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Rheology, spontaneous whey separation, microstructure and sensorial characteristics of probiotic yoghurts enriched with passion fruit fiber

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ABSTRACT

Beyond demonstrated beneficial health attributes, passion fruit rinds are a by-product of the fruit pulp industry, rich in total dietary fiber, particularly pectin. The aim of this study was to evaluate the influence of the addition of passion fruit fiber on the whey formation, rheological parameters, microstructure and sensorial characteristics of probiotic yoghurts. Skim milk bases enriched with 1% of passion fruit fiber or not were heat treated and inoculated with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, and divided into four groups according to the probiotic strain added — *Lactobacillus acidophilus* strains L10 and NCFM and *Bifidobacterium animalis* subsp. *lactis* strains Bl04 and B94. Fermentations were performed until the pH reached 4.5. Rheological characteristics of yoghurts were determined by a rotational rheometer in two cycles of shear rate ranging from 0 to 15 s^{-1} in both upward and downward curves. Sensorial analysis of passion fruit fiber yoghurts, either without any probiotic or co-fermented by *L. acidophilus* L10 or *B. animalis* subsp. *lactis* Bl04, was evaluated against a control yoghurt without fiber. Photomicrographs of freeze-dried yoghurts were made by field-emission scanning electron microscope (SEM). Thixotropy of enriched yoghurts was higher than that of their respective controls in the two cycles of shear rate. Apparent viscosity was significantly higher in fiber yoghurts co-fermented by the lactobacilli than in their controls at the end of cold storage. Photomicrographs demonstrated that in passion fruit fiber yoghurts the casein gel was more compact and overlaid the fiber, while filaments of exopolysaccharides were more frequent in control yoghurts. Appearance, odor and color of the passion fruit fiber yoghurts received scores as 'good', and the intensity of the passion fruit flavor was considered weak by the sensory assessors. Results indicate that the passion fruit fiber is an almost neutral ingredient for the design of new high value-added yoghurt.

1. Introduction

During milk fermentation in yoghurt manufacture, the pH decreases as the lactic acid is produced by the starter culture — *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Casein begins to aggregate at pH 4.7, isoelectric point, forming a fragile gel net. At the end of fermentation, the gel of the set-type yoghurt is usually broken to produce stirred yoghurt and the subsequent operations of mixing, pumping and packaging impact in its structure, decreasing the apparent viscosity. However, during thawing to approximately 20 °C and cold storage at 4–5 °C, the stirred yoghurt recovers partially its structure and viscosity, thus behaving as a pseudoplastic material (Damin, Minowa, Alcantara, & Oliveira, 2008; Marafon, Sumi, Alcantara, Tamime, & de Oliveira, 2011; Marafon et al., 2011; Sodini, Remeuf, Haddad, & Corrieu, 2004; Tamime & Robinson, 2007).

Rheological and organoleptic properties, texture characteristics and microstructure of yoghurt depend on many factors such as milk base formulation, bacterial culture selection, production process, packaging and storage (Tamime & Robinson, 2007). For the development of new fermented milks, the influence of modifications in the milk base on texture, rheology and sensorial properties of products has been studied, concerning mainly the lipid content of milk (De Lorenzi, Pricl, & Torriano, 1995; Espírito-Santo, Perego, Converti, & Oliveira, 2012; Folkenberg & Martens, 2003), the addition of proteins to increase total solids (Gastaldi, Lagaude, Marchesseau, & Fuente, 1997; Marafon, Sumi, Granato, et al., 2011; Penna, Converti, & Oliveira, 2006; Sodini, Lucas, Tissier, & Corrieu, 2005), the total dietary fiber (DF) contents (Espírito-Santo et al., 2012; García-Pérez et al., 2005; McCann, Fabre, & Day, 2011; Staffolo, Bertola, Martino, & Bevilacqua, 2004), and the addition of prebiotics (Guggisberg, Cuthbert-Steven, Piccinah, Butikofer, & Eberhard, 2009; Kipa, Meyerb, & Jellema, 2006) or calcium (Ozcan-Yilsay, Lee, Horne, & Lucey, 2007; Singh & Muthukumarappan, 2008).

