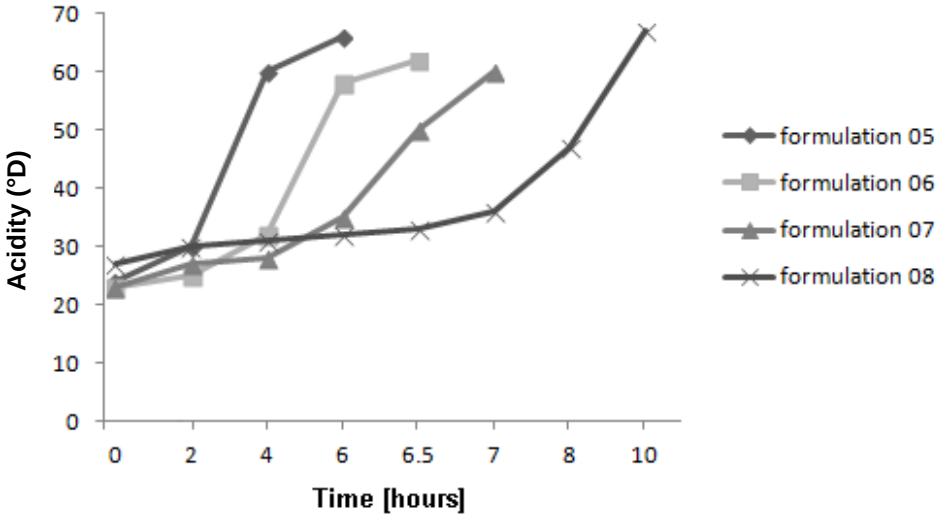

Figure 2. Fermentation time of the formulations with *S. thermophilus*, *L. acidophilus* and *Bifidobacterium* spp. (SAB).

Instituto Adolfo Lutz (2008). All samples were collected under aseptic conditions and were immediately taken to the laboratory.

Decimal dilutions were made serially, and 10 ml of sample was aseptically transferred into a sterile Erlenmeyer flask containing 90 mL of 0.1% sterile distilled peptone water. This solution was then homogenized and subsequent dilutions were made.

Each sample was serially diluted from 10^{-1} to 10^{-15} in peptone water. One milliter of each dilution was inoculated into triplicate plates containing MRS agar at each dilution (De Man et al., 1960). All the analyses were evaluated each week over 4 week storage at 4°C. The probiotic bacteria (*L. acidophilus*, *Bifidobacterium* spp. and *S. thermophilus*) were counted with MRS agar, followed by 72 h incubation at 37°C under anaerobic conditions. Formulations with *L. acidophilus* were counted with MRS agar with maltose using spread plate method, followed by 72 h incubation at 37°C under aerobic conditions. *L. casei* was determined with MRS agar and glucose using the spread plate method, followed by six days incubation at 20°C under aerobic conditions (Farnsworth et al., 2006). The lactic acid bacterial counts of the formulations were determined after 0, 7, 14, 21 and 28 days of storage. The counts of probiotics, performed in triplicate, were calculated from the colonies on agar plates and thus expressed as colony forming units per milliliter (CFU/ml).

Identification of lactic acid bacteria included the catalase test and the Gram staining of cells and was done according to Holt et al. (1994).

