J Food Sci Technol (November 2019) 56(11):5017–5026
https://doi.org/10.1007/s13197-019-03974-5

ORIGINAL ARTICLE

In vitro gastrointestinal evaluation of a juçara-based smoothie: effect of processing on phenolic compounds bioaccessibility

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Revised: 13 June 2019 / Accepted: 24 July 2019 / Published online: 1 August 2019

Abstract In the present work, the bioaccessibility of the main phenolic compounds of a juçara, banana and strawberry homogenized smoothie (control), subjected to pasteurization and sonication, was evaluated. The smoothie was also evaluated in terms of its main chemical and physical characteristics. Pasteurized smoothie showed higher apparent viscosity, as well as higher initial shear stress when compared to the control and sonicated samples. The increase in the apparent viscosity of the pasteurized smoothie was associated with the smaller particle size of this sample (68 μm). These characteristics conferred to the pasteurized smoothie higher physical stability than the control and sonicated smoothies. Phenolic compounds bioaccessibility was higher in the pasteurized and sonicated smoothies than in the control sample, which confirmed the positive effect of the treatments for the preservation of these compounds after gastrointestinal digestion. Compared to the sonication process, the pasteurization provided higher total phenolic compounds bioaccessibility (47%), as well as of ferulic (16%) and ellagic (80%) acids. Antioxidant capacity was higher in gastric digest for all the samples evaluated by ABTS assay. These results confirm the importance of processing on the physical stability and phenolic compounds bioaccessibility of the juçara-based smoothie, standing out the thermally treated product.

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Keywords *Euterpe edulis* Martius · Pasteurization · Sonication · Antioxidant compounds · Rheology

Introduction

Juçara (*Euterpe edulis* Martius), a typical palm of the Brazilian Atlantic Forest, produces fruits rich in antioxidant compounds, such as anthocyanins. However, juçara pulp has, in general, low sensory acceptance when consumed pure, mainly due to its low soluble solids content. Taking into account that anthocyanins act as a natural antioxidant and colorant, the use of juçara pulp as a functional ingredient in new formulations has been encouraged (Schulz et al. 2017; Ribeiro et al. 2018b).

Fruit-derived products such as smoothies are naturally rich in sugar and nutrients and are susceptible to microbial growth, which makes essential their microbiological stabilization (Santhirasegaram et al. 2014). Heat treatment, by reducing the microbial load, has traditionally been the most applied method for conservation of fruit juices, making them suitable for consumption. However, processing conditions can cause negative effects on the product, such as the cooked residual flavor, browning, and the reduction of the compounds potentially beneficial to health (Chitgar et al. 2016).

Thus, non-conventional technologies have been evaluated as alternatives to reduce microbial load, once they may cause a lower impact on antioxidant compounds and contribute to increase the supply of processed products with biological properties. In this sense, the use of acoustic waves in food processing has been widespread as an alternative for their conservation. Ultrasound, by the phenomenon of cavitation, can simultaneously assure the destruction of microorganisms and the enzymatic inactivation. However, its efficiency depends on several factors,

such as the physical and chemical characteristics of the product, the concentration and type of microorganisms present in the food, among others. For this reason, each product requires different processing conditions, and, therefore, specific studies are needed to increase the knowledge of the effects of sonication on foods' quality (Chemat et al. 2011).

The evaluation of the effects of digestion on potential bioactive compounds present in natural and processed foods is an activity that has contributed to the interpretation of the results of technological researches associated with human nutrition. This evaluation has been strongly encouraged by the scientific community because little is known about the behavior of the bioactive compounds present on food along the gastrointestinal tract. Bioaccessibility, one of the responses obtained by in vitro gastrointestinal evaluation, is defined as the amount of an ingested compound that is available for absorption into the intestine after digestion. Thus, through an in vitro digestion simulation it is possible to understand the effects of bioactive compounds on the human organism (Palafox-Carlos et al. 2011).

In vitro digestion models have been widely used to study the structural alterations, digestibility, release and bioaccessibility of compounds in foods, through the simulation of gastrointestinal conditions. These models generally comprise a digestion with gastric pepsin at acidic pH, followed by digestion with pancreatin and bile salts under alkaline conditions (Minekus et al. 2014).

In vitro gastrointestinal evaluation allows simulating the main effects of digestion in the food matrix without involving the ethical issues typically present in the in vivo tests, making it a potential technique for this purpose (McClements and Li 2010).

In this context, the objective of this work was to evaluate the in vitro bioaccessibility of the main phenolic compounds present in a juçara, banana and strawberry smoothie subjected to pasteurization and sonication processes, as well as to characterize the products in terms of their main chemical and physical characteristics.

