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Tropical fruit by-products water extracts as sources of soluble fibres and phenolic compounds with potential antioxidant, anti-inflammatory, and functional properties

Marcela Albuquerque Cavalcanti de Albuquerque[a,b], Romina Levit[c], Carolina Beres[d], Raquel Bedani[a,b], Alejandra de Moreno de LeBlanc[c,*,1], Susana Marta Isay Saad[a,b,*,1], Jean Guy LeBlanc[c,1]

[a] Department of Biochemical and Pharmaceutical Sciences, School of Pharmaceutical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 580, 05508-000 São Paulo, SP, Brazil
[b] Food Research Center, University of São Paulo, São Paulo, Brazil
[c] CERELA-CONICET, C.P. T4000ILC, San Miguel de Tucumán, Argentina
[d] Brazilian Agricultural Research Corporation (EMBRAPA), Américas Avenue 29501, 23020-470 Guaratiba, Rio de Janeiro, Brazil

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ABSTRACT

Fibre content, phenolic content, antioxidant and anti-inflammatory activities were evaluated for water extracts of fruit by-products (passion fruit, orange, acerola, and mango). The impact of these extracts on microbial growth, folate production, and adhesion ability of *Streptococcus thermophilus* TH-4 and *Lactobacillus rhamnosus* LGG was investigated. Mango water extract (MWE) presented the highest phenolic content and antioxidant activity. Orange water extract (OWE) and MWE showed the best anti-inflammatory potential by decreasing the highest nitric oxide levels. When TH-4 and LGG were grown together, folate production was only stimulated by MWE. Passion fruit water extract and OWE increased the TH-4 adhesion whereas acerola water extracts and MWE improved LGG adhesion when strains were used individually. These results showed that fruit by-product water extracts (FWE), especially from mango, presented potential beneficial biological and functional properties. These FWE could be used to develop new functional antioxidant foods and natural pharmaceutical ingredients.

1. Introduction

Brazil produces and processes an impressive amount of tropical fruits, such as orange, passion fruit, mango, and acerola. As a result, large amounts of fruit by-products (peel, pulp, and seeds) are generated from the fruit processing industry and represent an important environmental problem (Vieira, Bedani, Albuquerque, Bíscola, & Saad, 2017). In contrast, these residues are a natural source of nutrients and different bioactive molecules. These biocompounds have great potential to be used as functional food ingredients or for application as phytochemical pharmaceutical substances for the prevention or treatment of human diseases (Kowalska, Czajkowska, Cichowska, & Lenart, 2017; O'Shea et al., 2015; Oliveira, Angonese, Gomes, & Ferreira, 2016).

In this context, dietary fibres (DF) are important plant components present in large amounts in fruit by-products. DF are not hydrolysed by the endogenous enzymes in the small intestine of humans (Joint FAO/WHO, 2010) and their consumption is associated with beneficial health effects, including, regulation of the intestinal transit, prevention or treatment of cancer, diabetes, and cardiovascular diseases (Beres et al., 2016; Wang et al., 2015).

Phenolic compounds are known to be widely present in fruits and their by-products, and they are the most important group of natural antioxidants in the diet. These bioactive molecules act as reducing agents and can improve human health. Besides the structural diversity of phenolic compounds, it is crucial to determine their biological properties such as anti-inflammatory activity, microbial growth

* Corresponding authors at: Department of Biochemical and Pharmaceutical Sciences, School of Pharmaceutical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 580, 05508-000 São Paulo, SP, Brazil (S.M.I. Saad).
E-mail addresses: malbuquerque17@gmail.com (M.A.C. Albuquerque), rlevit@cerela.org.ar (R. Levit), carolberes@gmail.com (C. Beres), raquelbedani@yahoo.com.br (R. Bedani), demoreno@cerela.org.ar (A. de Moreno de LeBlanc), susaad@usp.br (S.M.I. Saad), leblanc@cerela.org.ar (J.G. LeBlanc).
[1] Authors participated equally in this work.

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stimulation, and their impact on the production of beneficial metabolites by different microorganisms, especially by probiotics (LeBlanc et al., 2017; Oh et al., 2012; Rocchetti, Chiodelli, Giuberti, & Lucini, 2018; Santos et al., 2017). Probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill et al., 2014).

DF from fruits by-products may be good sources of bound phytochemicals including phenolic compounds (Haminiuk, Maciel, Plata-Oviedo, & Peralta, 2012). In some cases, the complex DF-phenolic compounds is considered as being an antioxidant dietary fibre, a category defined as "a dietary fibre concentrate containing significant amounts of natural antioxidants associated with non-digestible compounds" (Quirós-Sauceda et al., 2014).

According to Ajila and Rao (2013), DF associated with polyphenols may enhance the antioxidant effect of these bound phenolic compounds in the human intestine when compared to the non-bound ones. Considering that the DF-phenolic complexes are not hydrolysed by the human digestive enzymes (Velderrain-Rodríguez et al., 2016), they may exert a positive impact on the gut microbiota modulation (Tomás-Barberán, Selma, Espín, 2016). In addition, they contribute to maintain a balanced oxidant status in the intestinal environment, in that manner, protecting the intestinal epithelium against inflammatory processes, protecting the intestine against the colonization by pathogens, and increasing the adhesion of probiotics microorganisms to intestinal cells (Ajila & Rao, 2013; Dueñas et al., 2015).

Inflammatory processes may start in the gut due to an unbalanced intestinal microbiota promoting the action of pathogens and improvement of inflammation since the Toll-like receptors of macrophages are able to recognize lipopolysaccharides (LPS), an endotoxin produced by pathogenic microorganisms (Ambriz-Pérez, Leyva-López, Gutierrez-Grijalva, & Heredia, 2016). Different mechanisms of action might be related to the anti-inflammatory properties of the phenolic compounds. Among them, up/downregulation of transcriptional factors (e.g, NF-kβ), inhibition of pro-inflammatory mediators (e.g, interleukin IL-6), inhibition of activated immune cells (e.g, macrophages), and inhibition of the inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) are discussed (Ambriz-Pérez et al., 2016).

The use of fruit by-products with prebiotic potential to improve the growth of beneficial microorganisms and the production of beneficial metabolites such as the B-group vitamins by probiotic and gut commensal microorganisms has been investigated (Albuquerque, Bedani, Vieira, LeBlanc, & Saad, 2016; LeBlanc et al., 2017; Vieira, Bedani, Albuquerque, Biscola, & Saad, 2017). According to Gibson et al. (2017), prebiotic is defined as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" extending the prebiotic effect to other substrates such as non-carbohydrate substrates, which could include phenolic compounds.

