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Beneficial microorganisms viability and sensory acceptance of a potentially synbiotic dairy-based tomato spread

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ABSTRACT

Nowadays, important features for the development of new food products include their convenience, taste, and ability to promote beneficial effects to human health. This study aimed to develop a potentially synbiotic dairy-based tomato spread and to evaluate the viability of the microorganisms employed, as well as the sensory acceptability of the product throughout its storage for 28 days. Three different trials were studied, all containing the prebiotic inulin and the starter culture *Streptococcus thermophilus* ST-M6: **T1** (control); **T2** with the probiotic strains *Lactobacillus acidophilus* NCFM and *Bifidobacterium animalis* subsp. *lactis* Bb-12, and **T3** with Bb-12 and the bacteriocinogenic and potentially probiotic strain *Lactobacillus sakei* subsp. *sakei* 2a. The product was stored at 4 °C and analysed after 1, 7, 14, 21, and 28 days of storage. For all trials, the pH decreased throughout storage, the viability of all strains was high (≥7.9 log cfu/g), and sensory evaluation with consumers showed mean scores above 7 during the storage period evaluated (up to 21 days), which demonstrates the product has potential as a functional food.

1. Introduction

The recent trend in the development of new food products is their convenience, taste, and human health promoting functionality. A functional synbiotic product combines probiotic and prebiotic ingredients, which may improve the gastrointestinal tract (GIT) function. Probiotics are defined as live microorganisms, which when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2001). Prebiotics are selectively fermented ingredients that promote specific changes in the composition and/or activity of the GIT microbiota and confer benefits to the host (Gibson, Probert, Van Loo, Rastall, & Roberfroid, 2004).

Lactobacillus acidophilus NCFM and *Bifidobacterium animalis* subsp. *lactis* Bb-12 are commercial probiotic cultures used in a variety of food products and dietetic supplements. Dairy products are considered good vehicles for probiotics delivery and, among them cheese is the most appropriate product, due to its buffering ability. In general, cheeses show higher pH when compared to fermented milks and, therefore, are able to protect the microorganisms against the harsh conditions found in the GIT, helping to maintain high cell viability rates (Cruz, Buriti, Souza, Faria, & Saad, 2009). Tomato (*Lycopersicon esculentum*) is an interesting natural ingredient to be added to a dairy-based product. It contains different antioxidants, including lycopene, β-carotene, and vitamin C. Lycopene has been shown to be useful to prevent different types of cancer by reducing the side effects of free radicals and unstable molecules, which could damage the body's healthy cells (Lugasi, Hóvári, Biró, Brandt, & Helyes, 2004).

Some probiotic and non-probiotic lactic acid bacteria (LAB) strains are able to produce antimicrobial compounds, known as bacteriocins (Nishie, Nagao, & Sonomoto, 2012). Bacteriocins are ribosomally synthesized antimicrobial peptides and a promising technological alternative for preventing the growth of spoilage and pathogenic microorganisms in foods, a process called biopreservation (Snyder & Worobo, 2014). *Lactobacillus sakei* subsp. *sakei* 2a is an aerotolerant LAB isolated from a Brazilian fresh sausage (De Martinis & Franco, 1998) and there is only one study available in the scientific literature about its application in a dairy product (Villarreal et al., 2013). *Lb. sakei* subsp. *sakei* 2a is able to produce bacteriocins (Carvalho et al., 2010) and its probiotic potential was shown in gnotobiotic mice challenged with the foodborne pathogen *Listeria monocytogenes* (Bambirra et al., 2007).

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Therefore, this study aimed to develop a particularly innovative synbiotic dairy-based tomato spread and to evaluate the viability of the microorganisms employed, as well as the sensory acceptability of the product throughout its refrigerated storage for up to 28 days.

2. Material and methods

2.1. Experimental design and microbial cultures

Experimental design was randomized and consisted of three combinations (trials) (Table 1). Every trial was manufactured as three repetitions (triplicates) and samples from the food product were obtained at 1, 7, 14, 21, and 28 days of storage for further analysis.

The strains *Streptococcus thermophilus* ST-M6, *B. animalis* subsp. *lactis* Bb-12 (Christian Hansen, Hoersholm, Denmark), and *Lb. acidophilus* NCFM (Danisco, Dangé, France) used in the present study consisted of freeze-dried commercial cultures powders for direct vat inoculation (DVS) and were added at 0.01 g/100 mL, 0.03 g/100 mL, and 0.05 g/100 mL, respectively. *Lb. sakei* subsp. *sakei* 2a was kept in De Man Rogosa Sharpe (MRS) broth (Oxoid, Basingstoke, UK) added of glycerol (20 mL/100 mL) and stored at − 80 °C. The microorganism was grown in MRS broth at 30 °C for 24 h, re-cultivated (inoculum of 4 mL/100 mL) at 30 °C for approximately 16 h, centrifuged (5000 × *g* for 10 min) and finally washed twice (NaCl 0.9 g/100 mL) to obtain the pellet. This washed pellet was inoculated directly in the 18 L of milk employed for the cheese production.

