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Synbiotic potential of fresh cream cheese supplemented with inulin and *Lactobacillus paracasei* in co-culture with *Streptococcus thermophilus*

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Abstract

The influence of the addition of *Lactobacillus paracasei* and *Streptococcus thermophilus* on the fructan content at the beginning and at the end of storage at 4 ± 1 °C of a potentially synbiotic fresh cream cheese manufactured with inulin was investigated. Three cheese-making trials were prepared, all supplemented with a lactic culture of *S. thermophilus* (T1, T2 and T3). *L. paracasei* subsp. *paracasei* was added in T1 and T2. Inulin was added in T2 and the fructan content was measured after 1 and 21 days of storage. Samples of T2 possessed similar mean concentrations of fructans after 1 and 21 days of storage, 7.32% and 7.27%, respectively, and no significant difference was observed. These results indicated that the metabolism of starter and probiotic bacteria did not degrade the fructans present in those cheeses. Additionally, synbiotic cheeses possessed a fructan content higher than 7 g per 100 g, sufficient to confer prebiotic potential during the entire storage period of these products.

Keywords: Fructan; Inulin; *Lactobacillus paracasei*; *Streptococcus thermophilus*; Synbiotic; Cheese

1. Introduction

The definition of the term probiotic has evolved through the years (Sanders, 2003). The most recent definition says that probiotics are live microorganisms administered in amounts that positively affect the health of the host (FAO/WHO, 2001; Sanders, 2003). *Lactobacillus casei/paracasei* strains have been widely studied with respect to their 'health-promoting' properties, being currently employed as probiotic or mixed cultures in dairy products (Felis, Dellaglio, Mizzi, & Torriani, 2001; Itsaranuwat, Al-Haddad, & Robinson, 2003; Medici, Vinderola, & Perdigón, 2004; Stanton et al., 1998; Stiles & Holzapfel, 1997).

In addition to the probiotic approach of directly introducing live bacteria to the colon through dietary supplementation, another approach to increase the number of beneficial bacteria, such as bifidobacteria, in the intestinal microbiota is through the use of prebiotics. Prebiotics are non-digestible dietary components that pass through to the colon and selectively stimulate the proliferation and/or activity of populations of desirable bacteria *in situ* (Mattila-Sandholm et al., 2002). Pre- and probiotics may be combined in a food product, called a synbiotic (Holzapfel & Schillinger, 2002). Fructooligosaccharides (FOS) and inulin-type fructans have been those most studied as prebiotics (Fooks, Fuller, & Gibson, 1999; Gilliland, 2001; Roberfroid, 2005). Fructans are carbohydrates, in which most of the glycosidic bonds are made of fructosyl-fructose bonds, and usually have a terminal glucose unit. Inulin is a linear β-(2 → 1)-linked fructose polymer that occurs in garlic, asparagus root, Jerusalem artichoke, dahlia tubers or chicory root (Rocha, Catana, Ferreira, Cabral, & Fernandes, 2006).

Fresh cream cheese is a cheese obtained from the homogenisation of a fresh cheese base with further ingredients, including gums and hydrocolloids, salt and other

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spices. Fresh cream cheese is a versatile food that permits addition of other ingredients, including fibres like inulin. It has a spreadable structure, and is used as a spread on bread, in sandwiches and as a salad dressing. Due to its manufacturing process, fresh cream cheese appears to be ideally suited to serve as a carrier for probiotic bacteria. It is an unripened cheese, stored at refrigeration temperatures, and the shelf life is rather limited (Heller, Bockelmann, Schrezenmeir, & deVrese, 2003).

A fresh cream cheese could possess synbiotic effects, due to the simultaneous incorporation of *Lactobacillus paracasei* and inulin. Nonetheless, the possible degradation of inulin added to this cheese, by the metabolism of probiotic and starter bacteria, should be investigated.

The aim of the present study was to evaluate the prebiotic potential of a synbiotic fresh cream cheese manufactured with inulin and supplemented with a potentially probiotic *L. paracasei* subsp. *paracasei* culture in co-culture with *Streptococcus thermophilus*, and to compare the physicochemical characteristics of the product with probiotic and control fresh cream cheeses during storage at 4 ± 1 °C for up to 21 days.