Specific strains as well as composition of the bacterial cultures used in fermentation, especially those releasing exopolysaccharides or combinations of the starter culture with one or more probiotics, also play an important role in the development of yoghurt structure

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(Espírito-Santo et al., 2012; Laws & Marshall, 2001; Prasanna, Grandison, & Charalampopoulos, 2012; Rawson & Marshall, 1997; Reid et al., 2003; Staffolo et al., 2004; Tamime & Robinson, 2007).

Formulation of new food products with ingredients from fruit by-products rich in total DF has increased in recent years, being convenient to their association with probiotic bacteria for the promotion of the intestinal health (Lamsal & Faubion, 2009; Sendra et al., 2008). Dietary fiber can be fractioned into two major groups of components, the water-insoluble and the water-soluble fraction. While the insoluble fraction stimulates the intestinal peristalsis, the soluble one promotes the selective growth of the indigenous microbiota, acting as a prebiotic (Sembries et al., 2003). Nawirska and Kwasniewska (2005) reported the importance of DF intake on a daily basis to prevent obesity, atherosclerosis, heart diseases, gut cancer and diabetes. Therefore it is healthier to consume the total dietetic fiber, instead of just its prebiotic fraction. One of the promising fruit by-products is the passion fruit peel, which, in addition to its functional properties such as the reduction of cholesterol and glucose in blood serum (Barbalho et al., 2011; Janebro et al., 2008; Medeiros et al., 2009; Parkar, Stevenson, & Skinner, 2008), it was shown, in the recent study of our group, the improvement in fatty acid profile and increase in the conjugated linoleic acid content of probiotic yoghurts added with passion fruit peel (Espírito Santo et al., 2012a). Moreover, passion fruit peel fiber enhanced the texture parameters of skim yoghurts during cold storage (Espírito-Santo et al., 2012b). Based on this background, the present study aimed to evaluate some other important aspects of the rheology, spontaneous whey separation, microstructure and sensorial characteristics of probiotic yoghurts enriched with passion fruit fiber.

2. Materials and methods

2.1. Preparation of passion fruit fiber and determination of water and oil holding capacity

Passion fruit by-product was obtained from a fruit pulp manufacturer, located in the city of Jundiai, São Paulo State, Brazil, and maintained in freezer at −26 °C until processing.

The peels of the passion fruit were dried in an oven (Quimis® Q314M-293) at 60 °C under airflow at 12 air changes·min^{-1} until constant weight. The dry peels were reduced to fine powder in a Bimby processor, TM 31 (Vorwerk, Wuppertal, Germany). Fiber particle size was standardized to less than 17.7 μm, measured through sieves (Granutest, São Paulo, Brazil), and fiber powder was stored in glass pots maintained under refrigeration at 4 °C until use.

The water and oil holding capacities of the passion fruit fiber (PFF) were determined by centrifugation (multispeed PK 131, ALC Porta, Italy) at 25 °C, according to Chau and Huang (2004).

2.2. Milk preparation

Skim milk powder (Molico®, Nestlé, Araçatuba, SP, Brazil) was reconstituted to 12 g·100 mL^{-1} in potable water filtered by a water purifier, FR600 (IBBL, São Paulo, Brazil) and divided into two portions: (i) enriched with PFF powder at 1.0 g·100 mL^{-1} of milk and (ii) not enriched and designed as control. The milk bases were heat treated at 85 °C for 15 min in a bath thermostat (A100, Lauda, Königshofen, Germany) under agitation at 300 rpm provided by steel two-blade propeller connected to an agitator (Q250M1 (Quimis, Diadema, Brazil)). Afterwards, the milk bases for the analysis of rheology, syneresis and microstructure were divided into sterile glass flasks (500 mL) and those for the sensorial analysis were conditioned in sanitized white polypropylene containers (15 L), cooled in an ice bath and stored at 4 °C for 24 h until inoculation.

