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ORIGINAL RESEARCH

Pasteurised, microfiltered and lactose-hydrolysed skimmed milk with added probiotics: Development and storage stability

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Recently the food industry has been attempting to innovate its products to meet consumer demands for health benefits from their food. Thus, the objective of this research was to study the combination of technologies to obtain a pasteurised, microfiltered, and lactose-hydrolysed skim milk with an extended shelf life and with added probiotics (PMLHSP). This PMLHSP was subjected to physico-chemical, microbiological and sensory evaluations, plus its shelf life was estimated at 5 °C. The viability of the probiotics in this system was also evaluated during storage at 5 °C and indicated a shelf life of about 28 days. The probiotic culture added to the microfiltered skim milk presented good viability in the product throughout refrigerated storage, with counts of above 8 log CFU/mL.

Keywords Microfiltration, Lactose-hydrolysed milk, Probiotic.

INTRODUCTION

According to Sloan (2014), healthfulness is the reflection of a combination of attributes such as fresh, avoidance of certain substances, inclusion of positive aspects and high quality. Wellness is seen as positive and a shift away from illness. One of the five most important tendencies concerning food is that of healthfulness and well-being (FIESP/ITAL 2010). Lactose-free or lactose-reduced dairy products with the addition of functional compounds such as probiotic micro-organisms fulfil this tendency.

Lactose, the predominant sugar in milk, is a disaccharide whose absorption requires hydrolysis by β-galactosidase (lactase) in the small intestine. A deficiency of this enzyme leads to inadequate digestion and consequently to intolerance. Approximately 75% of the world population loses the ability to digest lactose at some point (Mattar *et al.*, 2012). The incidence can be up to 80% amongst Africans, Arabs, Greeks, Chinese, Koreans, Eskimos, Canadians, Jews and Indians (Shukla, 1997; Rusynyk and Still 2001).

Low-lactose milks have been developed for lactose-intolerant individuals who wish to continue consuming milk and dairy products, as this product represents one of the main sources of calcium. Enzymic hydrolysis is the preferred method to reduce lactose content in dairy products as it does not modify the concentrations of the other milk components (Heng and Glatz 1994). Lactose-free or lactose-hydrolysed milk is normally sold as an ultrapasteurised product (Adhikari *et al.* 2010). In Brazil, the only lactose-hydrolysed milk available on the market is a UHT (ultra high temperature) product. The lactose hydrolysis of milk increases the amount of reducing monosaccharides (glucose and galactose), which are more reactive in the Maillard reaction, affecting the colour and lack of freshness of the product (Adhikari *et al.* 2010). Moreover, the higher proteolytic activity in lactose-hydrolysed milk

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increases the level of free amino terminals and amino groups from lysine on the side chain, which may also contribute to the Maillard reaction and loss of availability of lysine, and therefore, the nutritional value of the protein may be lower in this type of milk (Jansson *et al.* 2014). As a consequence, these UHT lactose-hydrolysed milk products tend to darken in colour, which could also prejudice their acceptability, especially when consumed alone. Of the alternatives aimed at minimising changes in flavour, colour and nutrition due to the use of high temperatures, are the milk conservation methods that apply milder temperatures, such as pasteurisation and/or the use of microfiltration.

For pasteurisation, the milk is heat-treated between 72 and 75 °C for 15–20 sec and then cooled to 4 °C or less. This type of heat treatment, known as HTST (high temperature short time), eliminates the pathogenic microbial microbiota but not all the deteriorative micro-organisms, resulting in a shelf life of just a few days. On the other hand, microfiltration allows one to reduce the microbial load by way of a mechanical separation using membranes under mild temperature conditions (Hoffmann *et al.* 2006). This technology has no negative impact on the organoleptic, functional and nutritional properties of the product (Cianci, *et al.*, 2005; Creamer *et al.* 2002; Ferreira, 2001). In addition to reducing the microbial load and somatic cells and prolonging the shelf life, microfiltration maintains the natural, fresh flavour characteristics. The combination of pasteurisation and microfiltration makes it possible to obtain pasteurised milk with a longer shelf life. Antunes *et al.* (2014) combined pasteurisation, microfiltration and lactose hydrolysis and obtained milk with an extended shelf life of 50 days with respect to the total aerobic mesophilic count and titratable acidity, and shelf life of 21–27 days (at 5 °C ± 1 °C) in terms of the sensory quality and proteolysis index. After 28 days of storage, the sensory panelists described the samples as only slightly more astringent, bitter and acid than the controls indicating that the sensory changes were not extensive.