Materials and methods

Material

Frozen strawberry pulp (Sempre Viva, Brazil) was acquired in a local market in Rio de Janeiro. Frozen juçara pulp (Juçaí, Brazil) was provided directly from the manufacturing company in the state of Rio de Janeiro. Banana pulp was obtained by depulping the fruits in horizontal depulper (Bonina 0.25 dF, Itametal, Itabuna, Brazil) with 1.5 mm sieve and polyethylene blades, packed in plastic bags and immediately frozen. Fruits from Nanica variety in ripeness degree 6 were used. Before using, pulps were defrosted and juçara pulp was pre-treated by centrifugation in basket centrifuge (Model K7165, Thermo Scientific IEC, Bellport, EUA) provided with 150 μm nylon filter, aiming at the reduction of suspended solids and lipid fraction that could affect the final product quality due to oxidation and, consequently, rancidity.

Smoothie processing

Smoothie formulated with juçara (20%), banana (40%) and strawberry (40%) pulps was standardized in a pilot blender. This product was developed according to Ribeiro et al. (2018a), which defined the formulation based on an experimental design where sensory acceptance and antioxidant capacity of the smoothie were the main response parameters. After mixing, the smoothie was homogenized in an APV homogenizer (Gaulin, USA) at 60 MPa (control). Control sample was then subjected to pasteurization (pasteurized smoothie) and sonication (sonicated smoothie) as described below.

The smoothie was pasteurized on a scraped surface heat exchanger (FT25D, Armfield, England) at 90 °C for 35 s. Cold filled was carried out in ultra-clean container chamber.

Sonication was performed in batch on a Hielscher Ultrasonic UIP-1000hd processor (Teltow, Germany) (frequency 20 kHz—220 V), equipped with IP65 titanium transducer, Buster B4 1.8 with area of 2.5 cm^2, 20 mm submerged in the sample and sonotrode BS2d18. A volume of 200 mL of the smoothie was packed in a jacketed glass reactor (250 mL), coupled to a thermostatic bath set at 10 °C, in which the product was sonicated for 7 min at 220 W.

Products were stored under freezing until further chemical and physical analysis as well as in vitro gastrointestinal digestion assays.

In vitro gastrointestinal evaluation

In vitro gastrointestinal evaluation was performed according to the method described by Minekus et al. (2014). Briefly, the steps evaluated in this work are described below. All assays were performed in 50 mL falcon tube, kept in a thermostatic bath shaken at 120 rpm at 37 °C.

In the oral phase, 5 g of samples were mixed with 4 mL of salivary fluid, 25 μL of 0.3 M $CaCl_2$, 0.5 mL of 75 U mL^{-1} α-amylase aqueous solution and 0.475 mL of water Milli-Q grade. This mixture was incubated for 2 min. After this period, the gastric digestion step was started, by adding 8 mL of gastric solution, 1 mL of 2000 U mL^{-1} pepsin aqueous solution and 0.5 μL of 0.3 M $CaCl_2$ in the

same tube. After stirring the mixture, the pH was corrected to 3.0 with 1 M HCl and final volume (20 mL) was reached by the addition of Milli-Q grade water. The samples were then incubated for 2 h. For the intestinal step, to the resulting mixture from the oral and gastric phases (20 mL), after the incubation period, 11 mL of intestinal solution, 5 mL of pancreatin solution diluted in the intestinal solution (100 U mL^{-1}), 2.44 mL of 10 Mm bile, also diluted in the intestinal solution, and 40 µL of 0.3 M $CaCl_2$ were added. After mixing the solutions, the pH was corrected to 7.0 with 1 M HCl and final volume (40 mL) was reached by the addition of Milli-Q grade water. At this phase, the samples were also incubated for 2 h.

Enzymatic activity was stopped using an enzyme inhibitor (1 mM Pefabloc, 10 µL for each mL of solution). Samples were centrifuged at 4 °C at 5000 g for 15 min. Supernatants were separated and stored under freezing until the compounds analysis.

Bioaccessibility (B) was expressed in percentage of the target compound present in the sample after the in vitro gastrointestinal digestion simulation, as shown in Eq. (1). The remaining antioxidant capacity (RAC) was also calculated (Eq. 2).

$$B(\%) = \frac{C_{digested}}{C_{undigested}} \quad (1)$$

where B is the bioaccessibility expressed in percentage, $C_{digested}$ is the concentration of the target compound in digested sample and $C_{undigested}$ is the concentration of the target compound in undigested sample.

$$RAC(\%) = \frac{AC_{digested}}{AC_{undigested}} \quad (2)$$

where RAC is the remaining antioxidant capacity after digestion expressed in percentage, $AC_{digested}$ is the antioxidant capacity of digested sample and $AC_{undigested}$ is the antioxidant capacity of the undigested sample.

Analytical methods

Total phenolic compounds (TPC)

For the reactions, 30 µL of each extract were mixed with 120 µL of sodium carbonate solution at 7.5% (w/v) and 150 µL of 10% Folin–Ciocalteu reagent. The absorbance was measured at 760 nm. TPC content was expressed in mg gallic acid equivalent 100 g^{-1} (Georgé et al. 2005).

