

**In vitro bioaccessibility and identification of antioxidant compounds in clarified cashew apple juice ‘cajuína’****Bioaccessibilidade e identificação in vitro de compostos antioxidantes no suco de caju clarificado ‘cajuína’**

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**ABSTRACT**

The objective of this study was to determine the in vitro bioaccessibility and identity of the phenolic compounds, and total antioxidant activity of two commercial brands of cajuína. The simulated gastrointestinal digestion caused a reduction in the total phenolic content, total flavonoids, and antioxidant activity in both cajuína brands. However, the content of all compounds identified by High performance liquid chromatography after the simulated digestion process increased, in particular ellagic and gallic acids in brand A. Such compounds may be involved in processes of transformation and release of the food matrix, a fact that generated an increase in the bioaccessible fraction. However,

there is a reduction in the total bioaccessible fraction and antioxidant activity, indicating that most of the present compounds are unstable, and they underwent degradation after the simulated digestion process. Simulated gastrointestinal digestion affected the profile and content of phenolic compounds, and, as expected, antioxidant activity. It is worth mentioning the increase in the bioaccessible fraction of acids ellagic, gallic, p-coumaric and the epicatechin flavonoid.

**Keywords:** Phenolic compounds. Simulated gastrointestinal digestion. Flavonoids. *Anacardium occidentale* L.

## RESUMO

O objetivo deste estudo foi determinar a bioacessibilidade e identidade *in vitro* dos compostos fenólicos e a atividade antioxidante total de duas marcas comerciais de cajuína. A digestão gastrointestinal simulada causou uma redução no conteúdo fenólico total, flavonóides totais e atividade antioxidante nas duas marcas de cajuína. No entanto, o conteúdo de todos os compostos identificados por cromatografia líquida de alta eficiência após o processo de digestão simulado aumentou, em particular os ácidos elágico e gálico da marca A. Esses compostos podem estar envolvidos em processos de transformação e liberação da matriz alimentar, fato que gerou um aumento na fração bioacessível. No entanto, há uma redução na fração bioacessível total e na atividade antioxidante, indicando que a maioria dos compostos presentes é instável e sofreu degradação após o processo de digestão simulado. A digestão gastrointestinal simulada afetou o perfil e o conteúdo de compostos fenólicos e, como esperado, a atividade antioxidante. Vale ressaltar o aumento da fração bioacessível dos ácidos elágico, gálico, p-cumarico e epicatequina flavonóide.

**Palavras-chave:** Compostos fenólicos. Digestão gastrointestinal simulada. Flavonóides. *Anacardium occidentale* L.

## 1 INTRODUCTION

Tropical regions host a wide variety of fruit species, the consumption of which gained much interest in recent years owing to their high content of bioactive compounds (Da Silva et al., 2014; Rufino et al., 2010; Abreu et al., 2019). The cashew tree (*Anacardium occidentale* L.) is a tropical plant native to Brazil, and is distributed in various tropical regions, including Mozambique, Tanzania, Kenya, Guinea Bissau, Indonesia, Thailand, Vietnam, Nigeria, and India (Paramashivappa et al., 2001).

The cashew pseudofruit, which is known as cashew apple, is a fibrous and juicy fruit. Since the cashew pulp is rich in ascorbic acid (Queiroz et al., 2011), phenolic compounds and minerals, it can be described as a functional food. The cashew has good potential for processing owing to its fleshy pulp, smooth skin, high sugar content, and exotic flavor being widely consumed in the form of juice, nectar, sweets, and jelly among others (Lima et al., 2014; Sivagurunathan et al., 2010).

The clarified juice of cashew apple (cajuína) is defined by the Brazilian legislation (Brasil, 2000), as an undiluted and unfermented drink, arising from the edible part of the apple, obtained through an appropriate technique, and subjected to a physical clarification process. This juice must display certain characteristics: color varying from colorless to yellow translucent; specific flavor being slightly acid and astringent; and specific aroma. The law recommends the use of authorized

technology adjuvants in physical clarification, and the product obtained should correspond to the Standards of Identity and Quality (PIQ) fixed for fruit juice.

