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Dried Tomato-Flavored Probiotic Cream Cheese with *Lactobacillus paracasei*

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Abstract: A dried tomato-flavored probiotic cream cheese (P) containing *Lactobacillus paracasei* Lpc-37 was developed for the purpose of this study. The same product, but without probiotic addition (C) was used as control. *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* were used as lactic starter cultures. Chemical composition analyses and sensory tests were performed on days 1 and 7, respectively. Titratable acidity, pH value and *L. paracasei* population were determined every 7 d during the refrigerated storage (21 d) of the cream cheeses. The experiment and analyses were performed in triplicate, using standard methods. Probiotic population remained greater than $10^7$ CFU/g throughout the storage period, thereby characterizing the product as potentially probiotic. Cream cheeses C and P did not differ on the sensory tests, both obtaining good overall acceptance by the consumers, of which 82.6% stated that they certainly or probably would buy the product.

Keywords: cream cheese, functional food, *Lactobacillus paracasei*, probiotics

Practical Application: *Lactobacillus paracasei* Lpc-37 is a probiotic bacterium and clinical studies have shown that this microorganism beneficially affects its host. In general, dried tomato-flavored products and cream cheese are products with good acceptance by the consumers. Thus, regular consumption of the probiotic cream cheese developed in this study may have positive effects on health and well being of people if incorporated into their diet.

Introduction

When consumers are asked about factors that influence their food choices, one reason they mention is health. Functional foods offer a positive health message for people, once they can produce a positive effect in their organism or, even, avoid a negative one (Lähteenmäki 2003).

Probiotics are defined as “live microorganisms that when administered in adequate amounts confer health benefit on the host” (FAO 2002). These effects include the promotion of gastrointestinal resistance to colonization by pathogens; reduction of pathogen populations through the production of acetic and lactic acids, besides bacteriocins and other antimicrobial compounds; promotion of lactose digestion in lactose intolerant individuals; stimulation of the immune system; alleviation of constipation and increase of minerals and vitamins absorption (Tuohy and others 2003; Sullivan and Nord 2005; Minocha 2009).

Cheese appears to be a good substrate for the development of new probiotic foods, particularly some types of fresh cheeses, which, due to their technological characteristics, have a series of advantages over other products (Roupas and Williams 2007; Ribeiro and others 2009), such as: pH values that do not inhibit probiotic multiplication and relatively high water activity and fat content (Stanton and others 1998).

Different types of cheeses have been successfully tested as vehicles for probiotic strains of *Lactobacillus* and *Bifidobacterium*: Malaysian Crescal cheese (Souza and Saad 2009), Petit-suisse cheese (Cardarelli and others 2008), Cheddar (Sharp and others 2008), Cottage (Blanchette and others 1996), Argentinian fresh cheese (Vinderola and others 2009), Turkish Beyaz cheese (Kilic and others 2009) and goat cheese (Gomes and Malcata 1998).

Fresh cream cheese is an unripened cheese, stored at refrigeration temperatures, obtained by blending and homogenizing a fresh cheese base with other ingredients, like hydrocolloids, salt, and spices. It can be used as a spread on bread and as a salad dressing. Because of its manufacturing process, this cheese appears to be an ideal vehicle for probiotic bacteria (Heller and others 2003).

The addition of probiotics in cheese manufacturing faces many challenges, the most important being maintaining the sensory characteristics of the traditional cheese (Bruhn and others 2002) and the survival of the probiotic bacteria throughout the cheese making process (Fortin and others 2011).

The current Brazilian food legislation sets forth that, a product should contain a minimum number of viable probiotic cells between $10^7$ and $10^9$ CFU/d per daily portion up to the end of its shelf-life in order to produce the claimed probiotic benefits (Brazil 2008). However, it is consensus for the international scientific community that the effects of probiotic microorganisms can vary depending on the strain, quantity ingested as well as the
physiological characteristics of the host (FAO 2002; Isolauri and others 2004; Tiihonem and others 2010).