2.2. Dairy-based tomato spread production

Quark cheese was produced with 18 L of skimmed pasteurised milk (Salute, Descalvado, Brazil; high temperature short time [HTST] process), which was heated to 37–38 °C and inoculated with the appropriate microbial cultures, according to the experimental design shown in Table 1 and described above. Next, calcium chloride (0.25 g/L) was added in all trials. As soon as the pH reached values in the range of 6.3–6.5, commercial rennet Ha-la (100% chymosin identical to the bovine enzyme; Christian Hansen, Valinhos, Brazil; 5 mg/L) was added to the cheese-milk, which was allowed to set again for approximately 1 h, until a curd was formed and the pH reached values between 5.6 and 5.8. The gel formed was cut into cubes (20 × 20 × 20 mm), placed in sterilised cotton cheesecloth, and allowed to drain at 15 °C for 15 h. After draining, the cheese-base was placed in sterilised beakers, covered with a PVC film and stored at 4 ± 1 °C, until the final mixture took place. For the final mixture, an industrial blender (Lucre, Catanduva, Brazil, 15 L capacity) was employed for mixing the following ingredients: quark cheese; pasteurized tomato pulp; dried tomato; commercial sterilized milk cream; dried basil; grated parmesan cheese; salt; olive oil; commercial sucrose; stabilisers guar gum, xantan gum, carrageenan gum, prebiotic fibre inulin, and the whey obtained during cheese production. After the mixture step, a smooth salmon cream was formed. Samples containing approximately 40 g of the food product were packaged in individual plastic pots for food products (68 mm diameter, 32 mm high, 55 mL total volume, Tries Aditivos Plásticos, São Paulo, Brazil). The plastic pots were sealed with aluminium covers, and stored at 4 ± 1 °C for up to 28 days. Table 2 shows the dairy-based spread overall formulation.

Table 1
Description of the three different trials (T1, T2, and T3) of the dairy-based tomato spread studied, according to the microbial cultures employed.

Trials	*St. thermophilus* ST-M6[a]	*B. animalis* subsp. *lactis* Bb-12[b]	*Lb. acidophilus* NCFM[c]	*Lb. sakei* subsp. *sakei* 2a[d]
T1*	+	−	−	−
T2	+	+	+	−
T3	+	+	−	+

* control; + = addition; - = no addition.
[a] *Streptococcus thermophilus* ST-M6 (Christian Hansen, Hoersholm, Denmark).
[b] *Bifidobacterium animalis* subsp. *lactis* Bb-12 (Christian Hansen).
[c] *Lactobacillus acidophilus* NCFM (Danisco, Dangé, France).
[d] *Lactobacillus sakei* subsp. *sakei* 2a (Laboratory of Food Microbiology, School of Pharmaceutical Sciences/University of São Paulo, São Paulo, Brazil).

2.3. Determination of pH

The pH values of the dairy-based tomato spreads were determined for triplicate samples with a Thermo Orion Three Stars pH metre (Thermofisher Scientific, Waltham, MA, USA) equipped with a penetration electrode model 2AO4 (Analyser, São Paulo, Brazil) after 1, 7, 14, 21, and 28 days of storage at 4 ± 1 °C and also during the steps of the quark cheese preparation.

2.4. Microbiological analysis

Microbial counts of *Lb. acidophilus* NCFM, *B. animalis* subsp. *lactis* Bb-12, *Lb. sakei* subsp. *sakei* 2a, and *St. thermophilus* were conducted throughout the food product storage (after 1, 7, 14, 21, and 28 days) for all trials. For the microbiological analysis, portions of 25 g of the food product were transferred to aseptic bags (Nasco, Whirl-Pak®, USA), added of 225 mL of 0.1 g/100 mL peptone (Oxoid) water, blended in a Bag Mixer 400 device (Interscience, St. Nom, France), and then submitted to further ten-fold serial dilutions using the same diluent.

St. thermophilus ST-M6 was counted by pour-plating 1 mL of each dilution in agar M17 (Oxoid) supplemented with 10 g/100 mL of lactose (Oxoid), followed by aerobic incubation at 37 °C for 48 h (Richter & Vedamuthu, 2001). *Lb. acidophilus* NCFM was enumerated by pour-plating 1 mL of each dilution in formulated MRS agar, modified by the replacement of glucose by maltose, followed by aerobic incubation at 37 °C for 48 h (International Dairy Federation, 1995). *B. animalis* subsp. *lactis* Bb-12 populations were determined by pour-plating 1 mL of each dilution in MRS agar (Oxoid)

Table 2
Ingredients and formulation of the dairy-based spreads studied.

Ingredients	Amount (g/100 g)
1. Quark cheese	50.00
2. Tomato pulp	14.00
3. Milk cream	13.00
4. Dried tomato	11.00
5. Inulin	4.00
6. Whey	2.60
7. Parmesan cheese	1.51
8. Olive oil	1.50
9. Sugar	1.00
10. Salt	0.80
11. Guar gum	0.22
12. Basil	0.15
13. Xantan gum	0.11
14. Carrageenan gum	0.11
Total	**100.00**

2. Pasteurized tomato pulp (5.8 °Brix, Pomodoro, Goiânia, Brazil); 3. Commercial sterilized milk cream (25% lipids, Nestlé, Araçatuba, Brazil); 4. Dried tomato (Agro Industrial Itaguaçú, Itaguaçú da Bahia, Brazil); 5. Prebiotic inulin (GR, Orafti, Oreye, Belgium); 7. Grated parmesan cheese (Faixa Azul, Vigor, São Paulo, Brazil); 8. Olive oil (Gallo, Mairinque, Brazil); 9. Commercial sucrose (União, Limeira, Brazil); 10. Salt (Cisne, Cabo Frio, Brazil); 11. Guar gum (Grindsted® 250, Danisco, Cotia, Brazil); 12. Dried basil (Fuchs, Itupeva, Brazil); 13. Xantan gum (Grindsted® 80, Danisco), and 14. Carrageenan gum (Grindsted® CY500, Danisco).