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The impact of fruit and soybean by-products and amaranth on the growth of probiotic and starter microorganisms

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A B S T R A C T

The ability of different fruit by-products, okara, and amaranth flour, to support the growth of probiotic and non-probiotic strains was evaluated. The tests were conducted with three commercial starter cultures (*Streptococcus thermophilus*), ten probiotic strains (seven *Lactobacillus* spp. and three *Bifidobacterium* spp. strains), and two harmful bacteria representative of the intestinal microbiota (*Escherichia coli* and *Clostridium perfringens*). *In vitro* fermentability assays were performed using a modified MRS broth supplemented with different fruits (acerola, orange, passion fruit, and mango), and soy (okara) by-products or amaranth flour. Orange and passion-fruit by-products were the substrates that most promoted the growth of bacterial populations, including pathogenic strains. On the other hand, the acerola by-product was the substrate that showed the highest selectivity for beneficial bacteria, since the *E. coli* and *Cl. perfringens* populations were lower in the presence of this fruit by-product. Although the passion fruit by-product, okara, and amaranth stimulated the probiotic strains, the growth of the pathogenic strains studied was higher compared to other substrates. Different growth profiles were verified for each substrate when the different strains were compared. Although pure culture models do not reflect bacterial interaction in the host, this study reinforces the fact that the ability to metabolize different substrates is strain-dependent, and acerola, mango, and orange by-products are the substrates with the greatest potential to be used as prebiotic ingredients.

1. Introduction

For more than two decades, Brazilian agriculture has registered strong growth. Brazil has become a major exporter of agricultural products, with a surplus of USD 78.6 billion in 2013. The country is one of the largest fruit exporters and the largest exporter of processed citrus, particularly concentrated frozen orange juice. In addition to citrus fruits, the main fruits produced include bananas, apples, grapes, melons, and tropical fruits, particularly papayas, mangoes, avocados, and pineapples. These last three are the most important in terms of volume. In the grain sector, soybeans are expected to continue to be one of the most important agricultural products. Currently, Brazil is the second largest producer, only behind the USA, but this scenario is expected to change by 2024, with Brazil overtaking the USA (OECD/FAO, 2015).

By-products generated in the fruit and vegetable processing industries are also an important environmental problem, resulting in significant economic losses for the sector. These facts have increased the interest of the food industries in discovering and applying strategies to improve the sustainability of food processing, such as the use of these by-products for livestock feeding and fuel production (Villanueva-Suárez, Pérez-Cózar, & Redonco-Cuenca, 2013). Even though they are frequently treated as industrial waste, they might be good sources of nutrients and bioactive compounds and improve the nutritional and functional properties of food products. A good example of this is okara, a by-product of soymilk and tofu (bean curd) processing, which presents high amounts of dietary fibres, proteins, lipids, and minerals, along with unspecified monosaccharides and oligosaccharides (Jiménez-Escrig, Tenorio, Espinosa-Martos, & Rupérez, 2008; Mateos-Aparicio, Mateos-Peinado, Jiménez-Escrig, & Rupérez, 2010). In general, okara may be considered a good and cheap source of dietary fibres, since they are its major component (Lu, Liu, & Li, 2013), and could be used to increase the content of high-value compounds in different products (Bedani, Campos, Castro, Rossi, & Saad, 2014; Villanueva-

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Suárez et al., 2013).

The majority of by-products generated by the fruit industry are made up of peel, seeds, and skins (O'Shea, Arendt, & Gallagher, 2012). Peel constitutes around 15–20% of the fresh fruit, and some studies have demonstrated that this is also a promising source of health-promoting compounds and could be used in functional foods (Ajila, Aalami, Leelavathi, & Rao, 2010; Barros, Ferreira, & Genovese, 2012). Passion fruit peel, for example, is rich in dietary fibre (DF), especially pectin (Zeraik, Pereira, Zuin, & Yariwake, 2010). By-products from orange juice production have also been widely studied and applied as a DF source in various products, such as bakery products (Romero-Lopez, Osorio-Diaz, Bello-Perez, Tovar, & Bernardino-Nicanor, 2011), functional dairy products (Sendra et al., 2008), and meat products (Sayas-Barberá, Viuda-Martos, Fernández-López, Pérez-Alvarez, & Sendra, 2012). Acerola, as well as passion fruit and orange by-products, presents significant amounts of DF, especially pectin, polyphenols, anthocyanins, phenolic compounds, and α-glucosidase inhibitors (Assis, Lima, & Faria Oliveira, 2001; Paz et al., 2015; Rochette et al., 2013). Mango by-products have important bioactive compounds to improve health and reduce the risk of diseases, and studies have demonstrated their use as food ingredients in many products (Ajila et al., 2010; Jahurul et al., 2015).

Dietary fibres are largely present in fruit by-products generated during processing and their potential to increase the population of beneficial bacteria, including *Lactobacillus* spp. and *Bifidobacterium* spp. has been recognized (Silva, Cazarin, Bogusz Junior, Augusto, & Maróstica Junior, 2014). Soluble fibres may form gels in the gastrointestinal tract, which contribute to their fermentability by the gut microbiota. The products generated during this fermentation may be associated with different effects on the host's health and well-being, such as increasing colon bacteria biomass and reducing colonic pH through the production of short-chain fatty acids. These compounds are important for the nutrition of enterocytes and the inhibition of pathogenic bacteria (Gibson, 2004).

