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Influence of cofermentation by amylolytic *Lactobacillus* strains and probiotic bacteria on the fermentation process, viscosity and microstructure of gruels made of rice, soy milk and passion fruit fiber

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ABSTRACT

Gruels tailored to school-age children and made of soy milk and rice flour with or without total dietary fiber from passion fruit by-product were fermented by amylolytic lactic acid bacteria strains (*Lactobacillus fermentum* Ogi E1 and *Lactobacillus plantarum* A6), by commercial probiotic bacteria strains (*Lactobacillus acidophilus* L10, *Lactobacillus casei* L26 and *Bifidobacterium animalis* subsp. *lactis* B94) and by co-cultures made of one amylolytic and one probiotic strain. The influence of ingredient composition and bacterial cultures on kinetics of acidification, α-amylase activity of the bacteria, apparent viscosity and microstructure of the fermented products was investigated. During fermentation of the gruels, α-amylase activity was determined through the Ceralpha method and apparent viscosity, flux behavior and thixotropy were determined in a rotational viscometer. Rheological data were fitted to Power Law model. The combination of amylolytic and probiotic bacteria strains reduced the fermentation time of the gruels as well as increased the α-amylase activity. The addition of passion fruit fiber exerted less influence on the apparent viscosity of the fermented products than the composition of the bacterial cultures. Scanning electron microscopy provided evidence of exopolysaccharide production by amylolytic bacteria strains in the food matrices tested. The co-cultures made of amylolytic and probiotic bacteria strains are suitable to reduce the fermentation time of a soy milk/rice matrix and to obtain a final product with pH and viscosity similar to yoghurt.

1. Introduction

According to Food Processing (2009) and Granato, Branco, Cruz, Faria, and Shah (2010), the market of food products containing functional ingredients such as probiotics, prebiotics, soy derivatives and dietary fiber, grows approximately 5% per year worldwide and the selling of these products is expected to be over US$19.6 billion in 2013. About 65% of the sales of functional foods correspond to probiotic products (Granato et al., 2010; Stanton, Ross, Fitzgerald, & Van Sinderen, 2005). FAO/WHO (2001) defines probiotics as live microorganisms that when administered in adequate amounts, are able to colonize the gastrointestinal tract conferring health benefits to the host. Prevention of diarrhea caused by rotavirus (common in schoolchildren), lowering of serum cholesterol, modulation of the immune system response and prevention of colon cancer are amongst the benefits commonly attributed to probiotics consumption (Farnworth, 2008). Fermented dairy products have been the most utilized food matrix for probiotic intake, but the development of probiotic vegetable beverages has been increasing as an alternative to attend to the needs of individuals with lactose intolerance or milk allergy (Espírito-Santo et al., 2012). Besides, the great majority of disadvantaged populations cannot afford a dairy-based functional food. In Brazil, the National Program for Nutrition in the School (PNAE) stipulates that 30% of the recommended daily nutritional requirements for school-age children should be provided by the school meal service, challenging the formulation of a diversified menu (Brasil, 2009).

The use of a vegetal food matrix with high protein quality such as hydrosoluble extract of soybeans (soy milk) instead of a milk base is a cheaper way to develop probiotic beverages, which can be more affordable to poor communities in soybean producer countries, such as those of South America and South Asia (Tou et al., 2007). Beyond the health benefits of probiotics themselves, fermentation by probiotic bacteria has been noticed as beneficial to increase functional aspects of soy milk by generating antihypertensive peptides (Donkor, Henriksson, Vasiljevic, & Shah, 2005), promoting normobiosis in the intestinal tract (Cheng et al., 2005) and reducing the content of nondigestible

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oligosaccharides (LeBlanc, Garro, & de Giori, 2004). Fermentation of soy milk can also reduce the beany off-flavor of the soybean (LeBlanc et al., 2004; Wang, Zhou, & Chen, 2008).

