Food Chemistry 196 (2016) 251–258

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

In vitro fermentation of juçara pulp (*Euterpe edulis*) by human colonic microbiota

Karla Bigetti Guergoletto [a,b,*], Adele Costabile [a,d], Gema Flores [c], Sandra Garcia [b], Glenn R. Gibson [a]

[a] Department of Food and Nutrition Sciences, The University of Reading, RG6 6AP Reading, UK
[b] Department of Food Science and Technology, State University of Londrina, Celso Garcia Cid Road, Km 380, C.P.:6001, 86051-970 Londrina, Brazil
[c] Consejo Superior de Investigaciones Científicas – CSIC, Madrid, Spain
[d] Health Sciences Research Centre, Life Sciences Department, Whitelands College, University of Roehampton, London, UK

ARTICLE INFO

Article history:
Received 17 April 2015
Received in revised form 3 August 2015
Accepted 14 September 2015
Available online 15 September 2015

Keywords:
Juçara
Batch culture fermentation system
Faecal microbiota
Phenolic compounds

ABSTRACT

This study was carried out to investigate the potential fermentation properties of juçara pulp, using pH-controlled anaerobic batch cultures reflective of the distal region of the human large intestine. Effects upon major groups of the microbiota were monitored over 24 h incubations by fluorescence *in situ* hybridisation (FISH). Short-chain fatty acids (SCFA) were measured by HPLC. Phenolic compounds, during an *in vitro* simulated digestion and fermentation, were also analysed. Juçara pulp can modulate the intestinal microbiota *in vitro*, promoting changes in the relevant microbial populations and shifts in the production of SCFA. Fermentation of juçara pulp resulted in a significant increase in numbers of bifidobacteria after a 24 h fermentation compared to a negative control. After *in vitro* digestion, 46% of total phenolic content still remained. This is the first study reporting the potential prebiotic effect of juçara pulp; however, human studies are necessary to prove its efficacy.

1. Introduction

Juçara (*Euterpe edulis*) is a palm tree that belongs to the Arecaceae family and the genus *Euterpe*, widely distributed in the Atlantic forest. This plant produces a noble type of palm heart, with higher quality and superior flavour, compared to other *Euterpe* species, which are widely consumed and has economic importance in Brazil (Borges et al., 2011). Besides the palm heart, juçara also produces a round fruit, with a glossy black pulp covering the seed. It has a varied harvest and production period, depending on geographic and climatic origins (De Brito et al., 2007). To be consumed, juçara berries are usually macerated with water and separated from their seeds to obtain a thick and purple pulp, which is consumed as such or further used in different kinds of beverages, ice creams, sweets and sauces (Borges et al., 2011). The juçara pulp has an important nutritional value, with proteins, sugars, fibre and a lipid fraction with high content of polyunsaturated fatty acids, oleic acid and a lower content of saturated lipids. Besides, juçara is rich in phenolic compounds, with a content of total monomeric anthocyanins varying from 14.8 to 410 mg cy-3 glu.100/g and total phenolic around 2611 mg 100/g GAE according to the growth region and climate conditions of plant production (Borges et al., 2011).

High levels of phenolic compounds with antioxidant activity present in fruits, especially berries, have often been associated with reduced risk of several chronic diseases caused by oxidative stress (Manach, Scalbert, Rémésy, & Jiménez, 2004). Different studies have demonstrated that the *in vivo* effect of phenolic compounds is also dependent on absorption and metabolism in the gastrointestinal tract. It has been estimated that only 5–10% of the total polyphenol intake is absorbed in the small intestine. The remaining polyphenols (90–95% of total polyphenol intake) may accumulate in the large intestinal lumen (up to the millimolar range) where the colonic microbiota are responsible for breakdown of the original polyphenolic structures into a series of low-molecular-weight metabolites that, being absorbable, may actually be responsible for health effects derived from polyphenol-rich food consumption, rather than the original compounds found in foods (Cardona, Andres-Lacuerva, Tulipania, Tinahonesb, & Queipo-Ortuno, 2013).

The human large intestine is an extremely active fermentation site and is inhabited by over 1000 different bacterial species, which reach numbers of around 10^{14} colony-forming units in total (Gibson & Roberfroid, 1995). Through the process of fermentation, the gut microbiota is able to produce a wide range of compounds

* Corresponding author at: Department of Food Science and Technology, State University of Londrina, Celso Garcia Cid Road, Km 380, C.P.:6001, 86051-970 Londrina, Brazil.
E-mail address: karla2901@gmail.com (K.B. Guergoletto).

http://dx.doi.org/10.1016/j.foodchem.2015.09.048
0308-8146/

that have both positive and negative effects on gut physiology, as well as systemic influences. The balance among these bacterial species has been linked to both beneficial and detrimental effects in the large intestine. *Bifidobacterium* spp. and *Lactobacillus* spp. are genera that have been shown to exert beneficial effects in the colon, whilst others, have been associated with deleterious bacterial species, such as certain members of the *Clostridium* group (Rastall et al., 2005). Diet is considered a major driver for changes in the functional relationship between microbiota and the host. At present, non-digestible food ingredients represent a useful dietary approach for influencing composition of the human gut microbiota and/or activity of the gastrointestinal microbiota, thus conferring benefits upon host health (Maccaferri et al., 2012). Previous *in vitro* studies have been used to better understand the influence of phenolic compounds on the bacterial metabolism, although much work has been conducted on the isolated effect of these compounds on the composition and activity of the human gut microbiota (Cueva et al., 2013; Hidalgo et al., 2012; Keppler & Humpf, 2005). Recently, a few studies have focussed on the impact of whole plant foods on gut microbial species composition and relative abundance (Carvalho-Wells et al., 2010; Tuohy, Conterno, Gasperotti, & Viola, 2012).

