International Food Research Journal 23(6): 2631-2637 (December 2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Viability of the probiotic *Lactobacillus acidophilus* La-5 in ice cream: effect of lactose hydrolysis and overrun

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Article history

Received: 27 October 2015
Received in revised form: 17 January 2016
Accepted: 22 February 2016

Keywords

Functional food
β-galactosidase
Lactase
Lactose intolerance
Dairy product

Abstract

The viability of *Lactobacillus acidophilus* La-5 incorporated into ice cream produced with and without hydrolysis of lactose was evaluated. Furthermore, the effects of La-5 and lactose hydrolysis on the physicochemical characteristics of the final product were analyzed. Three formulations were produced: T1 (without La-5 and lactose hydrolysis); T2 (La-5 and without lactose hydrolysis), and T3 (La-5 and lactose hydrolysis). La-5 viability, pH and titratable acidity were evaluated weekly during 28 days. The overrun was evaluated during manufacturing process. The lactose hydrolysis did not alter physicochemical properties of ice cream. Regarding La-5 populations, no significant differences were detected between T2 and T3 during storage ($p>0.05$). T2 and T3 presented La-5 counts of 7.71 and 7.67 log CFU/g on day 28, respectively. The La-5 strain has adapted to the ice cream matrix, and was resistant to the incorporation of air during the process, since 43.6 and 43.8% of overrun was obtained to T2 and T3, respectively. The manufacture of a probiotic ice cream with 56% of hydrolyzed lactose was possible. The hydrolysis of lactose contributed to obtaining a functional ice cream that could be consumed for individuals with intolerance to this carbohydrate, considering the variable degrees of lactase deficiency for each individual.

Introduction

Ice cream is a product of high nutritional value, but it may cause adverse reactions in individuals who have restrictions on consumption due to lactose intolerance caused by absence or deficiency in the production of β-galactosidase, which is the enzyme responsible for the lactose hydrolysis in the intestine (Ingram and Swallow, 2009). Hypolactasia and lactose malabsorption accompanied with clinical symptoms, such as bloating, flatulence, nausea, abdominal pain and diarrhea, are termed lactose intolerance. Symptoms are caused by undigested lactose in the large intestine, where lactose is fermented by intestinal microbiota and osmotically increases the water flow into the lumen (Vasiljevic and Shah).

β-galactosidase can be incorporated into milk products such as frozen milk, condensed milk, and ice cream in order to avoid lactose crystallization, resulting in dairy products with a mealy or gritty texture. Also, the β-galactosidase can improve some technological properties, such as increasing digestibility, softness and creaminess (Grosová *et al.*, 2008), besides enabling consumption by lactose-intolerant individuals, considering the variable degrees of lactase deficiency.

Likewise, probiotics microorganisms ("live microorganisms that, when administered in adequate amounts, confer a health benefit on the host") (Hill *et al.*, 2014), can alleviate the symptoms of lactose intolerance, in addition to their several positive health effects, such as stabilization of the intestinal microbiota, competition with pathogens for binding sites on mucosal epithelial cells nutrients, and stimulation of the immune system (O'Flaherty and Klaenhammer, 2010; Wang *et al*., 2010; Vandenplas *et al*., 2015).

Probiotic microorganisms are mainly incorporated into dairy products (Souza and Saad, 2009; Pereira *et al.*, 2010). However, for use in foods, probiotic microorganisms must be resistant to the processing operations. Thus, for incorporation in ice creams, probiotics must be resistant to the processing conditions such as the beating step, air incorporation, and remain viable during frozen storage (Homayouni *et al*., 2012).

The aim of this study was to evaluate the viability of *Lactobacillus acidophilus* La-5 incorporated into ice cream produced with and without hydrolysis of lactose. Furthermore, the effects of the presence of La-5 and lactose hydrolysis on the physicochemical characteristics of the final product were evaluated.