2. Materials and methods

2.1. Fresh cream cheese manufacture

Three pilot-scale fresh cream cheese-making trials, denoted T1, T2 and T3, were performed in quadruplicate (and a fifth batch of each trial was produced for determinations of pH, moisture, fat, protein and ash), all of them using *S. thermophilus* (TA 040, Danisco, Dangé, France) as starter culture for the production of the cheese base. Cream cheeses T1 and T2 contained the potential probiotic culture of *L. paracasei* subsp. *paracasei* (LBC 82, Danisco), and the *L. paracasei* subsp. *paracasei* probiotic strain, together with the prebiotic fibre inulin (Raftiline® HP-Gel, Orafti, Oreye, Belgium), respectively. Cream cheeses T3 (control fresh cream cheeses) were only supplemented with *S. thermophilus*.

Cheese base was manufactured in 10–l vats from commercial pasteurised milk (Xandô, Fazenda Colorado, Araras, Brazil; high temperature short time [HTST]) heated to 42–43 °C, after which addition of cultures proceeded. Both cultures employed were freeze-dried commercial cultures for direct vat inoculation and they were added at 30 mg l^{-1}, in order to achieve 10 log cfu l^{-1} (starter culture) and, at 10 mg l^{-1}, in order to achieve 9 log cfu l^{-1} (probiotic culture). Calcium chloride (0.25 g/l) was also added in all trials. In the next step, all vats were allowed to set at 42 °C. As soon as the pH reached values of 6.3–6.4, commercial rennet Ha-la (88–92% bovine pepsin + 8–12.5% bovine chymosin; Christian Hansen, Valinhos, Brazil, 50 mg l^{-1}) was added to the cheese-milk. When the curd was formed, the gel was gently cut into cubes, placed in sterilised cotton cheesecloth, and allowed to drain at 15 °C for 6 h. Part of the whey removed during draining of the cheese base, for production of fresh cream cheese T2, was placed in sterilised screw-cap-flasks and immediately refrigerated at 4 ± 1 °C, for subsequent use for dissolving inulin. After draining, the cheese base was cut and placed in sterilised beakers, covered with a PVC film and incubated at 13 °C overnight, to acidify and achieve pH values of 5 or less. On the following day, the beakers containing the cheese base were stored at 4 ± 1 °C, until the homogenisation stage, which took place after the addition of the further ingredients. Proportions of 98.7% and of 74.7% of cheese base were used in the production of cheeses T1 and T3, and of cheese T2, respectively. The three fresh cream cheese-making trials (T1, T2, and T3) were prepared through the addition of salt (NaCl; 0.8% of final product) and xanthan gum (Rhodigel 80, Rhodia, Melle, France, 0.5% of final product) to the fresh cream cheese. For cheeses T2, the inulin Raftiline® HP-Gel was previously dissolved in whey heated at 55–60 °C, in the proportion of one part of inulin to two parts of whey, to achieve 24% of the total ingredients of this trial, and immediately incorporated into the cheese base through homogenisation, during which a smooth and homogeneous cream was formed.

After homogenisation, cheeses were packaged in individual plastic cups, each one containing 40 g of cheese, sealed with a metallic cover, and stored under refrigeration (4 ± 1 °C) for up to 21 days. On the following day, cheeses were sampled for microbiological and physicochemical analysis of the final product.

2.2. Sample collection

Fresh cream cheeses from each batch were used for analysis of the final product (day 1) and after 21 days of storage. At each sampling day, at least six cups containing fresh cream cheese from the same batch and trial were used for analysis. For microbiological analysis, portions of 25 g were collected aseptically from two cups, after a quick mixing of the fresh cream cheese inside each cup with a sterilized spoon. Portions of fresh cream cheese from the other cups were collected for physicochemical analysis. Samples of the fresh cream cheese T2 after 1 and 21 days of storage were freeze dried for fructan determination.

