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

Karla Bigetti Guergoletto<sup>a,b\*</sup>; Adele Costabile<sup>a</sup>; Gema Flores<sup>c</sup>; Sandra Garcia<sup>b</sup>; Glenn R. Gibson<sup>a</sup>

<sup>a</sup>Department of Food and Nutrition Sciences. The University of Reading RG6 6AP, Reading, UK

<sup>b</sup>Permanent adress: Departament of Food Science and Technology; State University of Londrina, Celso Garcia Cid Road, Km 380; C.P.:6001; 86051-970; Londrina, Brazil

<sup>c</sup>Consejo Superior de Investigaciones Científicas – CSIC, Madrid, Spain.

\*Corresponding author. Tel + 55 43 3371-5966, fax + 55 43 3371-4080; Email address: karla2901@gmail.com

15    **ABSTRACT**

16

17    This study was carried out to investigate the potential fermentation properties of juçara pulp using  
18    24-h pH-controlled anaerobic batch cultures reflective of the distal region of the human large  
19    intestine. Effects upon major groups of the microbiota were monitored over 24 h incubations by  
20    fluorescence *in situ* hybridisation (FISH). Short-chain fatty acids (SCFA) were measured by HPLC.

21    The metabolism of phenolic compounds during an *in vitro* simulated digestion and fermentation  
22    were also investigated. Juçara pulp can modulate the intestinal microbiota *in vitro*, promoting  
23    changes in the relevant microbial populations and shifts in the production of SCFA. Fermentation of  
24    juçara pulp resulted in a significant increase in numbers of bifidobacteria after 24h fermentation  
25    compared to a negative control. After *in vitro* digestion, almost all the anthocyanin were degraded  
26    and 46 % of total phenolic were still remaining. This is a first study reporting the potential prebiotic  
27    effect of juçara pulp, however human studies are necessary to prove its efficacy.

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29    **Keywords:** Juçara; batch culture fermentation system; faecal microbiota; Phenolics compounds

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## 1. INTRODUCTION

Juçara (*Euterpe edulis*) is a palm tree that belongs to Aracaceae family and the genus *Euterpe*, widely distributed to 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 varied harvest and production period depending on geographic and climatic origins (De Brito, De Araujo, Alves, Carkeet, Clevidence, Noyotny, 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 beverage, 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 like 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 14.84 to 409.85 mg cy-3 glu.100/g and total phenolic around 2610.86 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, has been often associated with reduced risk of several chronic diseases caused by oxidative stress (Manach, Scalbert, Remesy, Jiménez, 2004). Different studies have been 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

67 effects derived from polyphenol rich food consumption, rather than the original compounds found  
68 in foods (Cardona, Andres-Lacuerva, Tulipania, Tinahonesb, Queipo-Ortuno, 2013).

69 The human large intestine is an extremely active fermentation site and is inhabited by over 1000  
70 different bacterial species which reaches number around  $10^{14}$  colony-forming units in total (Gibson,  
71 Roberfroid, 1995). Through the process of fermentation, gut microbiota is able to produce a wide  
72 range of compounds that have both positive and negative effects on gut physiology as well as  
73 systemic influences. The balance among these bacterial species has been linked to both beneficial  
74 and detrimental effects in the large intestine. *Bifidobacterium* spp. and *Lactobacillus* spp. are genera  
75 that have been shown to exert beneficial effects in the colon, whilst others, have been associated  
76 with deleterious bacterial species such as certain members of the *Clostridium* group (Rastall et al.,  
77 2005). Diet is considered a major driver for changes in the functional relationship between  
78 microbiota and the host. At present, non-digestible food ingredients represent a useful dietary  
79 approach for influencing composition of the human gut microbiota and/or activity of the  
80 gastrointestinal microbiota, thus conferring benefits upon host health (Maccaferri et al., 2012).

81 Previous *in vitro* studies have been used to better understanding the influence of phenolic  
82 compounds on the bacterial metabolism although much work has been conducted on the isolated  
83 effect of these compounds on the composition and activity of the human gut microbiota. Recently,  
84 few studies have focused on the impact of whole plant foods on gut microbial species composition  
85 and relative abundance.

