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ABSTRACT

This study was carried out to investigate the potential fermentation properties of juçara pulp using 24-h 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. The metabolism of phenolic compounds during an *in vitro* simulated digestion and fermentation were also investigated. 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 24h fermentation compared to a negative control. After *in vitro* digestion, almost all the anthocyanin were degraded and 46 % of total phenolic were still remaining. This is a first study reporting the potential prebiotic effect of juçara pulp, however human studies are necessary to prove its efficacy.

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

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

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 reaches number around 10^{14} colony-forming units in total (Gibson, Roberfroid, 1995). Through the process of fermentation, gut microbiota is able to produce a wide range of compounds 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 understanding 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. Recently, few studies have focused on the impact of whole plant foods on gut microbial species composition and relative abundance.

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 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 *Euterpe 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 acai and juçara pulp in industrial demands (Cohen, Alves, 2006). Lyophilised juçara pulp nutritional profile was characterized 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), and the results are presented in Table 1.

2.2 Simulated in vitro human digestion

Prior to being added into the batch culture systems, the lyophilised juçara pulp were digested in *vitro* under appropriate conditions according to the procedures describes by Maccaferri et al. (2012) Sixty grams of pulp was mixed with 150 mL of sterile distilled water and homogenized 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 37ºC for 30 min, under shaking. After incubation, pH was adjusted to 2.0 and pepsin (2.7g) in 0.1 M HCl (25 mL) was added, prior to a further incubation cycle, under shaking conditions, at 37ºC for 2 h. Finally, bile (3.5 g) and pancreatin (560 mg) were mixed with 0.5 M $NaHCO_3$ (125 mL) at pH 7.0 and then with lyophilised juçara pulp. Dialyses with membrane of 100-200 a cut-off Spectra/por 100-200 Da WCO dialysis membranes (Spectrum Europe B. V., Breda, The Netherlands) were used to remove

monosaccharides from the pre-digested juçara. 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 were used immediately. The samples were diluted 1:10 (w/v) with anaerobic phosphate buffered saline (0.1 M; pH 7.4) and homogenised in a stomacher for 2 min (460 paddle/min). 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), $MgSO_4.7H_2O$ (0.01 g/L), $CaCl_2.6H_2O$ (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 distille 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). Before addition of faecal slurries, temperature of the basal nutrient medium was set to 37ºC 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 ran under anaerobic conditions, 37ºC and pH 6.8 for a period of 24h 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 4ºC 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 Eppendorff tubes (1.5 mL) at -20ºC until 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 – Orafiti, Tienen, Belgium) and basal nutrient media with no substrate added, 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, Stolcker, 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 13000g 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 ºC until used for hybridization. Oligonucleotides 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, Bruh, Amann, Schleifer, Wagner, 1999); Bif164 specific for *Bifidobacterium* spp. (Langendijk et al. 1995); Lab158 for *Lactobacillus-Euterococcus* spp. (Harmsen, Elfferich, Schut, Welling, 1999); Bac303 specific for the *Bacteroides-Prevotell*a group (Manz, Amann, Ludwig, Vancanneyt, Schleifer, 1996); Chis150 for the *Clostridium histolyticum* subgroup; Erec482 for most members of *Clostridium cluster* XIVa (Franks, Harmsen, Raangs, Jonsen, Schut, Welling, 1998) and Prop 853 (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 ion exclusion high performance liquid chromatography (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 x 7.8 mm) at a temperature of 84 ºC 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 was 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 x 250 mm) and the mobile phases: A (95 % HPLC water, 5 % methanol and 1 % formic acid); mobile phase 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 in 5 min and from 100% B to 5% B in 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 were previously extract with 5 mL 1 % formic acid with methanol, vortex for 1 min and centrifuged at 3500 rpm. Batch cultures samples were centrifuged (13000g / 10 min), and both supernatants were filtered through a 0.22 µm filter and autoinjected into HPLC system.

2.8 *Total Phenolic analysis*

The total phenolic content were analyzed according to the Folin-Ciocalteu method adapted to 96-well plate microliter 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 sodium carbonate saturated solution was added and the solution kept in a shaker for 25 min at room temperature. The absorbance of the samples were measured at 650 nm using a GENios pro microplater reader (Tecan, Theale, Berks, UK**)** equipped to a Magellan Software. Methanolic solutions of gallic acid (Sigma-Aldrich, Poole, Dorset, UK) with concentration of 0 to 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 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 using GraphPad Prism 5.0 (GraphPad Software, LaJolla, CA, USA).