This study aimed to investigate the potential of juçara pulp to influence the growth of specific bacterial groups in a pH-controlled, stirred, batch-culture fermentation system that is reflective of the environmental conditions of the distal region of the human large intestine. We also investigated the metabolism of anthocyanins and phenolics compounds in the juçara pulp during an *in vitro* simulated digestion and fermentation by the gut microbiota.

2. Materials and methods

2.1. Juçara pulp

Juçara grains were collected during the 2014 harvest from *E. edulis* palm trees produced at the Bimini Farm (Rolândia, Paraná, Brazil). To obtain the pulp, the fruits were washed with clean water and sanitised for 30 min in water containing chlorine at a concentration of 200 mg/kg. Then, the grains were rinsed and pulped with clean water (1:1), using a depulper (Macanuda DM-Ji-05, Brazil). The seeds were separated for planting and the extract pasteurised in a water bath at 80 °C for 1 min, followed by cooling in an ice bath, and lyophilised. This extraction followed the methodology used to produce açaí and juçara pulp in industrial demands (Cohen & Alves, 2006). Lyophilised juçara pulp nutritional profile was characterised by official reference methods (ashes: UNI ISO 2171; proteins: UNI 10274 831/12/93 and ISO 1871 (15/12/75); total dietary fibre: AOAC 985.29; RS: AOAC 2002.02; glucans: AOAC 995.16 2005) (Table 1).

Table 1
Composition and nutritional profile of the lyophilised juçara pulp used in the present study.

Analysis	Result
Energy (kcal)	403 kcal/100 g
Protein	7.6 g/100 g
Total carbohydrate (by difference)	63.0 g/100 g
Carbohydrate	28.8 g/100 g
Total sugars *	3.8 g/100 g
Fat	21.0 g/100 g
Fibre total	34.2 g/100 g
Sodium	28 mg/100 g
Moisture	4.1 g/100 g
Ash	4.31 g/100 g

* Total sugars are the sum of glucose, sucrose and fructose expressed as monosaccharides.

2.2. Simulated in vitro human digestion

Prior to being added to the batch culture systems, the lyophilised juçara pulps were digested in *vitro*, under appropriate conditions, according to the procedures described by Maccaferri et al. (2012). Sixty grammes of pulp was mixed with 150 ml of sterile distilled water and homogenised in a stomacher (Seward, Worthing, U.K), at high speed for 5 min. α-Amylase (20 mg) was mixed with 1 mM $CaCl_2$ (6.25 ml, pH 7.0) and added to the juçara solution, then incubated at 37 °C for 30 min, with shaking. After incubation, pH was adjusted to 2.0 and pepsin (2.7 g) in 0.1 M HCl (25 ml) was added, prior to a further incubation cycle, under shaking conditions, at 37 °C for 2 h. Finally, bile (3.5 g) and pancreatin (560 mg) were mixed with 0.5 M $NaHCO_3$ (125 ml), the pH was adjusted to 7.0, and the mixture was incubated at 37 °C for 3 h. After this period, the sample solution was transferred to 1 kDa molecular weight cut-off regenerated cellulose dialysis tubing (Spectra/Por® 6, Spectrum Europe, Netherlands) and dialysed against NaCl (0.01 M at 5 °C) to remove monosaccharides from the pre-digested juçara. After 15 h, the dialysis fluid was changed and the process continued for an additional 2 h. Afterwards, the samples were lyophilised until the samples were completed dried (~7 days).

After each step of the digestion, 5 ml samples were collected and lyophilised for anthocyanins and phenolic compounds analysis by HPLC.

2.3. Faecal sample preparation

Faecal samples were collected from three separate individuals. All donors were in good health and had not had antibiotics for at least 6 months before the study. Samples were collected, on site, on the day of the experiment and placed in an anaerobic jar (Anaerojar™ 2.5L, Oxoid Ltd), including a gas-generating kit (AnaeroGen™, Oxoid), in order to reproduce the anaerobic conditions, for no longer than 1 h. Then, samples were weighed and diluted, 1:10 (w/v), with anaerobic sterile phosphate-buffered saline (0.1 M; pH 7.4) and homogenised in a stomacher for 2 min (460 paddle/min). To maintain the anaerobic conditions, the phosphate-buffered saline was maintained in anaerobic cabins until the time of use. Resulting faecal slurries from each individual were used to inoculate the batch-culture systems.