2.3. Physicochemical analysis of fresh cream cheeses

The pH values of cheeses were determined on triplicate samples with a pH meter (Analyser Model 300 M; Analyser, São Paulo, Brazil) equipped with a penetration electrode model 2AO4 GF (Analyser). Water activity (a_w) at 25 °C was determined on triplicate samples using the Novasina a_w-Center AWC 503-C equipped with a three-compartment a_w box (Novasina, Pfäfikon, Switzerland). Moisture content was determined in 5 g samples by drying at 70 °C under vacuum (Marconi MA030112, Piracicaba, SP, Brazil) for 24 h.

The mean composition of fresh cream cheeses (ash, fat and protein content) was determined in the final product (after one day of storage at 4 ± 1 °C) in triplicate grated samples. Ash was determined gravimetrically by heating the 2 g dried samples at 550 °C, until completely ashed. Protein was estimated by measuring the N content of cheeses by the Kjeldahl method and multiplying by a conversion factor (6.38), after drying 5 g of cheese samples. Fat was determined through extraction of lipids with ethyl ether, using the Soxhlet device. Analytical procedures followed the appropriated standard methods (Instituto Adolfo Lutz, 1985).

2.4. Microbiological analysis of fresh cream cheeses

Counts of *L. paracasei* for probiotic cheeses T1 and T2 and of *S. thermophilus* for cheeses T1, T2 and T3 were monitored during the storage period. For microbiological analysis, 25 g portions of duplicate cheese samples were blended with 225 ml of 0.1% peptone water in a Bag Mixer 400 (Interscience, St. Nom, France) and serially diluted using the same diluent.

L. paracasei was counted by pour-plating 1 mL of each dilution in DeMan-Rogosa-Sharpe agar (MRS agar, Oxoid Ltd. Basingstoke, UK) acidified to pH 5.4 with acetic acid, after 3 days of anaerobic incubation (Anaerobic System Anaerogen, Oxoid) at 37 °C (Oliveira, Sodini, Remeuf, & Corrieu, 2001). *S. thermophilus* was counted by pour-plating 1 mL of each dilution in M17 agar with added lactose (Oxoid), followed by incubation at 37 °C (Oliveira et al., 2001; Richter & Vedamuthu, 2001), for 48 h.

2.5. Sugar and fructan determinations

Fructose, galactose, glucose, sucrose and fructan determinations were performed in fresh cream cheeses T2, freeze-dried after 1 and 21 days of storage, employing the ion-exchange chromatographic method, based on AOAC method 997.08, described by Hoebregs (1997). The fructan content of plain Raftiline® HP-Gel (standard) was also determined. The enzyme Fructozyme® L (Novozymes, Bagsvaerd, Denmark) was employed for enzymatic hydrolysis of samples. For this purpose, a Dionex® HPLC System chromatograph, equipped with an automatic injector A550, a pump EGP40, a pulsed electrochemical detector ED40, and a Carbopac PA1 column with a guard column (Dionex Corporation, Sunnyvale, USA) was used. The gradient employed started with 40% H_2O and 60% NaOH (18 mM), reaching 90% NaOH (18 mM) after 15 min and 100% NaOH (18 mM) after 25 min. The volume of injections was 25 µl. Determinations were carried out in duplicate.

2.6. Experimental design and statistical analysis

The experimental treatments and levels constituted a randomised complete block design replicated four times (five times for moisture, fat, protein and ash), with repeated measurements taken at two time points. The treatments had a factorial structure. Analysis of variance was used to determine significant differences ($P < 0.05$) for analysis of pH, a_w, moisture, and for population of *L. paracasei* and *S. thermophilus* between the different types of product, employing the mixed effects model (Neter, Kutner, Nachtsheim, & Wasserman, 1996). The paired *t*-test was used to determine significant differences ($P < 0.05$) for the sugars fructose, galactose, glucose and sucrose and for the fructan content between days 1 and 21 of storage of cheeses T2, using MINITAB™ Statistical Software 13.0 (Minitab Inc., State College, PE, USA).