Folate is a B-group vitamin required by humans for their metabolic activities such as DNA replication, repair, and methylation and for the biosynthesis of nucleic acids. As humans are not able to produce folate, this vitamin must be obtained from the diet or from supplements. In addition, some beneficial microorganisms such as probiotics, present the ability to produce folate *de novo* which could be used to improve folate content in foods or in the gut through the fermentation of vegetable substrates by the human microbiota (Albuquerque et al., 2016).

The aim of this study was to determine the dietary fibre fractions, phenolic content and composition, and the antioxidant activity of four different fruit by-products water extracts (FWE) (from passion fruit, orange, acerola, and mango) and evaluate their anti-inflammatory potential. Additionally, this work aimed to evaluate the impact of each FWE on the growth and folate production by *Streptococcus thermophilus* TH-4 and *Lactobacillus rhamnosus* LGG and on the *in vitro* adherence abilities of both microorganisms to intestinal human epithelial cells.

2. Materials and methods

2.1. Fruit by-products processing

Four different fruit by-products [passion fruit (*Passiflora edulis* f. Flavicarpa), orange (*Citrus sinensis*), acerola (*Malpighia emarginata*), and mango (*Mangifera indica*)] were kindly supplied by fruit industries located in the state of São Paulo (Brazil). Passion fruit, orange, and acerola by-products were constituted mainly by peels and seeds, while mango by-product was constituted mainly by peel and a small amount of pulp attached to the peel. These fruit by-products were processed (washed, blanched, dried, reduced to a fine powder, and sieves were used to standardize the powder to less than 42 μm) according to Albuquerque et al. (2016) and stored at −20 °C until analysis.

2.2. Hot-water extraction

A hot water extraction was performed to obtain soluble fibres from all fruit by-product powders according to Beres et al. (2016). Briefly, each fruit by-product powder (< 42 μm) was blended with distilled water (1:12 ratio) and boiled (95–100 °C) under agitation (150 rpm) for 1 h. Each hot boiled mixture was immediately filtered using a sterile 0.22 μm membrane. The filtered obtained from each hot boiled mixture containing each fruit by-product was denominated fruit by-product water extract (FWE) and stored at 4 °C until use.

2.3. Physico-chemical analysis

Enzymatic-gravimetric methods (991.43 and 991.43, AOAC, 2012) were used to determine the total dietary fibre, soluble fibre, and insoluble fibre contents of each FWE. Values were expressed as g 100 mL^{-1} FWE.

Total phenolic content of each FWE was quantified using the Folin-Ciocalteau assay according to Magalhães, Santos, Segundo, Reis, and Lima (2010). Gallic acid was used as standard and the total phenolic content expressed as GAE mg mL^{-1} FWE.

Antioxidant activity of each FWE was determined based on two methods: the ORAC (hydrophilic oxygen radical absorbance capacity) method according to Ou, Hampsch-Woodill, and Prior (2001) and DPPH (1,1-diphenyl-2-picrylhydrazyl radical) according to Brand-Williams, Cuvelier, and Berset (1995) and the results were expressed as μmol Trolox L^{-1} FWE.

2.4. Evaluation of phenolic composition of FWE by HPLC-DAD

High performance liquid chromatography (HPLC) of all samples were performed in a Waters® Alliance e2695 HPLC System (Waters®, Milford, USA), equipped with a diode array detector (DAD). An aliquot of 5 μL of each filtered FWE, prepared according to the item 2.2, was injected without further preparation. Separations were performed on a Nova-Pak C18 column (250 × 4.6 mm, Waters®, Milford, USA). Mobile phase A consisted of 0.15% phosphoric acid and 99.85% ultrapure water, mobile phase B was 100% acetonitrile. The gradient profile was as follows: 5% B (0 min), 5% B (6 min), 12%B (12 min), 20%B (18 min), 30%B (22 min), 60% B (25 min) and 5%B (26 min), with a post-time of 5 min. The flow rate was 1.2 mL min^{-1}. Compounds were identified by comparison to the retention time (Sigma-Aldrich, St. Louis, MO, USA): gallic acid (2.370 min); protocatechuic acid (4.105 min); vanillinic acid (9.760 min); syringic acid (10.767 min); epicatechin (11.409 min); rutin (17.535 min); quercetin (22.943 min). Quantification was performed based on each compound concentration curve and expressed as mg 100 g^{-1} dry FWE and purity evaluated by comparing the spectra of the peaks to those of the standards.

2.5. Microorganisms and growth conditions

The starter *Streptococcus* (*St.*) *thermophilus* TH-4 and the probiotic *Lactobacillus* (*Lb.*) *rhamnosus* LGG were both supplied by Christian Hansen (Hørsholm, Denmark). The streptococci strain was grown at 37 °C for 24 h in LAPTg broth (Laiño, Del Valle, De Giori, & LeBlanc, 2013) and the lactobacilli in de Man, Rogosa, and Sharp broth (MRS, Oxoid, Basingstoke, UK) at 37 °C for 24 h. Both strains were grown statically under aerobic conditions. Viable streptococci and lactobacilli were counted by plating serial dilutions in LAPTg and MRS agar, respectively. Both strains were incubated (37 °C/48 h) in aerobic atmosphere; however, when in co-culture, *Lb. rhamnosus* LGG was incubated in anaerobic conditions (Anaerogen Anaerobic System, Oxoid) to avoid the concomitant growth of the streptococci strain.

2.6. Cell cultures and growth conditions

Caco-2 cell line ATCC HTB-37 and RAW 264.7 macrophages belonging to the collection of the *Centro de Referencia para Lactobacilos* (CERELA-CONICET, Tucumán, Argentina) were used. Both cells were cultivated in Dulbecco's modified Eagle's medium (DMEM) containing high glucose and GlutaMAX™ (Gibco, Gran Island, NY, USA) supplemented with 10% (v/v) fetal bovine serum (FBS, Natocor, Córdoba, Argentina), 100 IU/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B (Gibco, Gran Island, NY, USA) and incubated at 37 °C in a 5% CO_2 atmosphere. Cells at 70–80% of confluence were washed three times with DMEM without antibiotics, resuspended using the same medium and 1 mL of the cell inoculum (5×10^5 cells mL^{-1} for Caco- 2 cells and 4×10^4 for RAW cells mL^{-1}) was seeded in each well of a 24-well plate. The microplate was incubated in the same environmental conditions described above until presenting 70–80% of cell confluence to evaluate the effect of FWE on nitric oxide (NO) production and bacterial adhesion (Nakajima et al., 2017). The concentration of each FWE solution (ratio 1:10, v/v, in cell culture medium) used to perform the anti-inflammatory and the adhesion assays was not cytotoxic to the cells used in this study (data not shown) and this result was obtained based on the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay according to Oh et al. (2012).