Probiotics have been defined by FAO/UNO (Food and Agriculture Organization/United Nations Organization) and WHO (World Health Organization) (Joint FAO/WHO 2002) as 'live micro-organisms that, when administered in adequate amounts, confer benefits on their hosts'.

Probiotic bacteria are widely known to reduce the symptoms of lactose malabsorption (Prasanna *et al.* 2014) although the mechanism of the effect of probiotics in aiding lactose hydrolysis is still not completely understood. However, it is known that the pH in the intestine changes and also the activity of the enzyme beta-galactosidase, promoting a positive effect on the intestinal microbiota and a consequent improvement in intestinal activity (He *et al.* 2007).

Yoghurts and fermented milks are the main vehicles for probiotic cultures (Cruz *et al.* 2009). However, the addition of these micro-organisms to nonfermented milk is feasible, as, according to Shihata and Shah (2000), these cultures have a slow metabolism, are not very proteolytic and grow slowly in milk (Prasanna *et al.* 2014). There are many examples of nonfermented dairy products with added probiotics on the world market, such as powdered milk for newborn infants, milk-based desserts, ice creams, etc. (Cruz *et al.* 2009). Concerning the addition of probiotics to nonfermented milk, the study of Jiang *et al.* (1996) showed that the intake of nonfermented milk containing *Bifidobacterium longum*, with counts of around 10^8 CFU/mL, improved lactose digestion *in vivo* as measured by the net breath hydrogen production. These authors highlighted that milk containing bifidobacteria was better tolerated by lactose maldigesters, as evidenced by reduced symptoms of flatulence. Mustapha *et al.* (1997) prepared nonfermented acidophilus milk using four strains of *Lactobacillus acidophilus* (B, N1, E and ATCC 4356). This work verified that nonfermented acidophilus milk containing *L. acidophilus* N1 was the most effective of the four acidophilus milks in improving lactose digestion and tolerance.

The shelf life of Brazilian pasteurised milk is approximately 3–5 days, making the addition of probiotics impracticable as it would be a value-added product with a short shelf life. Microfiltration is of great value in this case, permitting a longer shelf life for the pasteurised milk.

There are many articles about pasteurised microfiltered milk and about lactose-hydrolysed milk. On the other hand, there are few articles about nonfermented milk with added probiotics. To the best of our knowledge, no studies on the production and stability during storage of a microfiltered, lactose-hydrolysed probiotic milk have been reported. Besides, this product is still not available on the retail market, as far as we know.

Thus, the objective of this research was to study the combination of technologies to obtain a microfiltered, lactose-hydrolysed probiotic skim milk with an extended shelf life. The viability of the probiotic in this system during storage at 5 °C was also investigated.

MATERIAL AND METHODS

Processing and storage of the products

Two processing procedures were carried out in this project to obtain low-lactose functional microfiltered skim milk samples, each using a 120 L skim milk batch. It used a type A skim milk (from only one farm, pasteurised at 72–75 °C/15 to 20 s and bottled in the farm premises, according to Brasil 2011).

The samples from each processing were stored for 43 days at 5 °C and evaluated with respect to their microbiological, physicochemical and sensory parameters every 7 days.

Lactose hydrolysis

The enzyme used for lactose hydrolysis was the β-Galactosidase (EC 3.2.1.23) isolated from *Kluyveromyces lactis* (50 000 U/mL) Prozyn SP/Brazil (São Paulo, Brazil).

For each processing run, beta-galactosidase was initially added to the 120 L skim milk batch at a concentration of 0.4 g/L of milk and incubated for 21 h at 10 °C (preselected hydrolysis condition obtained from the results of the preliminary tests). The desired degree of hydrolysis (>90%) was determined by cryoscopy. The cryoscopic analysis was carried out using a Lactron model M90 digital cryoscope. To obtain the stipulated degree of hydrolysis, it was considered that the complete hydrolysis of a 5% lactose solution would result in a lowering of the freezing point by 0.273 °C (−0.282°H), according to Ramet *et al.* (1979).

