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Sapucaia nut (*Lecythis pisonis* Cambess) and its by-products: A promising and underutilized source of bioactive compounds. Part II: Phenolic compounds profile

Fernanda Demoliner[a], Priscila de Britto Policarpi[a], Lúcio Flavo Lopes Vasconcelos[b], Luciano Vitali[c], Gustavo Amadeu Micke[c], Jane Mara Block[a,*]

[a] Laboratory of Fats and Oils, Department of Food Science and Technology, Federal University of Santa Catarina, Florianópolis, SC, Brazil
[b] Empresa Brasileira de Pesquisa Agropecuária do Meio-Norte (EMBRAPA Meio-Norte), Piauí, Brazil
[c] Department of Chemistry, Federal University of Santa Catarina, Florianópolis, SC, Brazil

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ABSTRACT

In this study, the profile of the bioactive compounds of sapucaia nut (*Lecythis pisonis* Cambess) and its by-products have been investigated. The phenolic profile by LC-ESI-MS/MS, the total phenolic content, the condensed tannins and the antioxidant activity of the sapucaia nut and shell were determined. 14 phenolic compounds were identified in the sapucaia nut extract, primarily phenolic acids and flavonoids. Catechin, epicatechin, myricetin, ellagic acid and ferulic acid presented significant correlation to the antioxidant activity. The sapucaia shell contained 22 phenolic compounds, 13 of which were quantified. The sapucaia shell extract showed a high content of total phenolic compounds, a high condensed tannins content, and high antioxidant activity. The higher antioxidant activity of the shell can be associated with a higher content of phenolics. Overall, it can be concluded that the sapucaia nut is a raw material rich in phenolic compounds that present high antioxidant activity. The nuts and the cake may be used as a promising raw material for the food industry, while the shells could be an alternative source of natural antioxidants. Further use in the cosmetics and pharmaceutical industry may also be envisaged.

1. Introduction

Lecythis pisonis Cambess, popularly known as "sapucaia" or "cumbuca de macaco", belongs to the family Lecythidaceae. This nut originates from the Brazilian Amazon and it is widely distributed throughout Brazil, mainly in the Atlantic forest and in the Amazon

* Corresponding author at: University of Santa Catarina, Agricultural Science Center, Department of Food Science and Technology, Rodovia Admar Gonzaga, 1346, Itacorubi, CEP: 88034-001 Florianópolis, SC, Brazil.
E-mail address: jane.block@ufsc.br (J.M. Block).

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region (Brandão et al., 2013; Vallilo, Tavares, Aued-Pimentel, Campos, & Moita Neto, 1999). Our previous study has demonstrated that the sapucaia nut and cake are an excellent source of proteins, dietary fiber, and selenium. On the other hand, the sapucaia oil is a source of unsaturated fatty acids (oleic and linoleic acids), γ-tocopherol and β-sitosterol (Demoliner et al., 2018). Several parts of sapucaia plants such as the leaves, shell, and oil are also used in popular medicine (Agra, Freitas, & Barbosa-Filho, 2007; Franco & Barros, 2006; Wickens, 1995). The antipruritic effects and antinociceptive activity of *Lecythis pisonis* leaves have been reported (Brandão et al., 2013; Silva et al., 2012).

In the last decade, the phenolic compounds in natural nut products and by-products have been investigated (Gomes & Torres, 2016; Hilbig, Alves, Muller, Micke, Vitali, Pedrosa, and Block, 2018; John & Shahidi, 2010; Robbins, Gong, Wells, Greenspan, & Pegg, 2015). The phenolic compounds, including flavonoids, phenolic acids, tannins, stilbenes, lignans, and phenolic aldehydes play an important role in human health, mainly due to their antioxidant properties (Taş & Gökmen, 2017). Several studies reported that the antioxidant activity of phenolic compounds is associated with the protection against diseases such as diabetes, cancer, hypercholesterolemia, and cardiovascular diseases (Alasalvar & Bolling, 2015; Hilbig, Policarpi, de Souza Grinevicius, Santos Mota, Toaldo, Luiz, Pedrosa, and Block, 2018; Kaliora, Kogiannou, Papassideri, & Kalogeropoulos, 2014; Shahidi & Ambigaipalan, 2015).

The sapucaia nuts and its by-products are not well known yet and it is still an underutilized raw material. Studies on bioactive compounds and antioxidant activity of sapucaia nuts and its by-products are still scarce in the literature. Therefore, the use of this native nut from Brazil in diet may be considered as an alternative to improve the diet of the Brazilian population and its by-products as well an alternative source for natural antioxidants. Based on this context, in order to divulge the phenolic and antioxidant potentialities of sapucaia nut and its by-products, the aim of this research was to identify and quantify the phenolic compounds of the extracts obtained from the sapucaia nuts and shell using LC-ESI-MS/MS.