Total anthocyanins (TA)

Anthocyanins content was quantified using cyanidin-3-glucoside as reference, with molar extinction coefficient of 26,900 M^{-1} cm^{-1} and molecular weight of 449.2 g mol^{-1}. Extracts were diluted in pH 1.0 and after 15 min of stabilization, absorbance was read at 510 and 700 nm in 96-well microplate reader (Synergy HT, Bio-Tek, USA) (Giusti and Wrolstad 2001).

ABTS assay

Antioxidant capacity was determined by the reduction method of the $ABTS^{\cdot+}$ radical (Sigma-Aldrich®, Spain) according to Gião et al. (2007), adapted to a 96-well microplate reader (Synergy HT, Bio-Tek, USA). For the reactions, 10 µL of each filtered and diluted extract were mixed with 200 µL ABTS·+ radical. Results were expressed as micromoles Trolox (Sigma-Aldrich®, Spain) equivalents per gram (µmol TE g^{-1}).

DPPH assay

DPPH (Sigma-Aldrich®, Spain) radical scavenging capacity of samples was determined according to the method described by Hidalgo et al. (2010). For the reactions, 10 µL of each properly diluted extract was added to 290 µL of DPPH solution (6×10^{-5} M in methanol). The absorbance was measured at 517 nm in a spectrophotometric microplate reader (Synergy HT, Bio-Tek, USA) using methanol as blank. Results were expressed as µmol TE g^{-1}.

Phenolic compounds analysis by ultra-high performance liquid chromatography (UHPLC)

Samples were analyzed in a Shimatzu Nexera X2 UHPLC chromatograph equipped with Diode Array Detector (Shimadzu, SPD-M20A). Separation was performed on a reversed-phase Aquity UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 µm particle size; from Waters) and a pre-column of the same material at 40 °C. The flow rate was 0.4 mL min^{-1}. HPLC grade solvents used were water/formic acid (0.1%) as solvent A and acetonitrile as solvent B. The elution gradient for solvent B was as follows: from 0.0 to 5.5 min eluent B at 5%, from 5.5 to 17 min a linear increase to 60%, from 17.0 to 18.5 min a linear increase to 100%, then column equilibration from 18.5 to 30.0 min again at 5%. Phenolic compounds were identified by comparing their UV spectra and retention times with that of corresponding standards.

Rheological behavior

Rheological assays in steady-state were performed on a Discovery HR-1 rheometer (TA Instruments, New Castle, USA). For this, a plate-plate geometry (40 mm) and 1 mm gap were used. Experiments were performed at 25 °C. Flow curves were obtained by an up–down–up steps

program with shear rate ranging from 0 to 600 s^{-1}. Models of the Power Law (Eq. 3) and Herschel–Bulkley (Eq. 4) were adjusted to the data using linear regression.

$$\tau = k \cdot \gamma^n \quad (3)$$

$$\tau = \tau_0 + k \cdot \gamma^n \quad (4)$$

where τ is the shear stress (Pa), τ_0 is the initial shear stress (Pa), k is the consistency index (Pa s^n), γ is the shear rate (s^{-1}) and n is the behavior index.

Mean particle size

Mean particle size was determined by laser diffraction using a Coulter (Beckman, LS230, USA). Samples were introduced into a tank filled with water until it reached 10% obscuration. The particle size was calculated in terms of the mean diameter of the sphere of equal volume ($D_{4.3}$) (Wang et al. 2014).

Optical microscopy

Samples of smoothies, diluted five times in ultra-pure water, were analyzed in a light microscope (Olympus BX51) coupled with a DP71 digital camera (Olympus Portugal SA, Porto, Portugal). All images were acquired using Olympus CellSens software.

Data analysis

Data obtained were statistically evaluated in the software Statistica 12, using analysis of variance (ANOVA), considering the Tukey test to verify the existence of significant differences among the means, using a 95% confidence interval. Results, on wet basis, were expressed in mean ± standard deviation of assays performed in triplicate.

Results and discussion

Chemical and physical characteristics

The results of the anthocyanins, phenolic compounds and antioxidant capacity of the smoothies are shown in Table 1. It is possible to observe that the control, pasteurized and sonicated smoothie samples did not statistically differ regarding their total anthocyanins and phenolic compounds contents. However, the antioxidant capacity of the samples, expressed by the inhibition of the DPPH radical, was affected by pasteurization and sonication ($p < 0.05$), with a higher negative influence of the heat treatment. This is, probably, due to the effect of heat, which can significantly affect the structure of some phenolic compounds, enabling the formation of new compounds by cleavage or complexation with reduced antioxidant potential, once heat favors the interaction phenolic-nutrients (Rawson et al. 2011; Li et al. 2017).