2.7. Evaluation of FWE anti-inflammatory properties using LPS-stimulated macrophages

The RAW 264.7 macrophage cell line was cultured in DMEM as described in 2.6. The experiment was performed according to Cha, Seo, Chung, Cho, and Youn (2014) with modifications. RAW cells at 70–80% of confluence in 24 well plate were washed three times with PBS and then co-incubated for two hours with the fruit extracts re-suspended in DMEM without antibiotic. After that, the cells were stimulated with LPS from *Salmonella* Typhimurium (Sigma, St. Louis, MO, USA) at a final concentration of 100 ng mL^{-1} in serum free-DMEM for 20 h at 37 °C and 5% CO_2. The NO concentrations were determined by the Griess reaction using a 96 well-plate. Briefly, 50 μL of each culture supernatant was first mixed with an equal volume of Griess reagent A (1% sulfanilamide in 5% phosphoric acid, Britania, Buenos Aires, Argentina) for eight minutes at room temperature in the dark. Next, 50 μL of Griess reagent B (0.1% N-1-naphtylenediaminedihydrochloride, Britania, Buenos Aires, Argentina) were added and incubated for eight minutes at room temperature in the dark. The absorbance was measured at 550 nm in a microplate reader (VERSAmax, Molecular devices, Sunnyvale, CA, USA). The NO concentration was calculated using a sodium nitrite standard curve and the results were expressed as μMol of nitric oxide.

2.8. Influence of FWE on the growth of microorganisms and their folate production during fermentation

The ability of *St. thermophilus* TH-4 and *Lb. rhamnosus* LGG to ferment each FWE was performed according to Albuquerque et al. (2016) with modifications. Briefly, 5 log CFU mL^{-1} of *St. thermophilus* TH-4 and *Lb. rhamnosus* LGG strains (in pure culture and in co-culture) were inoculated in a modified MRS broth (mMRS) supplemented with 10% (v/v) of each FWE. Viable cell counts were performed before and after (24 h) fermentation as described in 2.1 and the results were expressed as log CFU mL^{-1}. mMRS broth without FWE inoculated with the strains was used as control.

Total folate content was determined using a microbiological method with the indicator strain *Lactobacillus casei* subsp. *rhamnosus* NCIMB 10463 according to Albuquerque et al. (2016). Samples were taken before (0 h) and after (24 h) the fermentation process. The total folate content was calculated using a folic acid standard curve and expressed as ng mL^{-1}.

2.9. Effect of FWE on the adhesion of microorganisms to Caco-2 cells

The effect of each FWE on *St. thermophilus* TH-4 and *Lb. rhamnosus* LGG adhesion to Caco-2 cells was investigated according to Parkar et al. (2010) with modifications. The FWE solution was prepared as described in Section 2.2. Caco-2 cells were washed using PBS (pH 7.4); then, 500 μL of each FWE solution and 500 μL of each bacterial inoculum (6 log CFU mL^{-1}), previously washed 3 times with PBS and re-suspended in DMEM without antibiotics, were added to each well. Both microorganisms were also tested as co-culture. The 24-wells microplate was incubated at 37 °C in the presence of 5% of CO_2 for 2 h. After that, each well was washed twice using PBS to eliminate unattached bacteria and 1 mL of 1% Triton X-100 solution (Sigma, ST. Louis, MO, USA) was added to each well to release the microorganisms adhered to Caco-2 cells. Serial dilutions of these samples and also of the initial inoculum were performed and the viable cells counted as described in 2.1. Anaerobic conditions were used for *Lb. rhamnosus* LGG when in co-culture with *St. thermophilus* TH-4. Wells containing FWE solution, each bacterial strain, bacterial co-culture or DMEM were used as controls. The experiment was performed in duplicate and the percentage of adhesion was calculated using the following formula:

$$\% \text{ adhesion} = (\text{CFU after adhesion x initial CFU}^{-1}) \text{ x } 100$$

The percentage change in the number of viable adherent bacteria was calculated as the average CFU in treated cells × (average CFU in untreated control)$^{-1}$ × 100.

2.10. Statistical analysis

Total dietary fibre, soluble fibre, insoluble fibre, total phenolic content, and antioxidant activity of each FWE and their effect on the growth of the microorganisms and their folate production were determined in triplicate. The anti-inflammatory activity of each FWE and the adhesion of the microorganisms to Caco-2 cells in the presence of each FWE were performed in duplicates with three independent repetitions of each assay. The results were expressed as mean ± standard deviation. 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. Pearson's correlation was performed to assess the correlation between TPC/ORAC, TPC/DPPH, and ORAC/DPPH. The differences between the samples were considered as statistically significant at $p < 0.05$. Principal Components Analysis was performed to evaluate the relationship between the physico-chemical

Table 1
Soluble fibre, total phenolic content, phenolic composition, and antioxidant activity of fruit by-products water extracts (FWE).

	Fruit by-products water extracts			
	PFWE	OWE	AWE	MWE
Soluble fibres (g/100 mL)	0.12 (0.0) ^{D}	0.39 (0.01) ^{B}	0.26 (0.01) ^{C}	0.47 (0.01) ^{A}
Total phenolics (mg GAE mL^{-1} FWE)	0.14 (0.00)^{D}	0.52 (0.01)^{C}	0.95 (0.06)^{B}	1.31 (0.03)^{A}
Phenolic profile (mg L^{-1} FWE)				
Phenolic acids				
Vanillic acid	1.0	8.0	2.0	0
Siringic acid	1.0	16.0	0	0
Gallic acid	1.0	38.0	0	199.0
Protocatechuic acid	0	4.0	0	1.0
Flavonoids				
Rutin	7.0	17.0	8.0	29.0
Quercitin	4.0	10.0	0	4.0
Epicatechin	0	0	0	2.0
Antioxidant activity				
ORAC (μmol Trolox L^{-1} FWE)	46.95 (0.65)^{B}	13.62 (0.48)^{C}	47.03 (1.86)^{B}	61.56 (2.16)^{A}
DPPH (μmol Trolox L^{-1} FWE)	304.7 (36)^{D}	898.9 (94)^{CD}	4431.7 (2 7 4)^{B}	18708.7 (1987)^{A}

PFWE: passion fruit water extract, OWE: orange water extract, AWE: acerola water extract, MWE: mango water extract. ^{A,B} Different capital letters in the same row denote significant differences between fruit by-products ($P < 0.05$).

characteristics of each extract and their respective biological effects to determine which extract presented the best prebiotic potential. For this analysis, the R-Studio version 1.1.453 was employed.

3. Results

3.1. Fibre and total phenolic contents, antioxidant activity, and phenolic composition of each FWE

Fruit by-product water extracts (FWE) were used as sources of dietary fibre associated with phenolic compounds. The total dietary fibres, soluble fibres, insoluble fibres, total phenolic content, phenolic composition, and the antioxidant activity of all FWE were evaluated. Insoluble fibres were not detected in any of the FWE since all hot boiled mixtures described in Section 2.2 were filtered. Thus, the total dietary fibres amount did not differ from the soluble fibres content. Regarding Table 1, significant differences between the soluble fibres values of the FWE ($p < 0.05$) were observed. Mango water extract (MWE) presented the highest soluble fibres content (0.47 ± 0.01 g 100 mL^{-1}) while passion fruit water extract (PFWE) (0.12 ± 0.0 g 100 mL^{-1}) presented the lowest content.