The probiotic strain used in this work, has demonstrated excellent adhesion to human epithelial cell lines (Caco-2), the strain induced the inhibition of selected pathogens in in vitro studies. Moreover, the studies in vitro and in animal model, Lactobacillus paracasei Lpc-37 demonstrated ability to modulate the immune system, confirming its ability to interact to the balance the intestinal mucosal immune response (Technical memorandum TM 56-Ie, Danisco).

In addition, L. paracasei Lpc-37 was tested for its ability to stabilize the human intestinal microbiota during and after antibiotic therapy (Engelbrekton and others 2006). The authors observed that this strain was able to reduce the antibiotic-induced disturbance of the total microbiota and maintained bifidobacteria at significantly higher levels than that found in the placebo group 2 wk after the cessation of antibiotic therapy.

The international regulations, recommended that the following information should be described on the label of probiotic foods: genus, species and strain designation; minimum viable numbers of each probiotic strain at the end of the shelf-life; health claim(s); suggested serving size related to the health claim; proper storage conditions; and corporate contact details for consumer information (FAO 2002). This study aimed to develop and characterize a dried tomato–flavored cream cheese with the addition of probiotic microorganisms.

Materials and Methods

Production of dried tomato–flavored cream cheese

After preliminary tests, 2 cream cheese formulations were defined: probiotic cream cheese, added with 2% of probiotic microorganism L. paracasei Lpc-37 (LYO 50 DCU, Danisco, Dangé-Saint-Romain, France) (Technical memorandum TM 56-Ie 2012) and control cream cheese, without the probiotic addition, designated as P (probiotic) and C (control), respectively. Commercial skimmed milk (Primesa, Marechal Cândido Rondon-PR, Brazil) was heated up to 37 °C, when then calcium chloride (0.25 g/L) and starter cultures (1% Lactococcus lactis subsp. lactis and L. lactis subsp. cremoris, Danisco Dangé-Saint-Romain, France) were added. L. paracasei Lpc-37 was added at the same time as the starter cultures in the probiotic cream cheese. After homogenization, commercial rennet (0.05 g/L; HÁ-LA, Niebüll, Germany) was incorporated. Upon the formation of a firm curd (pH 5.6 to 5.8), it was gently diced, separated from the whey and left to stand for 15 min to allow remained whey to drain off. The cheese curd was then transferred to sterilized cotton bags and kept for 14 h at refrigeration temperature (5 °C) until all the residual whey had drained off. Next, the curd was homogenized with 29.6% pasteurized cream (20% of fat, Nestlé, Araçatuba, Brazil), 0.5% xanthan gum (Cargill, Cosmópolis, Brazil), 18.4% sliced dried tomato (Speciale, Limeira, Brazil), and 1.5% sodium chloride (Cisne Salt, Cabo Frio, Brazil), using a kitchen mixer (Britânia, Camaçari, Brazil). After homogenization, the products were packaged in polypropylene pack (600 g) with lids and stored under refrigeration (5 °C). The experiment was done in triplicate, as well the physicochemical and microbiological analyses.

Storage and sampling days

The cheese creams were analyzed for ash, protein, fat, carbohydrate, and moisture contents after 1 d of refrigerated storage (5 °C). L. paracasei Lpc-37 counts, pH and acidity were determined on days 1, 7, 14, and 21 of storage. Sensory analysis was performed 7 d after manufacture, as this is considered to be the minimum time necessary for the finished product to develop the balanced flavor produced by the blend of the different added flavoring components.

Analyses

Physicochemical analyses. All analytical procedures were performed according to official standard methods (AOAC 2003). The moisture content was determined in a forced air-drying oven (105 °C for 16 h). Ash was gravimetrically determined by incineration in a muffle furnace at 550 °C. The protein level of the samples was estimated by measuring the total nitrogen content with the Kjeldahl method, followed by multiplying the result by a conversion factor (6.38). Fat was determined by the Gerber method. The carbohydrate content was calculated by difference. The pH values were determined with a digital potentiometer (Tecnal, Piracicaba, Brazil) at room temperature. Acidity was measured by titration and expressed as percentage lactic acid.