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Materials and Methods

Chemical compounds and culture

For the manufacture of different ice cream formulations, the following ingredients were used: 74% of UHT whole milk (Frimesa, Capanema, Brazil), 9.75% condensed sweetened milk (Mococa, Aracatuba, Brazil), 3.7% refined sugar (União, São Paulo, Brazil), 9.85% UHT milk cream (Nestlé, Aracatuba, Brazil), 1.2% emulsifier (Duas Rodas Industrial Ltda, Jaraguá do Sul, Brazil), 1.2% stabilizer (Duas Rodas Industrial Ltda, Jaraguá do Sul, Brazil), 0.06% β-galactosidase Maxilactis enzyme (DSM, São Paulo, Brazil), 0.18% vanilla flavor (Mix, São Bernardo do Campo, Brazil), and probiotic culture *Lactobacillus acidophilus* La-5 (Christian Hansen, Hoersholm, Denmark). The probiotic culture employed to probiotic ice cream manufacture was freeze-dried commercial culture for direct vat inoculation (DVS culture). The probiotic culture of *L. acidophilus* La-5 was added at 0.05%, in order to achieve the minimum of 6.00 log CFU/g during the production of ice cream.

Ice cream manufacture

The variables involved in the production of ice cream formulations are presented in Table 1. Three pilot-scale ice cream-making trials, denoted T1, T2 and T3 were produced (three repetitions of each trial were produced on different days). The ice cream manufacturing steps are described in Figure 1. The hydrolysis of lactose was carried out as reported by (Campos *et al*., 2009).

Storage period and sampling

The formulations were stored frozen at -18°C for a period of 28 days before analysis. During this period, microbiological (enumeration of *Lactobacillus acidophilus* La-5) and physicochemical (pH and titratable acidity) determinations were performed once a week. Portions of each ice cream after 1 day of storage were also collected for subsequent chemical composition analysis of the final product.

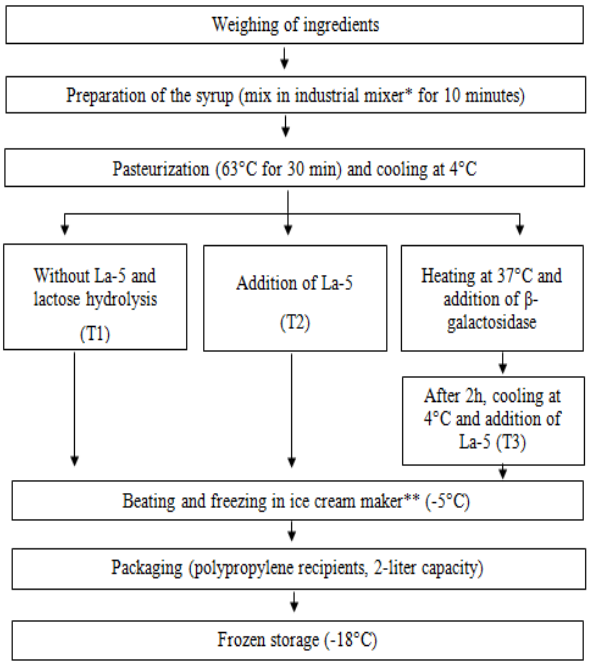
Enumeration of Lactobacillus acidophilus *La-5*

After the storage period described previously, portions of 25 g of ice cream were homogenized with 225 ml 0.1% peptone water using a Bag Mixer (Interscience, St Nom, France). Subsequent decimal dilutions were prepared using the same diluent. *L. acidophilus* La-5 was counted by pour-plating 1 ml of each dilution in DeMan-Rogosa-Sharpe (MRS) agar, after 2 days of aerobic incubation at 37°C for 48 hours (International Dairy Federation, 2012). La-5 determination was carried out in duplicate and the results were expressed in colony-forming units per gram of ice cream (CFU/g).

Table 1. Variables employed in the manufacture of ice cream

Ice cream	Probiotic culture[a]	Lactose hydrolysis[b]
T1[c]	-	-
T2	+	-
T3	+	+

+ = Presence. - = Absence.
[a] *Lactobacillus acidophilus* La-5 (Christian Hansen, Hoersholm, Denmark).
[b] Hydrolysis carried out with the addition of β-galactosidase Maxilactis enzyme (DSM, São Paulo, Brazil).
[c] Control formulation.



* Mixer 15 (Finamac, São Paulo, Brazil).
** PVS50 (Polo Sul, São Carlos, Brazil).

