Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9291
ISSN on-line: 1807-8648
Doi: 10.4025/actascihealthsci.v35i1.11939

Microbiological, physical, chemical and sensory characteristics of milk fermented with *Lactobacillus plantarum*

Aloisio Henrique Pereira de Souza[1,3*], Giselle Aparecida Nobre Costa[2], Lucia Helena da Silva Miglioranza[2], Luciana Furlaneto-Maia[3] and Ana Flavia de Oliveira[3]

*[1]Centro de Ciências Agrárias, Programa de Pós-graduação em Ciência de Alimentos, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, Paraná, Brazil. [2]Departamento de Ciência e Tecnologia de Tecnologia, Universidade Norte do Paraná, Londrina, Paraná, Brazil. [3]Universidade Tecnológica Federal do Paraná, Londrina, Paraná, Brazil. *Author for correspondence. E-mail: aloisio_rick17@yahoo.com.br*

ABSTRACT. This study developed a fermented milk with *Lactobacillus plantarum* and evaluated its microbiological, physical-chemical and sensory characteristics during 70 days of storage at 10ºC. The study analyzed the counts of total viable cells, total and thermotolerant coliforms, yeast and mold; acidity, pH, ash, fat and total solids; sensory evaluation and purchase intention of the final product by consumers. Nutrition information was compared with seven commercial brands of fermented dairy products. The final formula contained 10% sugar, 6% milk powder and 4% microbial inoculum. The final product was fat-free. Acidity, ash content and total solids were stable during storage, unlike pH. No total or thermotolerant coliforms, yeast or mold were detected. *L. plantarum* counts ranged from 10.1 Log_{10} CFU mL^{-1} at the beginning to 8.9 Log_{10} CFU mL^{-1} at the end of the storage period. The product had good acceptance and high purchase intent. The nutrition information of fermented milk was similar to those of commercial brands evaluated. *L. plantarum* demonstrated good viability in fermented milk, and although not considered a probiotic food in Brazil, it is promising for the production of foods with functional properties and/or health claims.

Keywords: fermented dairy products, viability of lactic acid bacteria, shelf life of fermented milk products, probiotic.

Caracterização microbiológica, físico-química e sensorial de leite fermentado com *Lactobacillus plantarum*

RESUMO. Desenvolveu-se uma formulação de leite fermentado com *Lactobacillus plantarum* e avaliou-se seu comportamento microbiológico, físico-químico e sensorial durante 70 dias de armazenamento em refrigeração. Foi analisada a contagem total de células viáveis de *Lactobacillus plantarum*, coliformes totais e termotolerantes, e bolores e leveduras; acidez titulável, pH, teor de cinzas, gordura e extrato seco total; análise sensorial e intenção de compra do produto final. A informação nutricional do produto foi elaborada e comparada a sete leites fermentados. A formulação final conteve 10% de açúcar, 6% de leite em pó e 4% de inóculo microbiano. O produto final foi isento de gordura. A acidez, os teores de cinzas e o extrato seco total foram estáveis durante a estocagem, diferentemente do pH. Não foi detectada a presença de coliformes totais e termotolerantes, bolores e leveduras. A contagem do *L. plantarum* variou de 10,1 a 8,9 Log10 UFC mL^{-1}, no início e final da estocagem. Obteve-se boa aceitação e intenção de compra do produto final. A informação nutricional do leite fermentado foi semelhante às marcas comerciais avaliadas. *L. plantarum* apresentou boa viabilidade em leite fermentado, e, embora não seja considerado um alimento probiótico no Brasil, o mesmo é promissor na produção de alimentos com propriedades funcionais e/ou de saúde.

Palavras-chave: produtos fermentados do leite, viabilidade de culturas láticas, vida útil de lácteos fermentados, probiótico.

Introduction

Fermented milk is defined as a dairy product that during the fermentation process had its nutritional aspects as well as its physical and chemical sensory aspects changed. This process is a result of the activity of several microorganisms that use milk as substrate, as well as the different dairy types, like whole milk, skim, partially skimmed, fortified or modified milk (FERREIRA, 1999). According to Brazilian legislation, its definition and parameters of identity and quality are defined by the Ministry of Agriculture, Livestock and Food Supply (BRASIL, 2000a).

