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Tropical fruit pulps decreased probiotic survival to *in vitro* gastrointestinal stress in synbiotic soy yoghurt with okara during storage

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A B S T R A C T

The effect of mango and guava pulps on *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* Bb-12 viability in a soy yoghurt (SY) and on probiotic survival under simulated gastrointestinal conditions were investigated throughout 28 days of storage at 4 °C. The impact of fruit pulps on SY sensory acceptability was also assessed. Three formulations were produced from soymilk fermented with ABT-4 culture (La-5, Bb-12, and *Streptococcus thermophilus*) and supplemented with inulin and okara: SYC (control), SYM (with mango pulp), and SYG (with guava pulp). All formulations showed probiotic viabilities ranging from 8 to 9 log cfu/g, and fruit pulps did not affect the probiotic viabilities. However, the fruit pulps decreased probiotic survival significantly to simulated gastrointestinal stress. Acceptability was higher for SYM and this difference was significant at 21 days. Therefore, the improved acceptability of SY through the addition of fruit pulps might lead to a reduction in probiotic functionality.

1. Introduction

The incorporation of soybean as part of the human diet is increasing due to its nutritional characteristics such as high quality proteins, polyunsaturated fatty acids, dietary fibres, besides its important role in the reduction of the risk of cardiovascular diseases, type 2 diabetes, cancer, and osteoporosis (Chen, Li-Jun, Jian-Jun, Bo, & Rui, 2010; Rinaldoni, Campderrós, & Padilla, 2012; Xiao, 2008). In addition, studies have also suggested that okara, a soybean waste generated from soymilk production, might be used in the food industry to confer increased nutritional and functional properties to products (Jiménez-Escrig, Tenorio, Espinosa-Martos, & Rupérez, 2008). However, soy consumption has been limited owing to its undesirable beany flavour and the presence of oligosaccharides (stachyose and raffinose) that often lead to flatulence and stomach discomfort (Yeo & Liong, 2010).

Soymilk fermentation, especially with lactic acid bacteria, for the production of soy yoghurt (SY), may improve its flavour and texture, as well as enhance its beneficial health properties (Cruz et al., 2009; Donkor, Henriksson, Vasiljevic, & Shah, 2005). Several studies have shown that soy products, particularly soy yoghurt, may be good vehicles for probiotic microorganisms (Bedani, Rossi, & Saad, 2013; Champagne, Green-Johnson, Raymond, Barrette, & Buckley, 2009; Donkor, Henriksson, Vasiljevic, & Shah, 2007a,b; Farnworth et al., 2007; Wang et al., 2009). In addition to the use of probiotic microorganisms, prebiotic ingredients like inulin may lead to a protective effect, improving the survival and activity of probiotic bacteria during storage of probiotic food products, as well as during the passage through the gastrointestinal tract (GIT) (Akin, Akin, & Kirmaci, 2007; Buriti, Castro, & Saad, 2010; Hernandez-Hernandez et al., 2012).

The probiotic microorganisms most widely used are strains belonging to the *Lactobacillus* and *Bifidobacterium* genera (Saxelin, Tynkkynen, Mattila-Sandholm, & de Vos, 2005). Several beneficial effects have been attributed to *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* Bb-12, among them: prophylactic activity against infectious rotavirus diarrhoea in children (Weichert, Schroten, & Adam, 2012; Weizman, Asli, & Alsheikh, 2005); relief of clinical symptoms of atopic dermatitis in children (Isolauri, Arvola, Sütas, Moilanen, & Salminen, 2000); intestinal microbiota

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modulation (Savard et al., 2011), and hypocholesterolemic effect (Abd El-Gawad, El-Sayed, Hafez, El-Zeini, & Saleh, 2005).