Figure 1. Protocol employed for the manufacture of ice creams T1, T2 and T3

Physicochemical analysis of ice cream

The pH values of the ice creams T1, T2, and T3 were determined in triplicate using a pHmeter Model Tec 3MP (Tecnal, Piracicaba, Brazil) equipped with a penetration electrode (Tecnal, Piracicaba, Brazil). Titratable acidity was determined with Dornic solution (Merck, Darmstadt, Germany) in the presence of phenolphthalein indicator, and the results were expressed in percentage of lactic acid. The chemical composition of the ice creams was determined in the final product (after one day of storage at -18°C). Ash was determined gravimetrically by incineration at 550°C in muffle furnace (FDG, São Paulo, Brazil). Protein was estimated by measuring the N content of

ice cream by the Kjeldahl method and multiplying by a conversion factor (6.38). Fat was determined through lipids extraction by Mojonnier method. Moisture content was determined by oven drying method at 105°C (Nova Ética, Vargem Grande Paulista, Brazil). All determinations were carried out in triplicate and according to standard methods of AOAC (Association of Official Agricultural Chemists, 2005). Carbohydrates content was calculated by difference to achieve 100% of total contents.

Determination of overrun

Overrun was determined for all ice cream from each batch, in duplicate samples, using the following equation: overrun (%) = $\rho_{syrup} - \rho i_{ce\ cream} \times 100 / \rho_{ice\ cream}$, where ρ = weight of 250 ml sample (Muse and Hartel, 2004).

Lactose hydrolysis

The lactose hydrolysis was performed in ice cream syrup (T3) after the pasteurization step. β - galactosidase was added (0.24 ml enzyme / 300 ml milk) and the ice cream syrup was gently homogenized during 2 minutes. The ice cream syrup was maintained at 37°C during 2 h (Campos *et al.*, 2009). After this period, the ice cream syrup was cooled to 4°C to probiotic addition.

Determination of lactose hydrolysis

In order to determine the percentage of lactose hydrolysis, the glucose concentration was determined in ice cream T3 (from each batch), in triplicate, by the glucose oxidase method, using the Glucose PP Kit (Gold Analisa Diagnóstica Ltda, Belo Horizonte, Brazil). The absorbance was measured in a spectrophotometer at 505 nm (Thermo Scientific, Waltham, United States of America). The results were calculated as mg/dl glucose and expressed in percentage of lactose hydrolysis (Campos *et al*., 2009).

Experimental design and statistical analysis

The experimental treatments and levels constituted a randomized complete block design replicated three times, with repeated measures at two or three time points. Statistical analysis was carried out using STATISTICA v.8.0 software (Statsoft Inc., Tulsa, United States of America). Data were checked for the normality and homogeneity of variances, using the Shapiro-Wilks and Brown-Forsythe tests, respectively, with α value = 0.05. When homogeneity of variances was verified, analysis of variance (ANOVA) was used to determine significant differences ($p < 0.05$) among different trials (T1, T2 and T3) and different days of storage (1, 7, 14, 21 and 28), using repeated measures. When ANOVA was significant ($p < 0.05$), differences between means were detected using post hoc Tukey's test. When homogeneity of variances was not verified, the equivalent non-parametric tests were applied: Kruskal Wallis test, followed by post hoc Mann Whitney U (different ice cream trial in the same storage period), or Friedman tests, followed by post hoc LSD rank (different storage period for a same ice cream trial). Post hoc Mann Whitney U and LSD rank tests were applied only when Kruskal Wallis and Friedman tests detected significant difference (Bower, 1997; Bower 1998a; Bower, 1998b).

Results and Discussion

Chemical composition, pH and titratable acidity

Table 2 shows the chemical composition of the ice cream samples. No significant differences were observed in the formulations T1, T2, and T3 for the parameters protein, ash, and total carbohydrates. However, significant difference was detected to moisture and fat, when T3 was compared to ice cream T1 and T2 ($p < 0.05$). The presence of the probiotic strain and hydrolysis of lactose are factors that not influence chemical characteristics of ice cream, specifically fat content. Several factors may affect milk chemical composition such as season and climate, diet, age, stage of lactation, and animal health (Farkye, 2004). This may explain the variation in this parameter, once it was not possible to use the same milk batch for all ice cream productions.

The pH values and the titratable acidity of the ice creams samples are shown in Table 3. No significant differences were detected when T1, T2 and T3 were evaluated between day 7 and 28 ($p > 0.05$). When all samples were compared in each day of storage, significant differences were found only when T1 was compared with T2 and T3 on day 14 ($p < 0.05$). Silva Junior and Lannes (2001) found pH values of 6.47 and 6.60 for ice cream prepared without addition of probiotics. Regarding the titratable acidity values, all formulations showed no significant differences during the storage period (28 days) ($p > 0.05$). The titratable acidity of the formulation containing the probiotic microorganism (T2) was statistically higher ($p < 0.05$) than the values observed for T1 and T3. However, these changes did not affect the viability of the microorganism *Lactobacillus acidophilus* La-5, since the pH values of the ice cream samples remained close to neutrality, which is considered optimal for survival of probiotics in this type of food matrix (Homayouni *et al*., 2012).