Several microorganism used in production of fermented milks are currently considered as probiotics, i.e., live microorganisms that, when ingested at a given amount, promote, beyond basic nutrition, health benefits (BOYLSTON et al.,

2004). Among these, stood out the improvement of the defenses of intestinal mucosa against pathogens and the balance of microbiota after taking antibiotics, and help in lactose digestion in intolerant individuals, stimulate immune system, relieve constipation, increase absorption of minerals, and promote the production of vitamins. Some studies have reported the decrease in risk of colon cancer, cardiovascular disease, decrease in cholesterol, antihypertensive effect, reduction in the ulcerative activity of *Helicobacter pylori*, control of colitis caused by rotavirus or *Clostridium difficile* and prevention of urogenital infections (TUOHY et al., 2003).

In Brazil, the species approved by the National Health Surveillance Agency (ANVISA), for purposes of functional and/or health claims, with probiotics characteristics, are: *Lactobacillus acidophilus*, *Lactobacillus casei* Shirota, *Lactobacillus casei* variety *rhamnosus*, *Lactobacillus casei* variety *defensis*, *Lactobacillus paracasei*, *Lactococcus lactis*, *Bifidobacterium animallis* (including the subspecies *B. lactis*), *Bifidobacterium longum* and *Enterococcus faecium* (BRASIL, 2008).

Lactobacillus plantarum belongs to the group of lactic acid bacteria that have heterofermentative metabolism (CLAESSON et al., 2007). This microorganism has a long history of natural occurrence and safe use in a variety of food products, and clinical studies emphasize these desirable characteristics for its use in human beings (VRIES et al., 2006). However, the ANVISA does not recognize it as a probiotics, although this microorganism is used in several European Union countries like Germany, France, Italy, among others, and by the United States.

The consumption of fermented milk is widely associated with the presence of lactobacilli in the formulation, due to desirable sensory characteristics promoted by these microorganisms, associated with the benefits to consumer health. In this way, the goal of this study was to develop a formulation of fermented milk using *L. plantarum*, and to evaluate its microbiological behavior, as well as its physical, chemical and sensory features along 70 days of refrigerated storage, since to date there are no reports of its use in dairy products in domestic market.

Material and methods

We developed a formulation of fermented milk containing *L. plantarum* with a sample of reconstituted milk plus sugar. During the storage period, we evaluated: total count of viable cells of *L. plantarum*, total and thermotolerant coliforms, yeast and mold count, titratable acidity, pH, ash content, fat and total solids, acceptance and purchase intent of the final product, and nutritional information and comparison with seven trademarks.

Development and processing of the fermented milk

For developing the fermented milk a commercial strain of *Lactobacillus plantarum* BG112 (Sacco® - Brazil) was used along with skimmed milk powder. Fifteen different formulations (Table 1) were developed, which were sensorially analyzed to survey the main characteristics of the product, to select the promising formulation, and to evaluate the shelf life of the product. The formulation with the best sensory characteristics was the one with 4% of inoculum, 6% of skimmed milk powder (SM) and 10% of refined sugar, this being the final formulation.

The preparation of the inoculum was performed with the activation of the culture in 100 mL of sterile 10% SM, with subsequent addition of 1.5 g of the freeze-dried culture, followed by incubation for 6-8h at 37ºC or until clot formation.

Table 1. Formulations tested for the elaboration of the final product.