Table 1 Bioactive compounds and antioxidant capacity of juçara, banana and strawberry smoothie

Parameter	SMC	SMP	SMS
TA[1] (mg 100 g^{-1})	24 ± 0[a]	23 ± 1[a]	23 ± 1[a]
TPC[2] (mg 100 g^{-1})	278 ± 10[a]	264 ± 13[a]	277 ± 23[a]
ABTS (μmol TE g^{-1})	12 ± 1[a]	10 ± 0[b]	12 ± 0[a]
DPPH (μmol TE g^{-1})	9 ± 0[a]	7 ± 0[b]	8 ± 0[c]
Ferulic acid (mg L^{-1})	21 ± 1[a]	20 ± 1[a]	21 ± 1[a]
Gallic acid (mg L^{-1})	1823 ± 17[a,b]	1727 ± 61[b]	1852 ± 52[a]
Ellagic acid (mg L^{-1})	246 ± 11[a]	280 ± 32[a]	407 ± 23[b]

Results expressed in mean ± standard deviation. Means followed by equal letters in the same line do not statistically differ ($p > 0.05$)

TA total anthocyanins, *TPC* total phenolic compounds, *SMC* control smoothie, *SMP* pasteurized smoothie, *SMS* sonicated smoothie, *TE* Trolox equivalents

[1]Expressed in cyanidin-3-glucoside

[2]Expressed in gallic acid

In accordance to our findings, Azofeifa et al. (2015) also observed the decrease in the antioxidant capacity of blackberry juice after pasteurization at 92 °C for 10 s. For theses authors, this decrease could be explained by changes in the chemical structures of the phenolic compounds due to heat treatment, which causes cleavage of covalent bonds and modifications in the number and arrangements of hydroxylated groups and glycosylation of anthocyanins. Thus, the severity and nature of these chemical re-arrangements determine the loss of anthocyanins' bioactivity. In the same way, Chitgar et al. (2016) verified the negative effect of heat on antioxidant capacity of barberry juice subjected to pasteurization at 90 °C for 1 min.

It is important to note that sonication, by means of the collapse of the cavitation bubbles, can break the structure of the fruit cells, releasing phenolic compounds, which can balance the loss of these compounds during the processing (Zinoviadou et al. 2015).

Muzaffar et al. (2016) reported the increase in the total phenolic compounds content of sweet cherry fruit after ultrasound treatment. The fruits were treated at 60 W for time range of 0–60 min. As processing time increased from 0 to 40 min, a significant increase of phenolic compounds was observed. According to the authors, it is due to the disruption of cell wall material, which corroborates the significant increase in the ellagic acid content of sonicated sample observed in our work.

The main differences observed in the smoothie samples occurred in their physical parameters. As shown in

Table 2, the apparent viscosity of the samples at shear rates of 50 s^{-1} and 100 s^{-1}, which, in general, correspond to human swallowing and food industry processes, respectively (Steffe 1996), were significantly different ($p < 0.05$). Pasteurized sample presented higher absolute value for the apparent viscosity in comparison to the other evaluated samples.

Good fit to the data was obtained by Power Law and Herschel–Bulkley models, presenting R^2 higher than 0.99. For both models, the value of the behavior index (n) was less than 1, indicating that the samples had non-Newtonian fluid behavior with pseudoplastic characteristics, typical behavior of fruit products (Gouvêa et al. 2017).

Consistency index (k), which indicates the sensitivity of the fluid to the temperature gradient, was higher in the pasteurized smoothie as observed in the apparent viscosity values discussed above. In addition, this sample presented higher resistance to flow as compared to control and sonicated smoothies, since it presented higher absolute value of initial shear stress (Herschel–Bulkley model, Table 2).

Pasteurization favored the reduction of smoothie particle size as it can be seen in Fig. 1 and Table 2. It may be due to the collision of the smoothie particles during the heat treatment in the scraped surface heat exchanger conducted in continuous mode. It made possible a higher solubilization of macromolecules, such as starch and pectin, and of macromolecular aggregates, due to the breakdown of these into smaller particles (Ahmed et al. 2005; Bi et al. 2015).