3. Results and discussion

3.1. Physico-chemical analysis of fresh cream cheeses

Content of ash, fat, protein, fructan and total dry matter of cheeses T1, T2 and T3 on day 1 of storage (final product) are shown in Fig. 1. Due to addition of inulin, cheeses T2 contained lower amounts of ash, fat and protein, compared to cheeses T1 and T3. Changes in pH, moisture and a_w values for fresh cream cheeses T1, T2 and T3 after 1 and 21 days of storage are shown in Table 1. Cheeses T2 presented pH values significantly lower ($P < 0.05$) than cheeses T1 and T3, due to initial lower values in the fresh cheese base. Nonetheless, in spite of the different initial values of pH, the changes in pH, a_w and moisture were similar for cheeses T1, T2 and T3, which all showed a significant reduction of pH and a_w between 1 and 21 days of storage ($P < 0.05$). Moisture values of cheeses T1, T2 and T3 did not change during storage. In addition, cheeses T1, T2 and T3 were not significantly different in moisture on the first day and after 21 days of storage ($P > 0.05$).

The reduction of pH values observed during storage of the fresh cream cheeses in the present study is a natural process caused by the continuous production of lactic acid and other organic acids by the starter and the probiotic cultures, as reported in previous studies with Minas fresh cheeses (Buriti, Rocha, Assis, & Saad, 2005; Buriti, Rocha,

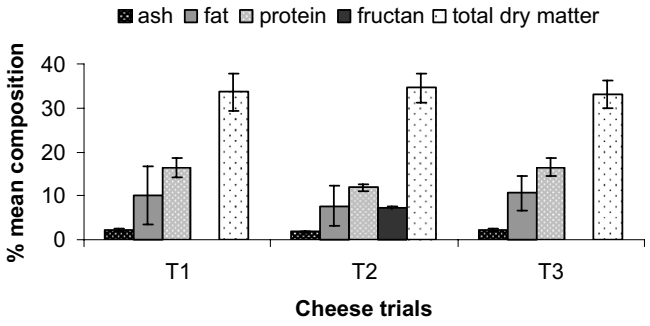

Fig. 1. Content (wet weight) of ash, fat, protein, fructan and total solids (mean of 5 batches for each cheese) in fresh cream cheese (T1 = *Lactobacillus paracasei* added; T2 = *Lactobacillus paracasei* + inulin added; T3 = control).

Table 1
Changes in pH, moisture and a_w values (mean ± standard deviation)[A] of 3 different trials of fresh cream cheese studied, after 1 and 21 days of storage at 4 ± 1 °C

Cheeses trials	Storage days	pH	Moisture (%)	a_w
T1	1	5.05 ± 0.21[a]	66.39 ± 4.18[a]	0.979 ± 0.003[a]
	21	4.80 ± 0.15[b]	66.03 ± 4.30[a]	0.977 ± 0.003[b]
T2	1	4.85 ± 0.08[b]	65.38 ± 3.30[a]	0.980 ± 0.004[a]
	21	4.69 ± 0.05[c]	65.05 ± 3.37[a]	0.976 ± 0.003[b]
T3	1	5.01 ± 0.28[a]	66.95 ± 3.20[a]	0.981 ± 0.006[a]
	21	4.83 ± 0.18[b]	66.46 ± 2.69[a]	0.978 ± 0.002[b]

[A] Mean of 5 batches (repetitions) of each cheese for pH and moisture, and of 4 batches for a_w.
[a–c] For each trial, within a column, different superscripts denote significant differences ($P < 0.05$) between different days of storage.

& Saad, 2005). In the present study, the higher production of acid in cheeses T1, T2 and T3 was probably caused by *S. thermophilus* and, to a lesser extent, by *L. paracasei* in cheeses T1 and T2. Similarly, Mendoza, García, Casas, and Selgas (2001) did not observe changes in a_w of dry fermented sausages supplemented with inulin Raftiline® ST (of the same family of inulin Raftiline® HP-Gel used in this study, although with a lower degree of polymerisation) and verified that the evolution of pH and a_w was similar to conventional products.

3.2. Viability of L. paracasei and of S. thermophilus during fresh cream cheese production and storage

Fresh cream cheeses T1, T2 and T3 contained similar populations of *S. thermophilus* after 1 and 21 days of storage, and always above 9.5 log cfu g^{-1}. To provide health benefits, the suggested concentration for probiotic bacteria is at least 6 log cfu ml^{-1} or g^{-1} of a product during its shelf life (Shah, 2000). Probiotic cheeses T1 and T2 revealed mean populations of *L. paracasei*, respectively, of 7.20 and of 7.12 log cfu g^{-1} in the first day of storage and of 7.31 and of 7.39 log cfu g^{-1} after 21 days of storage. No significant differences were detected either between populations of *S. thermophilus* obtained for cheeses T1, T2 and T3, or of *L. paracasei* for T1 and T2 ($P > 0.05$). In the present study, the presence of inulin in cheeses T2 had no implications on growth and viability of neither *L. paracasei* nor *S. thermophilus* in cheeses.