Experimental design				
Groups	Ingredients	Inoculum	Acidity (% lactic acid)	Sensory characteristics
	4% SM+ + 8% Sugar	4%	0.60-0.70	Little sweet
	4% SM + 7% Sugar	4%	0.60-0.70	Tasteless/Unstable/Lumpy
	4% SM + 6% Sugar	4%	0.60-0.70	Sour flavor
	5% SM + 8% Sugar	4%	0.60-0.70	Little sweet
1	5% SM + 7% Sugar	4%	0.60-0.70	Tasteless / Lumpy
	5% SM + 6% Sugar	4%	0.60-0.70	Less acidic flavor
	6% SM + 8% Sugar	4%	0.60-0.70	Little sweet
	6% SM + 7% Sugar	4%	0.60-0.70	Consistent/Viscous
	6% SM + 6% Sugar	4%	0.60-0.70	Pleasant acidity
	10% SM + 10% Sugar	4%	0.60-0.70	Sweet / Pleasant acidity
2	10% SM + 10% Sugar	4%	0.85-0.90	Sour flavor
	10% SM + 10% Sugar	4%	1.45-1.50	Spoiled flavor
	4% SM + 10% Sugar	4%	0.60-0.70	Tasteless / Sweet / Lumpy
3	5% SM + 10% Sugar	4%	0.60-0.70	Sweet / Lumpy
	6% SM + 10% Sugar	4%	0.60-0.70	Consistent/ Viscous /Pleasant acidity

+ SM – Skimmed milk powder.

The production of the fermented milk was made through weighing the dry ingredients (sugar and skimmed milk powder), addition of distilled water and mixing the solution, and the filling was done using high density polyethylene bottles, previously washed and sanitized with hypochlorite solution at 200 mg kg^{-1} of active chlorine. Then, the product was sterilized in an autoclave at 121ºC for 15 min. and subsequent cooling at 10ºC. It was inoculated 4% of milk culture of *L. plantarum* at room temperature and incubated at 37ºC for 10h.

Microbiological analysis

The total count of viable cells of *L. plantarum* was made to monitor this organism over storage time. Also total and thermotolerant coliforms were quantified aiming the safety for sensory analysis, and the count of yeast and mold, in order to evaluate possible contaminations in the final product.

For the analysis of total count of viable cells, an aliquot of 1 mL was taken from the final formulation at the times T0, T14, T28, T42, T56 and T70, followed by serial dilution (in peptone water – Himedia) and seeded in De Man-Rogosa-Sharpe agar (MRS - Himedia). The plates were incubated at 37ºC for 72h under aerobic conditions. All platings were performed in quadruplicate.

The analysis of total and thermotolerant coliforms was carried out using the multiple tube technique. The inoculations took place directly in series of three tubes containing lactose bile brilliant green broth (CLVBB - Himedia) and EC broth (Himedia), with Durhan tubes, followed by incubation at 37ºC and 45ºC for 24-48h, respectively, according to Silva et al. (2001). These analyses occurred at time zero (T0), at the end of fermentation, and after seven days (T7), to verify the presence of these microorganisms.

Yeast and mold count was undertaken on potato dextrose agar medium (BDA - Himedia) acidified with solution of tartaric acid (10%) and incubated at 25ºC for 120h (SILVA et al., 2001). It was performed at T0 and then every 14 days, by the end of the experiment.

Physical and chemical analyses

Physical and chemical analyses occurred at distinct times. It was examined the titratable acidity, pH, content of ash and fat, according to standards established by the *Instituto Adolfo Lutz* (IAL, 2008). The titratable acidity and the pH were performed in triplicate with two repetitions, during fermentation and at all the storage times (T0, T14, T28, T42, T56 and T70), for monitoring and process control.

The determination of total solids and ash content were made at T0; T28; T56 and T70 at 4ºC in triplicate. At first, it was analyzed the total solids by the gravimetric method for milk, according to Pereira et al. (2001). The fat analysis was determined only at T0, once this nutrient was absent from the formulation.

Sensory analysis

For sensory analysis during the storage, we proceeded with the acceptance test at the storage times T0 and T70, being analyzed the attributes: color, sweetness, acidity, viscosity and overall evaluation. A team of 50 volunteers untrained tasters and possible potential consumers of the product evaluated the samples through a structured 7 point-hedonic scale (1 = dislike very much, and 7 = like very much) (FERREIRA et al., 2001). The samples were presented in randomized complete blocks design, aiming to enhance the comparison between the samples. We calculated the acceptability index (AI) of the product (mean of overall acceptance x 100 / 7) and a minimum of 70% of AI was used as a threshold to consider that the product was well accepted by the consumers (DUTCOSKY, 1996).