Table 2 Rheological characteristics and particle size of juçara, banana and strawberry smoothie

	SMC	SMP	SMS
Apparent viscosity (Pa s)			
γ (50 s^{-1})	0.071 ± 0.050^a	0.221 ± 0.007^b	0.050 ± 0.000^c
γ (100 s^{-1})	0.044 ± 0.002^a	0.135 ± 0.004^b	0.030 ± 0.001^c
Power law model parameters			
n	0.433 ± 0.015^a	0.363 ± 0.006^b	0.523 ± 0.015^c
k (Pa s^n)	0.597 ± 0.059^a	2.523 ± 0.108^b	0.273 ± 0.021^c
R^2	0.99	0.99	0.99
Herschel–Bulkley model parameters			
τ_0 (Pa)	1.857 ± 0.117^a	5.703 ± 0.155^b	1.057 ± 0.035^c
n	0.650 ± 0.010^c	0.563 ± 0.006^b	0.700 ± 0.017^c
k (Pa s^n)	0.127 ± 0.006^a	0.567 ± 0.032^b	0.083 ± 0.006^c
R^2	0.99	0.99	0.99
Particle size*			
$D_{4.3}$ (μm)	104^a	68^b	287^c

Results expressed in mean ± standard deviation. Means followed by equal letters on the same line do not statistically differ ($p > 0.05$)

SMC, control smoothie; SMP, pasteurized smoothie; SMS, sonicated smoothie; γ, shear rate; n, behavior index; k, consistency index; τ_0, initial shear stress; R^2, coefficient of determination

*Mean values

This phenomenon contributed to increase the apparent viscosity in this sample. Thus, with the reduction of particle size there is an increase in surface area, favoring stronger interactions (Zhou et al. 2017).

Sonication was performed in batch and without stirring. Thus, the zones closer to the probe were, possibly, more affected by the cavitation caused by the acoustic waves, contributing to a lower homogeneity of this sample. Heterogeneous suspensions contribute to phase separation during storage due to the sedimentation of larger particles (Kubo et al. 2013). Therefore, larger particle size of the smoothie subjected to sonication had a negative impact on its physical stability.

In vitro gastrointestinal digestion

Bioaccessibility of total anthocyanins after the main phases of in vitro gastrointestinal digestion is shown in Fig. 2a. It varied from 5 to 25% among the samples, being the highest percentage observed in gastric phase. This behavior was also observed by Correa-Betanzo et al. (2014), when evaluating the bioaccessibility of wild blueberry anthocyanins during in vitro digestion.

This behavior was expected, since the stability of anthocyanins is strongly influenced by the pH of the medium. According to Castañeda-Ovando et al. (2009), pH promotes changes in the structure of these compounds, and values of pH above 7.0 can lead to the degradation of this pigment, which is of great importance from the point of view of health due to its antioxidant potential.

Evaluating the effect of gastrointestinal digestion on anthocyanins of the smoothie samples, it can be stated that the processing favored the bioaccessibility of this pigment in the gastric phase. In the intestinal phase, the sonicated smoothie presented higher bioaccessibility for this pigment, differing statistically from the other samples (Fig. 2a). This behavior may have been influenced by the particle size of this sample, as discussed in the previous section. Possibly, the formation of agglomerates in the sonicated sample protected the anthocyanins when compared to the pasteurized sample, which presented smaller particle size.

Bioaccessibility of the smoothie phenolic compounds varied from 20 to 47% between gastric and intestinal digests (Fig. 2b). Bioaccessibility in the intestinal digest of the pasteurized smoothie was significantly higher compared to the intestinal digests of the sonicated and control smoothies. This corroborates the positive effect of the heat treatment on bioaccessibility of these bioactive compounds. Data reported by He et al. (2016) showed that heat-treated fruit juices also presented higher bioaccessibility of phenolic compounds than those treated by high hydrostatic pressure, a non-thermal conservation method. According to