Several factors contribute to the development of studies that add value to cajuína. Recently, the National Institute of Industrial Property (INPI) awarded the title of Geographical Indication (GI) of Cajuína of Piauí to the Union of Associations, Cooperatives and Producers of Cajuína of Piauí (Procajuína), which will be responsible for administering and supervising the stamp use. The GI is a registration granted to products and services linked to a certain region, it may be an Indication of Source or Appellation of Origin. In the case of Cajuína of Piauí, the GI will be an Indication of Source, which is related to the geographic name of the region, protecting and ensuring the identity, as well as standardizing a way to expand and control the production process (Sebrae, 2016).

Previous studies have revealed high levels of phenolic compounds in cashew (Bataglioni et al., 2015; Lima et al., 2014), and antioxidant and antimutagenic properties in cajuína (Melo-Cavalcante et al., 2003; Melo-Cavalcante et al., 2008). In humans, the antioxidant effect of phenolic compounds helps protect cells against oxidative damage caused by free radicals, stabilizing or deactivating free radicals before they damage the cells (Pisoschi & Pop, 2015). Thus, these compounds have attracted attention as potential agents for reducing the risk of many diseases related to oxidative stress (Mosele et al., 2016).

However, in functional terms, the determination of the total content of bioactive compounds in foods is not sufficient because their bioaccessibility, i.e., the amount released from the matrix during gastrointestinal digestion and available for absorption in the intestine, is more important. To our knowledge, no study has reported on the bioaccessibility of cajuína. Therefore, the determination of the bioaccessibility of cajuína phenolic compounds is important because its constituents may be bioaccessible and exert beneficial effects. Accordingly, the objective of this study was to identify the phenolic compounds, determine their bioaccessibility, and assess the total antioxidant activity.

## **2 MATERIAL AND METHODS**

### **2.1 CHEMICALS**

ABTS<sup>+</sup> (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), pancreatin, pepsin, bile extract, ultra-pure phenolic standards (Ferulic, gallic, caffeic, chlorogenic, elagic and p-coumaric acids; quercetin and epicatechin) were purchased from Sigma Aldrich (Saint Louis, USA). Methanol and HPLC grade acetic acid were purchased from Merck (Darmstadt, Germany), Folin-ciocalteu reagent from Vetec Chemistry (Brazil). All chemicals used in the

experiments were of analytical reagent grade. Deionized water was obtained from a Milli-Q Plus system (Millipore, Bedford, USA).

## 2.2 SAMPLES

Two commercial brands of cajuína were analyzed. These were selected because they had Geographical Indication (GI) registration granted by the Brazilian National Institute of Industrial Property. The samples (16 units in each lot) were supplied by the producers located in the state of Piauí, Brazil, in 500-mL packages, in two different lots. The packages were sealed and stored in the Laboratory of Bromatology and Food Biochemistry, Department of Nutrition, Federal University of Piauí, Brazil, at temperature of 4°C until analysis.

## 2.3 SAMPLE PREPARATION

Cajuína samples (3 mL) were extracted with methanol (8 mL) in an ultrasonic bath (USC-1400 Unique) for 15 min at room temperature (20 °C). The extract was centrifuged (Eppendorf Centrifuge 5702) at  $2,000 \times g$  for 15 min and the supernatant was used to determine the profile of individual phenolic compounds, total phenolic compounds, flavonoids, and antioxidant activity.

## 2.4 DETERMINATION OF TOTAL PHENOLIC COMPOUNDS

The total phenolic content was determined spectrophotometrically (Hewlett-Packard 8452 Spectrophotometer) using the Folin Ciocalteu reagent according to the methodology of Singleton & Rossi (1965). The absorbance was read at 765 nm and the concentration of the phenolic compounds was estimated from standard curve of gallic acid (50, 100, 150, 250, 500, 750, 1,000 mg·mL<sup>-1</sup>). The results were expressed in mg of gallic acid equivalent (GAE) per 100 mL of sample.