To complement the study, a questionnaire was applied to the tasters for characterization. At the end of sensory test, a purchase intent test was done, using a 5 points-scale (1 = certainly would not buy, and 5 = certainly would buy) to verify the possible purchase intent of the developed formulation.

Development of the nutrition label and comparison with commercial formulations

The nutrition label of the product was elaborated and compared with nutrition information on labels of fermented milks of trademarks established in Brazilian market. For the calculation of nutrition information we used the nutrition data of sugar available in the Brazilian Food Composition Table (NEPA, 2004) and of the milk powder, with the data available on the product label. The serving of the product was taken from the Resolution RDC 359 (BRASIL, 2000b) and the calculation and presentation of nutrition information followed the one proposed by the RDC 360 (BRASIL, 2000c).

Ethical aspects

The present study was approved by the Research Bioethics and Ethics Committee of the Santa Casa of Londrina, under the protocol number 028/2009, to accomplish sensory tests. All the tasters signed the consent form before performing sensory analyses.

Statistical analysis

The results were tested by an analysis of variance (Anova) and the mean values were compared using the Tukey's test, with the aid of the software BioEstat®, version 5.0 (2007). The significance level adopted was $p < 0.05$ to reject the null hypothesis.

Results and discussion

As presented in experimental design, the preliminary tests were divided into three groups to guarantee desirable sensory characteristics for fermented milk, and to establish production parameters with the present strain (BG 112). In the first group, prototypes were developed to test different concentrations of skimmed milk powder (SM – first variable) and of sugar (second variable). It was verified that within the same concentration of milk, no difference was found as for sweetness, physical aspect and acidic taste. The formulation containing SM 6% presented desirable texture

In the second group, it was evaluated the influence of the content of lactic acid in the fermented milk, as a criterion in the processing control and its maintenance during storage. The higher the acidity of the sample, the lower its acceptance, being required a product with 0.60-0.70% of lactic acid (Table 1).

In the third group we sought to verify the difference in the concentration of milk, on the sensory characteristics of the product. The formulation with SM at 6 and 10% of sugar had pleasant sensory features, probably due to higher concentration of sugar and low acidity in relation to the other studied formulations, in addition to characteristics close to the similar products available in market (Table 1).

The fermentation kinetics was determined by preliminary tests, with monitoring of fermentation time through the analyses of pH and acidity, being checked the time of 10h in continuous process to get the product. Antunes et al. (2004) verified that it was necessary a fermentation time of about 20h, with incubation at 21°C until achieving pH 5.00 ± 0.10, in a product fermented with *B. animalis lactis*. A reduced fermentation time results in lower manufacturing costs and thus higher interest by food industry and technology.

The count of viable cells in relation to the Log_{10} CFU mL^{-1}, pH and the acidity were monitored during the fermentation, and later in storage, as presented in Table 2. The number of cells of *L. plantarum* was 8.43 and 10.11, at the start and end of fermentation, respectively, in addition to pH and acidity of 0.17 and 0.70%; 6.26 and 4.19, respectively in cited times. There was no growth of yeast and mold, total and thermotolerant coliforms in the analyzed storage times, indicating good hygienic and sanitary conditions in the beginning and along the storage time.

Table 2. Mean value[1] and standard deviation of the count of *L. plantarum*, acidity and pH at the different storage times of the fermented milk.

Storage time (days)	[2]Count of Log_{10} (CFU mL^{-1})	[3]Acidity (% lactic acid)	[3]pH
0	$10.12^{a} \pm 0.02$	$0.70^{a} \pm 0.02$	$4.19^{a} \pm 0.02$
14	$10.52^{b} \pm 0.05$	$0.67^{a} \pm 0.04$	$3.78^{b} \pm 0.01$
28	$9.02^{c} \pm 0.22$	$0.67^{a} \pm 0.02$	$4.03^{c} \pm 0.01$
42	$9.10^{c} \pm 0.13$	$0.66^{a} \pm 0.02$	$4.11^{d} \pm 0.04$
56	$9.36^{d} \pm 0.13$	$0.67^{a} \pm 0.02$	$4.02^{c} \pm 0.02$
70	$8.92^{d} \pm 0.17$	$0.68^{a} \pm 0.03$	$4.05^{c} \pm 0.02$

[1]Mean values followed by the same letters are not different by Tukey's test ($p < 0.05$); [2]Data analyzed in quadruplicate; [3]Data analyzed in quadruplicate.