2.3. Microbial cultures

The freeze-dried starter yoghurt culture (CY340, DSM, Moorebank, NSW, Australia) composed of *Streptococcus thermophilus* (St) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Lb) and two *Lactobacillus acidophilus* strains (LAFTI® L10, DSM, and NCFM, HOWARU™ Dophilus, Danisco, Madison, WI, USA) and two *Bifidobacterium animalis* subsp. *lactis* strains (Bl04, Danisco and LAFTI® B94, DSM) were used in this study. The lyophilized cultures were diluted in 50 mL of sterilized milk — inoculums, according to the recommendations of the manufacturer. The amount (g) of the lyophilized cultures was previously determined to reach counts around 6 Log CFU mL^{-1} of each microorganism in the inocula. The counts of probiotic bacteria increase during the fermentation of milk and are maintained above 9 Log CFU mL^{-1} during 2 weeks of cold storage of the yoghurts, as previously reported by Espírito Santo et al. (2012a) and Espírito-Santo et al. (2012b).

2.4. Experimental procedure

Four types of probiotic yoghurts were prepared according to the experimental design presented in Table 1. For the rheology, syneresis and microstructure analysis, each yoghurt type was prepared in duplicate in two independent batch fermentations (N = 4). The flasks containing 500 mL of the heat treated milk base were inoculated with 1 mL of yoghurt starter cultures and 1 mL of probiotic culture. Thereby, each milk flask had, before fermentation, an average count of 6.4 Log CFU mL^{-1} of St and 5.9 Log CFU mL^{-1} of Lb, and the probiotic counts were ~6.9 Log CFU mL^{-1}. Afterwards, the flasks with the samples were transferred to a water bath at 42 °C and connected to a CINAC (*Cynetique d'acidification*, Ysebaert, Frépillon, France) system (Spinnler & Corrieu, 1989) which recorded the pH decrease.

At pH 4.5, fermentation was stopped and the flasks were capped and cooled to 20 °C in an ice bath. Then, the flasks were transferred to a laminar flow cabinet and the coagulum was broken with regular upward and downward movements for 2 min with a sterile perforated disk on a stainless steel rod. The coagulum of the yoghurts in the 15 L containers was broken under agitation at 100 rpm by a sterile steel propeller connected to the agitator already described in Section 2.2. The stirred yoghurts were distributed into 50 mL polypropylene cups, thermally sealed and stored in refrigerator at 4 °C.

2.5. Spontaneous whey separation

After 24 h of fermentation, four cups of each yoghurt type were carefully homogenized (N = 32) and an aliquot (8 mL) of yoghurt was collected throughout a 10 mL (1:10) sterile plastic pipette (Sterilin®, Barloworld Scientific, Staffordshire, UK), placed in a shelf pipette holder and maintained in a perpendicular position at 4 °C. The whey formed in the upper phase of the yoghurt was read in the pipette scale. This procedure was repeated for yoghurts stored at 7

Table 1
Experimental design to study the rheology, spontaneous whey separation, microstructure and sensorial characteristics of probiotic yoghurts enriched with passion fruit fiber.

Yoghurt	Probiotic	Fiber
Control	*Lactobacillus acidophilus* NCFM	−
Control	*L. acidophilus* L10	−
Control	*Bifidobacterium animalis* subsp. *lactis* Bl04	−
Control	*B. animalis* subsp. *lactis* B94	−
Passion fruit fiber	*L. acidophilus* NCFM	+
Passion fruit fiber	*L. acidophilus* L10	+
Passion fruit fiber	*B. animalis* subsp. *lactis* Bl04	+
Passion fruit fiber	*B. animalis* subsp. *lactis* B94	+

−: without passion fruit fiber; +: with passion fruit fiber.