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

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92

## 93 2. MATERIALS AND METHODS

94

### 95 2.1 *Juçara* pulp

96 Juçara grains were collected during the 2014 harvest from *Euterpe edulis* palm trees produced at the  
97 Bimini Farm (Rolândia, Paraná, Brazil). To obtain the pulp, the fruits were washed with clean water  
98 and sanitised for 30 min in water containing chlorine at a concentration of 200 mg/kg. Then, the  
99 grains were rinsed and pulped with clean water (1:1) using a depulper (Macanuda DM-Ji-05,  
100 Brazil). The seeds were separated for planting and the extract pasteurised in a water bath at 80 °C  
101 for 1 min, followed by cooling in an ice bath, and lyophilised. This extraction followed the  
102 methodology used to produce acai and juçara pulp in industrial demands (Cohen, Alves, 2006).  
103 Lyophilised juçara pulp nutritional profile was characterized by official reference methods (ashes:  
104 UNI ISO 2171; proteins: UNI 10274 831/12/93 and ISO 1871 (15/12/75); total dietary fibre: AOAC  
105 985.29; RS: AOAC 2002.02; glucans: AOAC 995.16 2005), and the results are presented in Table  
106 1.

107

### 108 2.2 *Simulated in vitro* human digestion

109 Prior to being added into the batch culture systems, the lyophilised juçara pulp were digested in  
110 *vitro* under appropriate conditions according to the procedures describes by Maccaferri et al. (2012)  
111 Sixty grams of pulp was mixed with 150 mL of sterile distilled water and homogenized in a  
112 stomacher (Seward, Worthing, U.K), at high speed for 5 min.  $\alpha$ -amylase (20 mg) was mixed with 1  
113 mM  $\text{CaCl}_2$  (6.25 mL, pH 7.0) and added to the juçara solution, then incubated at 37°C for 30 min,  
114 under shaking. After incubation, pH was adjusted to 2.0 and pepsin (2.7g) in 0.1 M HCl (25 mL)  
115 was added, prior to a further incubation cycle, under shaking conditions, at 37°C for 2 h. Finally,  
116 bile (3.5 g) and pancreatin (560 mg) were mixed with 0.5 M  $\text{NaHCO}_3$  (125 mL) at pH 7.0 and then  
117 with lyophilised juçara pulp. Dialyses with membrane of 100-200 a cut-off Spectra/por 100-200 Da  
118 WCO dialysis membranes (Spectrum Europe B. V., Breda, The Netherlands) were used to remove

119 monosaccharides from the pre-digested juçara. After each step of the digestion, 5 mL samples were  
120 collected and lyophilised for anthocyanins and phenolic compounds analysis by HPLC.

121

### 122 2.3 Faecal sample preparation

123 Faecal samples were collected from three separate individuals. All donors were in good health and  
124 had not had antibiotics for at least 6 months before the study. Samples were collected, on site, on  
125 the day of the experiment and were used immediately. The samples were diluted 1:10 (w/v) with  
126 anaerobic phosphate buffered saline (0.1 M; pH 7.4) and homogenised in a stomacher for 2 min  
127 (460 paddle/min). Resulting faecal slurries from each individual were used to inoculate the batch-  
128 culture systems.

129

### 130 2.4 Batch Culture Fermentation

131 Batch-culture fermentation vessels (working volume 100 mL) were sterilised and filled with 45 mL  
132 of basal nutrient medium (peptone water (2 g/L), yeast extract (2 g/L), NaCl (0.1 g/L), K<sub>2</sub>HPO<sub>4</sub>  
133 (0.04 g/L), NaCO<sub>3</sub> (2 g/L), MgSO<sub>4</sub>.7H<sub>2</sub>O (0.01 g/L), CaCl<sub>2</sub>.6H<sub>2</sub>O (0.01 g/L), Tween 80 (2 mL/L),  
134 haemin (50 mg/L), vitamin K1 (10 µl/L), L-cysteine (0.5 g/L), bile salts (0.5 g/L), resazurin (1  
135 mg/L) and distille water). The pH of basal medium was adjusted to 7 and autoclaved before  
136 dispensing into vessels. Medium was then gassed overnight with O<sub>2</sub>-free N<sub>2</sub> (15 mL/min). Before  
137 addition of faecal slurries, temperature of the basal nutrient medium was set to 37°C using a  
138 circulating water bath and the pH was maintained at 6.8 using a pH controller (Electrolab, U.K.).  
139 The vessels were inoculated with 5 mL of faecal slurry (1:10, w/v), and in order to mimic  
140 conditions located in the distal region of the human large intestine the experiment was ran under  
141 anaerobic conditions, 37°C and pH 6.8 for a period of 24h h. During this period, samples (4 mL)  
142 were collected at four time points (0, 4, 8 and 24 h) for fluorescence *in situ* hybridization (FISH)  
143 and SCFAs and at seven time points (0, 1, 2, 4, 6, 8, 24 h) for phenolic compounds by HPLC.  
144 Before FISH analysis, duplicate samples were fixed for a minimum of 4 h at 4°C with 4% (w/v)