The count of viable cells was performed every 14 days after processing. According to Brasil (2008), the minimum count required is from 10^8 to 10^9 CFU mL^{-1} or CFU g^{-1} in daily recommendation of the product ready for consumption, in order to it be characterized as probiotics. None of the storage times presented count below this threshold. However it was verified a drop of two log cycles in 70 days of storage.

The fermented milk developed with *L. plantarum* could claim functional and/or health properties, since it met the existing legislation in relation to its microbiological viability during the study period under refrigeration. Nevertheless, the microorganism despite having a history of safe use in the industry and be linked with food products in countries belonging to European Union, it is not yet considered as probiotics by Brasil (2008).

No significant difference ($p < 0.05$) was detected in acidity along storage time of the fermented milk (Table 2). Zacarchenco and Massaguer-Roig (2004) evaluated the post-acidification effect during the storage of milk fermented with *Streptococcus thermophillus*, *B. longum* and *L. acidophilus,* and the authors verified variations in titratable acidity and pH during 21 storage days. Oliveira and Damin (2003) developed different formulations of fermented milk using blends containing 50% *S. thermophilus* and 50% (v v^{-1}) *L. delbrueckii bulgaricus*, *L. acidophilus* and *L. rhamnosus*, being verified post-acidification at one and seven days of storage.

The pH throughout the experiment ranged from 3.78 to 4.19 with extremes at the 0 and 14 days of storage (Table 2), which commonly occurs in studies with fermented products. According to Ferreira (1999), pH is important from technological point of view because it allow determining some parameters of processing, as for example at pH 4.7

occurs the casein precipitation once its isoelectric point is reached, but a sudden pH decrease around 3.70 indicates the proteolysis of the product. However, these characteristics are able to inhibit the development of pathogenic microorganisms (VRIES et al., 2006).

As listed in Table 3, the content of ash and total solids was not significantly different between assessed storage times ($p < 0.05$). Nevertheless, the fat content was absent in the product. Thamer and Penna (2006) elaborated different formulations of probiotic fermented milk drinks plus prebiotic and observed that the experiment, with a formulation of 8% of milk base of which 45-55% corresponded to whey powder, 6% of sugar and 1% of fructooligosaccharide, obtained, on average, 0% fat, 15.70% total solid and 0.61% ash, values similar to those obtained in the present study, although being different products.

Table 3. Mean value[1] and standard deviation of ash and total solids in the different storage times of fermented milk.

Storage time (days)	[2]Ash (%)	[2]Total solids (%)
0	$0.58^a \pm 0.01$	$15.40^a \pm 1.20$
28	$0.58^a \pm 0.01$	$14.96^a \pm 0.02$
56	$0.57^a \pm 0.01$	$15.13^a \pm 0.03$
70	$0.57^a \pm 0.01$	$15.05^a \pm 0.03$

[1]Mean values followed by the same letters in the columns are not different by Tukey's test ($p < 0.05$); [2]Data analyzed in quadruplicate.

Determinations made by Venturoso et al. (2007), with three samples of commercial fermented milk and in three different batches, obtained values between 11-19.7 and 0%, respectively, of fat free dry extract, and lipid. Most studies involving the development of milk-based fermented beverages, like fermented milk, yogurt, and milk drink, has found reduced content or even the absence of fat (ANTUNES et al., 2004; THAMER; PENNA, 2006; VENTUROSO et al., 2007). Considering the percentage of minerals, similar contents (0.60-0.73%) were found in commercial yogurt (RODAS et al., 2001).