The percentage of hydrolysis was confirmed by high-performance liquid chromatography (HPLC), carried out according to Burgner and Feinberg (1992). The analytical conditions were as follows: chromatograph equipped with a Nucleosil 100-5 NH_2 column, 250 × 4.6 mm, 5 μm (Macherey-Nagel); column temperature of 40 °C; mobile phase: acetonitrile + deionised water (75:25 v/v) – filtered and degassed; mobile phase flow rate of 1 mL/min (constant); Reodyne injection valve with a 20 μL loop; Varian ProStar model 350 refractive index detector at 40 °C; Varian ProStar model 210 isocratic pump; Cromacon Ciola HotColumn column oven; and Borwin version 1.50 data collection software. The detection limit of the method was 0.2 g lactose in 100 mL milk.

Microbial cultures

A preliminary test was carried out employing the following probiotic monocultures in an isolated way: *Lactobacillus acidophilus* LA-5 and *Bifidobacterium animalis* subsp *lactis* BB-12, to determine which was most suitable for the nonfermented milk. The samples of microfiltered milk with the addition of LA-5 or BB-2 were stored at 5 °C and evaluated weekly in relation to the parameters of pH, total count and culture viability. In these tests, the culture showing the best performance was *B. animalis* subsp *lactis* BB-12 (data not shown), which was therefore chosen to continue the research.

The experiments were conducted with a DVS (direct vat set) culture of *Bifidobacterium animalis* subsp. *lactis* BB-12 (Chr. Hansen/Valinhos/Brazil). The probiotic culture was suspended in 1L sterile skim milk before the use.

Milk microfiltration

After hydrolysis of the lactose, the skim milk was submitted to microfiltration using a microfiltration-MFS-1 pilot unit (Tetra Laval, Paris). This unit was equipped with a uniform transmembrane pressure (UTP) ceramic membranes (Membralox, Societe des Ceramiques, Bazet, France), with a membrane area of 0.24 m^2 (mean pore size of 1.4 μm), corresponding to a capacity of approximately 150 L of skim milk/h. The following parameters of the process were chosen: permeate flux of 120 L/h, retentate flux of 6.3 L/h, volumetric concentration factor (VCF) of 20 and temperature of 48 ± 1 °C. An UTP of 60 kPa was used to minimise membrane fouling. The microfiltered skim milk was filled into sterile 1-L glass bottles using an automatic doser inside a laminar flow chamber, and then, the probiotics added under aseptic conditions. The DVS (direct vat set) culture of *Bifidobacterium animalis* subsp. *lactis* BB-12 was inoculated into the microfiltered milk to achieve an inoculum of 10^8 CFU/mL.

Microbiological analyses of the just-processed functional microfiltered milk samples and during storage

The samples were submitted to the following analyses before and immediately after microfiltration: total aerobic mesophiles, total aerobic psychrophiles, coliforms at 30 °C and at 45 °C, coagulase-positive staphylococci, *Salmonella* spp and yeast and moulds. In addition, the following analyses were carried out every 7 days during storage (43 days): total mesophilic count, coliforms at 30 °C, coliforms at 45 °C, yeasts and moulds.

The total aerobic mesophilic count was done on standard plate count agar (Difco, Detroit, MI) containing triphenyltetrazolium chloride (TTC) (Merck, Whitehouse Station, NJ) incubated at 32 ± 1 °C/48 h (Frank and Yousef 2004). The most probable number procedure (MPN) was used to determine coliforms at 30 °C and at 45 °C with lauryl sulphate tryptose broth (Difco, Detroit, MI) and brilliant green bile lactose broth (Difco, Detroit, MI), incubating at 30 ± 1 °C for 24–48 h for coliforms at 30 °C (ISO 48312006) and *Escherichia coli* broth (Difco, Detroit, MI) incubating at 44 ± 1 °C for 24 h (ISO 72512005) for the heat-tolerant coliforms. Dichloran rose bengal chloramphenicol agar (Difco, Detroit, MI) was used for the yeast and mould counts, incubating at 25 ± 1 °C for 5 days (ISO and IDF 2004; number ISO6611). PCA plate count agar, incubating at 7 ± 1 °C for 7 days, was used for the aerobic psychrotrophic count (Frank and Yousef 2004). The presence of coagulase-positive staphylococci and *Salmonella* were searched according to the procedures recommended by Henning *et al.* (2004). The results for the microbial counts were expressed in log CFU/mL, with the exception of the coliform counts which were expressed in MPN/mL and the *Salmonella* analyses, expressed as present or absent.