2.4. Batch culture fermentation

Batch culture fermentation vessels (working volume 100 ml) were sterilised and filled with 45 ml of basal nutrient medium (peptone water (2 g/l), yeast extract (2 g/l), NaCl (0.1 g/l), K_2HPO_4 (0.04 g/l), $NaCO_3$ (2 g/l), $MgSO_4{\cdot}7H_2O$ (0.01 g/l), $CaCl_2{\cdot}6H_2O$ (0.01 g/l), Tween 80 (2 ml/l), haemin (50 mg/l), vitamin K1 (10 μl/l), L-cysteine (0.5 g/l), bile salts (0.5 g/l), resazurin (1 mg/l) and distilled water). The pH of basal medium was adjusted to 7 and autoclaved before dispensing into vessels. Medium was then gassed overnight with O_2-free N_2 (15 ml/min) to maintain the anaerobic condition. Before addition of faecal slurries, temperature of the basal nutrient medium was set to 37 °C, using a circulating water bath, and the pH was maintained at 6.8, using a pH controller (Electrolab, U.K.). The vessels were inoculated with 5 ml of faecal slurry (1:10, w/v), and, in order to mimic conditions located in the distal region of the human large intestine, the experiment was run under anaerobic conditions [the system was permanently gassed with O_2-free N_2 (15 ml/min)], 37 °C and pH 6.8, for a period of 24 h. During this period, samples (4 ml) were collected at four

time points (0, 4, 8 and 24 h) for fluorescence *in situ* hybridization (FISH) and SCFAs, and at seven time points (0, 1, 2, 4, 6, 8, 24 h) for phenolic compounds by HPLC. Before FISH analysis, duplicate samples were fixed for a minimum of 4 h at 4 °C with 4% (w/v) filtered paraformaldehyde at 4% (w/v). For HPLC analysis, samples were centrifuged for 10 min at 1300*g* and fermentation supernatant fractions were removed and stored in sterile Eppendorf tubes (1.5 ml) at −20 °C prior to analysis.

2.5. Inoculation of juçara pulp in the batch culture fermentation systems

The lyophilised digested juçara pulp (1% w/v) was inoculated in stirring batch-culture vessels (one vessel per donor) containing faecal slurry (1%). The prebiotic FOS (1% w/v) (Raftilose P95 – Orafti, Tienen, Belgium) and basal nutrient media, with no substrate added (negative control), were also included in the experiment as positive and negative controls, respectively.

2.6. In vitro enumeration of bacterial population by FISH

FISH was performed as described by Daims, Stoecker, and Wagner (2005). Briefly, aliquots (375 μl) of batch culture supernatant samples were fixed in 1.125 μl of 4% (w/v) filtered paraformaldehyde, then centrifuged at 13,000*g* for 5 min and washed twice with 1 ml of sterile PBS (0.1 M; pH 7.0). The cell pellet was resuspended in 300 μl of PBS-99% ethanol mixture (1:1, v/v), and stored at −20 °C until used for hybridization. Oligonucleotide probes, designed to target specific regions of 16S rRNA, were commercially synthesized and labelled with the fluorescent dye Cy3 (Sigma–Aldrich, UK). Bacterial groups enumerated were: Eub I–II–III, for the total number of bacteria (Daims, Brühl, Amann, Schleifer, & Wagner, 1999); Bif164, specific for *Bifidobacterium* spp. (Langendijk et al., 1995); Lab158, for *Lactobacillus–Enterococcus* spp. (Harmsen, Elfferich, Schut, & Welling, 1999); Bac303, specific for the *Bacteroides–Prevotella* group (Manz, Amann, Ludwig, Vancanneyt, & Schleifer, 1996); Chis150, for the *Clostridium histolyticum* subgroup; Erec482, for most members of *Clostridium cluster* XIVa (Franks et al., 1998) and Prop853, for *C. cluster* IX (Walker, Duncan, McWilliam Leitch, Child, & Flint, 2005).

2.7. High-performance liquid chromatography analysis

Samples were taken from the batch culture vessels at each time point and cell-free culture supernatants obtained by centrifugation of 1 ml at 13,000*g* for 10 min, followed by filter sterilisation (0.22 μm; Millipore, Cork, Ireland) to remove particulate matter. SCFA content was quantified by an ion exclusion high performance liquid chromatograph (HPLC) (LaChrom Merck Hitachi, Poole, Dorset UK) equipped with pump (L-7100), RI detector (L-7490) and autosampler (L-7200). Samples (20 μl) were injected into the HPLC, operating at a flow rate of 0.5 ml/min with column prepacked Rezex ROA – Organic Acid H+ 80% (300 × 7.8 mm) at a temperature of 84 °C and wavelength of 210 nm. H_2SO_4 (2.5 mM) was used as eluent and the organic acids (formic, lactic, acetic, propionic and butyric), were calibrated against standards at concentrations of 12.5, 25, 50, 75 and 100 mM. Internal standard of 2-ethylbutyric acid (20 mM) was included in the samples and external standards. All chemicals were provided from Sigma–Aldrich (Poole, Dorset, UK).