145 filtered paraformaldehyde at 4% (w/v). For HPLC analysis, samples were centrifuged for 10 min at  
146 1300 g and fermentation supernatant fractions were removed and stored in sterile Eppendorff tubes  
147 (1.5 mL) at -20°C until analysis.

#### 148 149 *2.5 Inoculation of juçara pulp in the batch culture fermentation systems*

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

#### 154 155 *2.6 In vitro enumeration of bacterial population by FISH*

156 FISH was performed as described by Daims, Stolcker, Wagner, (2005). Briefly, aliquots (375 µl) of  
157 batch culture supernatant samples were fixed in 1.125 µL of 4% (w/v) filtered paraformaldehyde,  
158 then centrifuged at 13000g for 5 min and washed twice with 1 mL of sterile PBS (0.1 M; pH 7.0).  
159 The cell pellet was resuspended in 300 µL of PBS-99% ethanol mixture (1:1, v/v), and stored at -20  
160 °C until used for hybridization. Oligonucleotides probes designed to target specific regions of 16S  
161 rRNA were commercially synthesized and labelled with the fluorescent dye Cy3 (Sigma-Aldrich,  
162 UK). Bacterial groups enumerated were Eub I-II-III for the total number of bacteria (Daims, Bruh,  
163 Amann, Schleifer, Wagner, 1999); Bif164 specific for *Bifidobacterium* spp. (Langendijk et al.  
164 1995); Lab158 for *Lactobacillus-Euterococcus* spp. (Harmsen, Elfferich, Schut, Welling, 1999);  
165 Bac303 specific for the *Bacteroides-Prevotella* group (Manz, Amann, Ludwig, Vancanneyt,  
166 Schleifer, 1996); Chis150 for the *Clostridium histolyticum* subgroup; Erec482 for most members of  
167 *Clostridium* cluster XIVa (Franks, Harmsen, Raangs, Jonsen, Schut, Welling, 1998) and Prop 853  
168 (Walker, Duncan, McWilliam Leitch, Child, Flint, 2005).

#### 169 170 *2.7 High-performance liquid chromatography analysis*

171 Samples were taken from the batch culture vessels at each time point and cell-free culture  
172 supernatants obtained by centrifugation of 1 mL at 13,000 g for 10 min followed by filter  
173 sterilisation (0.22 µm; Millipore, Cork, Ireland) to remove particulate matter. SCFA content was  
174 quantified by ion exclusion high performance liquid chromatography (HPLC) (LaChrom Merck  
175 Hitachi, Poole, Dorset UK) equipped with pump (L-7100), RI detector (L-7490) and autosampler  
176 (L-7200). Samples (20 µL) were injected into the HPLC, operating at a flow rate of 0.5 mL/min  
177 with column prepacked Rezex ROA – Organic Acid H+ 80% (300 x 7.8 mm) at a temperature of 84  
178 °C and wavelength of 210 nm. H<sub>2</sub>SO<sub>4</sub> (2.5 mM) was used as eluent and the organic acids: formic,  
179 lactic, acetic, propionic and butyric, were calibrated against standards at concentrations of 12.5, 25,  
180 50, 75 and 100 mM. Internal standard of 2-ethylbutyric acid (20 mM) was included in the samples  
181 and external standards. All chemicals were provided from Sigma-Aldrich (Poole, Dorset, UK).

182 Analysis of anthocyanins and other phenolic compounds was also evaluated in the lyophilised  
183 juçara extract before, during and after digestion, and in the batch-culture vessels, using an Agilent  
184 1100 series liquid chromatograph equipped with a quaternary pump and a photodiode array detector  
185 (Hewlett–Packard Agilent, Bracknell, UK). A Nova Pak C18 4-µm column (4.6 x 250 mm) and the  
186 mobile phases: A (95 % HPLC water, 5 % methanol and 1 % formic acid); mobile phase B (50 %  
187 HPLC water, 50 % acetonitrile and 1 % formic acid), at a flow rate of 0.7 mL/min were used in the  
188 experiment. Starting with 5% B, the gradient was as follows: from 5% B to 50% B in 40 min, from  
189 50% B to 100% B in 10 min, isocratically 100% B in 5 min and from 100% B to 5% B in 5 min.

