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Quality parameters of probiotic yogurt added to glucose oxidase compared to commercial products through microbiological, physical–chemical and metabolic activity analyses

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ABSTRACT

The performance of probiotic yogurt with the addition of glucose oxidase (250 and 500 ppm, GOX1 and GOX2, respectively) compared to commercial products available in the Brazilian market was investigated. Microbiological (probiotic bacteria count), physical–chemical (pH, proteolysis, minerals) and metabolic activities (production of organic acids, flavor and aroma compounds and fatty acid profile) were performed. Glucose oxidase yogurt presented suitable viability of lactic acid and probiotic cultures (>6 log CFU/g), as well as lower pH values. On the other hand, higher values of proteolysis, diacetyl, acetaldehyde, conjugated linoleic acid (CLA) and polyunsaturated fatty acids (PUFAs), and similar values of lactic acid and acetic acid were found. In a functional food perspective, the addition of glucose oxidase to probiotic yogurts may be an interesting technological option for small and medium-size dairy enterprises to enter to the market of functional dairy foods.

1. Introduction

Fermented milks, in particular yogurt, are the most frequently used food matrices for the addition of probiotic bacteria worldwide, with decisive consumer preference (Vecchio and Annuziata, 2013), which has been proven by many studies as providing potential benefits to health (Ejtahed et al., 2011; Kimoto-Nira et al., 2014; Lollo et al., 2013, 2015; Nabavi, Rafraf, Somi, Homayouni-Rad, & Asghari-Jafarabadi, 2014; Wang et al., 2012).

Incorporation and viability of probiotic bacteria in foods throughout their storage, resulting in benefit to consumer health, are a constant challenge for the food industry, requiring the understanding of factors intrinsic and extrinsic to processing (Granato, Branco, Cruz, Faria, & Shah, 2010). Particularly in the case of yogurts, interaction with the added starter culture, low proteolytic activity, post-acidification, exposure to oxygen and low temperatures during storage may cause negative impacts on the survival of such microorganism, resulting in commercial products inability to provide the benefits to consumer health during shelf life at a daily intake (Granato, Branco, Cruz, Faria, & Shah, 2010).

The addition of glucose oxidase in yogurt processing has been reported as a potential technological solution to minimize the exposure to oxygen, also known as oxidative stress and thus maintaining stable counts of probiotic bacteria along the product storage (Cruz, Cadena, et al., 2013; Cruz, Castro, Faria, Bogusz, et al., 2012; Cruz, Castro, Faria, Lollo, et al., 2012; Cruz, Castro, et al., 2013; Cruz et al., 2010) with impaired sensory acceptance (Cruz, Cadena, et al., 2011; Cruz, Faria, et al., 2011). But probiotic yogurts are still pending an assessment of their intrinsic quality parameters compared to trademarks, processed by large companies with branches around the world, which have an already established formulation marketed throughout the Brazilian territory. In this context, the objective of this study is to evaluate the quality parameters of strawberry probiotic yogurt with the addition of glucose oxidase compared to commercial probiotic yogurts by using microbiological (probiotic bacteria viability), physical–chemical (pH, proteolysis,

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minerals) and metabolic activity analyses (production of organic acids, flavor and aroma compounds and fatty acid profile).

2. Material and methods

2.1. Yogurt processing

Yogurt probiotic processing is available (Cruz, Cadena, et al., 2011; Cruz, Cadena, et al., 2013; Cruz, Castro, Faria, Bogusz, et al., 2012; Cruz, Castro, Faria, Lollo, et al., 2012; Cruz, Castro, et al., 2013; Cruz, Faria, et al., 2011; Cruz et al., 2010). Standardized whole raw milk (3.2% w/w fat) with skim milk powder 3.5% w/v (Atilatti, Itatiba, Brazil) underwent heat treatment in a 50 L stainless steel tank (95 °C/15 min), cooled to 45 °C and inoculated with *Streptococcus thermophilus salivarius* spp. TA 040, *Lactobacillus delbrueki* spp. *bulgaricus* LB340, *Lactobacillus acidophilus* La 14 and *Bifidobacterium longum* Bl05 (Danisco, São Paulo, Brazil; 6.6 and 7.7, log CFU/mL, respectively). Subsequently, the inoculated milk underwent fermentation at 45 °C with pH monitoring up to 4.7–4.6. Then it underwent cooling to 10 °C and subsequent disruption of the gel by stirring with simultaneous addition of 250 and 500 ppm of glucose oxidase (Glucomax CO, Prozyn, São Paulo, Brazil, GOX1 and GOX2 yogurts) and 5% w/w strawberry pulp (Duas Rodas, São Paulo, Brazil) and 10% w/w sugar (União, Rio de Janeiro, Brazil). It was later placed in 100 mL polypropylene glasses (Dixie Toga, São Paulo, Brazil), sealed by induction (Ernercan, Viscosin, USA) and kept under refrigerated storage at 5 °C for 30 days in order to carry out the analyses.

At the same time, full fat commercial probiotic yogurt flavored strawberry available in Brazil, belonging to trademarks from large companies (PROB1, PROB2, PROB3, PROB4, PROB5) and indicating the presence of bacteria of the genus *Bifidobacterium* (*Bifidobacterium lactis*, *Bifidobacterium animalis*) and *Lactobacillus* (*Lactobacillus acidophilus*) in their labels was obtained from supermarkets in Campinas at the beginning of shelf life and kept under refrigerated storage at 5 °C for 30 days in order to carry out the analyses. The 30-day period to perform the experiment corresponds to the end of shelf life of commercial yogurts.