2. Materials and methods

2.1. Samples

Sapucaia nuts (*Lecythis pisonis* Cambess) were obtained from dry ripe sapucaia fruit from Teresina, Piauí, Brazil (samples A1, A2 and A3) and provided by the Brazilian Agricultural Research Corporation (EMBRAPA Meio-Norte). Another sample was sourced from native trees in the city of Viçosa, Minas Gerais, Brazil (sample B1). About 600 g of each sample (harvested in October 2016) were obtained.

2.2. Chemical reagents

Folin-Ciocalteau phenol reagent, gallic acid, vanillin, (+)-catechin hydrated, ABTS [2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid)], DPPH (2,2-diphenyl-1-picrylhydrazyl), 2,2′- 2,4,6-tripyridyl-triazine (TPTZ) and Trolox were obtained from Sigma-Aldrich (St. Louis, MO, USA). Stock solution (1000 mg L^{-1} in methanol 100%) of ultra-pure phenolic standards (Sigma-Aldrich, St. Louis, MO, USA) were prepared and stored in a freezer. They were used to prepare the calibration curves of phenolic compounds using appropriate dilution from the mixture. All other chemical reagents and solvents used in the experiment were of analytical grade (P.A.) and obtained from Vetec (Rio de Janeiro, Brazil) and Sigma-Aldrich (Darmstadt, Germany).

2.3. Obtaining of extracts

The extracts obtained from the nuts and shells were prepared following the methods reported by John and Shahidi (2010) and Hilbig, Alves, et al. (2018), with some modifications. The sapucaia nuts and shell were dried at 40 °C for 60 min in an oven equipped with air circulation (model 400/D, Nova Ética®, Vargem Grande Paulista, Brazil) to reduce their moisture. Then, the samples were milled using an analytical laboratory grinder (model Q298A, Quimis®, Diadema, Brazil) and sieved to 60-mesh size. The nuts and shells were defatted by blending with hexane (1:20 w/v, 30 min × 3) in a mechanical stirrer (model TE-139, Tecnal®, Piracicaba, Brazil) at a room temperature of 22 °C. The defatted samples were air-dried until total evaporation of the solvent and stored in amber bottles with a nitrogen atmosphere at −24 °C for the preparation of the extracts.

The extraction of phenolic compounds from the nut and shell was performed under optimal operational conditions (solvent, time and temperature) determined in preliminary studies (data not shown). The defatted samples of sapucaia nut (1 g) and sapucaia nut shell (1 g) were placed into Erlenmeyer flasks, following the addition of 30 mL of the solvent (water for defatted samples of sapucaia nut; and methanol:-water −80:20 for defatted samples of sapucaia nut shell). Then the samples were sonicated at 80 °C for 60 min in an ultrasonic cleaning bath (EGS 5HD, 40 kHz, 300 W, Enge Solutions®, São Paulo, Brazil). After sonication, the extracts were filtered with paper filter (Whatman no 541, 125 mm) and stored in amber bottles under nitrogen atmosphere at −24 °C for further analysis.

2.4. Extraction, identification and quantification of phenolic compounds by LC–ESI-MS/MS

2.4.1. Samples preparation

The sample preparation was performed following the methodology of Schulz et al. (2015), with some modification. Aliquots of 1 mL of the extract solution were mixed with 5 mL of HPLC grade methanol (99.9%) and 5 mL of hydrochloric acid (0.01 mol L^{-1}) and were subjected to acid hydrolysis in an oven (model 400/D200 °C, New Ethics®, São Paulo, Brazil) at 85 °C for 30 min. The solution was adjusted to pH 2 using 6 mol. L^{-1} NaOH. Then, a partition extraction (three times) with 10 mL of ethyl ether was carried out followed by centrifugation at 3000 *g* for 10 min. The supernatants were combined, and the organic solvent was removed using a rotary evaporator (model 558, Fisatom®, São Paulo, Brazil). The dried extract was resuspended in 1 mL of methanol and diluted 10 times with methanol:water (70:30, v/v) for injection in the LC-ESI-MS/MS system.