## 2.5 DETERMINATION OF TOTAL FLAVONOIDS

The flavonoid content was determined according to the method described by González-Aguilar et al. (2007). Briefly, 1 mL of extract was mixed with 4 mL of deionized water and 300 µL of 5% NaNO<sub>2</sub> for 5 min. After mixing, 300 µL of 10% AlCl<sub>3</sub> (methanolic solution) was added. After standing for 1 min, 2 mL of 1 M NaOH was added. Finally, water was added to a final volume 10 mL. The absorbance of the reaction was determined at 415 nm. The total flavonoid concentration was calculated using the standard curve of quercetin (20, 30, 40, 50, 100, 200, and 400 mg·mL<sup>-1</sup>) and expressed as mg equivalent of quercetin (QEs) per 100 mL of sample.

## 2.6 ANTIOXIDANT ACTIVITY

### 2.6.1 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS) method

The ABTS method is based on the inactivation of the antioxidant ABTS<sup>+</sup> radical cation, which is measured by the reduction in the absorbance at 734 nm. The method was conducted as described by Re et al. (1999). A solution containing 7 µM ABTS was prepared and mixed in the same proportion with a solution of 2.45 µM potassium persulfate to form the corresponding superoxide anion. The

working solution was obtained by diluting 1 mL of this radical-forming mixture with 50 mL of ethanol to obtain an absorbance of 0.70 ( $\pm$  0.02) at 734 nm. An aliquot (2.9 mL) of this solution was added to 60  $\mu$ L of extract and incubated at room temperature. The absorbance was read at 734 nm, 7 min after the addition of the extract. The total antioxidant activity was expressed as  $\mu$ mol in 100 mL of trolox equivalent antioxidant capacity (TEAC).

### 2.6.2 2,2-diphenyl-1-picrylhydrazyl (DPPH) method

The total antioxidant activity was analyzed by investigating the capacity to eliminate DPPH free radicals using a modified version of the method of Brand-Williams et al. (1995). A solution of 100  $\mu$ M DPPH in methanol was prepared and stored under refrigeration until use. The working solution was obtained by diluting the DPPH solution with 80% methanol to an absorbance of approximately 0.80 ( $\pm$  0.02) at 515 nm. An aliquot (2.9 mL) of this solution was mixed with 100  $\mu$ L of extract and incubated at room temperature for 30 min in the dark. The absorbance was measured at 515 nm and the total antioxidant activity was expressed as  $\mu$ mol in 100 mL<sup>-1</sup> of TEAC.

### 2.7 IN VITRO SIMULATED DIGESTION

The digestions were performed with simulated gastric and intestinal fluids, both of which were prepared in accordance with the procedures implemented by Moura & Canniatti Brazaca (2006). The simulated gastrointestinal digestion was performed with pepsin (0.032 g) solubilized in 3 mL of 0.1 M HCl for the gastric phase, and pancreatin (0.036 g) and bile salts (0.220 g) dissolved in 5 mL of 0.1 M NaHCO<sub>3</sub> for the intestinal phase. The sample (20 mL) was added 20 mL of 0.01 M HCl and the pH adjusted 2 using 2 M HCl. After adjusting the pH, 3.0 mL of pepsin solution was added and the sample stirred in a water bath at 37 °C for 2 h (Tecnal TE820) to simulate the digestion of food in the stomach. Then, the solution was cooled to 20 °C and titrated with 0.5 M NaOH 0.5 to pH of 7.5 to simulate the pH of the small intestine. Subsequently, 5.0 mL of pancreatin solution and bile salts was added, followed by agitation in a water bath at 37 °C for 2 h to simulate the digestion of food in the intestine. Finally, the sample was centrifuged at 6,800  $\times$  g for 15 min and the supernatant stored at -80 °C until analysis.

### 2.8 BIOACCESSIBILITY

After the *in vitro* digestion procedure, the supernatant was analyzed for total phenolics, total flavonoids, and antioxidant activity as described previously. For the identification of phenolic compounds by HPLC-UV, the supernatant was diluted in the mobile phase (1:2 v/v) and filtered through a 0.45- $\mu$ m membrane before injection. The bioaccessibility (%) was defined as the content of compound released during the simulated digestion process compared to the content of the compound in the sample, and the value was calculated according to the formula below (Leufroy et al., 2012).