Analysis of anthocyanins and other phenolic compounds were also evaluated in the lyophilised juçara extract before, during and after digestion, and in the batch-culture vessels, using an Agilent 1100 series liquid chromatograph equipped with a quaternary pump and a photodiode array detector (Hewlett-Packard Agilent, Bracknell, UK). A Nova Pak C18 4-μm column (4.6 × 250 mm) and the mobile phases, A (95% HPLC water, 5% methanol and 1% formic acid) and B (50% HPLC water, 50% acetonitrile and 1% formic acid), at a flow rate of 0.7 ml/min were used in the experiment. Starting with 5% B, the gradient was as follows: from 5% B to 50% B in 40 min, from 50% B to 100% B in 10 min, isocratically 100% B for 5 min and from 100% B to 5% B for 5 min. Detection wavelengths were 254, 280, 320, 365 and 520 nm. Peaks areas were referred to calibrations curves obtained with the corresponding standards for anthocyanin and other phenolic compounds. For lyophilised juçara extract, 0.4 g of each sample was previously extracted with 5 ml of 1% formic acid with methanol, vortexed for 1 min and centrifuged at 13,000*g*. Batch culture samples were centrifuged (13,000*g*/10 min), and both supernatants were filtered through a 0.22 μm filter and autoinjected into the HPLC system.

2.8. Total phenolic analysis

The total phenolic contents were analysed according to the Folin–Ciocalteu method adapted to 96-well plate microlitre assay, using gallic acid as the standard; 5 μl of the diluted extracts or standards were mixed with 145 μl of distilled water and 25 μl of Folin–Ciocalteu reagent. After 3 min at room temperature, 100 μl of saturated sodium carbonate solution were added and the solution kept in a shaker for 25 min at room temperature. The absorbances of the samples were measured at 650 nm, using a GENios pro microplater reader (Tecan, Theale, Berks, UK) equipped with a Magellan Software system. Methanolic solutions of gallic acid (Sigma–Aldrich, Poole, Dorset, UK) with concentrations of 0–500 mg/l were used for the calibration curve, and results were expressed as g gallic acid equivalents (GAE)/g or l of sample.

2.9. Statistical analysis

Differences between bacterial counts and SCFA profiles at 0, 4, 8 and 24 h of fermentation were tested for significance, using paired *t*-tests, assuming equal variance and considering a two-tailed distribution. To determine whether there were any significant differences in the effect of the substrates, differences at each time were tested, using 2-way ANOVA with Bonferroni post-test ($P < 0.05$). Significant differences were defined at $P < 0.05$. All analyses were performed by using a GraphPad Prism 5.0 (GraphPad Software, LaJolla, CA, USA).

3. Results

3.1. Changes in bacterial populations with in vitro batch culture fermentation

Numbers of the main bacterial groups constituting the core of the human intestinal microbiota were assessed by FISH during the experimental time course (0, 4, 8 and 24 h) (Fig. 1). Following juçara pulp fermentation, a significant increase in numbers of *Bifidobacterium* spp. (detected by Bif164 probe) was observed after 24 h of fermentation (log 7.67 ± 0.17 to log 8.5 ± 0.7) compared to FOS-Raftilose 95 (log 7.61 ± 0.31 to 7.6 ± 0.09) and the negative control (log 7.62 ± 0.31 to 6.5 ± 0.24) ($P < 0.001$). No significant differences were detected for *Lactobacillus/Enterococcus* spp. (detected by Lab158) at all time points. Cluster IX representatives (detected by Prop853) were increased by juçara pulp after 24 h of fermentation (log 7.50 ± 0.25) compared to FOS-Raftilose 95 (log 6.59 ± 0.07) ($P < 0.001$). Levels of the *Eubacterium rectale–Clostridium coccoides group* (enumerated by Erec482) and *Bacteroides* spp.*–Prevotella* group (enumerated by Bac303) significantly increased at 4 h ($P < 0.05$), 8 h ($P < 0.01$) and 24 h ($P < 0.001$),