2.4.2. Phenolic profile by LC-ESI-MS/MS

The identification and quantification of phenolic compounds were carried out using a high performance liquid chromatography (LC) system (model 1200 Series, Agilent Technologies®, Waldbronn, Germany) according to the method reported by Schulz et al. (2015). The analytes separation was performed in a Synergi™ Polar-RP 80 A column (4.0 µm particle size, 150 mm, 2.0 mm internal diameter, Phenomenex, USA) using a SecurityGuard Cartridges Polar-RP 80A (4.0 µm × 2.0 mm, Phenomenex, USA). Mobile phases were mixtures of methanol 95% and water 5% (v/v) (channel A), and water and formic acid 0.1% (v/v) (channel B). The separation was carried out at 30 °C, using segmented gradient elution as follows: 0 - 5 min, 10% A; 5–7 min, 90% A; 7–10 min, 90% A; 10–17 min, 10% A. Between the analyses, the column was conditioned for 5 min with the proportion of the initial mobile phase. The mobile phase flow rate was 150 µL min^{-1}, and the injected sample quantity was 10 µL.

The LC system was coupled with a mass spectrometry system consisting of a hybrid triple quadrupole/linear ion trap mass spectrometer Q Trap 3200 (Applied Biosystems/MDS Sciex, Concord, Canada). The mass spectrometer was operated in negative electrospray TurboIonSpray™ (Applied Biosystems/MDS Sciex, Canada) ionization mode. The MS/MS parameters were: capillary needle, maintained at −4500 V; curtain gas, 10 psi; temperature, 400 °C; gas 1, 45 psi; gas 2, 45 psi; and CAD gas, medium. Other parameters for: DP - Declustering Potential; EP - Entrance Potential; CEP - Collision Energy Potential; CE -

Collision Energy; CXP - Collision Cell Exit Potential of the 49 phenolic compounds tested are listed in supplementary material (Table S1). The software Analyst version 1.5.1 was used for the LC–ESI-MS/MS system control and data analysis.

2.5. Determination of total phenolic content (TFC)

The TFC was determined by the Folin-Ciocalteau method (Prado, Aragão, Fett, & Block, 2009; Singleton & Rossi, 1965), with some modifications. For the oxidation reaction, aliquots of 0.1 mL of the extract solution (0.033 g mL^{-1}) were transferred to 10 mL volumetric flasks. Then, 0.5 mL of Folin–Ciocalteau reagent and 1.5 mL of saturated sodium carbonate (75 g L^{-1}) were added, and the volume of each flask was completed with deionized water, shaken and left in the darkness at room temperature for 2 h. The absorbance of the resulting blue solution was measured at 764 nm with a spectrophotometer (SP 2000 UV, Bel Photonics®, Piracicaba, São Paulo, Brazil) and correlated to a calibration curve of gallic acid (50–1000 mg L^{-1}). The analysis was performed in triplicate and the results were expressed as gallic acid equivalent (mg GAE g^{-1}) of dry weight.

2.6. Antioxidant activity (AA)

2.6.1. FRAP assay

FRAP assay was carried out according to the method described by Benzie and Strain (1996) and Arnous, Makris, and Kefalas (2002), with modifications. The method is based on the reduction of a ferric 2,4,6-tripyridyl-s-triazine complex (Fe^{3+} − TPTZ) by antioxidants to the ferrous form (Fe^{2+} − TPTZ). Aliquots of 0.2 mL of the extract solution (0.033 g mL^{-1}) and 0.2 mL of $FeCl_3$ (3 mmol L^{-1} in 5 mol L^{-1} citric acid) were mixed in a tube and incubated for 30 min in a water bath at 37 °C. Then 3.6 mL of TPTZ solution (0.0780 g diluted in 250 mL of 0.05 M HCl) was added and the resulting solution was mixed by a vortex. After exactly 10 min, the absorbance (620 nm) was read using a spectrophotometer (SP 2000 UV, Bel Photonics®, Piracicaba, São Paulo, Brazil). The Trolox standard curve (20–1000 μM) was used to quantify the results, which were expressed as μmol TEAC g^{-1} of dry weight (μmol Trolox equivalent antioxidant capacity g^{-1} of sample).