Microbiological analyses. Portions of 25 g of each sample were aseptically collected, blended with 225 mL of 0.1% peptone water (Himedia, Mumbai, India) and serially diluted using the same diluent. L. paracasei Lpc-37 was enumerated by pour-plating 1 mL of each dilution on De Man–Rogosa–Sharpe agar (MRS agar, Himedia, Mumbai, India) acidified to pH 5.4 with acetic acid (Synth, Diadema, Brazil). The colonies were counted after 72 h of anaerobic incubation (Anaerobic System Anaerogen – Oxoid, Basingstoke, UK) at 37 °C, and the results were expressed as log colony forming units per gram of cream cheese (log CFU/g) (Oliveira and others 2001). The absence of cocci typical of the starter culture (Lactococcus species) on the plates was confirmed by sampling randomly the colonies and submitting them to Gram stain test.

The cream cheeses were evaluated by yeasts and moulds, fecal coliforms, Salmonella and coagulase-positive Staphylococcus analyses (APHA 2001), following international and Brazilian legislations recommendations to ensure their safety for consumption (Brazil 2001).

Sensory analyses. A triangle sensory test was carried out to evaluate whether there was an immediately perceptible difference between the control and the probiotic cream cheeses. Untrained panelists (42) were invited to the Universidade Norte do Paraná campus to participate in the test. This test was carried out in individual booths between 8:30 and 10:30 a.m., under white lighting equivalent to daylight. At the sensory session, 3 coded samples were presented simultaneously to the panelists, being 2 of them equivalent to daylight. The sensory session, 3 coded samples were presented simultaneously to the panelists, being 2 of them equal and 1 different. Each panelist had to indicate which sample was the different one. Sample presentation orders were balanced and randomized among the panelists.

The sensory acceptance test was carried out with 109 untrained panelists recruited from the staff and students of the University. This test was realized using the control cream cheese only, since the consumers did not detect difference between the samples in the triangle test (P > 0.05). Sensory sessions were performed in individual testing booths between 8:00 and 10:30 a.m. and from 6:00 to 7:30 p.m., under white lighting. Portions of approximately 20 g of the cream cheese were spread onto a cream cracker and served on disposable white polyethylene trays, coded with randomized 3-digit numbers. Each panelist used a ten point-numeric hedonic scale (0 = disliked extremely; 5 = neither liked nor disliked; 10 = liked extremely) to indicate his overall liking score (Villanueva and others 2000).
L. paracasei in flavored cream cheese...

Table 1–Means and standard deviation of the percentage composition of dried tomato-flavored cream cheeses.

<table>
<thead>
<tr>
<th>Components</th>
<th>Control cream cheese</th>
<th>Probiotic cream cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (%)</td>
<td>11.61 ± 1.41</td>
<td>10.35 ± 0.32</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>9.36 ± 0.18</td>
<td>9.06 ± 0.35</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>11.10 ± 0.90</td>
<td>9.97 ± 1.71</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>3.13 ± 0.29</td>
<td>3.27 ± 0.24</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>64.80 ± 1.14</td>
<td>67.36 ± 1.37</td>
</tr>
</tbody>
</table>

The same 109 people were asked to indicate their intention of purchase the product using a verbal numeric scale from 1 to 5 (1 = I would not buy certainly; 3 = Maybe I would buy, maybe not; 5 = I would buy certainly).

The Ethics Committee of the Universidade Norte do Paraná approved the study (protocol PP/0200/08) and terms of free and enlightened participation were signed by all consumers.

Experimental design and statistical analyses. The analyses of the physicochemical and microbiological data were carried out using a mixed model (PROC MIXED procedure of SAS 8.02 software) to determine the comparison of the means of the 2 different treatments. Transformations of outcomes were applied when the constant variance of the residuals assumptions were violated (Littel and others 1996). A table of the American Society for Testing and Materials (1968), based on the chi-square test, was used to analyze the triangular test data. Hedonic data were subjected to a two-way ANOVA (samples and consumers) and Tukey’s multiple means comparison test (HSD).