Contrary to what was observed in the present study, some authors reported that inulin-type prebiotics may aid survival of probiotic organisms during processing and storage of dairy products (Capela, Hay, & Shah, 2006; Desai, Powell, & Shah, 2004; Özer, Akin, & Özer, 2005). Desai et al. (2004) observed that seven *Lactobacillus* strains (*Lactobacillus casei* ASCC 1520, CSCC 2607, ASCC 290 and ASCC 279, *L. paracasei* ASCC 292, *Lactobacillus rhamnosus* ASCC 1521 and *L. zeae* ATCC 15820) achieved better viability when grown in reconstituted skim milk supplemented with inulin at 5% (w/v). Özer et al. (2005) reported no increase in the viability of the starters *S. thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, and of *Lactobacillus acidophilus* in yoghurt supplemented with inulin at 0.5% and at 1.0% (w/v). On the other hand, the authors reported an increased viability of *Bifidobacterium bifidum*. In addition, Capela et al. (2006) verified that the addition of 2% (w/v) of each one of the commercial fibres inulin Raftiline® ST, corn-resistant starch, and, particularly, FOS Raftilose® P95 improved the viability of *L. acidophilus* 33200, *L. casei* 279, *Bifidobacterium longum* 536 and *L. rhamnosus* GG in fresh yoghurt, when compared to control (no fibre added) yoghurt, during four weeks of storage at 4 °C. On the other hand, the authors reported that the same fibres were not capable of improving or maintaining the viability of probiotic microorganisms in freeze-dried yoghurts.

3.3. Sugar and fructan contents in the synbiotic cheeses T2

The fructose, galactose, glucose, sucrose and fructan contents of Raftiline® HP-Gel (standard) and of the synbiotic fresh cream cheese T2 samples, freeze-dried after 1 and

Table 2
Sugar and fructan content obtained for inulin Raftiline® HP-Gel and for samples of synbiotic fresh cream cheese T2, freeze-dried after 1 and 21 days of storage at 4 ± 1 °C

Carbohydrates[B] (%)	Samples[A]		
	Raftiline® HP-Gel	Cheese T2 – 1 day	Cheese T2 – 21 days
Fructose	0.108 ± 0.048	0.0113 ± 0.0048	0.0276 ± 0.0289
Galactose	0.000	0.914 ± 0.065[a]	1.03 ± 0.118[b]
Glucose	0.0057 ± 0.0037	0.255 ± 0.030[a]	0.146 ± 0.020[b]
Sucrose	0.0274 ± 0.0065	0.0012 ± 0.0014	0.0014 ± 0.0018
Fructan	95.2 ± 1.61	7.32 ± 0.392	7.27 ± 0.357

[A] Raftiline® HP-Gel with 98% of dry matter. Four batches of T2 – 1 day and three batches of T2 – 21 days.
[B] Content (wet weight).
[a–b] Within a row, different superscripts denote significant differences ($P < 0.05$) between different storage periods of T2.

21 days of storage at 4 ± 1 °C, are presented in Table 2. The mean content of 95.2% of fructan was observed in Raftiline® HP-Gel (97.14% of fructans on dry matter) whole samples.

In synbiotic cheeses T2, mean fructan contents after 1 and 21 days of storage were very close, 7.32% and 7.27%, respectively. These results were, however, 6% below the maximum content of fructan expected (7.77%) to be present in Raftiline® HP-Gel (whole matter). The fructan, fructose and sucrose content did not differ significantly after 1 and 21 days of storage of T2 ($P > 0.05$). On the other hand, a significant decrease in the glucose content ($P < 0.05$) and a small, but significant, increase in the galactose content ($P < 0.05$) of T2 was observed. This observation might be attributed to the fact that glucose is fermented by both microorganisms present, whereas most *S. thermophilus* strains are not able to metabolise galactose (Arena et al., 2006; Mozzi, Savoy de Giori, & Font de Valdez, 2003; Vaillancourt et al., 2004).