RESULTS AND DISCUSSION

Figure 1 shows the evolution of the acidity of the

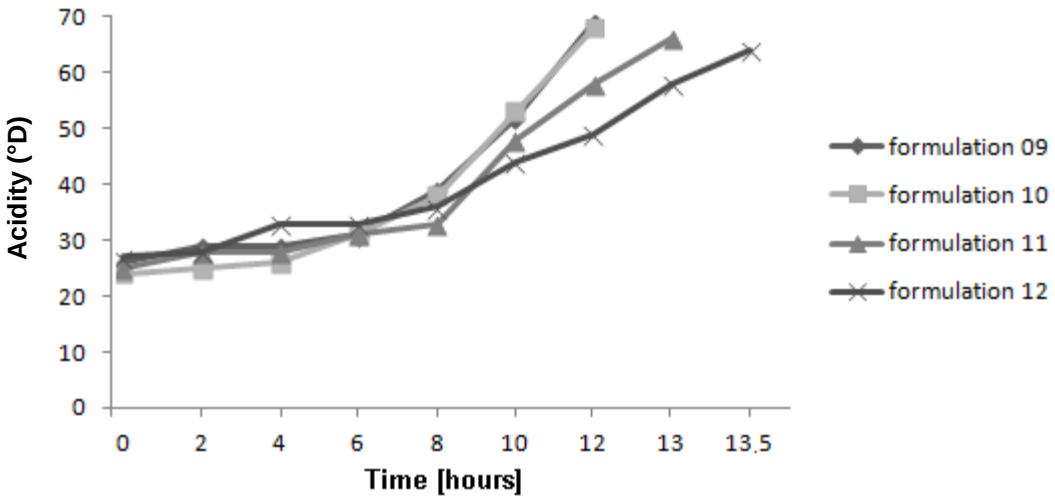

Figure 3. Fermentation time of the formulations with *L. acidophilus*.

Table 1. pH development in the formulations inoculated with *L. casei*.

Formulations/ time (h)	*F1	*F2	*F3	*F4
0	6.53	6.55	6.50	6.47
02:00	6.50	6.50	6.45	6.44
04:00	6.50	6.47	6.44	6.42
06:00	6.44	6.46	6.40	6.39
08:00	5.78	6.01	6.30	6.29
10:00	4.88	4.86	5.68	6.16
11:08	4.66	4.68	5.10	5.82
12:00			4.60	4.97
13:00				4.75

*Formulations 1, 2, 3 and 4.

Table 2. pH development in the formulations inoculated with SAB.

Formulations/time (h)	*F5	*F6	*F7	*F8
0	6.56	6.56	6.54	6.39
02:00	6.33	6.47	6.44	6.30
04:00	5.06	6.10	6.40	6.30
06:00	4.67	4.83	6.05	6.29
06:30		4.68	4.98	6.24
07:00			4.68	6.12
08:00				5.37
10:00				4.76

*Formulations 5, 6, 7 and 8.

formulations during fermentation, where it can be seen that the final values were within the limits established by the Brazilian legislation for lactic beverages which is 60 to 150°D (Brasil, 2005). Figure 1 shows the fermentation time of the formulations fermented with the lactic culture, *L. casei*.

It can be seen that formulations A1 and A2 (no supplementation and supplemented with sucrose) presented the shortest fermentation times of approximately 11 h. On the other hand, formulation A3 (supplemented with 50% sucrose and 50% glucose) required more than 12 h, and formulation A4, containing glucose, took more than 13 h. Since *L. casei* was a pure culture, fermentations using this culture took longer time to reach the ideal pH value (approximately 4.5) for fermented milk (Table 1).

For the formulations fermented using the SAB mixed lactic starter culture (composed of *S. thermophilus* with *L. acidophilus* and *Bifidobacterium* spp.), the fermentation time was shorter than that of the formulations prepared using a pure culture (Figure 2). Formulation A5 (with no added sugar) reached the final pH value in the shortest time, nearly 6 h (Table 2).

Formulations A6 and A7 (100% sucrose and 50% sucrose plus 50% glucose, respectively) showed fermentation times close to 7 h. On the other hand, formulation A8, containing the sugar glucose, showed the longest fermentation time of approximately 12 h.

The use of a mixed lactic culture favors the elaboration of fermented beverages, since according to Robinson (2002), the presence of the microorganism *S. thermophilus* favors the process, and it is a starter culture for fermented milks and yogurts. Thus, the fermentation process starts quicker and continues, in this case, with the inocula of *L. acidophilus* and *Bifidobacterium* spp., the optimum pH for the start of the activity of *L. acidophilus* being between 5.5 and 6.0 according to Gomes and Malcata (1999). Most lactic acid bacteria

Table 3. pH development in the formulations inoculated with *L. acidophilus*.