Results and Discussion

Chemical composition

Knowing the percentage composition of a product is important to help the consumers to make the most appropriate food choices for them. From Table 1, it can be observed that protein, fat, carbohydrates, ash, and moisture contents were similar (P > 0.05) for cheeses C and P.

At the present time, there is no specific regulation or standards stipulating minimum or maximum nutrient values for cream cheese in Brazil. However, the cheeses produced for the purpose of this study can be classified as semifat and extra-high moisture cheeses, according to the Brazilian Technical Regulation on Identity and Quality of Cheeses (Brazil 1996). In addition, the legal specifications for cream cheese and related products, published by the dairy division of the United States Dept. of Agriculture, classify this product as a light cream cheese with other foods (Type II, Class C). The standard establishes features of 70% maximum moisture, 16.5% maximum milk fat, and 1.4% maximum salt content (USDA 1994). The cream cheeses developed in this work accomplished these requirements, except for salt content, that was slightly higher (1.5%).

The cheeses developed for this study exhibited lower fat contents and higher carbohydrate levels than those commonly found in this type of product, a fact probably due to the addition of dried tomato, an ingredient that contains a high amount of sugar in its composition. On the other hand, probiotic and symbiotic creamy cheese developed by Buriti and others (2008) presented fat contents similar to those of this study.

Titratable acidity and pH

There was a nonsignificant (P > 0.05) increase in the pH values of the C and P cheeses in the 1st wk of refrigerated storage (Figure 1). A small reduction in these values was observed (P < 0.05) in the course of the following weeks, probably as a result of the production of lactic acid and other organic acids by both the starter cultures and the probiotic culture (Maruyama and others 2006). The acidity of cheese C showed a nonsignificant increase (P > 0.05) throughout time, while it increased significantly (P < 0.05) in cheese P up to the 14th d of storage.

A comparison between the formulations shows that the pH values of cheese C, up to the 14th d, were smaller than those of cheese P (P < 0.05). With regard to acidity, it was found that the control cheese showed values greater than those observed for the probiotic product (P < 0.05), except for the 14th d (Figure 1). Stanton and others (2001) also observed that cheeses containing probiotics exhibited higher pH values as compared to the cheeses that did not contain these microorganisms, and explained these higher pH values as being the result of the greater proteolytic activity, which may lead to the release of amines, which, on their turn, may exert a buffering effect.

L. paracasei Lpc-37 viability

The population of L. paracasei Lpc-37 (CFU/g) in cheese P throughout the 21 d of refrigerated storage investigated is depicted in Figure 2.
The initial *L. paracasei* Lpc-37 population in the cream cheese produced for this study was about 8 logarithmic cycles/g. This value declined during the 1st wk of storage, increased in the 2nd, and remained constant in the 3rd wk. Although this variation had been considered no significant statistically (P > 0.05), a slight growth trend was observed. Vinderola and others (2009) also observed a fluctuation in the number of viable cells of *L. paracasei* A13 from fresh cheese stored at 12 °C; nevertheless, at 5 °C the authors observed an increase of cell number during the shelf life.

The main challenge facing the use of probiotics in foods is maintaining their viability during manufacture and storage. In this study, the population of *L. paracasei* Lpc-37 remained stable throughout the storage period investigated, and presented population above 7.5 logarithmic cycles/g. The amount of viable cells, their species or strains are directly responsible for the beneficial effects on the hosts, although the minimum usually suggested for probiotic bacteria in foods is $10^8$ to $10^7$ CFU/g (Ross and others 2002; Souza and others 2008).

