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Passion fruit by-product and fructooligosaccharides stimulate the growth and folate production by starter and probiotic cultures in fermented soymilk

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ABSTRACT

Two starter cultures (*Streptococcus* (*St.*) *thermophilus* ST-M6 and TA-40) and five probiotic strains (*St. thermophilus* TH-4, *Lactobacillus* (*Lb.*) *acidophilus* LA-5, *Lb. rhamnosus* LGG, *Lb. fermentum* PCC, and *Lb. reuteri* RC-14) were used to ferment different soymilk formulations supplemented with passion fruit by-product and/or fructooligosaccharides (FOS) with the aim of increasing folate concentrations. Growth and folate production of individual strains were evaluated and the results used to select co-cultures. Both *St. thermophilus* ST-M6 and TH-4 were the best folate producers and were able to increase the folate content of all soymilk formulations when used alone or in co-culture with lactobacilli strains, especially in the presence of both passion fruit by-product and FOS. Thus, passion fruit by-product and FOS could be used as dietary ingredients to stimulate the folate production by selected bacterial strains during the fermentation of soymilk. It was also shown that vitamin production by microorganisms is strain-dependent and may also be influenced by nutritional and environmental conditions.

1. Introduction

Soymilk has been shown to be a good medium for the growth of lactic acid bacteria (LAB) and the ability of some *Lactobacillus* spp. and *Streptococcus thermophilus* strains in metabolizing oligosaccharides during the fermentation of soymilk has been shown in different studies (Bedani et al., 2013; Champagne et al., 2009; Donkor et al., 2007; Lee et al., 2013). The α-galactosidase activity is present in some LAB and this enzyme contributes to the growth of these microorganisms during the fermentation of soy-based products through the hydrolysis of some carbohydrates, such as raffinose and stachyose. This metabolic mechanism results on the production of short chain fatty acids by these microorganisms improving intestinal human's health and reducing non-desirable gastrointestinal side-effects caused by soy products (Fung and Liong, 2010; LeBlanc et al., 2008; LeBlanc et al., 2017). Thus, the α-galactosidase activity is an important physiological characteristic presented by lactobacilli and streptococci strains once humans are not able to metabolize soy oligosaccharides.

Additionally, it is known that the processing of soybeans may cause the loss of some water soluble nutrients such as folate, a soluble B-group vitamin (Arcot et al., 2002; Mo et al., 2013). On the other hand, the ability of some starter and probiotic cultures, belonging to the LAB's group, in producing folate during fermentative processes has been described (Albuquerque et al., 2016; Pacheco da Silva et al., 2016). Probiotics are “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Hill et al., 2014).

Previous studies have shown that selected LAB can be used to increase folate content during the fermentation of milks (Gangadharan and Nampoothiri, 2011; Holasová et al., 2005; Laiño et al., 2013; Laiño et al., 2014; Pompei et al., 2007). However, the ability of these microorganisms to produce folate during the fermentation of soymilk supplemented with fruit agro-industrial wastes has not been described yet. Moreover, the use of fermentation as a natural process to bio-enrich soymilks with natural folates produced by food-grade functional microorganisms may be considered as a promising alternative to provide health benefit to consumers and also to increase the economic value of these fermented foods.

Considering that the production of folate by microorganisms is

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strain-dependent and may depend on different growth conditions, studies have been investigating the impact of different dietary ingredients on folate production by microorganisms (Albuquerque et al., 2016; Espírito-Santo et al., 2015). In this context, passion fruit by-product may be used as fermentable carbohydrates source with prebiotic potential to improve not only the growth but also the production of beneficial metabolites by LAB, including folate, during soymilks fermentation (Corrêa et al., 2016; O'Shea et al., 2015; Albuquerque et al., 2016; Vieira et al., 2017). Prebiotics are defined as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Gibson et al., 2017) and, among them, fructo-oligosaccharides (FOS) are important compounds commonly used by food and pharmaceutical industries to modulate positively the human gut microbiota (Valdés-Varela et al., 2017). However, according to Padalino et al. (2012), the presence of FOS did not stimulate the folate production by the microorganisms during the fermentation of milk.

To the best of our knowledge, there is no report about the impact of passion fruit by-product and FOS supplementation on microbial growth and folate synthesis during soymilk fermentation. Therefore, considering the beneficial effect of fruit by-products and prebiotics on growth and beneficial metabolites production by LAB, this study aimed to evaluate the impact of passion fruit by-product and FOS on the growth and folate production by starter and probiotic strains individually and in co-culture to bio-enrich different fermented soymilks.

2. Material and methods

2.1. Microorganisms

The starters *Streptococcus* (*St.*) *thermophilus* ST-M6 (Christian Hansen, Hørsholm, Denmark) and TA-40 (DuPont Danisco, Dangé, France) and the probiotic strains *St. thermophilus* TH-4, *Lactobacillus (Lb.) acidophilus* LA-5, *Lb. rhamnosus* LGG, *Lb. fermentum* PCC, and *Lb. reuteri* RC-14 (Christian Hansen) were previously selected and used for their ability to produce folate in culture media supplemented with passion fruit by-product (Albuquerque et al., 2016).