**Equation 1.**

$$\text{Bioaccessibility (\%)} = \left( \frac{\text{Content of the compound released in the simulated digestion}}{\text{Contents of the compound in the sample}} \right) \times 100$$

**2.9 IDENTIFICATION OF PHENOLIC COMPOUNDS BY HPLC-UV**

The identification and quantification of phenolic compounds was performed according to an adapted method of Souza et al. (2018). The phenolic compounds were analyzed using a LC-20 AT high-performance liquid chromatograph (Shimadzu Corporation, Japan). The separation was performed using a Shimadzu GVP-ODS pre-column (10 mm × 4.6 mm) in line with a Shim-pack VP-ODS column (150 × 4.6 mm i.d., 5-μm particle size) (Sigma-Aldrich, St. Louis, MO, USA) equipped with a UV-Vis SPD-20A detector. The flow rate was maintained at 0.7 mL·min<sup>-1</sup> and the column temperature was maintained at 40 °C, with an injection volume of 10 μL. The gradient of the mobile phase was composed by (A) methanol with 1% acetic acid and (B) 1% acetic acid: from 0–1 min, 10% A; 1–5 min 15% A; 5–10 min, 20% A; 10–15 min, 25% A; 15–25 min, 30 % A; 25–30 min, 70 % A; 30–40 min, 80 % A; 40–50 min, 10 % A. The total run time was 50 min. The compounds were detected at 280 (i.e., gallic acid, epicatechin and ellagic acid), 320 (i.e., caffeic, p-coumaric, chlorogenic, and ferulic acids), and 360 nm (i.e., quercetin). The peaks were identified by comparison with the retention time of standards, and the quantification of the compounds was based on the areas of the respective peaks detected using the LabSolutions acquisition software version 5.57 SP1 Copyright (Shimadzu Corporation). The column calibration was performed by injecting the standards in triplicate at nine different concentrations (i.e., 0.014; 0.056; 0.225; 0.45; 7.81; 15.62; 31.25; 62.5, and 120.0 μg·mL<sup>-1</sup>). The levels of phenolic compounds were expressed as μg·mL<sup>-1</sup>.

**2.10 STATISTICAL ANALYSIS**

The Statistical Package for Social Science (SPSS) software, version 22, was used for the statistical analysis of the data. The results are presented as means of three replicates and their respective standard deviations. To verify a significant difference between the averages analyzed, the Student's t-test was used, with a significance level of 5% (p < 0.05) and a confidence interval of 95% (Siegel, 1995).

**3 RESULTS AND DISCUSSION**

The results of total phenolics and total flavonoids are shown in Table 1. Brand A contains a significantly higher content of total phenolics before and after the simulated digestion (187.43 ± 8.1; 105.25 ± 6.61 mg·100 mL<sup>-1</sup>, respectively) than brand B does (105.13 ± 5.14; 53.63 ± 8.75 mg·100 mL<sup>-1</sup>, respectively). Moreover, brand A displays a higher bioaccessibility (56.15%) than brand B does

(51.01%). On the other hand, there was no significant difference in the total flavonoid content between brand A ( $13.77 \pm 1.09$ ;  $11.57 \pm 1.07 \text{ mg}\cdot 100 \text{ mL}^{-1}$ ) and B ( $14.37 \pm 1.13$ ;  $12.81 \pm 0.28 \text{ mg}\cdot 100 \text{ mL}^{-1}$ ) before and after in vitro digestion, respectively, nor between treatments for each brand, i.e., the levels of these compounds remained stable during the in vitro digestion, displaying a high bioaccessibility (84.02 and 89.14%) for brands A and B, respectively.