Formulations/time (h)	*F9	*F10	*F11	*F12
0	6.52	6.52	6.45	6.45
02:00	6.43	6.40	6.35	6.38
04:00	6.43	6.40	6.35	6.30
06:00	6.40	6.30	6.27	6.16
08:00	4.74	4.70	4.67	5.21
10:00	4.41	4.52	4.50	4.94
12:00				4.78
13:00				4.51
13:30				4.46

*Formulations 9, 10, 11 and 12.

grow well at a pH below 6.5 (Raja et al., 2009). Lactic acid bacteria are neutrophilic, that is, they show optimum growth pH between 5 and 9, with the exception of a few *Lactobacillus* and *Leuconostoc* species (Granato et al., 2010).

Matijević et al. (2009) observed that the fermentation with *Bifidobacterium animalis* subsp*. lactis* BB-12 was shorter (approximately 11 h) than fermentations with *L. acidophilus* La-5 (approximately 13 h), suggesting the influence of the type of culture on the fermentation and survival of the microorganisms. The probiotic strains studied, such as *L. acidophilus*, *L. casei* ssp. *paracasei* and *Bifidobacterium bifidus*, in multiple combinations with mesophilic lactic bacteria (Flora Danica), also showed different behaviors and survival (Paraschiv et al., 2011).

Several factors that may influence the ability of the probiotics to survive in the product and become active when entering the consumer's gastrointestinal tract, must be considered when probiotics are added to fermented foods (Heller, 2001). These include the physiologic state of the probiotic organisms added (whether the cells are from the logarithmic or the stationary growth phase), the physical conditions of the product storage, such as temperature, chemical composition of the product to which the probiotics are added, and finally the possible interactions of the probiotics with the starter cultures, such as bacteriocin production, antagonism and also synergism. Several investigations have shown that bacteria from the logarithmic phase are much more susceptible to environmental stress than bacteria from the stationary phase (Heller, 2001).

Of the formulations fermented using the pure *L. acidophilus* culture, it can be seen that formulations A9, A10 and A11 presented the same fermentation time (Figure 3). On the other hand, formulation A12, containing the sugar glucose, only reached the ideal pH value after 14 h (Table 3).

Since this is a pure culture and the optimum pH value for fermentation by *L. acidophilus* is between pH 5.5 and 6.0, and considering that the beverage started to ferment at pH 6.53 on average, this microorganism took longer time to start its microbial activity.

Probiotic microbial count

The minimum cell count of a specific culture recommended for fermented lactic beverages at the moment of consumption is 1.0 x 10^6 viable cells/mL (Ostlie et al., 2003). Although quantitative standards vary from 10^6 to 10^7 cfu/g viable cells as minimum requirements, it is generally recommended that yogurt or fermented milk should contain at least one million viable cells per gram at the time of consumption (Damin et al., 2008).

The minimum concentration of probiotic bacteria necessary to cause a beneficial result has generally been accepted as 10^6 viable cells/ml at the moment of consumption (Sartor, 2004; Ostlie et al., 2003; Talwalkar and Kailasapathy, 2004; Shah, 2007). According to Cummings (2009), the dose of probiotics needed to exert a benefit is of the order 10^{10} or 10^{11} bacteria.

The final viable cell count in the fermented beverage after 28 days of storage was within the limits recommended for this product (Table 4), making it attractive as a supplement in the human diet. The formulations containing the *SAB* inoculum showed lactic bacterial counts above 1 x 10^{11} viable cells/mL at the end of storage. The same results were obtained for the other formulations containing other types of lactic culture.

In the beverage fermented by *L. casei*, the viable count increased during the first 14 days of storage and then started to decrease. This was because the microorganisms were still active during the first 14 days of storage, although at a decreased rate, as compared to the fermentation time in the fermenter. At the end of storage (28 days), the number of viable cells in the formulations varied between 10^{11} and 10^{12} viable cells/ml, demonstrating a high viability of the probiotic character.

The formulations inoculated with SAB showed lactic bacterial counts of above 1 x 10^{11} viable cells/ml at the end of storage. The same result could be observed for the other formulations that used other types of lactic culture.

For beverages fermented using *L. acidophilus*, the lactic acid bacterial counts at zero day were only above 10^{14} viable cells/ml for the formulation with 0% sugar, reaching a pH value of 4.4. However, during the 28 days of storage, the number of lactic acid bacteria fell to a level of 10^{11} viable cells/ml. As compared to the other formulations fermented using different lactic starters, it could be seen that these formulations showed a lower count after 28 days, but the probiotic characteristic remained viable. All the formulations presented significant differences ($p<0.01$).