Another potential substrate that could be used as a fermentable functional ingredient is amaranth (*Amaranthus* spp.) seed flour. Amaranth is a highly nutritional pseudocereal, which is an excellent source of proteins and other nutrients, including dietary fibre, vitamins, and minerals (Caselato-Sousa & Amaya-Farfán, 2012). *In vitro* and *in vivo* assays have shown that hydrolysis of amaranth proteins leads to the release of bioactive peptides with potential antithrombotic and antihypertensive activities (Fritz, Vecchi, Rinaldi, & Añón, 2011; Sabbione, Nardo, Añón, & Scilingo, 2016).

Therefore, as plant substrates may have compounds which might be promising in terms of stimulating the growth of beneficial bacteria, the present study aimed to evaluate the fermentation of different vegetable (fruit and soy) by-products, as well as amaranth flour by probiotic (lactobacilli and bifidobacteria) and starter (streptococci) strains and by harmful bacteria representative of the intestinal microbiota (*Escherichia coli* ATCC 8739 and *Clostridium perfringens* ATCC 13124).

2. Material and methods

2.1. Amaranth flour and the manufacture of the industrial by-products powder

Orange, acerola, passion fruit, and mango by-products were obtained from fruit processing companies located in the state of São Paulo, Brazil (in March, July, August, and December 2014, respectively) and stored at − 18 °C until their processing. Peel and seeds were the fruit by-products employed, except for mango, where only peel was used. All frozen fruit by-products were thawed at 4 °C for 48 h, washed, bleached (for 12 min), and dried in an air flow oven (60 °C/24 h). A blender (Magiclean, Arno, São Paulo, Brazil) was used to reduce the dry fruit by-products into a fine powder, and sieves were used to standardize the particle sizes (diameter below 0.42 mm). The final by-product powders were stored in polypropylene bags and kept at − 18 °C until use. Okara was supplied by UNIVERSOJA (Production and Development Unit for Soybean Derivatives) located at the School of Pharmaceutical Sciences of the São Paulo State University (Araraquara, São Paulo state, Brazil) according to Bedani, Rossi, & Saad (2013). The amaranth flour (Vida Boa, Produtos Naturais, Limeira, Brazil) was obtained from a local store (São Paulo, Brazil). The fruit and okara by-products powder (< 0.42 mm) and amaranth flour were processed and sterilized by gamma irradiation following Albuquerque, Bedani, Vieira, LeBlanc, & Saad (2016).

2.2. Physicochemical analysis of the by-products and amaranth flour

Total solids, lipids, protein (N × 6.25), and ash were determined according to the methods described by IAL (2008). Carbohydrate content was calculated by difference to reach 100 g of total contents. Total dietary fibre, soluble fibre, and insoluble fibre quantifications were conducted at the Physicochemical Laboratory of the Food Intelligence Laboratories Inc. (São Paulo, Brazil), using the enzymatic-gravimetric method 991.43 (AOAC, 2003).

2.3. Bacterial growth in the presence of fruit or soybean by-products, or amaranth flour

Thirteen food-grade strains normally used as probiotic or starter cultures by the food manufacturers, as well as two harmful bacteria representative of the intestinal microbiota, were evaluated using an *in vitro* fermentability test, according to García-Cayuela et al. (2014), Ryu, Kim, Park, Lee, & Lee (2007), and Watson et al. (2012). Briefly, fresh cultures of each strain (obtained at 37 °C for 24 h in the cultivation conditions described in Table 1), were individually inoculated (4–5 log CFU) in formulated modified DeMan-Rogosa-Sharpe (MRS) broth with phenol red (mMRS, Ryu et al., 2007), individually supplemented with 1% (w/v) of each by-product or amaranth flour. The enumeration of the bacterial populations was performed before (0 h) and after 24 h, and 48 h of incubation at 37 °C, using the appropriate agar media for each microorganism (Table 1).

2.4. Statistical analysis

The Shapiro-Wilk test was used to verify the data normality, and the Bartlett test was used to check assumptions of homoscedasticity. In order to evaluate the global effect of vegetable substrates on bacterial growth, in addition to chemical composition, data were submitted to Variance Analysis (ANOVA), and the Tukey test was used for means comparison. Data that did not show normality and homoscedasticity were submitted to Kruskal-Wallis and to means comparison by the Fisher LSD test. The statistical package Statistica 13.0 (StatSoft, Tulsa, OK, USA) was employed, and the results were presented as means ± standard deviation (SD). Principal Components Analysis was performed to evaluate the relationships between each bacterial strain and the vegetable substrates after 24 h and 48 h of incubation. Covariance was the matrix type used in the mapping. For this purpose, the XLSTAT 2017 software was employed.

3. Results

3.1. Chemical composition of the by-products and of amaranth flour

The chemical composition of each by-product and amaranth flour is presented in Table 2. A significant variation in total solids (TS) between all the by-products evaluated and amaranth flour ($p < 0.05$) after drying was observed. This variation in TS is probably due to the smaller size of the acerola and passion fruit fragments, compared to those of orange and mango, which resulted in a larger contact area, leading to higher mass transfer during the drying process. Regarding protein