In a similar study on cashew juice, Lima et al. (2014) obtained  $338.60 \pm 10.68$  and  $130.60 \pm 3.02 \text{ mg GAE}\cdot 100 \text{ g}^{-1}$  total phenolics before and after simulated gastrointestinal digestion, respectively, thus obtaining a bioaccessible fraction of 39%. Although the total phenolic content in the present study was lower than that in the previous study, higher bioaccessibility (56.15% for brand A and 51.01% for brand B) was measured. Therefore, although the clarification and pasteurization of cajuína reduce the phenolic content, they make the phenolic compounds highly bioaccessible.

**Table 1** - Determination of total phenolics and flavonoids in two brands of clarified cashew apple juice 'cajuína' before and after simulated gastrointestinal digestion.

Compounds	Samples	Before digestion	After digestion	Bioaccessible
		( $\text{mg}\cdot 100 \text{ mL}^{-1}$ )	( $\text{mg}\cdot 100 \text{ mL}^{-1}$ )	fraction
		Mean $\pm$ SD	Mean $\pm$ SD	(%)
Total phenolics	A	$187.43 \pm 8.1^{\text{aA}}$	$105.25 \pm 6.61^{\text{bA}}$	56.15
	B	$105.13 \pm 5.14^{\text{aB}}$	$53.63 \pm 8.75^{\text{bB}}$	51.01
Cashew apple				
juice				
(Lima et al., 2014)		$338.60 \pm 10.68$	$130.60 \pm 3.02$	38.57
Total flavonoids	A	$13.77 \pm 1.09^{\text{aA}}$	$11.57 \pm 1.07^{\text{aA}}$	84.02
	B	$14.37 \pm 1.13^{\text{aA}}$	$12.81 \pm 0.28^{\text{aA}}$	89.14

Mean of three replicates. Equal lowercase letters between before and after digestion and equal uppercase letters between the A and B brands represent not significant differences between the means according to the Student's *t*-test, at a significance level of 5% ( $p > 0.05$ ) and 95% confidence interval.

Total phenolics: results expressed in mg GAE·100 mL<sup>-1</sup> (Equivalent to Gallic Acid). Flavonoids: Results expressed in mg QE·100 mL<sup>-1</sup> (equivalent to quercetin). SD: standard deviation.

It is worth emphasizing that the reagent used for the determination of total phenolics (Folin Ciocalteu) also reacts with reducing compounds such as vitamin C and sugars, which are present in the sample studied. Thus, the reduction in the total phenolic content due exclusively to the digestive process might be less than what was actually determined because the presence of reducing agents could lead to overestimation of the total phenolic content.

The antioxidant activity results obtained with the ABTS and DPPH methods are shown in Table 2.

**Table 2** - Determination of the total antioxidant activity of two brands of clarified cashew apple juice 'cajuína' before and after simulated gastrointestinal digestion.

Compounds	Samples	Before digestion	After digestion	Bioaccessible
		μmol TEAC·100 mL <sup>-1</sup> Mean ± SD	μmol TEAC·100 mL <sup>-1</sup> Mean ± SD	fraction (%)
DPPH method	A	1,920.63 ± 77.85 <sup>a</sup> A	1,018.68 ± 11.05 <sup>b</sup> A	53.03
	B	919.27 ± 52.39 <sup>a</sup> B	376.55 ± 55.86 <sup>b</sup> B	40.96
ABTS method	A	1,911.00 ± 71.57 <sup>a</sup> A	881.49 ± 88.74 <sup>b</sup> A	46.12
	B	1,018.75 ± 163.83 <sup>a</sup> B	509.19 ± 165.41 <sup>b</sup> B	49.98

Mean of three replicates. Equal lowercase letters between before and after digestion and equal uppercase letters between the A and B brands represent not significant differences between the means according to the Student's *t*-test, at a significance level of 5% ( $p > 0.05$ ) and 95% confidence interval. TEAC: trolox equivalent antioxidant activity. SD: standard deviation

High antioxidant activity was measured, especially in brand A cajuína. With the DPPH method, average values of 1,920.63 ± 77.85 and 1,018.68 ± 11.05 μmol TEAC·100 mL<sup>-1</sup> before and after digestion, respectively, were obtained. Similar results were obtained for the same brand using the ABTS method (1,911.00 ± 71.57; 881.49 ± 88.74 μmol TEAC·100 mL<sup>-1</sup> before and after digestion, respectively). The bioaccessible fraction obtained with the DPPH method (53.03%) was



the highest in brand A, whereas that obtained with the ABTS method (46.12%) was the highest in brand B.