The Folin-Ciocalteu assay was used to determine the total phenolic content (TPC) of FWE and the results are shown in Table 1. MWE presented the highest TPC (1.31 ± 0.03 mg GAE mL^{-1} FWE) whereas PFWE (0.14 ± 0.00 mg GAE mL^{-1} FWE) presented the lowest one. Among the 15 phenolic standards employed (myricetin, quercitin, epicatechin, catechin, isorhamnetin, kampferol, ellagic acid, syringic acid, vanilic acid, p-coumaric acid, gallic acid, chlorogenic acid, caffeic acid, rutin, and protocatecuic acid), only 7 were identified (Table 1). Based on these standards, MWE presented the highest gallic acid content (199 mg L^{-1} FWE) and the highest content of rutin (29 mg L^{-1} FWE). In contrast, PFWE presented the lowest levels for all phenolic acids and flavonoids.

The antioxidant activity of each FWE was evaluated using the ORAC and the DPPH methods and the results are presented in Table 1. MWE presented the highest antioxidant activity when both ORAC and DPPH methods were employed (61.56 ± 2.16 μmol Trolox L^{-1} FWE and 18708.7 ± 1987 μmol Trolox L^{-1} FWE, respectively) followed by AWE (47.03 ± 1.86 μmol Trolox L^{-1} FWE and 4431.7 ± 274 μmol Trolox L^{-1} FWE, respectively). Considering the ORAC method, OWE (13.62 ± 0.48 μmol Trolox L^{-1} FWE) presented the lowest antioxidant activity, while PFWE (304.7 ± 36 μmol Trolox L^{-1} FWE) presented the lowest antioxidant activity when DPPH method was employed.

3.2. Effect of FWE on the NO production by LPS-stimulated macrophages

The potential anti-inflammatory effect of each FWE by reducing the NO levels is presented in Fig. 1. LPS-stimulated RAW 264.7

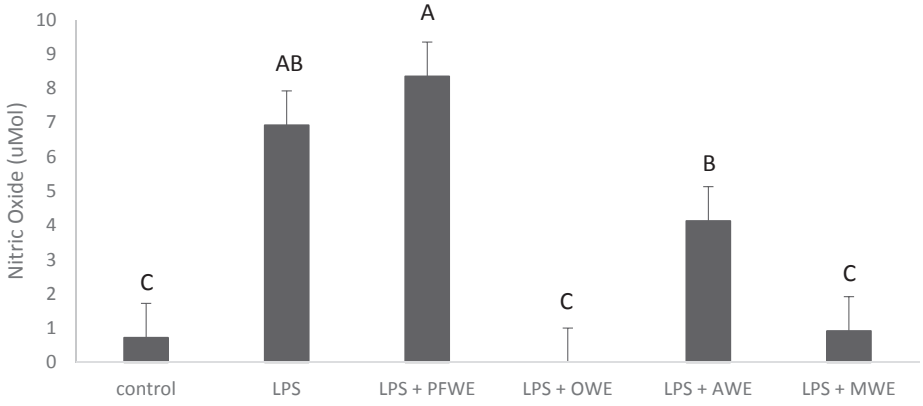

Fig. 1. Effect of fruit by-product water extracts on nitric oxide concentration produced by Lipopolysaccharides (LPS)-stimulated RAW 264.7 macrophages. ^{A,B} Different capital letters denote significant differences between different fruit water extracts on nitric oxide concentration ($P < 0.05$). PFWE: passion fruit water extract, OWE: Orange water extract, AWE: acerola water extract, MWE: mango water extract. Control: RAW 264.7 macrophages without LPS stimulation.

Table 2
Viable cell counts of *Streptococcus thermophilus* TH-4 and *Lactobacillus rhamnosus* LGG after 24 h of fermentation of mMRS broth supplemented with fruit by-product water extracts (FWE).

	Fruit by-products water extracts (log CFU mL^{-1})				
Strains	PFWE	OWE	AWE	MWE	Control
Pure culture					
St. thermophilus TH-4	6.8 ± 0.1^{Aa}	6.5 ± 0.2^{Aa}	2.2 ± 0.3^{Cb}	5.3 ± 0.3^{Bb}	2.1 ± 0.1^{Ca}
Lb. rhamnosus LGG	$7.4 \pm 0.2^{B\alpha}$	$7.6 \pm 0.1^{AB\alpha}$	$7.4 \pm 0.1^{B\alpha}$	$7.8 \pm 0.5^{A\alpha}$	$7.2 \pm 0.1^{B\alpha}$
Co-culture					
St. thermophilus TH-4	5.3 ± 0.3^{Bb}	6.5 ± 0.3^{Aa}	2.7 ± 0.2^{Ca}	6.6 ± 0.4^{Aa}	1.1 ± 0.1^{Db}
Lb. rhamnosus LGG	$7.5 \pm 0.2^{A\beta}$	$7.5 \pm 0.2^{A\alpha}$	$7.4 \pm 0.1^{A\alpha}$	$7.7 \pm 0.1^{A\alpha}$	$7.2 \pm 0.2^{B\alpha}$

^{A,B} Different capital letters in the same row denote significant differences between fruit by-products ($P < 0.05$). ^{a,b} Different small letters in the same column denote significant differences between *St. thermophilus* TH-4 growth in pure culture and in co-culture ($P < 0.05$). $^{\alpha,\beta}$ Different Greek letters in the same column denote significant differences between *Lb. rhamnosus* LGG growth in pure culture and co-culture ($P < 0.05$). PFWE: passion fruit water extract, OWE: Orange water extract, AWE: acerola water extract, MWE: mango water extract. Control: Modified MRS broth without any fruit by-product water extract.

macrophages produced high nitric oxide (NO) levels (6.9 ± 0.6 μMol) when compared to the negative control, LPS-non stimulated cells (0.7 ± 0.1 μMol). Orange water extract (OWE) showed the highest anti-inflammatory effect by completely decreasing the NO produced by the LPS-stimulated macrophages. There was no significant difference between the anti-inflammatory effect of OWE and MWE on NO reduction ($p > 0.05$). On the other hand, the results suggested that AWE and PFWE had no effect on decreasing NO levels produced by the LPS-stimulated macrophages, since there was no significant difference between the NO levels after the AWE and PFWE treatments and the LPS-stimulated control cells ($p < 0.05$).