190 Detection wavelengths were 254, 280, 320, 365 and 520 nm. Peaks areas were referred to  
191 calibrations curves obtained with the corresponding standards for anthocyanin and other phenolic  
192 compounds. For lyophilised juçara extract, 0.4 g of each sample were previously extract with 5 mL  
193 1 % formic acid with methanol, vortex for 1 min and centrifuged at 3500 rpm. Batch cultures  
194 samples were centrifuged (13000g / 10 min), and both supernatants were filtered through a 0.22 µm  
195 filter and autoinjected into HPLC system.

## 197 2.8 Total Phenolic analysis

198 The total phenolic content were analyzed according to the Folin-Ciocalteu method adapted to 96-  
199 well plate microliter assay, using gallic acid as the standard. 5  $\mu$ l of the diluted extracts or standards  
200 were mixed with 145  $\mu$ L of distilled water and 25  $\mu$ l of Folin-Ciocalteu reagent. After 3 min at  
201 room temperature, 100  $\mu$ l of sodium carbonate saturated solution was added and the solution kept in  
202 a shaker for 25 min at room temperature. The absorbance of the samples were measured at 650 nm  
203 using a GENios pro microplater reader (Tecan, Theale, Berks, UK) equipped to a Magellan  
204 Software. Methanolic solutions of gallic acid (Sigma-Aldrich, Poole, Dorset, UK) with  
205 concentration of 0 to 500 mg/L were used for the calibration curve, and results were expressed as g  
206 gallic acid equivalents (GAE)/g or L of sample.

207

## 208 2.9 Statistical analysis

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

215

## 216 3. RESULTS

### 217 3.1 Changes in bacterial populations in in vitro batch culture fermentation

218 Numbers of the main bacterial groups constituting the core of the human intestinal microbiota were  
219 assessed by FISH during the experimental time course (0, 4, 8 and 24 h) (Figure 1). Following  
220 juçara pulp fermentation, a significant increase in numbers of *Bifidobacterium* spp. (detected by  
221 Bif164 probe) was observed after 24 h fermentation ( $\log 7.67 \pm 0.17$  to  $\log 8.5 \pm 0.7$ ) compared to  
222 FOS-Raftilose 95 ( $\log 7.61 \pm 0.31$  to  $7.6 \pm 0.09$ ) and the negative control ( $7.62 \pm 0.31$  to  $6.5 \pm 0.24$ )

(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 fermentation ( $7.50 \pm 0.25$ ) compared to FOS-Raftilose 95 ( $6.59 \pm 0.07$ ) (P < 0.001). Levels of *Eubacterium rectale-Clostridium coccoides* group (enumerated by Erec482) and *Bacteroides* spp.- *Provetella* group (enumerated by Bac303) significantly increased at 4 h (P < 0.05), 8 h (P < 0.01) and 24 h (P < 0.001), whereas numbers of the domain bacteria (detected by EUB I-II-III) were increased after 24 h (P < 0.001) of fermentation ( $8.32 \pm 0.26$  to  $8.96 \pm 0.39$ ).

### 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 higher production of acetate and propionate but lower amounts of butyrate. As expected, the juçara fermentation resulted in 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 phenolics compounds of juçara pulp during digestion and fermentation by human faecal bacteria

In order to follow up the degradation of juçara during digestion, main flavonoids compounds were monitored by HPLC as reported in Table 3. The mainly anthocyanins detected in juçara pulp were cyanidin-3-rutinosideo followed by cyanidin-3-glucosideo, malvidin-3-glucosideo, pelargonidin and peonidin, and the flavonoids rutin, quercetin and p-cumaric. During the digestion, all anthocyanins were slowly degraded until the gastric phase. However, after the intestinal phase, anthocyanins were reduced in abundance compared to the original sample and after dialysis, any malvidin, peonidin and pelargonidin were detected. The detected flavonols quercetin and rutin were also degraded during the digestion in contrast with p-cumaric that slightly increased after dialysis.

