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Supplementation with fruit and okara soybean by-products and amaranth flour increases the folate production by starter and probiotic cultures

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ABSTRACT

The ability of two starter cultures (*Streptococcus* (*S.*) *thermophilus* ST-M6 and *St. thermophilus* TA-40) and eleven probiotic cultures (*St. thermophilus* TH-4, *Lactobacillus* (*Lb.*) *acidophilus* LA-5, *Lb. fermentum* PCC, *Lb. reuteri* RC-14, *Lb. paracasei* subsp. *paracasei*, *Lb. casei* 431, *Lb. paracasei* subsp. *paracasei* F19, *Lb. rhamnosus* GR-1, and *Lb. rhamnosus* LGG, *Bifidobacterium* (*B.*) *animalis* subsp. *lactis* BB-12, *B. longum* subsp. *longum* BB-46, and *B. longum* subsp. *infantis* BB-02) to produce folate in a modified MRS broth (mMRS) supplemented with different fruit (passion fruit, acerola, orange, and mango) and okara soybean by-products and amaranth flour was investigated. Initially, the folate content of each vegetable substrate was determined: passion fruit by-product showed the lowest folate content (8 ± 2 ng/mL) and okara the highest (457 ± 22 ng/mL). When the orange by-product and amaranth flour were added to mMRS, all strains were able to increase folate production after 24 h of fermentation. *B. longum* subsp *infantis* BB-02 produced the highest concentrations (1223 ± 116 ng/mL) in amaranth flour. Okara was the substrate that had the lowest impact on the folate production by all strains evaluated. *Lb. acidophilus* LA-5 (297 ± 36 ng/mL) and *B. animalis* subsp. *lactis* BB-12 (237 ± 23 ng/mL) were also able to produce folate after growth in mMRS containing acerola and orange by-products, respectively. The results of this study demonstrate that folate production is not only strain-dependent but also influenced by the addition of different substrates in the growth media.

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1. Introduction

Folate, an essential B-group vitamin, is the generic term for the naturally occurring folates and includes folic acid (FA), which is the fully oxidized synthetic form used in food fortification (Fajardo et al., 2012; Laiño et al., 2013a; LeBlanc et al., 2013; Rossi et al., 2011). This vitamin is involved in important metabolic activities such as DNA replication, repair and methylation and the biosynthesis of nucleic acids and some amino acids. It has also been shown to provide protection against certain types of cancers, and decrease in the risk of cardiovascular disease and is mostly known for its role in the development of the neural tubes of fetuses (Kariluoto et al., 2010; Laiño et al., 2013a).

Since humans are not able to synthesize folates, they need to acquire this vitamin exogenously from foods or dietary supplements (Laiño et al., 2014). Besides having a high cost of production, FA, the chemical form used by many countries for the mandatory fortification of foods, has shown to exert adverse secondary effects when consumed in large quantities, such as masking symptoms of vitamin B_{12} deficiency and possibly promoting certain types of cancer (Bailey and Ayling, 2009; Fajardo et al., 2012). In this sense, the bio-enrichment of foods with natural folates produced by selected microorganisms during the fermentative process has become a promising alternative to mandatory fortification with FA in order to prevent deficiencies that are present in a growing percentage of different populations throughout the world (Gangadharan and Nampoothiri, 2011; Iyer et al., 2009; Laiño et al., 2013a;. Laiño et al., 2013b; Laiño et al., 2014). Some strains of lactic acid bacteria (LAB) and bifidobacteria, mostly from the genus *Streptococcus*, *Lactobacillus*, and *Bifidobacterium*, widely used by the food industry to produce a variety of fermented foods, have been described as folate producers (Crittenden et al., 2003; Padalino et al., 2012; Pompei et al., 2007). In addition to the ability to produce folate, some bacterial strains possess other beneficial properties (such as immunological, neurological, endocrinological effects, can produce bioactive compounds, among others) which make them probiotic which are “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Hill et al., 2014). The ability of microorganisms to produce folate is a strain specific trait that can be influenced by the growth conditions

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including the presence or absence of carbohydrates, proteins or other important nutrients required for the microorganism multiplication (D'Aimmo et al., 2012; Kariluoto et al., 2006; Laiño et al., 2012; Laiño et al., 2013b; Padalino et al., 2012; Pompei et al., 2007; Sybesma et al., 2003). In this context, studies have suggested that different substrates may be used to stimulate folate production by bacteria and in turn increase the natural folate levels in the growth media (Gangadharan and Nampoothiri, 2011; Holasová et al., 2005; Padalino et al., 2012).