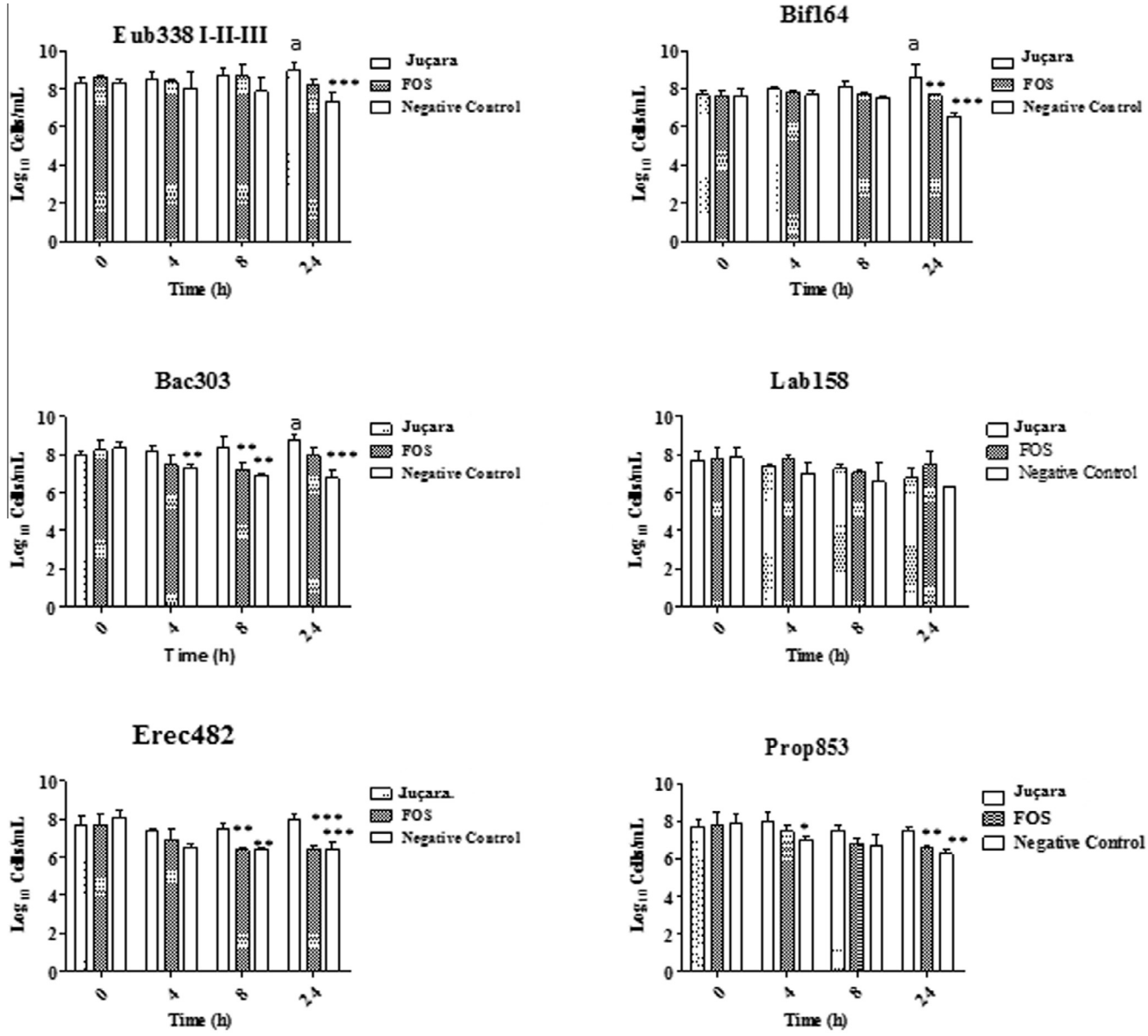

Fig. 1. Bacterial populations analysed by fluorescence *in situ* hybridisation (FISH) in a batch culture fermentation containing juçara pulp, FOS and negative control at 0, 4, 8 and 24 h of fermentation. Results are reported as means of the data ($n = 3$) log 10 CFU/ml ± standard deviations (SD); significant differences from juçara (using 2-way ANOVA Bonferroni post-tests to compare replicate means) $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Significant differences for the same vessels compared to 0 h within the same substrate (using *t*-test, $P < 0.05$), are indicated with letters.

whereas numbers of the domain bacteria (detected by EUB I–II–III) were increased after 24 h ($P < 0.001$) of fermentation (log 8.32 ± 0.26 to 8.96 ± 0.39). Very low numbers of the *C. histolyticum* group (Chis 150) were detected for all samples at time 0 h (log 5.73 ± 0.12). However, after 4 h of fermentation it was not possible to count any bacteria from this group, and a decrease below the limit of detection could have occurred.

3.2. SCFA production

Table 2 shows profiles of SCFA in the batch culture at 24 h of fermentation for juçara pulp, FOS-Raftilose 95 and negative control. Fermentation of juçara pulp resulted in a greater production of acetate and propionate but lower amounts of butyrate. As expected, the juçara fermentation resulted in a significantly higher amount of SCFA compared to the negative control; however, these amounts were lower for butyrate and similar for propionate and acetate when compared to the positive control (FOS) after 24 of fermentation.

3.3. Changes in phenolic compounds of juçara pulp during digestion and fermentation by human faecal bacteria

In order to follow up the degradation of juçara during digestion, major flavonoid compounds were monitored by HPLC, as shown in Table 3. The main anthocyanins detected in juçara pulp were cyanidin-3-rutinoside, followed by cyanidin-3-glucoside, malvidin-3-glucoside, peonidin-3-rutinoside and pelargonidin-3-glucoside, and the flavonoids rutin, quercetin and p-coumaric acid. At oral and gastric phases, only small amounts of anthocyanins were degraded and, after gastric phase, more than a half of these amounts were still present. However, after the intestinal phase, anthocyanins were reduced in abundance compared to the original sample and after dialysis malvidin, peonidin and pelargonidin

Table 2
Concentrations of major short-chain fatty acid during 0, 4, 8 and 24 h of fermentation of juçara, FOS and negative control in stirred pH controlled batch culture system. Values are means with standard deviations ($n = 3$).

Treatment	Time point (h)	Mean SCFA concentration (mM) in treatment (±SD)		
		Juçara	FOS (+ control)	− Control
Acetic	0	3.37 ± 0.35	2.51 ± 0.40	3.87 ± 0.2
	4	9.92 ± 3.31[a]	3.85 ± 0.36	2.26 ± 1.80*
	8	11.4 ± 2.22 [a]	18.1 ± 5.65[a]	3.15 ± 2.39*
	24	15.8 ± 3.81[a]	25.1 ± 14.84[a]	4.08 ± 3.22*
Propionic	0	2.72 ± 0.24	0.72 ± 1.58***	2.45 ± 0.09
	4	12.9 ± 0.74[a]	1.86 ± 2.63***	2.59 ± 2.15**
	8	14.5 ± 5.98[a]	3.24 ± 3.08*	2.63 ± 2.01**
	24	15.8 ± 5.06[a]	11.3 ± 8.22[a]	2.20 ± 1.38**
Butyric	0	1.16 ± 0.18	0.22 ± 0.08	1.19 ± 0.02
	4	1.15 ± 0.40	0.92 ± 0.70	0.96 ± 0.10
	8	0.9 ± 0.48	3.20 ± 3.74	0.90 ± 0.44
	24	1.10 ± 0.13	11.0 ± 11.15*[a]	0.57 ± 0.35

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, significantly different from juçara at the same time point using two-way ANOVA with Bonferroni post test. Significant differences compared to 0 h within the same substrate (using t-test, $P < 0.05$), are indicated with letters.

were not detected. The detected flavonols quercetin and rutin were also degraded during the digestion, in contrast with p-coumaric acid that slightly increased after dialysis.