2.2. Standardization of passion fruit by-product and fructo-oligosaccharide

Passion fruit (*Passiflora edulis* f. *Flavicarpa*) by-products (PF) were supplied by De Marchi, a processing fruit company located in the state of São Paulo (Brazil), and processed to a fine powder (< 42 μm) according to Albuquerque et al. (2016). FOS P95® (Beneo, Orafti®, Oreye, Belgium) was used as prebiotic ingredient. Both ingredients (PF and FOS) were irradiated to eliminate all contaminating microorganisms, which were verified by the lack of growth on BHI broth, plate count agar and potato dextrose agar plates according to Albuquerque et al. (2016).

2.3. Production of fermented soymilks

Ultra-high temperature (UHT) treated commercial soymilk (Pura Soja, Mais Vita, Yoki) was used to prepare four different formulations: soymilk (SM), SM supplemented with 1% (w/v) of passion fruit by-product (SM + PF), SM supplemented with 1% (w/v) of fructo-oligosaccharides (SM + FOS), and SM supplemented with 0.5% PF and 0.5% FOS (SM + PF + FOS).

An aliquot of each activated strain (grown in Hogg-Jago (HJ) glucose (Blomqvist et al., 2006) or MRS broth for streptococci or lactobacilli, respectively) was washed three times, suspended in sterile saline solution (0.85% NaCl, w/v), and used to inoculate each soymilk formulation (4–5 log CFU/mL). All SM were incubated at 37 °C and viable cell counts and folate content were determined before (0 h) and after 24 h of fermentation.

2.4. Microbiological analysis

Viable *St. thermophilus* strains were plate counted in M17 agar (Oxoid) supplemented with lactose (10%); *Lb. acidophilus* LA-5 on MRS agar containing maltose instead of glucose (Bedani et al., 2013); *Lb. rhamnosus* LGG on MRS agar acidified to pH 5.4 using acetic acid; and *Lb. fermentum* PCC and *Lb. reuteri* RC-14 on MRS agar (Oxoid). All strains were incubated aerobically at 37 °C for 48 h. When in co-culture with *St. thermophilus*, lactobacilli strains were incubated anaerobically in order to be able to differentiate streptococci and lactobacilli colonies.

2.5. Determination of folate

The folate content of all fermented soymilks was determined by a microbiological assay using the indicator strain *Lb. rhamnosus* NCIMB 10463, as described previously (Albuquerque et al., 2016). The advantage of this technique is that all folate forms can be quantified together (expressed as total folate concentrations). The technique has been used by numerous researchers because of this advantage and has been validated by the International Association of Official Analytical Chemists (AOAC, 2007) (AOAC Official Methods 944.12, 992.05, 960.46 and 992.05). Samples must be properly prepared and diluted sufficiently to fall within the linear range of standard curve and special care must be taken when analysing samples that might contain other compounds that could affect the growth of the indicator strain.

Additionally, a tri-enzymatic treatment was applied to all samples as described previously (Laiño et al., 2013). This procedure allows the release of folates bound to carbohydrates and proteins (simulating the digestion of the samples) and cleaves polyglutamyl folates (the main folate forms in foods) to smaller folate forms that can be consumed by the indicator strain *Lb. rhamnosus* NCIMB 10463 during the microbiological assay (Hyun and Tamura, 2005).

2.6. Statistical analysis

Statistical analysis was performed with Minitab 17 Statistical Software® (MINITAB Inc., USA) using one-way ANOVA followed by a Tukey's post hoc test. Student's *t*-test was used to assess differences between two different means. All data represent three analytical repetitions (triplicate) and were expressed as means ± standard deviations (SD). The differences among the samples were considered statistically significant at $p < 0.05$.

3. Results

3.1. Growth of microorganisms in fermented soymilk

All strains were able to grow in the different soymilk formulations (most of them reaching counts above 7 log CFU/mL), except for *Lb. reuteri* RC-14, which only grew when PF was added (Table 1). The growth of *Lb. acidophilus* LA-5 increased in the presence of PF, FOS or PF + FOS. All tested co-cultures used to ferment the different formulations of soymilk also reached counts above 7 log CFU/mL (Table 2).

In both SM + PF and SM + PF + FOS, there was a relevant decrease in pH of the samples fermented by *Lb. acidophilus* LA-5 grown individually or in co-culture with *St. thermophilus* ST-M6 and *St. thermophilus* TH-4 (Table 3). All soymilks fermented by each individual streptococci (ST-M6, TH-4, and TA-40) in the presence of PF and/or FOS presented poor acidification with final pH ranging from 5.9 ± 0.0 to 6.4 ± 0.1. Since *Lb. reuteri* RC-14 only grew in SM + PF, the pH values of the other soymilk formulations did not differ from their initial values (Table 3).