According to Makras, Van Acker, and De Vuyst (2005), inulin-type fructans are known for their so-called bifidogenic effect, meaning their ability to selectively increase the number of bifidobacteria in the human colon, as bifidobacteria are able to use inulin-type fructans as the sole energy source. Nonetheless, data on the growth of colonic microbiota other than bifidobacteria on inulin-type fructans have been reported (Duncan et al., 2003), including strains of *L. acidophilus* and *L. paracasei* (Kaplan & Hutkins, 2000; Makras et al., 2005). Kaplan and Hutkins (2000) verified that 12 of 16 *Lactobacillus* strains fermented fructans with 2–4 degrees of polymerization (FOS). On the other hand, the degree of polymerisation of inulin typically ranges from 3 to 60 (Murphy, 2001), which renders fermentation of this fructan by lactobacilli species difficult. Makras et al. (2005) investigated 10 strains of lactobacilli for their capacity to degrade oligofructose and inulin-type fructans. The authors verified that only two strains, *L. acidophilus* IBB 801 and *L. paracasei* subsp. *paracasei* 8700:2, were capable of degrading oligofructose, whereas only the human isolate *L. paracasei* subsp. *paracasei* 8700:2 grew rapidly using both oligofructose and inulin as energy sources at 37 °C under anaerobic conditions (atmosphere-controlled containing a mixture of 80% N_2, 10% CO_2, and 10% H_2).

The results obtained in the present study indicate that the metabolism of the starter *S. thermophilus* and of *L. paracasei* added to cheeses T2 did not reduce the fructan content during the storage period at 4 ± 1 °C in aerobic conditions (normal atmosphere). Nevertheless, it is important to emphasise that generally, *L. paracasei* strains are mesophilic bacteria with optimal growth temperature of 37 °C (Gardiner, Ross, Collins, Fitzgerald, & Stanton, 1998; Lynch, Muir, Banks, McSweeney, & Fox, 1999). However, in the present study, refrigerated storage temperature (4 ± 1 °C) was required for appropriate preservation of fresh cream cheese. This fact probably contributed to the absence of inulin degradation by *L. paracasei*. In addition, the solubility of inulin-type fructans (containing 10–65 degrees of polymerisation) in water decreases at low temperatures, being almost insoluble at 25 °C or less (Kim, Faqih, & Wang, 2001), whereas at room temperature, the solubility of FOS is up to 85%. According to Van-Loo (2004), this remarkable thermodynamic behaviour of inulin in solution is central to the explanation of differences in the way different fractions influence gastrointestinal fermentation. Possibly, the same behaviour might be observed in a food system, such as fresh cream cheese. Moreover, it is important to emphasise that the ability to ferment inulin-type fructans is strain-specific for lactobacilli (Makras et al., 2005).

Amounts of fructans between 4 and 5 g per day are considered effective in stimulating the growth of bifidobacteria of the colonic microbiota, thus resulting in the prebiotic effect (Manning & Gibson, 2004; Rao, 2001; Roberfroid, 1999). Coussement (1999) recommended up to 10 g inulin per food portion. In the present study, the fraction content of the synbiotic fresh cream cheeses T2 was above 7%, enough to confer a prebiotic potential during the whole storage of these products, assuming a daily consumption of 100 g of this cheese.

4. Conclusions

The current study shows that commercial inulin, at a proportion of 8%, may be applied in the manufacture of synbiotic fresh cream cheese supplemented with *S. thermophilus* (starter) and *L. paracasei* (probiotic). The cultures employed in this study were not able to degrade fructan and the concentration of this compound remained above 7% during the whole storage of the synbiotic cheese. This concentration of fructan, allied to the populations of potential probiotic *L. paracasei* above 7 log cfu g^{-1} throughout storage is sufficient to confer synbiotic functional properties to this cheese. Further application of inulin and/or oligofructose type fructans ought to be studied in other dairy systems and with other combinations of lactic and probiotic strains.

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