2.2. Microbiological analysis

1 mL of yogurt was transferred to a tube with screw cap containing 9 mL of sterile peptone water solution (Ventec, São Paulo, Brazil) 0.1% w/v and subsequent dilutions were made from this dilution for bacterial counts. The yogurt and probiotic bacteria were enumerated using a methodology published elsewhere (Cruz, Cadena, et al., 2013; Cruz, Castro, et al., 2013) in duplicate. The *S. thermophilus* count was performed using M17 Agar (Oxoid, São Paulo, Brazil), incubated aerobically at 37 °C/48 h, while the *L. bulgaricus* count was performed using MRS (Oxoid, São Paulo, Brazil) with pH 5.2 obtained by the addition of glacial acetic acid (Synth, São Paulo, Brazil), following anaerobic incubation at 45 °C/72 h. The *L. acidophilus* count was performed using 0.15% w/v bile salts-de Man, Rogosa, and Sharpe agar (Oxoid, São Paulo, Brazil), at 37 °C for 72 h under aerobic conditions while *B. longum*, *B. animalis* and *B. lactis* counts were performed in duplicate using Lithium chloride–sodium propionate agar (MRS-LP) containing concentrations of inhibiting agents (0.5 g/L of LiCl and 0.75 g/L of sodium propionate) after anaerobic incubation at 37 °C for 3 days. All the culture media were previously tested aiming to guarantee their selectivity towards the microorganism desired.

2.3. Physico-chemical analysis

2.3.1. pH and proteolysis

The pH of milk and yogurt samples was determined using a digital potentiometer (Micronal B-375, São Paulo, Brazil) through direct insertion of the electrode in the sample (Marshall, 1993). Proteolytic activity was quantified by measuring the amino acids and peptides released by probiotic cultures, using the reagent solution (OPA) containing the following reagents: sodium dodecyl sulfate, sodium tetraborate decahydrate, dithiothreitol, o-phthalaldehyde and ethanol. Proteolytic activity of cultures was expressed as absorbance of the OPA derivatives at 340 nm. Relative degree of proteolysis was determined as the difference between the proteolytic activity of yogurt and the proteolytic activity of unfermented milk (Cruz, Cadena, et al., 2013; Cruz, Castro, Faria, Bogusz, et al., 2012; Cruz, Castro, Faria, Lollo, et al., 2012; Cruz, Castro, et al., 2013).

2.3.2. Gross composition, sodium and calcium levels

Total solids were determined gravimetrically after 24 h of drying in an oven (Micronal, São Paulo, Brazil). The ashes were determined gravimetrically after heating 2 g of the sample using a muffle furnace at 550 °C. Protein was determined based on the total nitrogen, using the Kjeldahl method with subsequent multiplication by a factor of 6.38. Fat was determined using the Gerber method. All analyses followed standard procedures and were performed in triplicate (Brazil, 2006). Analysis of minerals was performed in duplicate using an ICP-OES with axial view, 1300 W, plasma gas flow rate of 15 L/min.

2.3.3. Lactic and acetic acid

Quantification of lactic acid and acetic acid was performed using high-performance liquid chromatography (model Varian 9010, Varian, Inc. Scientific Instruments, Palo Alto, CA, USA). The equipment consisted of ion exchange column Aminex HPX-87 H (Bio-RAD Laboratories, Richmond, CA, USA) kept at 65 °C, refractive index detector model RI 2000 for the determination of carbohydrates and a detector (with wavelength of 220 nm) for the determination of organic acids. The eluent was filtered and degassed using sulfuric acid solution prepared with ultrapure water using Milli-Q water purification system (Millipore Corporation, Billerica, MA), pH 2.8, and volumetric flow rate of 0.6 mL/min. The preparation of samples in duplicate consisted of mixing 3 mL of yogurt with 80 μL of 15.5 M nitric acid for further dilution with 1.0 mL of the mobile phase, 0.01 M sulfuric acid. The resulting mixture was centrifuged at 14,000 *g* for 30 min to remove proteins and the supernatant was filtered through 0.20 μm Millipore membranes. The column temperature was 65 °C and the mobile phase, 0.01 M sulfuric acid, had a flow rate of 0.6 mL/min (Cruz, Cadena, et al., 2013; Cruz, Castro, Faria, Bogusz, et al., 2012; Cruz, Castro, Faria, Lollo, et al., 2012; Cruz, Castro, et al., 2013). Quantification of carbohydrates and organic acids was carried out using standard curve of solutions of compounds of known concentrations. The injection volume was 25 μL, using an automatic injector, and integration of chromatographic peaks was performed using the Millenium software.

2.3.4. Flavor compounds

Quantification of diacetyl, acetaldehyde and ethanol was performed using solid-phase microextraction and gas chromatography. For the analyses, it followed the methodology described by Condurso, Verzera, Romeo, Ziino, and Conte (2008) with some modifications: 2 g of yogurt was weighed in 40-mL vials and 2 mL of saturated NaCl solution was added to it; the vials were then sealed with appropriate caps containing PTFE/silicone septa (Supelco — Bellefonte, PA, USA) and kept at 40 °C for 15 min for balance, and after 30 min for exposure of the fiber and extraction of the volatile compounds. During extraction the sample remained under stirring with the aid of a "*stir bar*" type magnetic stirrer at 750 rpm. After this extraction period, the fiber underwent thermal desorption at 250 °C in the gas chromatograph injector for a period of 7 min. To prevent the memory effect of fibers, a fiber blank was made among each extraction, in order to ensure the quality of the experiments. In the solid phase microextraction, it was used commercial SPME fiber (Supelco, Bellefonte, PA, USA) PN: 57328, 50/30 μm divinyl benzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS). For chromatographic analyses, it used a gas chromatograph (GC) brand Varian