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

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

256

257

#### 258 **4. DISCUSSION**

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

263 The prebiotic effects of fructooligosaccharides and galactooligosaccharides are thought to underpin  
264 certain functional outcomes in the large gut. However, there is limited information regarding the  
265 ability of other dietary components, including flavonoids, to influence the growth of selected  
266 intestinal bacteria (Tzounis et al., 2008). There is growing evidence from animal and human studies  
267 that dietary fibres and polyphenols might have an impact on the human gut microbiota, these  
268 compounds appear to modulate both species composition within the gut microbiota and metabolic  
269 profiles (Tuohy, Conterno, Gasperotti, Viola, 2012). The human colon contains a wide range of  
270 bacterial communities distributed in hundreds of distinct species and the balance among them, plays  
271 an important role in health and disease (Holzapfel, Haberer, Snel, Schillinger, Veld, 1998;  
272 Rigottier-Gois, Rochet, Garrec, Suaru, Dore, 2003).

273 Significant changes in bifidobacterial numbers were observed in response to juçara after 24 h of  
274 fermentation. Bifidobacteria are recognized as one of the most important bacterial groups associated

275 with human health providing beneficial effects in the large intestine (Gibson, Wang, 1994; Russel,  
276 Rossa, Fitzgerald, Stanton, 2011). It is well known that an increase in bifidobacteria number is  
277 favoured by the presence of mainly carbohydrates. However, recent studies have showed that some  
278 phenolics compounds, such anthocyanins and gallic acid have also the ability to promote the growth  
279 of the *Bifidobacterium* and *Lactobacillus* spp. (Gibson, 1999, Aura et al., 2005, Tzounis et al.,  
280 2008, Hidalgo et al., 2012). Following digestion, juçara pulp represented 3.2 g/100g of available  
281 sugar, 18.6 g/100g of fibre (data not shown) and 1613.8 mg GAE/100g of total phenolics that could  
282 be available for the beneficial bacteria growth.

283 The juçara fermentation also increased populations of *Bacteroides* spp. and *Clostridium* cluster  
284 XIVa, and although both groups may exert a detrimental effect on colon health due to an  
285 association with some metabolites, these groups also contain saccharolytic species which can  
286 produce large concentrations of beneficial SCFAs from sugars. Therefore, an increase in  
287 populations of *Clostridium* spp. and *Bacteroides* spp., is partially dependent on the precursor  
288 substrate to which they are exposed (Gibson, Roberfroid, 1995; Cummings, Macfarlane, 1997;  
289 Nyangale, Farmer, Keller, Chernoff, Gibson, 2014). Here, residual carbohydrates could be  
290 associated with saccharolytic species. In addition, low numbers of *Clostridium histolyticum* group  
291 were found in the present study (data not shown). This group have been associated to the production  
292 of negative effects by the fermentation of proteins and amino acids (Cummings, Macfarlane, 1997)  
293 and a decrease in its numbers can provide an addition information in terms of the specificity for  
294 beneficial bacteria during juçara fermentation. The antimicrobial effect on certain bacterial group  
295 for juçara fermentation, can be also related by the presence of phenolic compounds which, in  
296 accordance to previous studies, could inhibit the growth of potentially negative bacteria (Hidalgo et  
297 al., 2012).

298 Juçara fermentation in vitro induced a modulation of the colonic microbiome with increased acetate  
299 and propionate. Our results support the hypothesis that through an increase of some bacterial groups  
300 and the subsequent production of different amounts of SCFAs, juçara could exert a beneficial role

301 for the host. Both phenolics compounds and carbohydrates that escaped from juçara digestion could  
302 be responsible for such modulation (Vendrame, Gyglielnetti, Riso, Arioli, Klimis-Zacas, Porrini,  
303 2011; Tuohy et al., 2012).