In addition, considering the fact that a person would consume at least 10 g of cream cheese/day, this means that the product complied with the requirements of Brazilian food regulations, which require that, in order to be considered probiotic and approved to make health claims on the product label, the product should contain a minimum number of viable probiotic microorganisms between $10^8$ and $10^7$ CFU per daily portion (Brazil 2008) up to the end of its shelf life to deliver the claimed health benefits. The literature reports that probiotic effects are species and strain specific (FAO 2002; Isolauri and others 2004; Tiihonen and others 2010). *L. paracasei* Lpc-37 had already shown probiotic characteristics (Technical memorandum TM 56-1e, Danisco), and can be used to compose probiotic cream cheese and other foods.

Champagne and Gardner (2005) found that some probiotics have their viability reduced in cheeses during storage. However, our results corroborate those obtained by other authors, who found that cheeses are excellent vehicles for probiotic microorganisms (Sharp and others 2008; Mäkelainen and others 2009; Souza and Saad 2009; Araújo and others 2010). Some of the cream cheese characteristics can help to maintain the viability of probiotic microorganisms, such as a pH close to neutral, high water activity, low salt concentration, and a semisolid food matrix with a relatively high lipid concentration (Buriti and others 2005).

Sensory analyses

The results of the triangular test demonstrated that a significant part of the participants was not able to distinguish the control cheese from the probiotic cheese ($P > 0.05$), thereby evidencing that the high counts of *Lactobacillus* in the cheese did not cause any significant changes in the sensory characteristics of the traditional product.

According to Champagne and Gardner (2005), in general, the use of probiotic cultures does not drastically change the sensory properties and attributes of the foods to which they are added. In spite of the fact that this had not been observed in this study, the addition of these cultures to cheeses has been linked to an improvement of the sensory characteristics by others authors (Katsiari and others 2002; Souza and others 2008).

Given that no difference was verified between the control and probiotic cheese, only the control product was subjected to the overall acceptance and purchase intention tests by potential buyers. The cream cheese achieved good overall acceptance, with a mean liking score of 8.1, and with 60.36% of the participants assigning scores varying from 8.1 to 10.0 and 24.32% scores between 6.1 and 8.0. Liking scores between 2.0 and 6.0 represented only 15.32% of the consumers (Figure 3). None of the participants assigned a score lower than 2 to the product.

If the dried tomato-flavored cream cheese were commercialized, 82.56% of the sensory panelists would certainly or probably buy the product. Only 5.51% stated that they would probably or certainly not purchase the product (Figure 4).

The results also showed that 85.96% of the panelists who had stated “I would buy certainly” assigned liking scores between 8.1 and 10.0 in the overall acceptance test for the cream cheese, whereas 14.04% assigned scores between 6.1 and 8.0. The liking scores of the sensory panelists that “would buy probably” the product fell between 6.1 and 10.0. As for the choice “maybe I would buy, maybe not,” 30.77% of the panelists assigned scores between 6.1 and 8.0 and 69.23% between 4.1 and 6.0. With regard to those, who responded that they “probably would not buy” or “certainly would not buy,” 83.3% of the panelists assigned overall liking scores between 2.0 and 4.0. The data demonstrate a high level of acceptance of the product, which has been very few explored commercially.

Moreover, many panelists, using the comment field, classified the product as creamy, smooth and palatable. These features are in agreement with Johansen and others (2008), who analyzed the sensory properties of cream cheese, emphasizing that the most important part of the dynamic texture perception occurs in the mouth, during mastication. This general impression can be, probably, responsible to the high acceptance of the product in this study.

**Figure 3**–Scores assigned to dried tomato-flavored cream cheese in the overall sensory acceptance test. Scale: 0 = disliked a lot; 5 = neither liked, nor disliked; 10 = liked a lot.

**Figure 4**–Intention to purchase dried tomato-flavored cream cheese of the sensory panelists.
L. paracasei in flavored cream cheese . . .

Conclusion
Dried tomato-flavored cream cheese was proved to be an adequate vehicle for incorporating and carrying L. paracasei Lpc-37. The microorganism showed high level of survival during manufacture and refrigerated shelf life of the cream cheese. Furthermore, the product had a high index of sensory acceptance by the consumers.

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References