Despite anthocyanin degradation, 46% of total phenolic contents remained after juçara digestion, this being an important source of antioxidant activity that could reach the colon.

In order to evaluate the metabolism of phenolic compounds of digested lyophilised juçara pulp by human faecal microbiota, samples were collected at 0, 1, 2, 4, 6, 8 and 24 h and analysed, using HPLC and total phenolics analysis (Table 4). After 1 h of incubation, a small amount of benzoic acid started to appear, followed by gallic and syringic acid. No significant differences were found for rutin or p-coumaric acid and the total amount of phenolics was kept constant during *in vitro* fermentation.

4. Discussion

To date, scientific focus has been on the potential health benefits of *açaí* (*Euterpe oleracea*) and juçara (*E. edulis*) in terms of antioxidant activity (Borges et al., 2011; Cunha-Junior, Nardini, Khatiwada, Teixeira, & Walsh, 2015). This is the first study that has investigated the influence of juçara pulp fermentation on complex faecal microbiota *in vitro*.

The prebiotic effects of fructooligosaccharides and galactooligosaccharides are thought to underpin certain functional outcomes in the large gut and there is evidence regarding the ability of other dietary components, including flavonoids, to influence the growth of selected intestinal bacteria (Tzounis et al., 2008). The human colon contains a wide range of bacterial communities, distributed in hundreds of distinct species, and the balance among them plays an important role in health and disease (Holzapfel, Haberer, Snel, Schillinger, & Veld, 1998; Rigottier-Gois, Rochet, Garrec, Suau, & Dore, 2003).

Substrates that escape from human digestion can be used for beneficial bacteria growth. After digestion, juçara pulp presented 3.2 g/100 g of available sugar, or 18.6 g/100 g of fibre (data not shown) and 1614 mg GAE/100 g of total phenolics that could be available for gut bacteria metabolism. Carbohydrate fermentation is the chief energy source for the gut microbiota; however, as colonic carbohydrates are used up in the proximal colon, saccharolytic fermentation decreases as the concentration of substrate decreases. Microorganisms then switch to other energy sources, e.g. proteins, amino acids, and the end-products of these fermentations include SCFA but also branched-chain fatty acids, amines, indoles, sulfides, and phenols (Tuohy et al., 2012).

Significant changes in bifidobacterial numbers were observed in response to juçara after 24 h of fermentation. Bifidobacteria are recognised as one of the most important bacterial groups associated with human health, providing beneficial effects in the large intestine (Gibson & Wang, 1994; Russel, Rossa, Fitzgerald, & Stantona, 2011). It is well known that an increase in bifidobacteria number is favoured by the presence of (mainly) carbohydrates. However, recent studies have shown that some phenolic compounds, such as anthocyanins and gallic acid, also have the ability to promote the growth of *Bifidobacterium* and *Lactobacillus* spp. (Aura et al., 2005; Gibson, 1999; Hidalgo et al., 2012; Tzounis et al., 2008).

The juçara fermentation also increased populations of *Bacteroides* spp. and *C. cluster* XIVa and, although both groups may exert a detrimental effect on colon health due to an association with some metabolites, these groups also contain saccharolytic species which can produce large concentrations of beneficial SCFAs from sugars. Therefore, an increase in populations of *Clostridium* spp. and *Bacteroides* spp., is partially dependent on the precursor substrate to which they are exposed (Gibson & Roberfroid, 1995; Nyangale, Farmer, Keller, Chernoff, & Gibson, 2014). Here, residual carbohydrates could be associated with saccharolytic species. The increasing of the tested bacterial groups, showed that the

Table 3
Contents of anthocyanins and phenolic compounds detected by HPLC in lyophilised juçara pulp before, during and after simulated *in vitro* human digestion. Values are means with standard deviation ($n = 3$).