304 In the present study, the amount of acetate and propionate produced for juçara fermentation was  
305 similar to the positive control (FOS-Raftilose 95). Numerous *in vivo* and *in vitro* studies proved that  
306 this carbohydrate can modulate the gut through the selectively stimulate the gut microbiota (Gibson,  
307 Roberfroid, 1995, Gibson, Probert, Van Loo, Rastall, Roberfroid, 2004). The SCFA produced by  
308 gut microbiota in the colon have an important role. Butyrate is often associated as an energy source  
309 for the epithelium cells and acetate plays an important role in controlling inflammation and cobating  
310 pathogen invasion (Russel, Hoyles, Flint, Dumas, 2013). Also, acetate and propionate may have a  
311 direct role in central appetite regulation. The mechanism for propionate is in stimulating release of  
312 the anorectic gut hormones peptide YY (PYY) and glucagon like peptide-1 (GLP-1). They in turn  
313 are involved in the short-term signal of satiation and satiety to appetite centres of the brain,  
314 increasing satiety and reducing food intake in animals and man (Tolhurst et al., 2012; Cherbut et  
315 al., 1998; Brown et al., 2003; Chambers et al., 2014). Acetate administration is associated with  
316 activation of acetyl-CoA carboxylase and changes in the expression profile of regulatory  
317 neuropeptides favouring appetite suppression (Frost et al., 2014).

318 In this study, the degradation of anthocyanins and phenolics compounds during a simulated *in vitro*  
319 digestion was monitored and a decrease in these compounds was observed. This result could be due  
320 to anthocyanins being metabolized, oxidized, or degraded into other chemicals (Hidalgo et al.,  
321 2012). However, for those anthocyanins that do decompose, there seems to be a delay between the  
322 loss of anthocyanin aglycones and the formation of phenolic acids, probably due to the relatively  
323 stable transition products. This delay could further reduce concentrations of phenolic acids in the  
324 upper gastrointestinal tract (Fang, 2014), which explains a lower increase in the present study.  
325 Besides, from previous studies on simulated human gastrointestinal digestion have shown that  
326 anthocyanins are less stable at an elevated pH of the small intestine (Perez-Vicente, Gil-Izquierdo,

327 Garcia-Viguera, 2002; McDougall, Fyffe, Dobson, Stewark, 2007). Correa-Betanzo, Allen-Vercor,  
328 McDonald, Schroeter, Corredig, Paliyath (2014) observed by LC-ESI-MS analysis, a clear  
329 disappearance of the absorbance of the anthocyanin peaks from blueberries extract, when the  
330 incubation pH changed from acidic (pH 2) to alkaline (pH 8), indicating a ring cleavage for most  
331 anthocyanin.

332 Colonic bacteria are well known to act enzymatically on the polyphenolic backbone of the  
333 remaining unabsorbed polyphenols and produce different compound with physiological significance  
334 (Aura et al., 2005). Alongside the fermentation time, the amount of total phenolics was kept  
335 constant and similar results were also reported by Correa-Betanzo et al. (2014), who observed a  
336 constant level of the total phenolics compounds from blueberry extracts, both crude and digested,  
337 during simulated colon fermentation.

338 *In vitro* and *in vivo* intervention and epidemiological studies have shown new evidence on the wide  
339 range of health promoting activities of dietary polyphenols, including their anti-inflammatory,  
340 antioxidant, anticarcinogenic, antiadipogenic, antidiabetic and neuroprotective potentials,  
341 suggesting an association between the consumption of polyphenol-rich foods and a reduced risk of  
342 several chronic diseases (Jennings, et al., 2012; Hooper et al., 2012).

343

## 344 5. CONCLUSION

345 This study provides new insights about the numbers of bacteria population and the degradation of  
346 juçara phenolics compound during digestion and fermentation in the colon. The results suggest that  
347 *in vitro* digestion may cause a significant loss in the juçara anthocyanin, however the total phenolic  
348 compound is maintained during fermentation time. Following juçara pulp fermentation, beneficial  
349 modulations were seen in terms of bifidogenic effects. Future work will explore the role such  
350 changes play in human health.

351



352 **Significance of the study:** This is the first study reporting a potential prebiotic mode of activity for  
353 jucara and it does provoke changes in the bacterial metabolisms and bifidobacterial numbers which  
354 support a functional advantage to the host.

355

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359

## 360 7. REFERENCES

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## Figure Captions

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534 Figure 1- Bacterial populations analysed by FISH in a batch cultures fermentation containing  
535 juçara pulp, FOS and negative control. Results are reported as mean of the data (n = 3) Log10  
536 CFU/mL  $\pm$  standard deviations (SD); Significant differences from juçara (using 2-way ANOVA  
537 Bonferroni post-tests to compare replicate means) \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001.  
538 Significant differences for the same vessels compared to 0 h within the same substrate (using t-  
539 test, P < 0.05), are indicated with letters.

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