Lima et al. (2014) obtained similar antioxidant activity values when they analyzed cashew juice using the ABTS method, i.e.,  $1,810.00 \pm 1.92$  and  $480.00 \mu\text{mol TEAC} \cdot 100 \text{ mL}^{-1}$  before and after digestion, respectively. However, the cashew juice studied by these authors had a bioaccessible fraction (27%) lower than that obtained in this study (46.12% and 49.98%, brands A and B, respectively). This indicates that, despite processing, cajuína is an excellent option for cashew consumption.

The results of the identification and quantification of phenolic compounds in cajuína before and after *in vitro* gastrointestinal digestion are summarized in Table 3. Three phenolic acids (ellagic, gallic, and *p*-coumaric) and one flavonoid (epicatechin) were identified. Ellagic acid was the most abundant, especially in brand A ( $1,062.81 \pm 5.7 \mu\text{g} \cdot \text{mL}^{-1}$ ). After simulated gastrointestinal digestion, the content of ellagic acid increased in both brands, especially in brand A ( $1,898.14 \pm 31.42 \mu\text{g} \cdot \text{mL}^{-1}$ ), which presented a bioaccessible fraction of 178.59%.

The fruits of the Anacardiaceae family are rich in ellagitannins, which increase after *in vitro* digestion likely because of hydrolysis, releasing free ellagic acid. Previous studies simulating *in vitro* digestion showed that, generally, ellagitannins are fairly stable under the physiological conditions of the stomach. According to a previous study, the acidity (pH 1.8–2.0) and enzymes present in the stomach did not cause hydrolysis of ellagitannins and no degradation was observed (Tomás-Barberán et al., 2009). However, under intestinal physiological conditions, ellagitannins were hydrolyzed, freeing ellagic acid, likely because of the pH and effect of pancreatic enzymes and bile salts (Verotta et al., 2018).

Several studies have shown that ellagic acid has biological properties, including anti-inflammatory, antimutagenic, and anticarcinogenic activity, reducing the risk of cardiovascular diseases, atherosclerosis, and dyslipidemic disorders and stimulating wound healing and skin elasticity (Firdaus et al., 2018; Tomás Barberán et al., 2017).

Similarly, gallic acid content increased significantly after the simulated digestion in both brands, particularly in brand A ( $50.54 \pm 2.30$  and  $59.18 \pm 2.95 \mu\text{g} \cdot \text{mL}^{-1}$  before and after digestion, respectively). Several biological properties are associated with gallic acid, such as antioxidant and anti-inflammatory activity, anti-carcinogenic activity, and antihyperglycemic effects such as lowering glucose and glycosylated hemoglobin levels and increasing insulin levels in experimental diabetes models. This suggests the possibility of using gallic acid as a complementary therapy to hypoglycemic drugs (Oliveira et al., 2016; Pereira et al., 2018.).

**Table 3** - Content of phenolic compounds in two brands of clarified cashew apple juice 'cajuína' before and after simulated gastrointestinal digestion as determined by HPLC-UV.