3.3. The impact of FWE on the growth of microorganisms during fermentation

Results regarding the ability of *St. thermophilus* TH-4 and *Lb. rhamnosus* LGG to grow as pure cultures or in co-culture after 24 h of fermentation of each FWE are shown in Table 2. *Lb. rhamnosus* LGG was able to grow in the presence of all fruit extracts when in pure culture and in co-culture with *St. thermophilus* TH-4, reaching counts above 7 log CFU mL^{-1} after 24 h of fermentation. When in pure culture, LGG presented the highest growth in the presence of MWE (7.8 ± 0.5 log CFU mL^{-1}) and OWE (7.6 ± 0.1 log CFU mL^{-1}). Both FWE were significant different when compared to the control (7.2 ± 0.1 log CFU mL^{-1}) ($p < 0.05$). In contrast, *St. thermophilus* TH-4 was not able to grow in the absence of any FWE and, when compared to the growth of LGG, the streptococci strain presented the lowest counts in the presence of all FWE, especially in the presence of AWE when in pure culture (2.2 ± 0.3 log CFU mL^{-1}) and in co-culture (2.7 ± 0.2 log CFU mL^{-1}). The presence of *St. thermophilus* TH-4 had no effect on *Lb. rhamnosus* LGG growth.

3.4. Folate production by the microorganisms during FWE fermentation

The results regarding the production of folate by *St. thermophilus* TH-4 and *Lb. rhamnosus* LGG after 24 h of FWE fermentation are presented in the Fig. 2. The folate content of each FWE and of the mMRS broth were determined (PFWE, 14 ± 4 ng mL^{-1}; OWE, 18 ± 0 ng mL^{-1}; AWE, 22 ± 2 ng mL^{-1}; MWE, 14 ± 1 ng mL^{-1}, and mMRS broth, 7 ± 1 ng mL^{-1}). Among all FWE, AWE presented the highest amount of folate and the mMRS broth without FWE supplementation (control) presented the lowest vitamin content (7 ± 1 ng/mL). *St. thermophilus* TH-4 produced the highest amount of folate in the presence of MWE (41 ± 7 ng mL^{-1}), followed by OWE

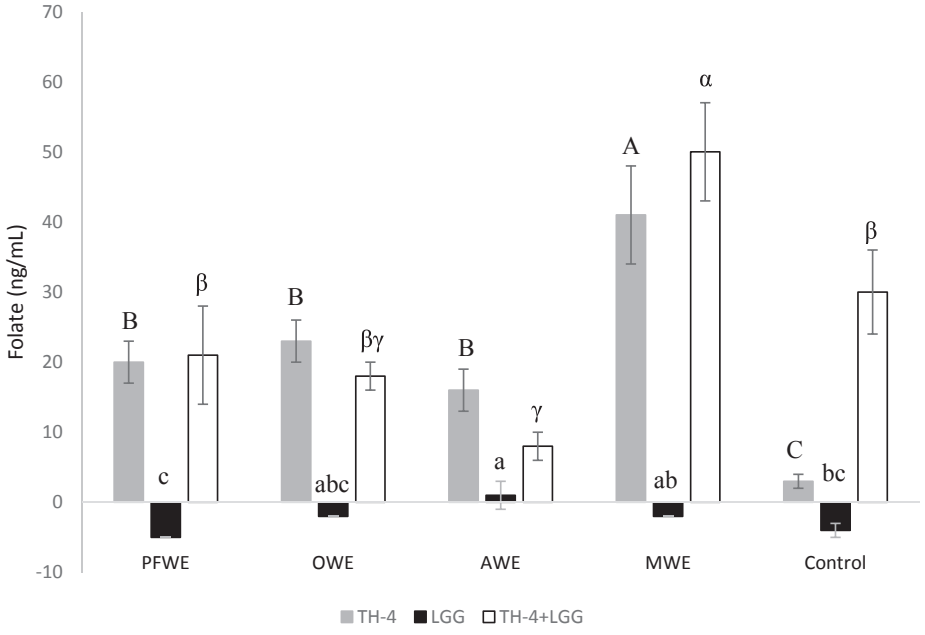

Fig. 2. Folate content after fruit water extracts fermentation. Grey bars present the production of folate by *Streptococcus thermophilus* TH-4. Black bars present the folate production by *Lactobacillus rhamnosus* LGG. White bars present the production of folate by the co-culture (TH-4 + LGG). ^{A,B} Different capital letters denote significant differences between folate production by *St. thermophilus* TH-4 among different fruit water extracts fermentation ($P < 0.05$). ^{a,b} Different small letters denote significant differences between folate production by *Lb. rhamnosus* LGG among different fruit water extracts fermentation ($P < 0.05$). $^{\alpha,\beta}$ Different Greek letters denote significant differences between folate production by the co-culture among different fruit water extracts fermentation ($P < 0.05$).

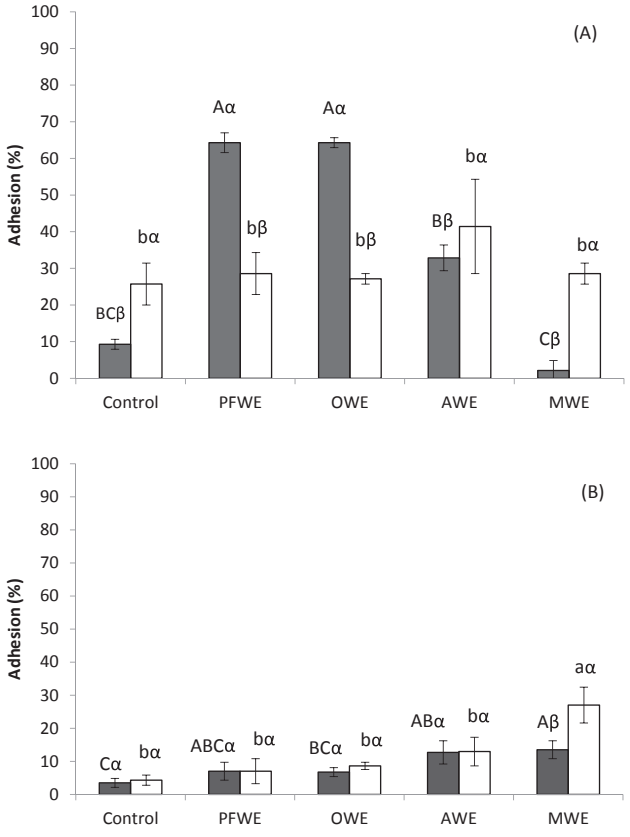

Fig. 3. Effect of fruit by-product water extracts (FWE) on the adhesion ability of *Streptococcus thermophilus* TH-4 and *Lactobacillus rhamnosus* LGG, as pure cultures and in co-culture in a model of human colon cell line (Caco-2). (A) Adhesion of *St. thermophilus* TH-4 as pure culture (grey bars) and in co-culture (white bars) with *Lb. rhamnosus* LGG. (B) Adhesion of *Lb. rhamnosus* LGG as pure culture (grey bars) and in co-culture (white bars) with *St. thermophilus* TH-4. ^{A,B} Different capital letters denote significant differences between *St. thermophilus* TH-4 or *Lb. rhamnosus* LGG adhesion to Caco-2 cells as pure culture ($P < 0.05$). ^{a,b} Different small letters denote significant differences between *St. thermophilus* TH-4 or *Lb. rhamnosus* LGG adhesion to Caco-2 cells as co-culture ($P < 0.05$). $^{\alpha,\beta}$ Different greek letters denote significant differences between *St. thermophilus* TH-4 or *Lb. rhamnosus* LGG adhesion to Caco-2 cells as pure cultures and co-culture ($P < 0.05$). PFWE: passion fruit water extract, OWE: orange water extract, AWE: acerola water extract, MWE: mango water extract. Control: without FWE supplementation.