3. RESULTS

3.1 Changes in bacterial populations in 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) (Figure 1). Following juçara pulp fermentation, a significant increase in numbers of *Bifidobacterium* spp. (detected by Bif164 probe) was observed after 24 h 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 (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 fermentation (7.50 ± 0.25) compared to FOS-Raftilose 95 (6.59 ± 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 ± 0.26 to 8.96 ± 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 degradated during the digestion in contrast with p-cumaric that slightly increased after dialysis.

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

In order to evaluate the metabolism of phenolics 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 phenolis 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, p-cumaric and the total amount of phenolics was kept constant during *in vitro* fermentation.

4. DISCUSSION

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

The prebiotic effects of fructooligosaccharides and galactooligosaccharides are thought to underpin certain functional outcomes in the large gut. However, there is limited information regarding the ability of other dietary components, including flavonoids, to influence the growth of selected intestinal bacteria (Tzounis et al., 2008). There is growing evidence from animal and human studies that dietary fibres and polyphenols might have an impact on the human gut microbiota, these compounds appear to modulate both species composition within the gut microbiota and metabolic profiles (Tuohy, Conterno, Gasperotti, Viola, 2012). 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, Suaru, Dore, 2003).

Significant changes in bifidobacterial numbers were observed in response to juçara after 24 h of fermentation. Bifidobacteria are recognized 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 showed that some phenolics compounds, such anthocyanins and gallic acid have also the ability to promote the growth of the *Bifidobacterium* and *Lactobacillus* spp. (Gibson, 1999, Aura et al., 2005, Tzounis et al., 2008, Hidalgo et al., 2012). Following digestion, juçara pulp represented 3.2 g/100g of available sugar, 18.6 g/100g of fibre (data not shown) and 1613.8 mg GAE/100g of total phenolics that could be available for the beneficial bacteria growth.

The juçara fermentation also increased populations of *Bacteroides* spp. and *Clostridium* 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; Cummings, Macfarlane, 1997; Nyangale, Farmer, Keller, Chernoff, Gibson, 2014). Here, residual carbohydrates could be associated with saccharolytic species. In addition, low numbers of *Clostridium histolyticum* group were found in the present study (data not shown). This group have been associated to the production of negative effects by the fermentation of proteins and amino acids (Cummings, Macfarlane, 1997) and a decrease in its numbers can provide an addition information in terms of the specificity for beneficial bacteria during juçara fermentation. The antimicrobial effect on certain bacterial group for juçara fermentation, can be also related by the presence of phenolic compounds which, in accordance to previous studies, could inhibit the growth of potentially negative bacteria (Hidalgo et al., 2012).

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

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

In the present study, the amount of acetate and propionate produced for juçara fermentation was similar to the positive control (FOS-Raftilose 95). Numerous i*n vivo* and *in vitro* studies proved that this carbohydrate can modulate the gut through the selectively stimulate the gut microbiota (Gibson, Roberfroid, 1995, Gibson, Probert, Van Loo, Rastall, Roberfroid, 2004). The SCFA produced by gut microbiota in the colon have an important role. Butyrate is often associated as an energy source for the epithelium cells and acetate plays an important role in controlling inflammation and cobating pathogen invasion (Russel, Hoyles, Flint, Dumas, 2013). Also, acetate and propionate may have a direct role in central appetite regulation. The mechanism for propionate is in 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 (Tolhurst et al., 2012; Cherbut et al., 1998; Brown et al., 2003; Chambers et al., 2014). 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).

In this study, the degradation of anthocyanins and phenolics compounds during a simulated *in vitro* digestion was monitored and a decrease in these compounds was observed. This result could be due to anthocyanins being metabolized, oxidized, or degraded into other chemicals (Hidalgo et al., 2012). 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 lower increase in the present study. Besides, from previous studies on simulated human gastrointestinal digestion have shown that anthocyanins are less stable at an elevated pH of the small intestine (Perez-Vicente, Gil-Izquierdo,

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

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

In vitro and *in vivo* intervention and epidemiological studies have shown new evidence on 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 (Jennings, et al., 2012; Hooper et al., 2012).

5. CONCLUSION

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

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

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Figure Captions

Figure 1- Bacterial populations analysed by FISH in a batch cultures fermentation containing juçara pulp, FOS and negative control. Results are reported as mean of the data (n = 3) Log10 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.