The sensory analysis was conducted by a team of judges consisting of 50 untrained volunteer tasters, mostly (76%) students, 66% females, and 50% aged 17-10 years. All the tasters were considered as possible potential consumers, once they affirmed to like fermented milk and consumed frequently this product. The results of the acceptance test can be observed in Table 4.

A significant difference ($p < 0.05$) was verified between the initial and final mean values of the attributes color, sweetness, acidity and overall grade of the product. However, when verified the mean grade in the scales, one can realize small differences, ranging from 'like slightly' to 'like moderately', which in practice means that the product had good acceptance of sensory characteristics in these attributes evaluated at T0 and T70, with good acceptability indices of 84.3% and 75.7%, respectively.

Table 4. Mean value[1] and standard deviation of the acceptance by attributes: color, sweetness, acidity viscosity, overall grade, and acceptability index of the product at T0 and T70.

Time (days)	Color	Sweetness	Acidity	Viscosity	Overall	AI[2]
0	$6.0^a \pm 1.17$	$5.8^a \pm 1.13$	$6.0^a \pm 1.14$	$5.7^a \pm 1.23$	$5.9^a \pm 1.00$	84.3%
70	$5.4^b \pm 1.31$	$5.0^b \pm 1.52$	$5.1^b \pm 1.36$	$5.4^a \pm 1.30$	$5.3^b \pm 1.02$	75.7%

[1]Mean values followed by the same letters in the columns are not different by Tukey's test ($p < 0.05$). [2]AI: acceptability index of the product, based on the overall grade (mean x 100/7). Scale used: 1 = dislike very much, and 7 = like very much.

In the purchase intent test, it was verified the potential consumption of fermented milk developed, and 68% of the tasters would certainly buy/probably would buy the product. This is an important result for marketing researches, because one can predict about a possible market niche for the product in question, since good tools are used to insert product into the market.

In the Table 5 is presented the nutrition information of the milk fermented with *L. plantarum*. It can be observed that the product has carbohydrate and proteins but is free of fat and fibers. Comparing the results of nutrition information of the formulation elaborated, in relation to the seven trademarks of fermented milks, in 100 g of product (Table 6), it is observed that the milk fermented with *L. plantarum* had nutrition information similar to other fermented milks available on the market, except for brands D and G that had fat in their nutritional composition.

The daily value (DV) of energy based on a 2.000 kcal diet of the milk fermented with *L. plantarum* was 7%, similar to other five brands assessed, except for the brands E and F, which presented lower energy values per 100 mL of the product.

Table 5. Nutrition information of the fermented milk developed with *L. plantarum*.

Nutrition information		
1 serving = 200 mL of fermented milk (1 medium cup)		
Amount per serving		% DV (*)
Energy value	127 kcal or 541 kJ	6%
Carbohydrate	26 g	9%
Protein	5.8 g	8%
Total fat	0 g	0%
Saturated fat	0 g	0%
Trans fat	0 g	**
Total fiber	0 g	0%
Sodium	85 mg	3.5%

*DV – Daily Values are based on a diet of 2.000 kcal or 8.400 kJ. Your daily values can be higher or lower depending on your calorie needs. **% DV Not established. Ingredients: Water, sugar, powdered milk, yeast milk. Does not contain gluten.

Table 6. Nutrition information of the fermented milk compared with seven trademarks already established in the market.

Nutrients in 100 g of the product	Trademarks							
	Control[1]	A	B	C	D	E	F	G
Calorie	64 kcal	74 kcal	64 kcal	68 kcal	79 kcal	31 kcal	38 kcal	82 kcal
Carbohydrate	13 g	16.5 g	14 g	14.5 g	13.5 g	4.9 g	7 g	14.5 g
Protein	2.9 g	2 g	2 g	2 g	2.7 g	2.5 g	3 g	2.7 g
Total fat	0 g	0 g	0 g	0 g	1.6 g	0 g	0 g	1.6 g
Saturated fat	0 g	0 g	0 g	0 g	1 g	0 g	0 g	1 g
Trans fat	0 g	0 g	0 g	0 g	0 g	0 g	0 g	0 g
Fiber	0 g	0 g	0 g	0 g	0 g	0 g	0 g	0.65 g
Sodium	42.5 mg	37.5 mg	32.5 mg	32 mg	46 mg	41 mg	43.5 mg	46 mg

[1]The fermented milk developed was considered as control to compare with commercial brands.