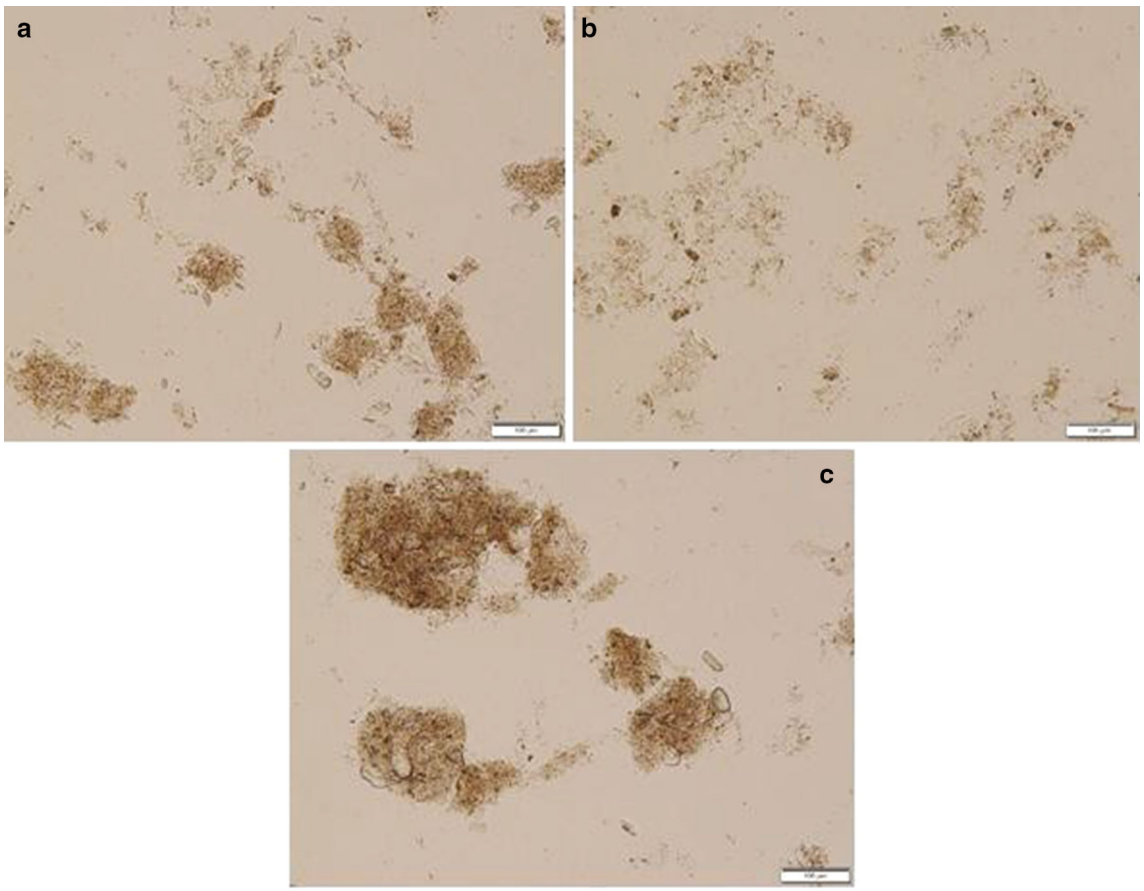

Fig. 1 Micrographies of the juçara, banana and strawberry smoothie [control (**a**), pasteurized (**b**) and sonicated (**c**)]. Scale bar (100 μm)

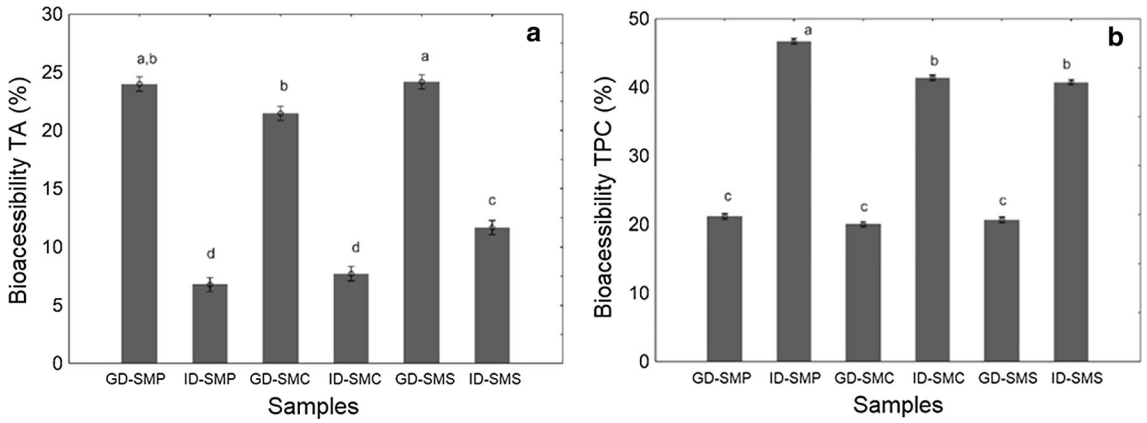

Fig. 2 Bioaccessibility of total anthocyanins (TA) (**a**) and total phenolic compounds (TPC) (**b**) of the juçara, banana an strawberry smoothie. Samples: control (SMC), pasteurized (SMP) and sonicated (SMS). *GD* gastric digest, *ID* intestinal digest

these authors, heat treatment weakens the cell wall of the fruits, favoring the release of phenolic compounds during gastrointestinal digestion.

The bioaccessibility of the phenolic compounds was higher in the intestinal digest for all evaluated samples, probably due to the increasing of pH in the intestinal phase, permitting the release of other phenolic compounds, which favors the hydrolysis of the cell wall. Furthermore, the increase of pH can cause the cleavage of anthocyanins that has phenolic acids as one of the degradation products (Sinela et al. 2017).

Cassani et al. (2018) reported that the increase of bioaccessibility of phenolic compounds of strawberry juice enriched with fibers was due to the release of ellagic acid,

one of the phenolic compounds present in strawberry, due to the hydrolysis of ellagitannins in function of alkaline pH of the intestinal environment.