In this line, some studies have evaluated the potential of by-products from fruit processing industries (peels, pulps, and seeds) as a source of dietary fibres and other bioactive compounds (Aguedo et al., 2012; López-Vargas et al., 2013; O'Shea et al., 2012; O'Shea et al., 2015). Additionally, there are reports that suggest that okara, a soybean by-product generated from soymilk and tofu (bean curd) industries, is also rich in nutritional and functional compounds (Jiménez-Escrig et al., 2008; Mateos-Aparicio et al., 2010; Stanojevic et al., 2013; Villanueva et al., 2011). The fruit and vegetable by-products generated by the Brazilian industry is either used as animal feed or discarded in the environment, causing environment contamination problems (Ayala-Zavala et al., 2010). A strategy to minimize this problem towards sustainable food processing is the use of these by-products in the development of new value-added products (Bedani et al., 2013; Espírito Santo et al., 2012a; Espírito Santo et al., 2012b). Furthermore, amaranth (*Amaranthus* spp.) is a pseudocereal that has attracted much interest of researchers in recent years, particularly due its excellent nutrient profile, providing good quality protein, dietary fibres, and lipids rich in unsaturated fats (Alvarez-Jubete et al., 2010; Tiengo et al., 2009). Thus, the aim of this study was to evaluate if the supplementation with fruit and okara by-products or amaranth flour affected the ability of two starter cultures (streptococci) and eleven probiotic cultures (streptococci, lactobacilli, and bifidobacteria) to produce folate in culture media.

2. Material and methods

2.1. Amaranth flour and the production of fruit and okara by-products

Passion fruit (*Passiflora edulis* f. *Flavicarpa*), orange (*Citrus sinensis*), acerola (*Malpighia emarginata*), and mango (*Mangifera indica*) by-products were supplied by fruit processing industries (on August, March, July and December 2014, respectively) located in the state of São Paulo (Brazil) and stored at -18 ± 2 °C until use to avoid enzymatic action and microbial contamination. Okara by-product was supplied by UNIVERSOJA (Production and Development Unit for Soybean Derivates) located at the School of Pharmaceutical Sciences of the São Paulo State University (Araraquara, São Paulo, Brazil) and was obtained as a fine powder (<42 mm) as described by Bedani et al. (2013). Commercial amaranth flour (Vida Boa — Produtos Naturais, Limeira, SP, Brazil) was obtained from a local store in the city of São Paulo (São Paulo, Brazil). All fruit by-products were processed according to the method described by Espírito Santo et al. (2012a,b) with some modifications. The fruit by-products were thawed at 4 ± 2 °C for 48 h, washed and bleached using clean water at 100 °C (12 min) followed by ice bath. Then, the fruit by-products were dried in oven under air flow at 60 °C for 24 h until completely dry. Afterwards, the dry material was reduced to fine powder in a blender (Magiclean, Arno, São Paulo, Brazil) and sieves (Granutest, São Paulo, Brazil) were used to standardize the particle size (<42 mm). All powders were stored in polypropylene bags and kept at -18 ± 2 °C until the analysis.

2.2. Irradiation of fruit and okara by-products powders and amaranth flour

Portions of 2.5 g of each powder were weighed in polypropylene bags, sealed and transported to Nuclear and Energy Research Institute (IPEN, São Paulo, Brazil) to perform the irradiation process of the samples using a modification of the method described by Rezende et al. (2014). Briefly, the samples were exposed to radiation (radioactive source ^{60}Co) in a Gammacell 220 irradiator (Atomic Energy of Canada Ltd., Ottawa, Canada) with an activity of 1287.6 Ci using a dose of 25 kGy at a rate of 1.089 kGy/h.