Contrary to the report by Scalabrini et al. (1998), soy was shown to be an optimum medium for the

Table 4. Viable cell counts in the fermented formulations during the storage period.

Sample	Storage period (days)									
	0		7		14		21		28	
	Average (CFU/ml)	sd	Average (CFU/ml)	sd	Average (CFU/ml)	sd	Average (CFU/ml)	sd	Average (CFU/ml)	sd
A1	6.33E+12^{ab}	2.17E+12	8.03E+12^{a}	5.69E+11	4.07E+13^{b}	1.80E+13	6.13E+12^{cd}	8.74E+11	6.93E+11^{ab}	1.63E+11
A2	2.03E+13^{abc}	2.31E+12	7.97E+12^{a}	8.33E+11	5.13E+12^{a}	9.61E+11	4.13E+12^{b}	5.86E+11	1.77E+12^{c}	6.11E+11
A3	1.03E+13^{ab}	5.77E+11	1.47E+13^{c}	3.06E+12	2.70E+13^{cd}	3.46E+12	7.20E+12^{d}	7.55E+11	1.83E+12^{c}	5.86E+11
A4	2.20E+13^{bc}	4.58E+12	9.03E+12^{ac}	7.02E+11	1.83E+13^{ac}	6.11E+12	5.23E+12^{bc}	3.51E+11	1.30E+12^{bc}	2.00E+11
A5	8.93E+12^{ab}	5.51E+11	8.23E+12^{a}	4.16E+11	6.80E+12^{a}	4.00E+11	2.07E+12^{e}	3.06E+11	8.07E+11^{ab}	7.51E+10
A6	8.13E+13^{e}	4.51E+12	7.90E+12^{a}	6.24E+11	5.90E+12^{a}	4.58E+11	9.13E+11^{ae}	2.52E+10	4.27E+11^{a}	7.77E+10
A7	3.20E+13^{cd}	3.00E+12	9.03E+12^{ac}	1.53E+11	6.90E+12^{a}	2.00E+11	3.80E+12^{b}	3.61E+11	7.87E+11^{ab}	5.03E+10
A8	2.00E+12^{a}	3.61E+11	9.40E+11^{b}	2.65E+10	8.60E+11^{a}	2.00E+10	5.93E+11^{a}	7.09E+10	1.33E+11^{a}	3.21E+10
A9	1.20E+14^{f}	1.73E+13	8.30E+13^{d}	6.56E+12	3.93E+13^{b}	9.07E+12	6.43E+12^{cd}	9.07E+11	6.40E+11^{ab}	2.31E+11
A10	4.00E+13^{cd}	5.00E+12	6.30E+12^{ab}	1.21E+12	3.33E+12^{a}	1.20E+12	6.00E+11^{a}	1.42E+11	2.03E+11^{a}	3.21E+10
A11	4.93E+13^{d}	8.62E+12	6.27E+12^{ab}	7.77E+11	2.00E+12^{a}	7.94E+11	4.20E+11^{a}	7.00E+10	8.40E+10^{a}	8.00E+09
A12	3.43E+13^{cd}	9.29E+12	7.33E+12^{ab}	7.09E+11	3.13E+12^{a}	7.02E+11	3.97E+11^{a}	8.62E+10	7.13E+10^{a}	8.33E+09
ANOVA *(p-value)	<0.001		<0.001		<0.001		<0.001		<0.001	

*p-value<0.01 means there are significant differences between the formulations. Means sharing the same letter within a column are not significantly different at 5% significance level.

development of the desired lactic cultures, the carbohydrates stachyose and raffinose which is available for fermentation. Soy milk is rich in soybean-oligosaccharides such as raffinose and stachyose and is an excellent growth medium for lactic acid bacteria (Kamaly, 1997; Wang et al., 2002, 2003; Beasley et al., 2003).