According to Alminger et al. (2014), the phenolic compounds are mainly found in glycosylated, esterified or polymerized forms. Thus, during gastrointestinal digestion they can be hydrolyzed as consequence of the acid environment of the stomach and of the alkaline environment of the intestine, as well as the action of digestive enzymes. These conditions result in several changes in the structure of these compounds, such as hydroxylation, methylation, dimerization and glycosylation, as well as in the formation of phenolic derivatives by the partial degradation of their original structure, as in the case of anthocyanins, becoming the bioaccessibility of these compounds highly dependent on their type and amount in the plant matrix.

Gastrointestinal digestion significantly reduced the antioxidant potential of the samples in both evaluated steps (Table 3). All the digested fractions presented antioxidant potential, being always higher in the gastric digest for all the smoothies.

According to Castañeda-Ovando et al. (2009), phenolic compounds, mainly anthocyanins, are dependent on the pH of the medium. Anthocyanins may present different structural conformations in function of pH, and therefore they have higher or lower antioxidant capacity as new structures are formed. Sui et al. (2014) showed that the increase in antioxidant capacity of anthocyanin-containing solutions was directly proportional to the increase of pH, as it varied from 2.2 to 6.0. Higher antioxidant capacity was also reported by Ribeiro et al. (2018b) in juçara extract (pH 5.75) rich in anthocyanins, as compared to that obtained at pH 1.0.

Rodríguez-Roque et al. (2013) submitted a soy drink rich in phenolic compounds to in vitro gastrointestinal digestion and observed a higher antioxidant capacity in the gastric digest as compared to intestinal digest. According to these authors, the reduction of the antioxidant capacity under intestinal conditions can be attributed to the structural reorganization of some compounds due to their sensitivity to alkaline pH. In addition, these compounds are capable of binding to other constituents of the food matrix, resulting in the formation of complexes, which may also contribute to the reduction of their antioxidant potential.

Although higher concentration of phenolic compounds was observed in the intestinal digest of smoothie samples, the alkaline pH of the intestine reduced their antioxidant potential, as previously mentioned. In spite of this result, potentially antioxidant compounds (ferulic, ellagic, vanillic and cinnamic acids) were also detected in the intestinal digest. These compounds can contribute to various beneficial actions to the human organism, once absorbed by the cells. Urias-Lugo et al. (2015) reported positive effects of extracts rich in phenolic acids and anthocyanins obtained from purple corn on the proliferation of several tumor cells, which corroborates the importance of the results obtained in this work.

In general, the processing contributed positively to the maintenance of the smoothie antioxidant capacity after in vitro gastrointestinal digestion. Gastric digest of pasteurized sample presented higher preservation of the antioxidant potential, measured by the reduction of the ABTS radical in comparison with the other gastric samples evaluated, particularly for that obtained after digestion of the control. Intestinal digest of pasteurized smoothie, as well as the gastric digest of sonicated smoothie, stood out regarding the retention of the antioxidant capacity, as measured by the DPPH radical, in comparison with intestinal digest of the control.

The behavior of the major phenolic compounds quantified in the smoothie samples subjected to gastrointestinal

Table 3 Antioxidant capacity of juçara, banana and strawberry smoothie submitted to in vitro gastrointestinal digestion

Samples	Digestion	ABTS[1]	RAC ABTS (%)	DPPH[1]	RAC DPPH (%)
SMC	UD	12 ± 1^{a}	–	9 ± 0^{a}	–
	GD	2.7 ± 0.1^{d}	23 ± 1^{b}	1.3 ± 0.1^{e}	14 ± 1^{c}
	ID	1.5 ± 0.3^{e}	$12 \pm 2^{c,d}$	1.1 ± 0.1^{f}	12 ± 1^{d}
SMP	UD	10 ± 0^{c}	–	7 ± 0^{b}	–
	GD	2.7 ± 0.1^{d}	28 ± 1^{a}	1.4 ± 0.1^{e}	19 ± 1^{b}
	ID	1.1 ± 0.3^{f}	11 ± 3^{d}	1.5 ± 0.2^{d}	21 ± 2^{a}
SMS	UD	12 ± 0^{b}	–	8 ± 0^{c}	–
	GD	2.8 ± 0.1^{d}	25 ± 1^{b}	1.6 ± 0.2^{d}	$20 \pm 2^{a,b}$
	ID	1.6 ± 0.3^{e}	14 ± 3^{c}	1.3 ± 0.2^{e}	15 ± 2^{c}

Results expressed in mean ± standard deviation. Means followed by equal letters in the same column do not statistically differ ($p > 0.05$)

SMC, control smoothie; SMP, pasteurized smoothie; SMS, sonicated smoothie; UD, undigested sample; GD, gastric digest; ID, intestinal digest; RAC, remaining antioxidant capacity

[1]Results expressed in μmol TE g^{-1}

Table 4 Bioaccessibility of the phenolic compounds of pasteurized and sonicated smoothies