Probiotic culture counts

The methodology found in Technical Bulletin P-12 from Chr-Hansen was used for *Bifidobacterium animalis* subsp. *lactis* counts, with adaptations of the standard IDF 4112007 methodology. An aliquot of 5 mL of a stock solution of dicloxacilin (Sigma, St. Louis, MO), 10 mL of a stock solution of LiCl (Merck, Whitehouse Station, NJ) and 5 mL of a stock solution of CyHCl (Merck, Whitehouse Station, NJ) were added to each litre of medium. The pour plate procedure was used with anaerobic incubation (Anaerogen, Oxoid, Basingstoke, UK) at 37 °C for 72 h.

Physicochemical analyses of the just-processed microfiltered probiotic milk samples during storage

On the first day of storage of the PMLHSP milk, the density, pH, titratable acidity, total dry extract, fat, nonfat solids, total nitrogen (TN), noncasein nitrogen (NCN), nonprotein nitrogen (NPN), total protein and ash contents were determined and the cryoscopic analysis applied. The pH was determined using a digital pHmeter (Micronal B-375, Micronal SA, Santo Amaro, Brazil). The density and total titratable acidity were determined according to the norms of the Instituto Adolfo Lutz (2005). The percentages of total dry extract (TDE) and fat (F) (Gerber method) and the lowering of the cryoscopic point were determined according to Brasil (2006). The nonfat solids (NFS) content was obtained from the relationship NFS = (TDE − F). The total nitrogen content was obtained by the Kjeldahl method (Association of Analytical Chemistry (AOAC) 1995), using a factor of 6.38. The noncasein nitrogen content was obtained by determining the total nitrogen in the supernatant after the isoelectric precipitation of the caseins (Association of Analytical Chemistry (AOAC) 1995). The nonprotein nitrogen content of the samples was determined by determining the total nitrogen in the supernatant after total precipitation of the proteins in the presence of 12% trichloroacetic acid (TCA), as described by Aschaffenburg and Drewry (1959). The mineral content was obtained according to Horwitz (2000).

The PMLHSP was evaluated weekly for proteolysis. Proteolysis was evaluated according to the proteolysis index (PI), which corresponds to the decrease in casein (CN) as a percentage of the true protein (TP) (CN%TP), where:

Casein (CN) = (TN-NCN) X 6.38

TP = (TN − NPN) X 6.38, the values for TN, NPN and NCN being obtained as described above.

Sensory acceptance test and storage stability

The PMLHSP milk was evaluated by acceptance test and difference from control. To evaluate the acceptance of the PMLHSP milk, the product was compared with pasteurised, microfiltered and lactose-hydrolysed skimmed milk from the same batch but without added probiotics. Pasteurised milk of the same brand used in processing was also evaluated (Meilgaard *et al.*, 2006a,b).

A sensory evaluation of the PMLHSP milk was carried out during refrigerated storage and compared with the control. The control sample (coded as 'C') consisted of commercial skimmed milk previously hydrolysed with lactase before each sensory evaluation session, without the addition of probiotics. The attributes evaluated were colour, sweetness, flavour, astringency, bitterness and acidity. The sensory panel consisted of 24 judges selected for their sensory acuity and used the difference from control by scales detailed below to evaluate the samples 7, 14, 21, 28, 35 and 43 days after manufacture. For the evaluations, each judge received the control, duly identified as such: two samples coded with random 3-digit numbers and also the control sample coded with a random 3-digit number. The samples were served according to a randomised complete block design. The results of judges who classified the control coded amongst the samples with scores below three or greater than five for colour and sweetness, below five for flavour or below four for astringency, bitterness and acidity were discarded. The research design was approved by the Research Ethics Committee (protocol 223/07, PUCCamp/Campinas/Brazil).

Statistical analysis

The results of sensory analysis were submitted to an analysis of variance and to Dunnett's means comparison test, as indicated to compare samples with a standard (ABNT 1995).