Since it is a tetrasaccharide with 2 molecules of galactose, one molecule of glucose and one molecule of fructose, stachyose [β-D-fructofuranosyl-*O*-α-D-galactopyranosyl-(1→6)-α-D-galactopyranoside-(1→6)-α-D-glucopyranoside], which is an indigestible oligosaccharide, provides 4 molecules of hexoses, whereas raffinose [β-D-fructofuranosyl-*O*-α-D-galactopyranosyl-(1→6)-α-D- glucopyranoside], which is a trisaccharide (and also an indigestible oligosaccharide) with 1 molecule each of glucose, fructose and galactose, (Suarez et al., 1999), provides 3 molecules of hexoses, making the medium rich in a substrate adequate for the development of probiotic cultures, as pointed out by Kamaly (1997), Wang et al. (2002, 2003) and Beasley et al. (2003).

Unlike the results obtained by Božanić et al. (2008), who showed that *L. casei* presented the best growth in soymilk with the addition of glucose, the present study indicated that *L. casei*, like the other strains in question, grew better in a substrate with the addition of 100% sucrose, although it also grew in a substrate with the addition of glucose, but presented a shorter fermentation time in the first substrate.

In conclusion, the samples supplemented with sucrose showed a more rapid evolution of acid as compared to the others. This was because sucrose is a disaccharide (formed of fructose and glucose), making more substrate available for the microorganism, in addition to that already in existent in the soybean besides the added inulin.

The formulation containing glucose presented a delayed evolution of acidity as compared to the others. When comparing the initial acidities of the mixtures at zero fermentation time, both should have presented the same acidity since the base mixture was of the same origin. The differences could have been occasioned by the physicochemical quality of the glucose (acid sugar) resulting from the storage conditions and exposition to other unknown factors.

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REFERENCES

Ahmed T, Kanwal R, Ayub N (2006). Influence of temperature on growth pattern of *Lactococcus lactis*, *Streptococcus cremoris* and *Lactobacillus acidophilus* isolated from camel milk. Biotechnology 5(4):481-488.

Ameshima T, Magome C,Takeshita K, Arihara K, Itoh M, Kondo Y(1998). Effect of intestinal *Lactobacillus* starter cultures on the