On the other hand, promoting a balanced daily energy intake for children, mainly those exposed to precarious economic situation, is a must according to the World Health Organization (Mouquet & Trèche, 2001; WHO, 1998). A cheap way to increase the energy value of soy milk is the addition of an amylaceous ingredient such as rice flour, which also acts as food texturing agent (Nguyen et al., 2007; Sabanis & Tzia, 2009). The nutritional quality of a rice based food can be improved through fermentation by amylolytic lactic acid bacteria (ALAB) which can increase the availability of lysine and improve the digestibility of starch in young children (Gobbetti, De Angelis, Corsettic, & Di Cagno, 2005; Lee, Gilliland, & Carter, 2001; Lee, Lee, Park, Hwang, & Ji, 1999). The fermentation of amylaceous matrix by ALAB offers a technological benefit by eliminating the need to add exogenous α-amylases for starch liquefaction (Haydersah et al., 2012; Mouquet & Trèche, 2001; Nguyen et al., 2007). Amylolytic activity was also observed in some probiotic strains of *Lactobacillus* and *Bifidobacterium* (Knudsen et al., 2013; Lee et al., 2001; Ryan, Fitzgerald, & van Sinderen, 2006). However, besides starch as carbon source, probiotic bacteria can be particularly demanding in amino acids, vitamins, minerals or other growth stimulant factors such as non-digestible carbohydrates, known as prebiotic fibers (Espírito-Santo et al., 2012). Many by-products from fruit processing industry, such as the yellow passion fruit rinds, are rich source of fibers and minerals which can be used as ingredient to support the bacteria growth during fermentation and to increase the nutritional and functional aspects of the food product (Cordova, Gama, Winter, Kaskantzis Neto, & Freitas, 2005; Espírito-Santo et al., 2013; Yapo & Koffi, 2008).

Considering the mentioned elements and the protein/energy daily needs of 2–6 years old children living in the poorest communities of Brazil (IBGE, 2006; Silva, Martins, Oliveira, & Miyasaka, 2010), our group formulated fermented products containing probiotic lactic acid bacteria as a base for a dessert that could be an alternative to probiotic yoghurt and integrate a school meal. To reach this purpose, gruels made of soy milk and rice flour with or without total dietary fiber from passion fruit rinds were fermented by amylolytic (*Lactobacillus fermentum* Ogi E1 and *Lactobacillus plantarum* A6) and commercial probiotic bacteria strains (*Lactobacillus acidophilus* Lafti L10, *Lactobacillus casei* Lafti L26 and *Bifidobacterium animalis* subsp. *lactis* Lafti B94) in single culture or in co-culture. The commercial probiotic bacteria selected have the status of GRAS (Generally Recognized as Safe) and QPS (Qualified Presumption of Safety), as reported by Jankovic, Sybesma, Phothirath, Ananta, and Mercenier (2010), and have been applied to the fermentation of soy milk (Donkor, Tsangalis, & Shah, 2007). The selected ALAB have been used in the fermentation of rice-based foods (Nguyen et al., 2007). The structural characteristics and rheological parameters of a fermented food are profoundly affected by the composition of the ingredients and the selection of bacteria strains (Donkor et al., 2007; Haydersah et al., 2012; Mouquet & Trèche, 2001; Nguyen et al., 2007; Wang et al., 2008). Thus, the co-cultures made of one amylolytic and one probiotic strain were employed in the fermentation of gruels made of rice flour and soy milk, in order to verify if they are more able than the single cultures in promoting an improvement of some biotechnological and physical aspects of the fermented product. The aim of this study was to evaluate the influence of the composition of ingredients of the food matrix and of bacterial culture on the apparent viscosity and flux behavior during fermentation of rice/soy milk gruels. In order to explain the findings on rheology, supplementary experiments were done on kinetics of acidification and α-amylolytic activity of the bacteria as well as the analysis of microstructure of the fermented products through scanning electron microscopy. To the best of our knowledge, it is the first time that amylolytic lactic acid bacteria isolated from vegetable matrices are used in co-culture with commercial and dairy-adapted probiotic bacteria strains in the fermentation of a soy milk/rice food matrix for the production of a yoghurt-like food.