(23 ± 3 ng mL^{-1}), and PFWE (20 ± 3 ng mL^{-1}), while *Lb. rhamnosus* LGG was not able to produce folate in the presence of any FWE. When both strains were tested in co-culture, the highest production of folate was observed in the presence of MWE (50 ± 7 ng mL^{-1}) followed by PFWE (21 ± 7 ng mL^{-1}), and OWE (18 ± 2 ng mL^{-1}). Although the co-culture was able to produce folate in the presence of PFWE and OWE, no significant difference was observed when both FWE were compared to the co-culture folate production observed for the non-supplemented mMRS broth. In the absence of FWE, the co-culture was able to produce the vitamin (30 ± 6 ng mL^{-1}); however, the folate production by the co-culture in the presence of MWE was significantly higher, when compared to the control (30 ± 6 ng/mL) ($p < 0.05$).

3.5. Adhesion of microorganisms in the presence of FWE

The results regarding the impact of each FWE on *St. thermophilus* TH-4 and *Lb. rhamnosus* LGG adhesion to Caco-2 cells are shown in Fig. 3. When in pure culture, the adhesion of *St. thermophilus* TH-4 was strongly enhanced by the presence of PFWE (64.3 ± 2.7%) and OWE (64.3 ± 1.4%). In contrast, MWE decreased the adhesion of TH-4 (2.1 ± 2.7%), even when compared to the control (9.3 ± 1.4%) ($p < 0.05$). The adhesion of *Lb. rhamnosus* LGG was positively influenced by the presence of MWE (13.5 ± 2.7%) and AWE (12.7 ± 2.7%) when compared to the control without FWE (3.5 ± 1.4%). In co-culture, AWE (41.4 ± 12.9%) and MWE (28.6 ± 2.9%) increased the adhesion of *St. thermophilus* TH-4 when compared to the adhesion of this strain in pure culture for the same both FWE (32.9 ± 3.5% and 2.1 ± 2.7%, respectively). In contrast, the adhesion of *Lb. rhamnosus* LGG in co-culture with the streptococci strain was enhanced only by the presence of MWE (27 ± 5.4%) when compared to the adhesion of the lactobacilli strain individually (13.5 ± 2.7%) and to the control (4.3 ± 1.5%). According to Fig. 3, *St. thermophilus* TH-4 did not interfered with *Lb. rhamnosus* LGG adhesion. In contrast, LGG negatively affected the TH-4 adhesion in the presence of PFWE and OWE.

3.6. Effect of physicochemical parameters of FWE on biological effects

To determine which FWE presented the best biological effect considering the physicochemical parameters evaluated, a Principal Components Analysis (PCA) was performed and the results are presented in Fig. 4. The closer to 1 is the eigenvalue of a given parameter tested, the greater its influence on the result obtained in the PCA. To interpret a PCA, it is necessary to establish a minimum eigenvalue. In our study, the eigenvalue was determined considering the percentages presented by the two main principal components: PC1 (61.65%) and PC2 (27.74%). PC1 explained 61.65% of the variation of the parameters evaluated, which represents between 10 and 11 parameters from a total of 17. The same evaluation was required for PC2. This principal component explained 27.74% of the parameters variation, which represents between 6 and 7 from the total analysed.

Thus, according to the table of eigenvalues generated by the software (R Studio), the value 0.23 to proceed the PCA was established. This means that the parameters that influenced the PCs at most were determined with eigenvalues that are the same or higher than the eigenvalue of 0.23 established. It was observed that soluble fibres, TPC, flavonoids, phenolic acids, DPPH, production of folate by TH-4, folate production by the co-culture, adhesion of TH-4, adhesion of LGG when in co-culture with TH-4, the growth of LGG, and the growth of LGG when in co-culture with TH-4 were the parameters that most influenced the PC1. This principal component was influenced by the parameters mentioned in the last sentence and explains the variation between the FWE samples in relation to these parameters. In this case, phenolic acids and folate production by TH-4 had the greatest influence on PC1, since they were the parameters that presented the highest eigenvalues.

Regarding the PC2, the parameters that most influenced this principal component were: TPC, ORAC, adhesion of TH-4, adhesion of LGG, adhesion of TH-4 when in co-culture with LGG, growth of TH-4, and growth of TH-4 when in co-culture with LGG. PC2 explains the variation between the FWE samples in relation to these parameters. In this case, the growth of TH-4 and the growth of TH-4 when in co-culture with LGG had the greatest influence on PC2, since they were the parameters that presented the highest eigenvalues.

Considering the distribution of the samples of FWE in Fig. 4 according to the parameters evaluated in this study, it was observed that the antioxidant characteristics of FWEs, according to their respective TPC values, favoured in a positive manner the adhesion of LGG to Caco-2 cells when this strain was tested individually and in co-culture with TH-4. In addition to the antioxidant characteristic, the other parameters, such as soluble fibre content, phenolic acids, and flavonoids, presented a tendency to favour the other biological and functional effects, such as the anti-inflammatory potential (considering the decrease in the nitric oxide concentration produced by the LPS-stimulated macrophages), production of folate by TH-4, production of folate by the co-culture, the growth of LGG individually and when in co-culture with TH-4. In contrast, the antioxidant characteristics, related to TPC, did