RESULTS AND DISCUSSION

Results of the microbiological analyses

For each batch, immediately after the microfiltration of the milk, counts for total aerobic mesophilic and total aerobic psychrotrophic bacteria were both <1 log CFU/mL; counts for coliforms at 30 °C and coliforms at 40 °C were both <0.3 MPN/mL; *Salmonella* spp was absent, and counts for coagulase-positive staphylococci, yeasts and moulds were <1 log CFU/mL. During the storage of the samples, counts were made for total aerobic mesophilic bacteria, coliforms, yeasts and moulds and the viability of the probiotic added.

Table 1 shows the mean results obtained for the microbiological analyses of the two processings during storage of the product. The counts for coliforms and for yeasts and moulds were below the limits of detection for up to 43 days of refrigerated storage. These results indicate the good microbiological stability of the PMLHSP as recommended by the Codex Alimentarius Commission (2004).

The criterion used by Elwell and Barbano (2006) to determine the expiry date for microfiltered milk was an aerobic mesophilic bacterial count of 20 000 CFU/mL (corresponding to 4.3 log CFU/mL). The shelf life of the product was estimated for them as a function of storage temperature (0.1; 2.0; 4.2; and 6.1 °C) of the product and varied between 16 days (at 6.1 °C) and 66 days (at 0.1 °C). The authors considered a total aerobic mesophilic count of 4.30 log CFU/mL to be the end of the shelf life of the product (Elwell and Barbano 2006). Caplan and Barbano (2013) achieved a refrigerated shelf life of 60–90 days at both 1.7 and 5.7 °C for microfiltered, pasteurised milk containing 2% of fat. According to their work, no containers of this milk exceeded 20 000 CFU/mL during the 90 days of storage at 5.7 °C. In the present study, the total aerobic

Table 1 Results for the microbiological analyses of the processing (n = 2) of pasteurised, lactose-hydrolysed, microfiltered milk samples with added probiotics, during storage at 5°C

	Microbial count (log CFU/mL or MPN/mL) during refrigerated storage (days)						
Micro-organism	*0*	*7*	*14*	*21*	*28*	*35*	*43*
Total aerobic mesophiles	<1	1 ± 0.08	2 ± 0.1	2.5 ± 0.5	2.7 ± 0.7	1.1 ± 0.1	3.8 ± 2.8
Coliforms at 30 °C	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
Coliforms at 45 °C	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
Yeasts and moulds	<1	<1	<1	<1	<1	<1	<1
Bifidobacterium animalis	8.6 ± 0.08	8.2 ± 0.01	8.3 ± 0.1	8.9 ± 0.01	8.3 ± 0.1	8.2 ± 0.2	8.3 ± 0.03

[Correction added on 29 July 2015, after first online publication: The value in Yeasts and moulds under 43 days was changed from "<14.3" to "<1".]

mesophilic counts were below these values up to 43 days of storage.

The probiotic culture (*B. animalis* subsp. *lactis*) added to the product showed good viability throughout refrigerated storage, with mean counts of 8.4 log CFU/mL for up to 43 days of refrigerated storage of the milk, whereas Saarela *et al.* (2006) observed 2 weeks of stability for *B. animalis* subsp. *lactis* BB-12 added to semiskimmed milk (1% fat) with storage at 4 °C.

Physicochemical composition of the PMLHSP on the 1st day of storage

Table 2 shows the data for the mean physicochemical composition (n = 2) of the pasteurised, microfiltered and lactose-hydrolysed skimmed milk samples with added probiotics, on the 1st day of storage. The results obtained for PMLHSP complied with all the physical and chemical requirements established by the literature (Walstra *et al.* 1999; Brasil 2011) for pasteurised skimmed milk.

The cryoscopy of milk is a physical property related to the substances dissolved in it, that is, mainly lactose and mineral salts. When the lactose is hydrolysed via the action of β-galactosidases, the number of free molecules in the milk increases, and on increasing the amount of substances dissolved in the milk, there is a tendency for the freezing point to lower. This explains the result for cryoscopy presented in Table 2, showing a greater depression (−0.822 ± 0.006 °H) than the maximum limit determined by the legislation for nonhydrolysed milk, which is −530°H (−0.512 °C) (Brasil 2011).