As for the content of total, saturated, and trans fats, only two commercial brands had the two first, and the last sort of fat was absent in all brands. Due to changes in eating habits and the rise in overweight of Brazilian population, the trend is to exclude or reduce the intake of fat-rich foods, in this way, the product developed in this study met this purpose.

Among all fermented milks examined available on the marked, only two claimed functional and/or health properties, two contained phenylalanine, three had gluten and trace amounts of cashew nut informed in the label. The presence of phenylalanine, gluten and cashew nut may limit the consumption of these drinks, by excluding population groups, potential consumers of the product. It is worth emphasizing that none of these products met the legislation in relation to individual serving recommended for fermented milk, because according to Brasil (2000b) the serving should be 200 mL, but all the products brought the label information considering the sales volume.

Conclusion

It was possible to develop a formulation of fermented milk with *L. plantarum* stable in relation to physical and chemical characteristics. Besides that, the cell viability of *L. plantarum* was above the minimum required by Brazilian food legislation with claims of functional and/or health properties to be characterized as a probiotic food, for a period of 70 storage days at 4ºC. There was no development of total and thermotolerant coliforms, nor yeast and mold. The product had good sensory acceptance from beginning to end of shelf life, good purchase intent by consumers, in addition to nutritional characteristics similar to fermented milks sold in Brazil. Additionally, *L. plantarum* featured a good viability in fermented milk, and although not considered a probiotics by Brazilian law, it is a promising microorganism to produce foods with functional and/or health properties.

Acknowledgements

To Sacco® Company, for donating the commercial strain of *Lactobacillus plantarum* BG112; to Dr. Marly Sayuri Katsuda, for her assistance during the research, and to the State University of Londrina and Federal Technological University of Paraná (Londrina Campus), for providing resources and technology to develop the study.

References

ANTUNES, A. E. C.; CAZETTO, T. F.; BOLINI, H. M. A. Skim yogurts added by whey protein concentrate: texture profile, syneresis and sensorial analysis. **Alimentos e Nutrição**, v. 15, n. 2, p. 107-114, 2004.

BOYLSTON, T. D.; VINDEROLA, C. G.; GHODDUSI, H. B.; HEINHEIMER, J. A. Incorporation of bifidobacteria into cheeses: challenges and rewards. **International Dairy Journal**, v. 14, n. 5, p. 375-387, 2004.

BRASIL. Ministério da Agricultura, Pecuária e do Abastecimento. Resolução n. 5, de 13 novembro de 2000. Aprova os Padrões de identidade e qualidade de leites fermentados (declarações relacionadas aos padrões de identidade e qualidade), constantes do anexo desta Resolução. **Diário Oficial [da] República Federativa do Brasil**. Brasília: Poder Executivo, 2000a.

BRASIL. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução n. 359, de 22 de dezembro de 2000. Aprova o Regulamento Técnico referente às Porções de Alimentos Embalados para Fins de Rotulagem Nutricional, constantes do anexo desta Resolução. **Diário Oficial [da] República Federativa do Brasil**. Brasília: Poder Executivo, 2000b.

BRASIL. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução n. 360, de 23 de dezembro de 2000. Aprova o Regulamento Técnico referente à Rotulagem Nutricional de Alimentos Embalados, constantes do anexo desta Resolução. **Diário Oficial [da] República Federativa do Brasil**. Brasília: Poder Executivo, 2000c.

BRASIL. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. **IX - Lista de alegações de propriedade funcional, atualizado em julho de 2008**. Aprova a lista de Alimentos com Alegações de Propriedades Funcionais e/ou de Saúde, Novos Alimentos/Ingredientes, Substâncias Bioativas e Probióticos, constantes nesta lista. Available from: <http://www.anvisa.gov.br/alimentos/comissoes/tecno_lista_alega.htm>. Access on: Dec. 10, 2009.