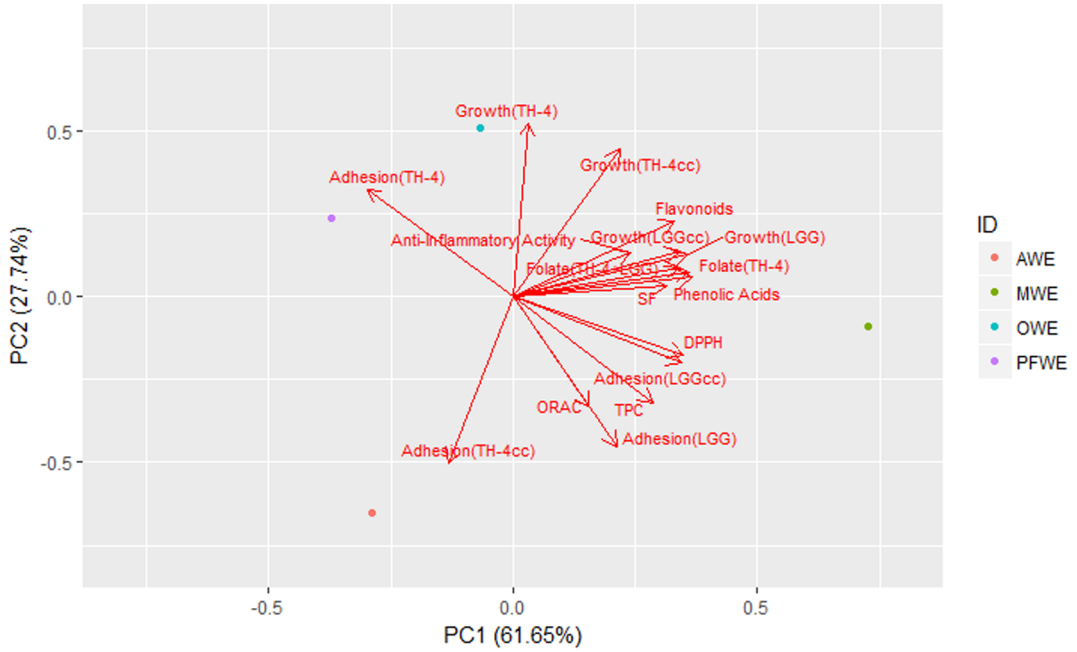

Fig. 4. Principal Component Analysis (PCA) graph of the influence of physico-chemical parameters of fruit by-product water extracts (FWE) on the biological and functional effects evaluated. *Streptococcus thermophilus* TH-4; *Lactobacillus rhamnosus* LGG. PFWE: passion fruit by-product water extract; OWE: orange by-product water extract; AWE: acerola by-product water extract; MWE: mango water extract. SF: soluble dietary fibres content; TPC: total phenolic compounds; Folate_TH-4: folate produced by TH-4; Folate_LGG: folate produced by LGG; Folate_TH-4 LGG: folate produced by the co-culture TH-4 + LGG; Adhesion_TH-4: adhesion of TH-4 to Caco-2 cells; Adhesion_LGG: adhesion of LGG to Caco-2 cells; Adhesion_TH-4 cc: adhesion of TH-4 in co-culture with LGG to Caco-2 cells; Adhesion_LGGcc: adhesion of LGG in co-culture with TH-4 to Caco-2 cells; Growth_TH-4: growth of TH-4 during fermentation of FWE; Growth_LGG: growth of LGG during fermentation of FWE; Growth_TH-4 cc: growth of TH-4 in co-culture with LGG during fermentation of FWE; Growth_LGGcc: growth of LGG in co-culture with TH-4 during fermentation of FWE.

not favour the adhesion of TH-4 individually or when in co-culture with LGG. Besides, the growth of TH-4 was not related to its ability to adhere to Caco-2 cells when in co-culture with LGG.

4. Discussion

It has been shown that fruit by-products possess bioactive molecules that can be used to develop new food products and/or new pharmaceutical preparations (Beres et al., 2016; Da Silva et al., 2014). Dietary fibres obtained from fruit are one of the most interesting substrates for the food industry due to their solubility, viscosity, water holding capacity, and fermentability (Macagnan et al., 2015). In this study it was shown that a hot water extraction was effective in obtaining similar amounts of soluble fibres as described previously by Vieira et al. (2017). Although high concentrations of soluble fibres were obtained in some fruit-by-products, such as mango, it is important to state that hot water extraction may not have extracted all of the fibres of the fruit by-products which could explain the lower total/soluble fibre content found in this study. Previous studies presented higher fibre contents for different fruit by-products (Macagnan et al., 2015; Martinez et al., 2012); however, pre-treatments were employed during the processing of the fruit by-products before the hot water extraction, such as the washing and bleaching treatments probably decreasing the amount of the soluble fibres contents of the fruit by-products evaluated (Chantaro, Devahastin, & Chiewchan, 2008). Additionally, we did not consider the exclusion of the moisture from our fruit by-products as we aimed to evaluate the original phenolic content and the antioxidant activity of the FWE in order to see if unprocessed fruit-by-products could be used directly in food applications without the need for expensive heating or concentration methods.

Regarding the association of dietary fibres with antioxidant bioactive molecules and other free antioxidant compounds, the total phenolic content of each FWE was evaluated and considerable amounts of total phenolic were observed especially for MWE. We observed that the hot water extraction significantly increased the total phenolic content compared to the extraction with methanol 70% of both OWE (0.2 mg GAE mL^{-1} FWE) and AWE (0.54 mg GAE mL^{-1} FWE) ($p < 0.05$). On the other hand, the hot water extraction did not present any significant difference for the MWE (1.33 mg GAE mL^{-1} FWE) and PFWE (0.12 mg GAE mL^{-1} FWE) total phenolic contents when the extraction with methanol 70% was used ($p > 0.05$). These data are in agreement with Kabir et al. (2015) and Martinez et al. (2012), who observed that hot water extractions yielded higher amounts of total phenolics than those with ethanol. From an ecological and economical point of view the use of water to perform extractions would be better and safer than the use of organic solvents.

The method employed for extracting phenolics used in our study was chosen based on a previous study (Beres et al., 2016). Temperature, solute:solvent ratio, and particle size were reported to influence the extraction of antioxidant fibers, which can be defined as polysaccharides bounded to phenolic compounds (Saura Calixto et al., 1998). Our data suggests that the FWE may be a good source of soluble fibres associated to phenolic compounds. Since the extraction of FWE were conducted at 100 °C during 1 h, free phenolics might be degraded, and phenolic compounds bound to polysaccharides could be protected during this treatment and be extracted from the matrix.

In fact, Beres et al. (2016) found that the variable that most influenced the extraction of antioxidant fibers was the temperature. The authors reported a positive relation between higher temperatures and extraction efficiency. Free phenolics are easily extracted from different matrix using conventional extraction methods. However, according to Chamorro, Viveros, Alvarez, Vega, and Brenes (2012), these compounds are not bio-accessible in the human intestine or even metabolized by the gut microbiota and, in this case, the benefits shown *in vitro* would not be reproducible *in vivo*. The main focus of our study was to obtain FWE that can be used by humans, considering that the bio-accessibility

and bioavailability of the compounds explored were of great physiological value. Chamorro et al. (2012) stated that bound phenolics are more bio-accessible as they are protected by polysaccharides. In addition, they can be used as substrates for gut microbiota fermentation producing several metabolites (e.g. folates) which will induce health benefits. Therefore, bound phenolics were the main focus of our study. Regarding the method for phenolic extraction, Dvořaková et al. (2008) used 3 different methods of extraction to extract phenolic compounds from a cereal matrix, as follows: free phenolic extraction, soluble ester phenolic extraction, and bound phenolic extraction. The last one was based on a hydrolytic procedure, similar to the hot water extraction method used in our study. According to these authors, the majority of free phenolics are flavanols, whereas the bound phenolics are mainly phenolic acids. Our results showed that the majority of bioactive compounds identified through HPLC were phenolic acids, which contributes to our statement that the majority of phenolic compounds extracted in our study were bound phenolics.