Compound	Juçara pulp	Oral phase	Gastric phase	Intestinal phase	After dialysis
Cyanidin-3-rutinoside	966 ± 54.9	720 ± 33.8	670 ± 24.4	13.0 ± 4.60	8.19 ± 1.99
Cyanidin-3-glucoside	322 ± 43.7	218 ± 21.7	188 ± 19.1	3.03 ± 0.43	2.29 ± 0.76
Malvidin-3-glucoside	33.2 ± 6.78	14.7 ± 3.50	15.1 ± 5.60	2.58 ± 0.09	N.D
Peonidin-3-rutinoside	16.2 ± 1.12	9.24 ± 1.67	9.37 ± 0.87	1.15 ± 0.41	N.D
Pelargonidin-3glucoside	15.6 ± 3.32	3.12 ± 0.56	2.73 ± 0.07	N.D	N.D
Rutin	9.4 ± 2.87	8.23 ± 1.45	9.22 ± 1.67	6.98 ± 1.22	1.2 ± 0.56
Quercetin	4.8 ± 1.09	3.73 ± 0.98	3.85 ± 0.93	1.39 ± 0.03	1.2 ± 0.69
p-Coumaric	6.96 ± 2.02	6.56 ± 1.06	8.26 ± 0.76	10.2 ± 0.84	8.39 ± 1.34
Gallic acid	N.D	N.D	N.D	N.D	N.D
Syringic acid	N.D	N.D	N.D	N.D	N.D
Benzoic acid	N.D	N.D	N.D	N.D	N.D
Total phenolic*	3474 ± 98.0	2205 ± 59.3	2206 ± 88.9	1673 ± 42.9	1614 ± 74.3

N.D = not detected.
* Data presented as mg of gallic acid equivalents (GAE)/100 g.

Table 4
Phenolic compound metabolism detected by HPLC during juçara fermentation in pH-controlled batch culture system for 24 h. Values are means with standard deviation ($n = 3$).

Incubation time (h)	Compounds						
	Rutin*	Quercetin*	p-Coumaric acid*	Gallic acid*	Syringic acid*	Benzoic acid*	Total phenolic**
0	0.17 ± 0.08	N.D	Tr	N.D	N.D	N.D	454 ± 62.5
1	0.20 ± 0.04	N.D	Tr	N.D	N.D	0.04 ± 0.03	430 ± 57.8
2	0.18 ± 0.09	N.D	Tr	Tr	N.D	0.02 ± 0.01	442 ± 23.6
4	0.18 ± 0.04	N.D	Tr	Tr	Tr	0.02 ± 0.01	424 ± 156
6	0.19 ± 0.04	N.D	0.001 ± 0.000	0.001 ± 0.000	Tr	0.02 ± 0.01	477 ± 122
8	0.20 ± 0.04	N.D	0.001 ± 0.000	0.003 ± 0.000	0.003 ± 0.001	0.01 ± 0.00	497 ± 108
24	0.18 ± 0.02	N.D	0.001 ± 0.000	0.003 ± 0.000	0.006 ± 0.001	0.006 ± 0.002	544 ± 157

N.D = not detected; Tr = traces.
* Data presented as mg/ml.
** Data presented as mg GAE/l.

anaerobic microbiota were well maintained in our system, and could ferment the substrates.

After 4 h of fermentation, numbers of the detrimental group *C. histolyticum* were too low to be detected and a decrease in its numbers can provide information in terms of the specificity for beneficial bacteria during juçara fermentation. The antimicrobial effect on certain bacterial groups during juçara fermentation, can also be related to the presence of phenolic compounds which, in accordance with previous studies, could inhibit the growth of potentially negative bacteria (Hidalgo et al., 2012).

From the present study it is not possible to distinguish whether the changing microbial population and the SCFA production are effects of fibres or polyphenols, but likely they could be a combination of both, since *in vitro* studies with the isolated compounds showed that both can be metabolised by gut microbiota and exert an increase in the beneficial species (Aura et al., 2005; Fleschhut, Kratzer, Rechkemmer, & Kulling, 2006; Gibson & Roberfroid, 1995). In the same way, there is some evidence from animal and human studies with whole plant foods, that they can also exert significant effects on the human gut microbiota (Tuohy et al., 2012; Vendrame et al., 2011); however, few studies have considered the possible synergistic activity of fibre and polyphenols (Tuohy et al., 2012).

Juçara fermentation *in vitro* induced a modulation of the colonic microbiome with increased acetate and propionate. The best known metabolic pathway for acetate and propionate production from gut bacteria, involves the metabolism of polysaccharides. Acetate production occurs mainly through the fructose-6-phospahte phosphoketolase pathway by bifidobacteria, and a greater production of this acid could be related to the increase of the bacteria (Miller & Wolin, 1996). According to Hosseini, Grootaert, Verstraete, and Wiele (2011), propionate can be produced from fermentable carbohydrates by two pathways. The first involves decarboxylation of succinate by the presence of *Bacteroides fragilis* and *Propionibacterium* spp groups, while the second is the acrylate pathway, in which pyruvate is first reduced to lactate by lactate dehydrogenase by the presence of Cluster IX of Clostridia groups, and an increase in those bacterial groups was indeed observed during juçara fermentation. Although the type of polysaccharides in juçara pulp has not been characterised, reports of açaí pulp (*Euterpe precatoria* and *E. oleracea*), show the presence of arabinogalactans, with a β-(1–3)-linked galactan, arabino-3-6-galactans, α-rhamnopyranose, β-galactopyranose, α-arabinofuranose and α-galacturonopyranose (Holderness et al., 2011), which are known to be degraded by the colonic microbiota to produce SCFA (Terpend, Possemiers, Daguet, & Marzorati, 2013).