CLAESSON, M. J.; SINDEREN, D.; O'TOOLE, P. W. 2007. The genus Lactobacillus - a genomic basis for understanding its diversity. **FEMS Microbiology Letters**, v. 269, n. 1, p. 22-28, 2007.

DUTCOSKY, S. D. **Análise sensorial de alimentos**. Curitiba: Champagnat, 1996.

FERREIRA, C. L. L. **Produtos lácteos fermentados**: aspectos bioquímicos e tecnológicos. Viçosa: UFV, 1999. (Cadernos didáticos).

FERREIRA, V. L. P.; ALMEIDA, T. C. A.; PETTINELLI, M. L. C. V.; SILVA, M. A. A. P.; CHAVES, J. B. P.; BARBOSA, E. M. M. **Análise sensorial**: testes discriminativos e afetivos. Campinas: SBCTA, 2001. (Manual: série qualidade).

IAL-Instituto Adolfo Lutz. Leites e derivados. In: ZENEBOM, O.; PASCUET, N. S.; TIGLEA, P. (Coord.). **Métodos físico-químicos para análise de alimentos**. 1. ed. digital. São Paulo: IAL, 2008. p. 819-877.

NEPA-Núcleo de Estudos e Pesquisas em Alimentação. **Table brasileira de composição de alimentos** (TACO). 1. ed. Campinas: Nepa; Unicamp, 2004.

OLIVEIRA, M. N.; DAMIN, M. R. Effect of total solids and sucrose contents on acidity, firmness and viability of yogurt and probiotic bacteria in fermented milk. **Ciência e Tecnologia de Alimentos**, v. 23, supl., p. 172-176, 2003.

PEREIRA, D. B. C.; SILVA, P. H. F.; COSTA JR., L. C. G.; OLIVEIRA, L. L. **Físico-química do leite e derivados**: métodos analíticos. 2. ed. Juiz de Fora: Epamig, 2001.

RODAS, M. A. B.; RODRIGUES, R. M. M. S.; SAKUMA, H.; TAVARES, L. Z.; SGARBI, C. R.; LOPES, W. C. C. Physico chemical, histologycal and viability of lactic bacteria in yogurts containing fruit. **Ciência e Tecnologia de Alimentos**, v. 21, n. 3, p. 304-309, 2001.

SILVA, N.; JUNQUEIRA, V. C. A.; SILVEIRA, N. F. A. **Manual de métodos de análises microbiológicas de alimentos**. 3. ed. São Paulo: Varela, 2001.

THAMER, K. G.; PENNA, A. L. B. Characterization of functional dairy beverages fermented by probiotics and with the addition of prebiotics. **Ciência e Tecnologia de Alimentos**, v. 26, n. 3, p. 589-595, 2006.

TUOHY, K. M.; PROBERT, H. M.; SMEJKAL, C. W.; GIBSON, G. R. Using probiotics and prebiotics to improve gut health. **Drug Discovery Today**, v. 8, n. 15, p. 692-700, 2003.

VENTUROSO, R. C.; ALMEIDA, K. E.; RODRIGUES, A. M.; DAMIN, M. R.; OLIVEIRA, M. N. Determination of the physical-chemical composition of dairy products: exploratory study to compare the results obtained by classic methodology and by ultra-sound. **Revista Brasileira de Ciências Farmacêuticas**, v. 43, n. 4, p. 607-613, 2007.

VRIES, M. C.; VAUGHAN, E. E.; KLEEREBEZEM, M.; VOS, W. M. *Lactobacillus plantarum*-survival, functional and potential probiotic properties in the human intestinal tract. **International Dairy Journal**, v. 16, n. 9, p. 1018-1028, 2006.

ZACARCHENCO, P. B.; MASSAGUER-ROIG, S. Microbiological, sensory and post-acidification evaluation during shelf-life of fermented milks containing *Streptococcus thermophilus*, *Bifidobacterium longum* and *Lactobacillus acidophilus*. **Ciência e Tecnologia de Alimentos**, v. 24, n. 4, p. 674-679, 2004.

Received on December 9, 2010.
Accepted on June 7, 2011.

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.