A strong correlation ($r^2 = 0.87$) was observed for TPC and DPPH. The antioxidant activity observed for all FWE using the DPPH method was directly proportional to the TPC amounts of the respective FWE. In contrast, TPC and ORAC ($r^2 = 0.52$) and ORAC and DPPH ($r^2 = 0.69$) presented a lower correlation. Considering the correlation observed between the TPC and the ORAC data, the low correlation value may indicate that some non-phenolic compounds (such as, antioxidant water-soluble vitamins) probably contributed to the total antioxidant activity, especially for OWE. This data is in agreement with Babbar, Oberoi, Uppal, and Patil (2011), who observed a low correlation between TPC and different methods to determine the antioxidant activity of some fruit by-product extracts.

The use of dietary fibres and antioxidants is recognized in the prevention of several chronic diseases (Saura-Calixto, 2011). According to Cha et al. (2014), NO produced by LPS-stimulated macrophages is a pro-inflammatory compound that plays an important role in the inflammatory response. Decreasing NO levels by using fruit extracts in an inflammatory context could be an important strategy to improve health. We observed that OWE and MWE present biological potential to be explored as natural anti-inflammatory ingredients by reducing NO to normal physiological levels. Additionally, the immunomodulatory effect of dietary fibres should also be considered (Wismar, Brix, Frøkiær, & Lærke, 2010). Macrophages present carbohydrate-binding pattern recognition receptors (PRRs) which can associate with different polysaccharide structures. Vogt et al. (2016) showed that the activation of immune cells by lemon pectin is Toll-like receptor dependent and that the epithelial barrier protective effect may be improved. The soluble fibre content of MWE, including its pectin content might be related to the positive biological and functional effects exerted by this fruit extract in our study, considering the PCA performed.

Our results suggest that the fruit by-products used to prepare their respective water extracts present great potential to act or to be source of antioxidant dietary fibres. FWE evaluated in the present study showed potential to act as prebiotic with antioxidant activity. Although dietary fibres may entrap some phenolic compounds in a core and reduce their antioxidant and/or prebiotic potential, the bio-accessibility and bioavailability of these molecules could be improved through fermentation by beneficial microorganisms from the intestinal microbiota as stated by Saura-Calixto (2011) and Tomás-Barberán, Selma, and Espín (2016). The extracts were able to improve the growth beneficial microorganisms and improve their folate-producing abilities showing that the soluble extracts might be responsible for the improved growth and folate production of these fruit-by-products when these were added in culture media or a food matrix (Vieira et al., 2017; Albuquerque, Bedani, LeBlanc, & Saad, 2017; LeBlanc et al., 2017).

Vieira et al. (2017) and Albuquerque et al. (2016) evaluated, respectively, the impact of different fruit by-products on the growth and folate production by starter and probiotic strains. Albuquerque et al. (2016) observed that the strains were able to produce the vitamin in the presence of different fruit by-products and that folate production cannot be associated to the growth ability of the strains once the vitamin production was strain-dependent and might be influenced by the nutritional and environmental conditions. Although most *St. thermophilus* and lactobacilli strains were reported as being able to ferment the fruit by-products, Vieira et al. (2017) also observed that *St. thermophilus* TH-4 did not grow in the presence of the acerola by-product after 24 h of fermentation which corroborates our findings. This fact was probably due to the absence of sucrose in AWE. In contrast, the other FWEs contained this sugar and stimulated the growth of the microorganisms (supplementary material). This fact was confirmed once TH-4 was not able to ferment the modified MRS broth without any FWE supplementation or other source of carbohydrate. Therefore, considering the fermentation of AWE, the absence of sucrose in this extract seems to inhibit the growth of *St. thermophilus* TH-4 and also the folate production by this microorganism when cultivated individually or in co-culture with *Lb. rhamnosus* LGG. Additionally, the AWEs phenolic content could also contribute to inhibit the growth of *St. thermophilus* TH-4, as stated by Vieira et al. (2017). It is important to point out that the concentration and the kind of the phenolics should be consider to confirm the negative impact on the growth of the microorganism.

Our results showed that MWE stimulated the folate production by *St. thermophilus* TH-4, in accordance with Albuquerque et al. (2016), and also by the co-culture (TH-4 + LGG), probably due to the phenolic and soluble fibres content and the synergic stimulation of LGG to TH-4 as indicated by PCA. In contrast, none of the FWE stimulated the production of folate by *Lb. rhamnosus* LGG which is in agreement with Laiño et al. (2013), who highlighted lactobacilli strains as usually folate consumers.

The adhesion ability to intestinal cells is another important characteristic of probiotic strains to promote their health benefits, including the immunoregulatory activity and the production of beneficial metabolites, such as different kind of vitamins, including vitamins from group B, once these patients present low nutrient absorption due to changes in their intestinal epithelium (Ambriz-Pérez et al., 2016; Parkar, Redgate, McGhie, & Hurst, 2014). Parkar et al. (2010) verified that monoK pectin was the most effective kiwi fruit pectin when compared to the prebiotic inulin, enhancing the adhesion of *Lb. rhamnosus* and decreasing the adhesion of *Salmonella* Typhimurium to Caco-2 cells. Additionally, the authors observed that depending on the kind and amount of carbohydrates, these compounds may have pro- or anti-adhesive effects. All these findings are in agreement with our results once we observed that the FWEs were able to improve the adhesion of *Lb. rhamnosus* LGG to Caco-2 cells.

5. Conclusion

This study showed that tropical fruit by-products water extracts, especially from mango by-products, are a source of biologically active compounds with anti-inflammatory potential and also can stimulate folate production by lactic acid bacteria. These water extracts could be used in the development of novel foods that can improve the health of the consumers. Taken together, the results highlight the use of mango by-product water extract as the most promising natural water extract from tropical fruit by-products considering the physicochemical, biological, and functional parameters evaluated. Further studies are required to characterize the different bioactive compounds and elucidate the mechanisms of action that allow them to modulate innate inflammatory processes and improve folate production.

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Declarations of statement

None.

Ethical statements

The authors state there are no Ethical matters involved in the study, since the study did not enroll human beings and, also, did not deal with animals.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2018.12.002.

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