Compounds	Samples	Before digestion	After digestion	Bioaccessible
		( $\mu\text{g}\cdot\text{mL}^{-1}$ )	( $\mu\text{g}\cdot\text{mL}^{-1}$ )	fraction
		Mean $\pm$ SD	Mean $\pm$ SD	(%)
Ellagic Acid	A	1062.81 $\pm$ 5.70 <sup>a</sup> A	1898.14 $\pm$ 31.42 <sup>b</sup> A	178.59
	B	390.90 $\pm$ 0.18 <sup>a</sup> B	683.55 $\pm$ 2.28 <sup>b</sup> B	174.85
Gallic acid	A	50.54 $\pm$ 2.30 <sup>a</sup> A	59.18 $\pm$ 2.95 <sup>b</sup> A	117.02
	B	34.22 $\pm$ 3.14 <sup>a</sup> B	41.69 $\pm$ 0.53 <sup>b</sup> B	121.63
<i>p</i> -Coumaric acid	A	2.99 $\pm$ 0.05 <sup>a</sup> A	6.56 $\pm$ 0.10 <sup>b</sup> A	224.13
	B	0.72 $\pm$ 0.01 <sup>a</sup> B	0.82 $\pm$ 0.00 <sup>b</sup> B	113.88
Epicatechin	A	14.54 $\pm$ 1.00 <sup>a</sup> A	32.39 $\pm$ 0.83 <sup>b</sup> A	222.75
	B	12.22 $\pm$ 0.05 <sup>a</sup> A	14.15 $\pm$ 0.33 <sup>a</sup> B	115.57

Mean of three replicates. Equal lowercase letters between before and after digestion and equal uppercase letters between the A and B brands represent not significant differences between the means according to the Student's *t*-test, at a significance level of 5% ( $p > 0.05$ ) and 95% confidence interval. SD: standard deviation.

Table 3 shows a significant variation between the levels of epicatechin in brand A before and after simulated digestion ( $14.54 \pm 1.0$  and  $32.39 \pm 0.83 \mu\text{g}\cdot\text{mL}^{-1}$ ), presenting a bioaccessibility of 222.75%. However, there was no significant difference between the levels of epicatechin before and after the *in vitro* digestion in brand B ( $12.22 \pm 0.05$  and  $14.15 \pm 0.33 \mu\text{g}\cdot\text{mL}^{-1}$ ), which also presented a lower bioaccessibility than brand A did. This difference between brands can be explained by the different processing procedure of cashew juice, pasteurization time, and differences between the raw materials, which are affected by several factors such as harvest time and climatic conditions.

Among the compounds identified, *p*-coumaric acid was present in the least amount, i.e.,  $2.99 \pm 0.05$  and  $6.56 \pm 0.1 \mu\text{g}\cdot\text{mL}^{-1}$  before and after digestion, respectively, for brand A, and  $0.72 \pm 0.01$  and  $0.82 \pm 0.0 \mu\text{g}\cdot\text{mL}^{-1}$  before and after digestion, respectively, for brand B. *p*-Coumaric acid has been associated with chemoprotective and antioxidant properties, antimicrobial activity, cardiovascular diseases antagonist, and anticancer activity (Kannan et al., 2013; Roy & Prince, 2013; Sharma et al., 2017).

In a previous study on cashew apple, Bataglioni et al. (2015) found that gallic acid and *p*-coumaric acid were present in the amount of  $148.53 \pm 1.56 \mu\text{g}\cdot\text{g}^{-1}$  and  $2.46 \pm 0.35 \mu\text{g}\cdot\text{g}^{-1}$ , respectively. Thus, it can be inferred that cajuína, when ingested regularly, may promote health benefits owing to the high content of bioactive compounds and antioxidant activity.

The observed mismatch between the bioaccessible fractions of the total phenolic content and antioxidant activity with the bioaccessible fraction of the compounds identified by HPLC indicates that these compounds behave differently when exposed to the conditions of digestion. The effects of flavonoids on intestinal digestion have been demonstrated in previous studies (Bouayed et al., 2011). While compounds such as ellagitannins, among others, may undergo hydrolysis, releasing free ellagic acid or other phenolic acids. Thus, it can be inferred that most of the phenolics present in cashew are unstable, causing a reduction in total phenolic content and antioxidant activity. However, it is noted that the four compounds identified may be involved in transformation and release processes which may have caused an increase in the bioaccessible fraction, thus, the regular consumption of clarified cashew apple juice (cajuína) provides antioxidant compounds that are important for health.

#### **4 CONCLUSION**

We conclude that cajuína is the source of phenolic compounds such as ellagic acid, epicatechin, *p*-coumaric acid, and gallic acid and has high antioxidant activity.

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