behaviour of *Staphylococcus aureus* in fermented sausage. Int. J. Food Microbiol. 41(1):1-7.
Angelov M, Kostov G, Simova E, Beshkova D, Koprinkova-Hristova P (2009). Proto-cooperation factors in yogurt starter cultures. Revue de Génie Industriel 3:4-12.
Axelsson L (2004). Lactic acid bacteria: classification and physiology. In: Salminen S, Wright A, Ouwehand A (eds) Lactic acid bacteria: microbiological and functional aspects. New York: Marcel Dekker, pp. 1-66.
Beasley S, Tuorila H, Saris PEJ (2003). Fermented soymilk with monoculture of *Lactococcus lactis.* Int. J. Food Microbiol. 81:159-162.
Božanić R, Lovković S, Jeličić I (2011). Optimising fermentation of soymilk with probiotic bacteria. Czech J. Food Sci. 29(1):51-56.
Božanić R, Brletić S, Lovković S (2008). Influence of temperature and sugar addition on soymilk fermentation by probiotic bacteria. Mljekarstvo 58:61-68.
Buckley ND, Champagne CP, Masotti AI, Wagar LE, Tompkins TA, Green-Johnson JM (2011). Harnessing functional food strategies for the health challenges of space travel—Fermented soy for astronaut nutrition. Acta Astronautica 68:731-738.
Brasil (2005). Ministério da Agricultura, Pecuária e Abastecimento. Gabinete do Ministro. Instrução Normativa Nº 16, de 23 de agosto de 2005. Aprova o Regulamento Técnico de Identidade e Qualidade de Bebida Láctea. Diário Oficial da União, Brasília, DF, 24 ago, seção 1:7.
Coeuret V, Gueguen M, Vernoux JP (2004). In vitro screening of potential probiotic activities of selected lactobacilli isolated from unpasteurized milk products for incorporation into soft cheese. J. Dairy Res. 71(4):451-460.
Cummings JH (2009). Probiotics: better health from 'good' bacteria? Br. Nutr. Found. Nutr. Bull. 34:198-202.
Damin MR , Minowa E, Alcântara MR, Oliveira MN (2008). Effect of cold storage on culture viability and some rheological properties of fermented milk prepared with yogurt and probiotic bacteria. J. Texture Stud. 39:40-55.
De Man JC, Rogosa M, Sharpe ME (1960). A medium for cultivation of lactobacilli. J. Appl. Bacteriol. 23:130-135.
Farnsworth JP, Li J, Hendricks GM, Guo MR (2006). Effects of transglutaminase treatment on functional properties and probiotic culture survivability of goat milk yogurt. Small Rumin. Res. 65:113-121.
Fric P (2007). Probiotics and prebiotics—renaissance of a therapeutic principle. Cent. Eur. J. Med. 2:237-270.
Food and Agriculture Organization of the United Nations (2011). Milk and milk products. Codex standard for fermented milks. Codex Stan 243-2003. 2nd ed. http://www.fao.org/docrep/015/i2085e/i2085e00.pdf. Accessed 4 September 2012.
Gibson GR, Scott KP, Rastall RA, Tuohy KM, Hotchkiss A, Dubert-Ferrandon A, Gareau M, Murphy EF, Saulnier D, Loh G, Macfarlane S, Delzenne N, Ringel Y, Kozianowski G, Dickmann R, Lenoir-Wijnkoop I , Carey Walker C, Buddington R (2010). Dietary prebiotics: current status and new definition. Food Sci. Technol. Bull. Functional Foods 7(1):1–19.
Gomes AMP, Malcata FX (1999). *Bifidobacterium* spp. and *Lactobacillus acidophilus*: biological, biochemical, technological and therapeutical properties relevant for use as probiotics**.** Trends Food Sci. Technol. 10:139-157.
Granato D, Branco GF, Cruz AG, Faria JAF, Shah NP (2010). Probiotic dairy products as functional foods. Compr. Rev. Food Sci. Food Saf. 9:455-470.
Hauly MCO, Fuchs RHB, Prudencio-Ferreira SF (2005). Suplementação de iogurte de soja com frutooligossacarídeos: características probióticas e aceitabilidade. Rev. Nutr. Campinas 18(5):613-622.
Heller KJ (2001). Probiotic bacteria in fermented foods: product characteristics and starter organisms. Am. J. Clin. Nutr. 73(suppl): 374S-379S.
Holt JC, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994). Bergeys Manual of Determinative Bacteriology. Philadelphia: Lippincott Williams and Wilkins, pp. 189-255.
Instituto Adolfo Lutz /São Paulo (2008). Métodos físico-químicos para análise de alimentos/coordenadores Odair Zenebon, Neus Sadocco Pascuet e Paulo Tiglea -- São Paulo:Instituto Adolfo Lutz.
Kaboosi H (2011). Antibacterial effects of probiotics isolated from yoghurts against some common bacterial pathogens. Afr. J. Microbiol. Res. 5(25):4363-4367.
Kamaly KM (1997). Bifidobacteria fermentation of soybean milk. Food Res. Int. 30:675-682.