Table 2. Chemical composition and moisture content (mean* ± standard deviation) of the formulations T1 (control – without addition of *L. acidophilus* and lactose hydrolysis), T2 (addition of *L. acidophilus* without lactose hydrolysis), and T3 (addition of *L. acidophilus* and lactose hydrolysis) in the final product, after 1 day of storage at -18°C

Ice cream	Moisture	Fat	Protein	Ash	Carbohydrates
T1	74.50±0.39^{A}	7.36±0.10^{A}	3.57±0.08^{A}	0.82±0.02^{A}	13.75±0.54^{A}
T2	76.28±0.41^{A}	7.23±0.26^{A}	3.68±0.50^{A}	0.83±0.00^{A}	11.99±0.85^{A}
T3	78.64±0.47^{B}	8.20±0.92^{B}	3.71±0.03^{A}	0.83±0.00^{A}	12.62±1.17^{A}

* results in percentage.
^{A,B}: Different lowercase superscript letters in the same column indicate significant differences ($p < 0.05$) between the different ice creams trials.

Table 3. Physicochemical parameters (pH and titratable acidity) of the formulations T1 (control – without addition of *L. acidophilus* and lactose hydrolysis), T2 (addition of *L. acidophilus* without lactose hydrolysis), and T3 (addition of *L. acidophilus* and lactose hydrolysis) after 7, 14, 21, and 28 days of storage at -18°C

Ice cream	Storage period (Days)	pH	Titratable acidity (% of lactic acid)
	7	6.68±0.26^{Aa}	0.14±0.01^{Aa}
	14	7.06±0.34^{Ab}	0.13±0.00^{Aa}
T1	**21**	6.94±0.35^{Aab}	0.14±0.01^{Aa}
	28	6.67±0.30^{Aa}	0.14±0.01^{Aa}
	7	6.52±0.06^{Aa}	0.19±0.05^{Ba}
	14	6.55±0.30^{Ba}	0.19±0.05^{Ba}
T2	**21**	6.60±0.22^{Aa}	0.17±0.04^{Ba}
	28	6.61±0.10^{Aa}	0.18±0.06^{Ba}
	7	6.52±0.28^{Aa}	0.16±0.01^{Aa}
	14	6.85±0.11^{Aa}	0.15±0.01^{Aa}
T3	**21**	6.24±0.13^{Aa}	0.14±0.01^{Aa}
	28	6.58±0.06^{Aa}	0.15±0.00^{Aa}

^{A,B}: For each day of storage, different uppercase superscript letters in the same column indicate significant differences ($p < 0.05$) between different trials.
^{a,b}: For each trial, different lowercase superscript letters in the same column indicate significant differences ($p < 0.05$) during the whole storage period.

Viability of L. acidophilus *La-5, overrun, and lactose hydrolysis*

The values found for the populations of the probiotic microorganism *Lactobacillus acidophilus* La-5 in the formulations T2 and T3 are presented in Table 4. To exert health benefits, probiotic bacteria must be viable and available in high concentrations, typically 10^6 CFU/g product (Shah, 2007). No significant differences were observed for T2 and T3 during the whole storage period ($p>0.05$). When the formulations were compared, no statistically significant differences were observed ($p>0.05$).

Regarding lactose hydrolysis and the effect over La-5 viability, for the formulation T3, 56% lactose was hydrolyzed. However, La-5 populations observed in these periods were not influenced due to the hydrolysis step, since the La-5 counts observed to T3 did not differ from T2 ($p > 0.05$). So, in this study La-5 did not use the hydrolysis products for metabolism during the period in which the syrup remained at 37°C. However, both formulations containing *L. acidophilus* La-5 showed satisfactory populations for a probiotic food, once populations above 10^7 CFU/g were observed throughout the storage period. Similarly, Abghari *et al*. (2011) observed populations above 10^7 CFU/g in unfermented ice cream produced with *Lactobacillus acidophilus*.