The flavouring agents also appears as a promising alternative to improve nutritional and sensorial properties of soy-based products (Behrens, Villanueva, & Silva, 2007; Granato, Ribeiro, Castro, & Masson, 2010). As the beany flavour of soybean is unacceptable to many consumers (Behrens et al., 2007), the addition of flavouring agents like fruit pulps and essences into soy yoghurt might help to mask these unpleasant sensory properties and potentially increase acceptability. Besides its nutritional appeal, fruit pulps such as mango and guava pulps have been used to increase the acceptability of soy-based products (Granato et al., 2010; Kumar & Mishra, 2003).

The consumption of probiotic yoghurts with fruits is increasing among consumers (Kailasapathy, Harmstorf, & Phillips, 2008). Nevertheless, most of the viability studies are done using natural or plain milk-based probiotic yoghurts and few investigated the impact of the incorporation of fruits on probiotic populations (Kailasapathy et al., 2008; Nualkaekul & Charalampopoulos, 2011; Ranadheera, Evans, Adams, & Baines, 2012; Vinderola, Costa, Regenhardt, & Reinheimer, 2002). The effect of natural fruit juices on the growth of probiotics and yogurt starter culture was reported to be strain-dependent (Vinderola et al., 2002). Studies that investigate the effect of fruit pulps on survival of probiotic microorganisms exposed to simulated gastrointestinal conditions in different food matrices are scarce. Moreover, to our knowledge, no information regarding the effect of tropical fruit pulps on gastric and enteric tolerance of probiotic strains in soy yoghurt matrix is available in the scientific literature. Besides food matrix, the storage period also seems to affect the probiotic resistance to simulated gastrointestinal stresses (Saarela, Virkajärvi, Alakomi, Sigvart-Mattila, & Mättö, 2006; Vinderola et al., 2011; Wang et al., 2009).

The resistance to simulated gastrointestinal juices is among the *in vitro* assays that are frequently suggested for the evaluation of the strain probiotic potential (Buriti et al., 2010; Gbassi, Vandamme, Yolou, & Marchioni, 2011). Checking the tolerance of probiotic microorganisms in the final product towards gastrointestinal conditions may help to select a suitable food matrix and contribute to probiotic survival and efficacy in the GIT (Buriti et al., 2010; Schillinger, Guigas, & Holzapfel, 2005).

Therefore, the aim of this study was to investigate the influence of the addition of the tropical fruit pulps mango and guava on the viability and resistance to simulated gastrointestinal conditions of *L. acidophilus* La-5 and *B. animalis* Bb-12 in synbiotic soy yoghurts with okara throughout 28 days of storage at 4 °C, and on the sensory acceptability of these products.

2. Material and methods

2.1. Production of okara flour

Okara was supplied by the Development and Production Unit for Soybean Derivatives located at the Faculty of Pharmaceutical Sciences of the São Paulo State University. The okara flour was obtained according to Bedani et al. (2013).

2.2. Synbiotic soy yoghurt manufacture

Three pilot-scale-making formulations of SY were produced, in triplicates (three different batches of the same formulation, using new soymilk batch and new inoculum), from soymilk enriched with calcium (120 mg/100 mL of soymilk), according to Table 1. The three types of products were fermented with an ABT-4 culture (Christian Hansen, Hørsholm, Denmark), containing the probiotic strains *L. acidophilus* La-5 and *B. animalis* subsp. *lactis* Bb-12, and the starter *Streptococcus thermophilus*: SYC (control), SYM (with mango pulp and essence), and SYG (with guava pulp and essence). The soy products were manufactured according to the procedure described by Rossi, Vendramini, Carlos, Pei, and Valdez (1999).

Table 1
Ingredients employed in the production of synbiotic soy yoghurt with okara.