Khurana HK, Kanawjia SK (2007). Recent trends in development of fermented milks. Curr. Nutr. Food Sci. 3:91-108.
Mathur S, Singh R (2005). Antibiotic resistance in food lactic acid bacteria-A review. Int. J. Food Microbiol. 105:281-295.
Matijević B, Božanić R, Tratnik L (2009). The influence of lactulose on growth and survival of probiotic bacteria *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 in reconstituted sweet whey. Mljekarstvo 59(1):20-27.
Nagpal R, Behare PV, Kumar M, Mohania D, Yadav M, Jain S, Menon S, Parkash O, Marotta F, Minelli E, Henry CJK, Yadav H (2012). Milk, milk products and disease free health: an updated overview. Crit. Rev. Food Sci. Nutr. 52(4):321-333.
Nagpal R, Kumar A, Arora S (2010). *In-vitro* probiotic potential of lactobacilli from indigenous milk products. Int. J. Probiotics Prebiotics 5(2):103-110.
Nagpal R, Yadav H, Puniya AK, Singh K, Jain S, Marotta F (2007). Potential of probiotics and prebiotics for synbiotic functional dairy foods. Int. J. Probiotics Prebiotics 2:75-84.
Omogbai BA, Ikenebomeh MJ, Ojeaburu SI (2005). Microbial utilization of stachyose in soymilk yogurt production. Afr. J. Biotechnol. 4(9):905-908.
Ostlie HM, Helland MH, Narvhus JA (2003). Growth and metabolism of selected strains of probiotic bacteria in milk. Int. J. Food Microbiol. 87:17-27.
Paraschiv D, Vasile A, Constantin M, Ciobanu A, Bahrim G (2011). Study of physiological properties of some probiotics in multiple cultures with mesophilic lactic acid bacteria by flora danica ch.hansen commercial starter. AUDJG – Food Technol. 35(2):56-65.
Raja A, Gajalakshmi P, Mohamed MRM, Mohamed IM (2009). Effect of *Lactobacillus lactis cremoris* Isolated from Kefir against food spoilage bacteria. Am. J. Food Technol. 4(5):201-209.
Rastall RA, Maitin V (2002). Prebiotics and synbiotics: towards the next generation. Curr. Opin. Biotechnol. 13:490-496.
Ringo E, Olsen RE, Gifstad TO, Dalmo RA, Amlund H, Hemre G I, Bakke AM (2010). Prebiotics in aquaculture: a review. Aquacult. Nutr. 16:117-136
Robinson RK (2002). Dairy microbiology handbook. New York: Wiley Interscience. p. 765.
Rrantes X, Railey D, Arias ML, Chaves C (2004). Evaluación del efecto de cultivos probióticos adicionados a yogurt comercial, sobre poblaciones conocidas de *Listeria monocytogenes* y *Escherichia coli* O157:H7. Arch. Latinoamericanos Nutr. 54(3):293-297.
Sakhare PZ, Narasimha RD (2003). Microbial profiles during lactic fermentation of meat by combined starter cultures at high temperatures. Food Control 14(1):1-5.
Sartor RB (2004). Probiotic therapy of intestinal inflammation and infections. Curr. Opin. Gastroenterol. 21:44-50.
Scalabrini P, Rossi M, Spettoli P, Matteuzzi D (1998). Characterization of *Bifidobacterium* strains for use in soymilk fermentation. Int. J. Food Microbiol. 39:213-219.
Schwenninger SM, Von AHU, Nieder B, Teuber M, Meile L (2005). Detection of antifungal properties in *Lactobacillus paracasei* subsp *paracasei* SM20, SM29, and SM63 and molecular typing of the strains. J. Food Prot. 68(1):111-119.
Shah NP (2007). Functional cultures and health benefits. Int. Dairy J. 17:1262-1277.
Shehata AI (2012). Molecular identification of probiotics *Lactobacillus* strain isolates by amplified ribosomal DNA restriction analysis (ARDRA). Afr. J. Microbiol. Res. 6(12):3034-3041.
Suarez FL, Springfield J, Furne KJ, Lohrmann TT, Kerr PS, Levitt MD (1999). Gas production in humans ingesting a soybean flour derived from beans naturally low in oligosaccharides1–3. Am. J. Clin. Nutr. 69:135-139.
Talwalkar A, Kailasapathy K (2004). Comparison of selective and differential media for the accurate enumeration of strains of

Lactobacillus acidophilus, Bifidobacterium spp. and *Lactobacillus casei* complex from commercial yoghurts. Int. Dairy J. 14:142-149.
Wang SH, Marinho CS, Carvalho EP (1994). Produção de iogurte de soja com diferentes associações de bactérias lácticas. Pesquisa Agropecuária Brasileira 29(10):1539-1601.
Wang YC, Yu RC, Chou CC (2002). Growth and survival of bifidobacteria and lactic acid bacteria during the fermentation and storage of cultured soymilk drinks. Food Microbiol. 19:501-508.
Wang YC, Yu RC, Yang HY, Chou CC (2003). Sugar and acid contents in soymilk fermented with *lactic acid bacteria* alone or simultaneously with bifidobacteria. Food Microbiol. 20:333-338.