Physicochemical composition of the PMLHSP during refrigerated storage

Figure 1 presents the mean results (n = 2) obtained in the evaluations of proteolysis index during refrigerated storage.

Santos *et al.* (2003) produced pasteurised microfiltered milk and determined that the end of the shelf life corresponded to the moment at which the decrease in per cent casein of the milk in relation to the total true protein was equal to or greater than 4.76% (PI ≥ 4.76%). This level was established as it was the level at which the defects in flavour due to proteolysis were detected by 50% of the sensory panel.

Table 2 Mean physicochemical composition (n = 2) of the pasteurised, lactose-hydrolysed, microfiltered milk samples with added probiotics, on the 1st day of storage

Composition	*Mean ± standard deviation*
Density (g/mL)	1.034 ± 0.000
pH	6.55 ± 0.04
Titratable acidity (°D)	17.56 ± 0.36
Total dry extract – TDE (%)	8.72 ± 0.10
Fat-F (%)	0.00 ± 0.00
NFS-NFS (%)	8.72 ± 0.10
Ash (%)	0.74 ± 0.00
Total protein (%)	3.29 ± 0.01
Cryoscopy (°H)	−0.822 ± 0.006

If the end of the shelf life adopted for the PMLHSP skimmed milk samples obtained in the present study was a PI equal to 4.76%, the shelf life would be about 28 days at a temperature of 5 ± 1 °C. Such a shelf life would be close to that obtained by Elwell and Barbano (2006), whose samples of pasteurised microfiltered skimmed milks stored at a temperature of 6.1 °C reached a PI of 4.76% after 36 days of storage.

The results obtained in the sensory evaluation of the PMLHSP elaborated in this study (Table 4) also showed that the microfiltered milks with added probiotics started being considered as inferior in an accentuated way as from the 28th day of storage, when compared to the control in terms of flavour, astringency and bitterness.

According to Jansson *et al.* (2014), lactose hydrolysis can be associated with increases in the proteolytic activity of the milk, probably due to the proteolytic side effects of the added lactase enzyme preparation. In that paper, they observed an important increase in free amino acids and free amines in the UHT lactose-hydrolysed milks, as compared to conventional UHT milk. In the present work, we

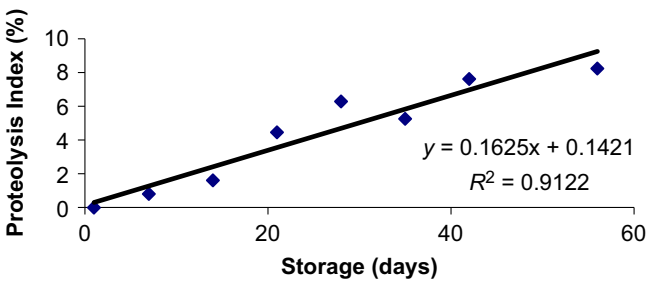

Figure 1 Mean values (n = 2) obtained for the proteolysis Index (%) of the pasteurised microfiltered lactose-hydrolysed skimmed milks with added probiotic during refrigerated storage.

observed a shelf life similar to that of Elwell and Barbano (2006), who evaluated pasteurised microfiltered skimmed milks. As a conclusion, the lactose hydrolysis procedure associated with microfiltration probably did not impair the sensorial characteristics of the milk. Moreover, a previous paper published by our team evaluated pasteurised, microfiltered and lactose-hydrolysed milk, and the shelf life of the product was 21–27 days (Antunes *et al.* 2014), similar to that obtained in the present study, indicating that the addition of a probiotic culture did not interfere in the sensory aspects of the product.

Acceptance test

The group that evaluated the sample was composed of 10 men and 40 women. Of the 50 consumers who took part in the evaluation, 34 replied that they consumed some type of functional food (with probiotic, prebiotic, fibre or others).

Table 3 shows the mean results obtained in the test for the evaluation of the milk samples. In the evaluation of colour, the sample of microfiltered milk with probiotic did not differ from the sample of commercial milk samples (skimmed and pasteurised milk) and the microfiltered milk with no probiotic.

In the evaluation of flavour, the commercial pasteurised milk was the most accepted and presented a mean close to 'liked', differing significantly ($P < 0.05$) from the microfiltered milk samples with and without probiotic, which presented means close to 'neither liked nor disliked' and did not differ between the two. It should be emphasised that the microfiltration process completely removes the remaining fat content and that the commercial sample contained about 0.5% fat. It should also be emphasised that the majority of consumers making up the taste panel consumed whole milk.