Samples		Ferulic acid[1]	B Ferulic acid (%)	Gallic acid[1]	B Gallic acid (%)	Ellagic acid[1]	B Ellagic acid (%)	Cinamic acid[1]	Vanillic acid[1]
SMP	UD	20 ± 1^{a}	–	1727 ± 61^{a}	–	272 ± 32^{b}	–	nd	nd
	GD	3.5 ± 0.1^{b}	18 ± 1^{a}	nd	nb	197 ± 7^{c}	73 ± 8^{a}	nd	5.9 ± 0.1^{a}
	ID	3.3 ± 0.1^{b}	$16 \pm 1^{a,b}$	nd	nb	216 ± 13^{c}	80 ± 10^{a}	4.6 ± 0.2^{a}	12 ± 1^{b}
SMS	UD	21 ± 1^{a}	–	1852 ± 52^{b}	–	407 ± 23^{a}	–	nd	nd
	GD	1.6 ± 0.0^{c}	7.5 ± 0.2^{c}	nd	nb	197 ± 15^{c}	49 ± 5^{c}	nd	5.9 ± 0.1^{a}
	ID	3.3 ± 0.0^{b}	15.2 ± 0.5^{b}	nd	nb	250 ± 14^{b}	62 ± 6^{b}	4.6 ± 0.2^{a}	nd

Results expressed in mean ± standard deviation. Means followed by equal letters in the same column do not statistically differ ($p > 0.05$)

SMP, pasteurized smoothie; SMS, sonicated smoothie; UD, undigested sample; GD, gastric digest; ID, intestinal digest; B, bioaccessibility; nd, not detected; nb, not bioaccessible

[1]Results expressed in mg L^{-1}

digestion was evaluated only in the processed samples, since these presented higher changes relating to the control, when evaluated by the spectrophotometric methods (Table 4).

Ferulic acid was more bioaccessible when the smoothie was thermally treated. The same behavior was verified in the bioaccessibility of the ellagic acid, although this compound was present in higher concentration in the sonicated smoothie. Gallic acid was not detected in the gastric and intestinal digests of both samples. This behavior was also observed by Schulz et al. (2017), when evaluating the bioaccessibility of the phenolic compounds present in the juçara fruits at several maturity stages.

As verified in this study, Schulz et al. (2017) also observed differences in bioaccessibility of phenolic compounds. According to these authors, different bioaccessibility values of phenolic compounds are due to the chemical diversity of this group, which vary from simple molecules to highly polymerized molecules. Most phenolic compounds are found in the glycosylated form as esters or polymers, which, during digestion, are hydrolyzed as cited by Alminger et al. (2014). It is also important to note that phenolic compounds can undergo structural changes during the gastrointestinal tract. In addition, degradation may give rise to other phenolic components. In our work, cinnamic and vanillic acids were detected only in the digested fractions of pasteurized and sonicated smoothies (Table 4), which is probably associated to the degradation of anthocyanins and formation of phenolic acids (Sinela et al. 2017).

Cinnamic acid was detected only in the intestinal digest of the evaluated smoothies. Vanillic acid was detected only in the intestinal digest of the pasteurized sample. Possibly, the physical characteristics of the pasteurized sample contributed to the maintenance of this compound after the intestinal digestion.

The effect of ferulic acid on the biochemical and histological properties of liver and heart of obese and diabetic rats was evaluated by Song et al. (2014). After 16 weeks of administration of this phenolic compound (60 mg/kg), the authors reported an increase in plasma antioxidant activity. In addition, they observed body weight control and a decrease in serum glucose and lipids in their liver and heart. Ellagic acid has also been associated with the antioxidant effect, which, together with the endogenous defense system, reduced the inflammatory response of hepatic injury induced by lipopolysaccharide/D-galactosamine in rats (Gu et al. 2014).

Thus, bioaccessible phenolic compounds of the smoothie can reinforce the body defenses, acting as antioxidant, when absorbed by the cells, emphasizing the importance of the evaluation performed in this work.

Conclusion

Pasteurized smoothie presented better physical characteristics with emphasis for lower particles' size among the evaluated samples. Bioaccessibility of the phenolic compounds was higher in the pasteurized and sonicated samples, and a higher influence of the heat treatment on this parameter was observed, since the bioaccessibility of ferulic and ellagic acids was significantly higher in the pasteurized sample. Thus, it is concluded that pasteurization of juçara, banana and strawberry smoothie conferred higher physical stability to the product, contributing to a higher preservation of phenolic compounds after in vitro gastrointestinal digestion.

Acknowledgements The authors are incredibly grateful to Federal University of Rio de Janeiro, Embrapa Agroindústria de Alimentos and University of Minho for support. Leilson O. Ribeiro acknowledge the Capes for his fellowship (88881.133775/2016-01) and Ana C.

Pinheiro acknowledge the Foundation for Science and Technology (FCT) for her fellowship (SFRH/BPD/101181/2014).

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