Ingredients (g/100 mL of soymilk)	Products		
	SYC	SYM	SYG
Sugar	8.00	8.00	8.00
Okara flour	5.00	5.00	5.00
Inulin	3.00	3.00	3.00
Skimmed milk powder	2.50	2.50	2.50
Lactose	1.00	1.00	1.00
Soybean oil	0.80	0.80	0.80
Gelatin	0.30	0.30	0.30
Probiotic culture[a]	0.50	0.50	0.50
Pasteurised and frozen mango pulp	0.00	12.5	0.00
Pasteurised and frozen guava pulp	0.00	0.00	12.5
Identical to natural mango flavour	0.00	0.05	0.00
Natural guava flavour	0.00	0.00	1.00

[a] ABT-4 culture (containing the probiotic strains *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* Bb-12, and the starter *Streptococcus thermophilus* Christian Hansen, Hørsholm, Denmark) with around 10^{10} cfu/g of La-5 and 10^{11} cfu/g of Bb-12. SYC: control soy yoghurt (SY); SYM: SY with mango pulp and essence; SYG: SY with guava pulp and essence.

Each formulation of SY was manufactured in batches of 4 L. Lactose (1.0 g/100 mL of soymilk) (Alibra®, Alibra Ingredientes, Campinas, Brazil) and soybean oil (0.8 g/100 mL of soymilk) (Liza, Cargill Agrícola, Uberlândia, Brazil) were added to calcium-enriched soymilk (Mais Vita, Yoki Alimentos, São Bernardo do Campo, Brazil) and mixed for 5 min using a blender (Magiclean, Arno, São Paulo, Brazil). The mixture was then heated to 50 °C for the addition of sugar (8.0 g/100 mL of soymilk) (da Barra®, Cosan, Barra Bonita, Brazil) and up to 80 °C for the incorporation of skimmed milk powder (2.5 g/100 mL of soymilk) (Molico, Nestlé, Araçatuba, Brazil), gelatin (0.3 g/100 mL of soymilk) (Royal, Gelco Gelatinas do Brasil, Pedreira, Brazil), and inulin (3.0 g/100 mL of soymilk) (Beneo® GR, Orafti, Oreye, Belgium). The mixture was again put into a blender for 5 min and heated to 95 °C for the addition of okara flour (5.0 g/100 mL of soymilk). Afterwards, the mixture was cooled to 37 °C for the addition of the ABT culture (Christian Hansen) at 0.5 g/100 mL of soymilk in order to achieve an inoculation level of 8 log cfu/g of each probiotic strain used (*L. acidophilus* La-5 and *B. animalis* Bb-12). Fermentation took place at 37 °C until reaching pH 5.0, in order to guarantee that the pH values during the storage were around 4.5. Next, the product was cooled in an ice-bath and refrigerated (4 ± 1 °C) for 24 h. Then, pasteurised and frozen mango pulp (12.5 g/100 mL of soymilk) (*Mangifera indica* L.; Icefruit, Icefruit Comércio de Alimentos, Tatuí, Brazil) and mango essence (0.05 g/100 mL of soymilk) (Duas Rodas, Jaraguá do Sul, Brazil) or pasteurised and frozen guava pulp (12.5 g/100 mL of soymilk) (*Psidium guajava*; Icefruit, Icefruit Comércio de Alimentos, Tatuí, Brazil) and guava essence (1.0 g/100 mL of soymilk) (Duas Rodas, Jaraguá do Sul, Brazil) were incorporated into SY after the breaking of coagulum. Portions of 50 g and 20 g (for sensory evaluation) of SY were packaged in polypropylene plastic pots for food products (Tries Aditivos Plásticos, São Paulo, Brazil) and sealed with metallic covers with varnish in a sealer (Delgo Nr. 1968, Delgo Metalúrgica, Cotia, Brazil). The batches were stored under refrigeration (4 ± 1 °C) for 28 days. Products from each formulation were used to determine the viability of the probiotic and starter cultures and pH after 1, 7, 14, 21, and 28 days of storage, the probiotic resistance to simulated gastrointestinal conditions after 1, 14, and 28 days of storage, and sensory acceptability after 7, 14, and 21 days of storage.