In another study of goat milk-based ice cream supplemented with three different probiotic strains (*Lactobacillus acidophilus* La-5, *Propionibacterium jensenii* 702, and B*ifidobacterium animalis* subsp. *lactis* BB-12), Ranadheera *et al.* (2013) found results that were consistent with the observed in this study, since La-5 populations ranged from 7.70 to 7.38 log CFU/g at day 1 and 52, respectively.

Lower populations were observed by several authors when compared to the present study. Nousia *et al*. (2011) studied ice cream containing *Lactobacillus acidophilus* LMGP-21381, and found populations of 6.87 log CFU/g during three weeks of storage at -15°C. Magariños *et al.* (2007) and Corrales *et al.* (2007) found La-5 populations of 10^6

Table 4. Populations of *Lactobacillus acidophilus* La-5 (mean ± standard deviation) of the formulations T2 (addition of *L. acidophilus* without lactose hydrolysis) and T3 (addition of *L. acidophilus* and lactose hydrolysis) after 7, 14, 21, and 28 days of storage at -18°C

	Populations of *Lactobacillus acidophilus* La-5 (log CFU/g)	
	Ice cream	
Storage period (days)	**T2**	**T3**
7	7.36 ± 0.11^{Aa}	7.61 ± 0.17^{Aa}
14	7.39 ± 0.18^{Aa}	7.60 ± 0.06^{Aa}
21	7.51 ± 0.44^{Aa}	7.57 ± 0.13^{Aa}
28	7.71 ± 0.24^{Aa}	7.67 ± 0.30^{Aa}

[A]: For each day of storage, same uppercase superscript letter in the same row indicate no significant differences ($p > 0.05$) between different trials.
[a]: For each trial, same lowercase superscript letters in the same column indicate no significant differences ($p > 0.05$) during the whole storage period.

CFU/g at the end of the storage period, of 60 to 85 days, respectively.

Akin *et al*. (2007) found *Lactobacillus acidophilus* counts of 6 log CFU/g in ice cream containing probiotics after 30 days of storage, and observed populations above 7 log CFU/g only when the ice cream samples were supplemented with inulin. In the present study, even without the addition of a prebiotic ingredient, the La-5 populations were greater than 7.00 log CFU/g throughout the frozen storage of the ice cream samples.

The ice cream manufacturing process promotes the incorporation of large amounts of air to the product through the beating process, which can result in cell death of the probiotic cultures due to oxygen toxicity (Homayouni *et al.*, 2012). Some studies have suggested that probiotic microorganisms could only survive in food matrices suffering oxygen incorporation during its production whether any protection against oxygen toxicity was used (Kailasapathy and Sultana, 2003; Gaudreau *et al*., 2013). The overrun values of the formulations T1, T2, and T3 were 44.1%, 43.6%, and 43.8%, respectively, with no significant differences for all formulations ($p>0.05$). These results demonstrated that the *Lactobacillus acidophilus* La-5 strain has advantages for use in ice cream, since even without protection against the action of oxygen on La-5, the incorporation of air did not affect the viability of this microorganism. The results are in agreement with Ferraz *et al.* (2012) that observed that 45% of overrun did not influenced *Lactobacillus acidophilus* populations. The authors reported that values of 60% and 90% of overrun resulted in decrease in probiotic viability. For the 90% overrun trial, a decrease of 2 log CFU/g was observed.

In addition to the oxygen incorporation, freezing can also affect the viability of probiotic microorganisms in ice cream resulting often in lower bacterial populations (Akin *et al*., 2007; Homayouni *et al*., 2012). However, in this study, no changes were observed for the La-5 populations in the ice cream samples (T2 and T3). Once it is a frozen product, its characteristics remained unchanged, and thus it can be a good matrix for supplementation with microorganisms such as *Lactobacillus acidophilus* La-5. Studies on longer storage periods are needed to determine the shelf life of this type of product.

Conclusion

The ice cream has proven to be an excellent matrix for delivery of *Lactobacillus acidophilus* La-5, with sufficient populations to be classified as probiotic food, without protection against oxygen toxicity. The hydrolysis of lactose did not influence the viability of this microorganism in the products. However, this step contributed to obtaining a functional ice cream that could be consumed for individuals with intolerance to this carbohydrate, considering the variable degrees of lactase deficiency for each individual.

Acknowledgements

The authors would like to thank to Conselho Nacional de Pesquisa e Desenvolvimento (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for fellowships.

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