2.3. Microbiological analyses of irradiated samples

After irradiation, each sample (2.5 g) was added to 100 mL of Brain Heart Infusion (BHI) broth (Oxoid, Basignstoke, UK) and incubated at 37 °C for 24 h. After the incubation period, 100 μL of each sample was transferred to 3 sterile plates which were filled with Plate Count agar (Oxoid) or Potato Dextrose agar (Oxoid) supplemented with tartaric acid 10% solution using *pour plate* technique to confirm the absence of any contaminating microorganism.

2.4. Microorganisms, culture media, and growth conditions

The microbial strains employed in this study as well as the culture media and incubation conditions are shown in Table 1.

For the *in vitro* test, a modified MRS medium (mMRS) containing peptone (10 g; Oxoid, Basignstoke, RU), 'LAB-LEMCO' Powder (8 g; Oxoid), yeast extract (4 g; Oxoid), Tween 80 (1 mL; Merck, Hohenbrunn, Germany), ammonium acetate (2 g; Labsynth, São Paulo, Brazil), $MgSO_4 7H_2O$ (0.18 g; Merck), $MnSO_4 H_2O$ (0.05 g; Merck), Na_2SO_4 (2 g; Labsynth), K_2SO_4 (1.25 g; Labsynth), Na_2CO_3 (0.2 g; Labsynth), $CaCl_2 \cdot 2H_2O$ (0.11 g; Labsynth), L(þ)-cysteine HCl (0.5 g; BioChemica, Sigma Aldrich, Switzerland), phenol red (0.18 g; Labsynth) and distilled water (1 L) was used.

2.5. In vitro fermentation assay

The effect of fruit and okara by-products powders and amaranth flour on folate production by different bacteria was evaluated using an *in vitro* model assay adapted from Ryu et al. (2007) and Buriti et al. (2014). Each strain was cultured twice in its respective culture broth and incubation conditions as described in Table 1 for 24 h at 37 °C. An aliquot of 1 mL was taken from the second growth, centrifuged (10,000 *g* for 5 min), washed three times using sterile saline solution (0.85 g NaCl/100 mL), resuspended at the same initial volume (1 mL) using sterile saline and used to inoculate (5 log colony forming units (CFU)/mL) mMRS supplemented with 1% (w/v) of each irradiated

Table 1
Starter and probiotic cultures tested and culture media and incubation procedures employed.

Strains	Code	Type of culture	Culture media	Incubation condition
Streptococcus (*St.*) *thermophilus*				
St. thermophilus	ST-M6*	1	HJ[a]	Aerobic
St. thermophilus	TH-4*	2		
St. thermophiles	TA-40**	1		
Lactobacillus (*Lb.*) spp.				
Lb. acidophilus	LA-5*	2	MRS[b]	Anaerobic[d]
Lb. fermentum	PCC*	2		
Lb. reuteri	RC-14*	2		
Lb. paracasei subsp. *paracasei L. casei*	431*	2		
Lb. paracasei subsp. *paracasei*	F-19*	2		
Lb. rhamnosus	GR-1*	2		
Lb. rhamnosus	LGG*	2		
Bifidobacterium (*B.*) spp.				
B. animalis subsp. *lactis*	BB-12*	2	MRS cysteine (0.05%)[c]	Anaerobic[d]
B. longum	BB-46*	2		
B. longum subsp. *infantis*	BB-02*	2		

*Christian Hansen; **Danisco; 1 — Starter cultures; 2 — Probiotic cultures; [a]Hogg-Jago (HJ) glucose broth (Blomqvist et al., 2006); [b]MRS broth (Oxoid, Basingstoke, UK) with L-cysteine (0.05% w/v, Sigma-Aldrich, St. Louis, USA); [c]MRS broth (Oxoid); [abc]Culture media used to prepare the inoculum. [d]AnaeroGen™ Anaerobic System (Oxoid).

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