With respect to the overall evaluation of the product, the microfiltered milk sample with probiotic did not differ significantly from the sample without probiotic, which, for its part, did not differ from the commercial milk sample. Thus, the presence of probiotics did not alter the sensory characteristics of the product in a perceptible way similar to that observed by Cruz *et al.* (2012), working with probiotic yoghurt.

When considered as pure milk, the sweetness intensity of the microfiltered milks with and without probiotics presented means close to 'the way I like it' and differed from the commercial skimmed milk which presented a mean between 'the way I like it' and 'slightly less sweet than I like it'.

It was concluded from the sensory analyses that the samples of pasteurised microfiltered lactose-hydrolysed skimmed milks with and without the addition of probiotics presented acceptability slightly inferior to the commercial milk sample, but did not differ one from the other with respect to acceptability. This demonstrated that the addition of probiotics to the product did not affect its acceptance by consumers.

Difference-from-control test

For each time evaluated, Table 4 shows the mean values obtained as from correct judgments, that is, where the Control (C) was correctly identified in the sensory evaluation of the samples.

In the first evaluation, the samples showed means close to 'equal to C for colour', not differing from the Control at an error level of 5%. This result confirms the advantage of using the combination of pasteurisation and microfiltration in the conservation of lactose-hydrolysed milk, as the use of

Table 3 Results obtained in the evaluation of the pasteurised, lactose-hydrolysed, microfiltered milk samples with respect to acceptance of the colour, aroma and flavour, the overall acceptance of the product, and evaluation of the sweetness intensity (considering it as pure milk)

	Milk samples			
Evaluation of the acceptability	*Pasteurised milk*	*Microfiltered without probiotic*	*Microfiltered with probiotic*	*M.S.D.*
Colour	6.7 ± 1.5 a	5.9 ± 1.8 b	6.1 ± 1.8 ab	0.69
Aroma	6.5 ± 1.2 a	6.3 ± 1.4 a	6.2 ± 1.4 a	0.59
Flavour	6.5 ± 1.6 a	5.5 ± 1.9 b	5.1 ± 2.0 b	0.73
Overall acceptance of the product	6.2 ± 1.7 a	5.7 ± 1.8 ab	5.3 ± 1.9 b	0.65
Sweetness intensity	2.6 ± 0.8 b	3.3 ± 1.0 a	3.0 ± 1.1 ab	0.37

Results expressed as the mean ± the standard deviation. M.S.D.: minimal significant difference at an error level of 5% according to Tukey's test. In each line, values followed by the same letters do not differ significantly at an error level of 5%. The results are means of the scores of the 24 judges.

Table 4 Mean values obtained in the evaluations of pasteurised, lactose-hydrolysed, microfiltered milk samples with added probiotic (PMLHSP) for colour, sweetness, flavour astringency, bitterness and acidity (1st test time) and for flavour, astringency, bitterness and acidity (subsequent testing times) as compared to commercial skimmed milk previously hydrolysed with lactase before each sensory evaluation session without the addition of probiotics (Control) and compared to commercial pasteurised skimmed milk