2. Material and methods

2.1. Preparation of the raw material

The fruits of yellow passion fruit (*Passiflora edulis* var. *flavicarpa* Deg., Passifloraceae) were acquired in a market of organic products in the city of Curitiba, Parana State, Brazil. The fruits were decontaminated by immersion into a solution of 5 ppm of chlorine active hypochlorite for 30 min and then thoroughly washed under running tap water. Afterwards, the peel and pulp were separated and the peels – which represented around 60% of the weight of the fruit – were dried in oven under air flow at 50 °C until constant weight, milled to fine powder (passion fruit fiber, PF) and the particle size was standardized to less than 42 μm.

Hydrosoluble soybean extract (soy milk) was prepared as described by Mandarino and Carrao-Panizzi (1999), with some modifications. Briefly, 600 g of soybeans were cooked in 1.5 L of boiling water for 10 min to inactivate the lipoxygenase, responsible for the color degradation and beany off-flavor of the soybean. Then, the cooking water was drained and the grains were washed, decorticated by rubbing them between the palms of the hands and cooked in 3 L of water for 5 min. After cooling, the soybeans were milled in a knives blender for 15 min. The dough obtained was cooked for 10 min under constant stirring, cooled and passed through sieves to obtain a final product with particle size smaller than 50 μm. The resulting soy milk was freeze-dried in a lyophilizer (Christ model Alpha 1–2 LD plus, Passau, Germany) and stored at 4 °C until use.

2.2. Preparation of the gruels

The dry matter content of the ingredients and of the different food matrices before and after fermentation was determined in an infrared moisture analyser (Sartorius, MA30, Gottingen, Germany). The dry matter contents of the rice flour, freeze-dried soy milk and total dietetic fiber from passion fruit rinds were of 88.4 ± 0.3, 95.1 ± 0.5 and 90.9 ± 0.2%, respectively. To obtain 100 g of base gruel (RS) with 20 g of dry matter (DM), white rice flour (Moulin des Moines, France) and lyophilized soy milk were mixed at 10 g of dry matter each in deionized water. Passion fruit fiber was added at 1 g of dry matter to the base formulation (RS) in order to obtain a fiber-enriched gruel (RSPF) with 21 g of dry matter in 100 g of product. A gruel made of rice flour at 10% of DM was also prepared. The starchy formulations were cooked at 90 °C under constant mixing for 15 min to obtain the total gelatinization of the starch and ensure a heat treatment to the food matrix. The gruels were then distributed in portions of 10 g into sterile falcon tubes, cooled and stored at 4 °C until inoculation.

2.3. Microorganisms and fermentation process

In this study, three freeze-dried commercial cultures of probiotic bacteria were used, specifically *L. acidophilus* LAFTI L10 (DSM, Moorebank, NSW, Australia), *L. casei* LAFTI L26 (DSM) and *B. animalis* subsp. *lactis* LAFTI B94 (DSM) and two amylolytic lactic acid bacteria (ALAB) strains from the collection of IRD, *L. fermentum* OgiE1 and *L. plantarum* A6 (LMG 18053, BCCM, Gent, Belgium), both isolated from fermented cereal-based foods. The bacterial strains were cultivated and activated in MRS broth (Difco™, Becton, Dickinson and Co, Le Point de Croix, France) as described by Nguyen et al. (2007).

The bacteria count in each inoculum was 8 log of CFU·mL^{-1} and the inoculation rate was 1 ml per 100 g of food matrices. Rice gruel and soy milk were fermented by single cultures (one of the 5 bacteria strains). RS and RSPF gruels were fermented by single bacteria cultures or by co-culture made of one amylolytic strain and one probiotic strain, performing 6 different bacteria co-cultures (PA, PB and PC = *L. plantarum* A6 in co-culture with *L. acidophilus* L10, *B. lactis* B94 and *L. casei* L26, respectively; FA, FB and FC = *L. fermentum* OgiE1 in co-culture with *L. acidophilus*