The amounts of acetate and propionate produced during juçara fermentation were similar to those of the positive control (FOS-Raftilose 95). Numerous *in vivo* and *in vitro* studies have proved that FOS can modulate the gut through the selective stimulation of the gut microbiota (Gibson, Probert, Van Loo, Rastall, & Roberfroid, 2004; Gibson & Roberfroid, 1995). The SCFA produced by gut microbiota in the colon has an important role. Butyrate is often associated as an energy source for the epithelial cells and acetate plays an important role in controlling inflammation and combatting pathogen invasion (Russel, Hoyles, Flint, & Dumas, 2013). Also, acetate and propionate may have a direct role in central appetite regulation. The propionate mechanism involves stimulating release of the anorectic gut hormones, peptide YY (PYY) and glucagon-like peptide-1 (GLP-1). They, in turn, are involved in the short-term signal of satiation and satiety to appetite centres of the brain, increasing satiety and reducing food intake in animals and man (Brown et al., 2003; Chambers et al., 2014; Cherbut et al., 1998; Tolhurst et al., 2012). Acetate administration is associated with activation of acetyl-CoA carboxylase and changes in the expression profile of regulatory neuropeptides favouring appetite suppression (Frost et al., 2014). This way, our results support the hypothesis that, through an increase of some bacterial groups and the subsequent production of different amounts of SCFAs, juçara could play a beneficial role for the host.

In this study, anthocyanins and phenolic compounds from juçara pulp, during a simulated *in vitro* digestion, were monitored, and a decrease in these compounds was observed. This result could be due to anthocyanins being metabolised, oxidised, or degraded into other chemicals (Perez-Vicente, Gil-Izquierdo, & Garcia-Viguera, 2002). However, for those anthocyanins that do decompose, there seems to be a delay between the loss of anthocyanin aglycones and the formation of phenolic acids, probably due to the relatively stable transition products. This delay could further reduce concentrations of phenolic acids in the upper gastrointestinal tract (Fang, 2014), which explains a smaller increase in the present study. Besides, from previous studies on simulated human gastrointestinal digestion, authors have shown that anthocyanins are less stable at the elevated pH of the small intestine (McDougall, Fyffe, Dobson, & Stewark, 2007; Perez-Vicente et al., 2002).

The juçara pulp is composed mainly of carbohydrates and fat (Table 1), and previous studies have reported that the food matrix can also influence phenolic compound bioavailability during digestion (Lesser, Cermak, & Wolfram, 2004; Sengul, Surek, & Nilufer-Erdil, 2014). Sengul et al. (2014) evaluated the consumption of pomegranate phenolic compounds with several foods or food components, including cellulose, citric acid, sugars and salt. The authors observed that these components could negatively affect the total phenolic content depending of the digestion stage. When the carbohydrates lactose, fructose and glucose were digested, together with phenolic, about 2-fold decreases in these compounds were detected in the dialysed fraction. On the other hand, the oil content during digestion can present a preserving effect on phenolic compounds (Sengul et al., 2014) and the higher quercetin content after juçara dialysis, can be related to the delay in absorption and metabolism of the oil content that resulted in more quercetin absorption as reported by Lesser et al. (2004).

Throughout the fermentation time, the amount of total phenolics remained constant and similar results were also reported by Correa-Betanzo et al. (2014), who observed a constant level of the total phenolic compounds from blueberry extracts, both crude and digested, during simulated colon fermentation.

Although, in the present study, the remaining phenolic compounds were not completely identified, colonic bacteria are well known to act enzymatically on the polyphenolic backbone of the remaining unabsorbed polyphenols and produce different compounds with physiological significance (Aura et al., 2005). According to previous studies, syringic and gallic acids are mainly products of malvidin-3-glycoside degradation (Fleschhut et al., 2006; Hidalgo et al., 2012) and ferulic and protocatechuic acids are from cyanidin biotransformation (Flores et al., 2015; Keppler & Humpf, 2005). Also, the heterocyclic C-ring of anthocyanins can be broken and degraded into phloroglucinol derivatives (from the A-ring) and benzoic acids (from the B-ring) and both phloroglucinol aldehyde and its oxidation product, phloroglucinol acid, have been detected following the degradation of anthocyanins (Cueva et al., 2013). However, future work will be done to identify the degradation products from juçara pulp phenolic compounds during fermentation by gut bacteria.

In vitro and *in vivo* intervention and epidemiological studies have shown evidence of the wide range of health-promoting activities of dietary polyphenols, including their anti-inflammatory, antioxidant, anticarcinogenic, antiadipogenic, antidiabetic and neuroprotective potentials, suggesting an association between the consumption of polyphenol-rich foods and a reduced risk of several chronic diseases (Hooper et al., 2012; Jennings et al., 2012), and these benefits could be related to the remaining phenolic components after degradation by the colonic microbiota, as observed in the present study, and reported by other authors (Cardona et al., 2013), as the hydrolysis by the gut microbiota can increase the bioavailability of polyphenols, breaking down many complex polyphenols into smaller phenolic acids, which can be absorbed across the intestinal mucosa (Tuohy et al., 2012).

5. Conclusion

This study provides new insights about the numbers of bacteria and the degradation of juçara phenolic compounds during digestion and fermentation in the colon. Following juçara pulp fermentation, beneficial modulations were seen in terms of bifidogenic effects. However, future works will explore which, polyphenols or fibres, constitute the main effect of juçara modulation and the role such changes play in human health.

Acknowledgements

We would like to thank Bimini Farm for the juçara berries used in the study. We also wish to thank CAPES (Brazil), for a scholarship to K.B.G. Process number 004163/2014-01.

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