		Sample			
Test time	*Attributes*	*Control**	*PMLHSP*	*Pasteurised milk*	*M.S.D.*
7 days	Colour	3.8 ± 0.4 a	3.5 ± 0.8 a	3.7 ± 0.7 a	0.49
	Sweetness	3.9 ± 0.5 a	4.0 ± 1.3 a	2.7 ± 1.2 b	0.87
	Flavour	6.0 ± 0.5 a	5.2 ± 1.6 a	5.8 ± 1.2 a	0.95
	Astringency	5.0 ± 0.4 a	4.6 ± 1.0 a	4.9 ± 0.8 a	0.65
	Bitterness	5.1 ± 0.4 a	4.6 ± 0.8 a	4.7 ± 0.7 a	0.56
	Acidity	5.0 ± 0.5 a	4.8 ± 0.6 a	4.8 ± 0.6 a	0.51
14 days	Flavour	5.8 ± 0.5 a	5.1 ± 1.2 b		0.68
	Astringency	5.1 ± 0.5 a	4.6 ± 0.8 b		0.43
	Bitterness	5.1 ± 0.4 a	4.6 ± 0.6 b		0.38
	Acidity	5.1 ± 0.4 a	4.8 ± 0.7 a		0.45
21 days	Flavour	5.9 ± 0.6 a	5.2 ± 1.4 b		0.70
	Astringency	5.1 ± 0.5 a	4.6 ± 0.8 b		0.48
	Bitterness	4.9 ± 0.4 a	4.7 ± 0.6 a		0.36
	Acidity	5.1 ± 0.5 a	4.9 ± 0.6 a		0.41
28 days	Flavour	5.9 ± 0.5 a	4.4 ± 1.5 b		0.72
	Astringency	5.1 ± 0.5 a	4.1 ± 1.1 b		0.56
	Bitterness	5.1 ± 0.5 a	4.5 ± 0.7 b		0.33
	Acidity	5.0 ± 0.5 a	4.6 ± 0.8 a		0.40
35 days	Flavour	5.8 ± 0.4 a	4.6 ± 1.9 b		1.00
	Astringency	4.9 ± 0.4 a	4.2 ± 1.2 b		0.63
	Bitterness	5.1 ± 0.4 a	4.6 ± 0.9 b		0.45
	Acidity	5.0 ± 0.5 a	4.4 ± 1.0 b		0.60
43 days	Flavour	5.8 ± 0.6 a	5.0 ± 1.4 b		0.76
	Astringency	4.6 ± 0.5 a	4.6 ± 0.7 a		0.36
	Bitterness	5.0 ± 0.5 a	4.8 ± 0.6 a		0.41
	Acidity	4.9 ± 0.5 a	5.0 ± 0.4 a		0.30

Values expressed as the mean ± standard deviation of correct judgments.

For each test time, for each attribute (line), means followed by letters different from the Control are statistically different from it at the 5% error level.

M.S.D. (5%): minimal significant difference according to Dunnett's test at the 5% error level.

*Commercial skimmed milk previously hydrolysed with lactase before each sensory evaluation session, without the addition of probiotics.

milder temperatures minimised the effects of the Maillard reaction, and consequently browning of the milk. This fact is important, as the darker colour that lactose-hydrolysed milk could present could compromise its acceptance by lactose-intolerant consumers.

With respect to sweetness, the commercial pasteurised skimmed milk sample, with a mean situated between 'slightly less sweet than C' and 'moderately less sweet than C', differed significantly from the Control at an error level of 5%. There was no significant difference between the other samples and the Control with respect to sweetness. These results are justified by the fact that in lactose-hydrolysed milk, the lactose (disaccharide) is converted into glucose and galactose, which are sweeter sugars than lactose. Thus, pasteurised skimmed milk tends to be less sweet than pasteurised lactose-hydrolysed skimmed milk (control C).

For the other attributes (flavour, astringency, bitterness and acidity), the commercial pasteurised skimmed milk sample, as also the PMLHSP samples, did not differ from the Control in the first evaluation.

In general, as from the 14th day, the PMLHSP sample differed significantly ($P < 0.05$) from the Control C with respect to flavour, astringency and bitterness.

On the 28th day, the sample showed means situated between 'inferior to C, without off-flavour' and 'with slight off-flavour'; and between 'equal to C' and 'slightly more astringent, bitter and acid than C' and hence did not show marked sensory alterations.

The sample evaluated on the 43rd day did not present results for astringency, bitterness and acidity consistent with those obtained for the previous test times.

In synthesis, the results of the sensory evaluation of the PMLHSP elaborated in this study showed that the microfiltered milks with added probiotics already started to be considered significantly inferior to the control in terms of flavour, astringency and bitterness after 2 weeks of storage. However, the difference only became accentuated as from 28 days of storage.

CONCLUSIONS

In the present study, the combination of technologies allowed one to obtain a microfiltered, lactose-hydrolysed probiotic skim milk with an extended shelf life. The probiotic culture added to the microfiltered skim milk presented good viability in the product throughout refrigerated storage, with counts above 8 log CFU/mL for up to 43 days of refrigerated storage at 5 °C. Based on the physicochemical, microbiological and sensory evaluations, a shelf life of 28 days was estimated for the product developed in this research.

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