2.6.2. ABTS assay

The ABTS [2,2′-azino-bis (3-ethylbenzotiazoline-6-sulphonic acid)] assay was carried out according to Re et al. (1999) with some modifications. The $ABTS^{\cdot+}$ radical (7 mM − 0.03836 g ABTS dissolved in 10 mL deionized water) was mixed with 10 mL of potassium persulfate solution (2.45 mM − 0,0331 g of potassium persulfate dissolved in 25 mL deionized water), homogenized and stored for 12–16 h at room temperature in the absence of light. For the sample analysis, the $ABTS^{\cdot+}$ radical solution was adjusted by mixing it with ethanol to achieve an absorbance value of (0.700 ± 0.02) at 734 nm ($A_{734} = A_0$). Then, 20 μL of extracts solutions (0.033 g mL^{-1}) and 980 μL of ABTS solution were added to the test tubes, and after 6 min the absorbance was read by a spectrophotometer (SP 2000 UV, Bel Photonics®, Piracicaba, São Paulo, Brazil) at 734 nm ($A_{734} = A_f$), using glass cuvettes. The radical inhibition percentage was calculated using the following equation: % radical inhibition = $(1–A/A_0) \times 100$, where, "A_0" is the initial absorbance and "A_f" is the final absorbance. A standard curve of Trolox stock solution (190, 390, 590, 790 and 1000 μmol mL^{-1}) was used to quantify the results. The results were expressed in μmol TEAC g^{-1} of dry weight (μmol Trolox equivalent antioxidant capacity g^{-1} of sample).

2.6.3. DPPH assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed according to Brand-Williams, Cuvelier, and Berset (1995), with modifications, as described by Mensor et al. (2001) and Prado et al. (2009). Initially, an aliquot of 2.9 mL of DPPH solution (0.1 mM–0,03943 g of DPPH dissolved in 10 mL of ethanol) was transferred to test tubes containing 0.1 mL of extract (0.033 g mL^{-1}). The samples were kept in the absence of light for 30 min, and then the absorbance was measured with a spectrophotometer (SP 2000 UV, Bel Photonics®, Piracicaba, São Paulo, Brazil) at 515 nm. The radical inhibition percentage was calculated using the following equation: % radical inhibition = $(1 - A_f/A_0) \times 100$, where, "A_0" is initial absorbance and "A_f" is the final absorbance. A standard curve of Trolox stock solution (80, 160, 320, 640 and 1280 μmol mL^{-1}) was used to quantify the results. The results were expressed in μmol TEAC g^{-1} of dry weight (μmol Trolox equivalent antioxidant capacity g^{-1} of sample).

2.7. Determination of condensed tannins (CT)

The determination of condensed tannins was carried out according to the method described by Price, Van Scoyoc, and Butler (1978) and adapted by Villarreal-Lozoya, Lombardini, and Cisneros-Zevallos (2007). Aliquots of 1 mL of the extract solution (0.033 g mL^{-1}) were collected and placed into two separate test tubes (one for the sample and one for the blank solution). Then, 5 mL of vanillin reagent (0.5 g reagent and 200 mL of 4% HCl in methanol) was added to each sample, and 4% HCl in methanol was added to the blank solution. The test tubes were kept in the absence of light for 20 min and the absorbance was measured at 500 nm using glass cuvettes in a spectrophotometer (SP 2000 UV, Bel Photonics®, Piracicaba, São Paulo, Brazil). A standard curve of catechin solution (0, 10, 37.5, 75, 150, 300, 600 and 1200 mg L^{-1}) was used for the quantification and the results were expressed in cathechin equivalent (mg CE g^{-1}) of dry weight.

2.8. Statistical analysis

The normal distribution and homoscedasticity of the data was evaluated by the Brown-Forsythe tests. Analysis of variance (ANOVA) and Tukey's test for comparison of means at a significance level of 5% ($p < 0.05$) were performed using the software Statistica® 7.0 (2008). Correlations between the antioxidant capacity and phenolic content of the samples were carried out using the Pearson's test. All analyses were carried out in triplicate and results expressed as the mean ± standard deviation (SD).

3. Results and discussion

3.1. Phenolic profile by LC-ESI-MS/MS

Table 1 shows the mass spectra limits of detection and quantification, as well as the retention times, the fitting equation, and determination coefficient obtained from the analysis of the phenolic compounds. 14 phenolic compounds were identified in the sapucaia nut extracts, including seven phenolic acids, six flavonoids and one phenolic aldehyde (Table 2). Myricetin and, vanillic, ferulic, and ellagic acids were the main compounds quantified in all samples of sapucaia nut. On the other hand, catechin was quantified in samples A1 and B1, and epicatechin only in sample A1. For sample A1, the concentration of ellagic acid, myricetin, and catechin were significantly higher ($p < 0.05$) when compared to samples A2, A3, and B1.

22 phenolic compounds were identified in the sapucaia shell extracts, corresponding to ten phenolic acids, ten flavonoids, and two phenolic aldehydes (Table 2). The gallic, protocatechuic, vanillic, ferulic, and ellagic acids, epigallocatechin, catechin, epicatechin, taxifolin, myricetin, and vanillin were quantified in all samples. Kaempferol was quantified only in samples A1, A3, and B1; and aromadendrin was quantified in samples A1 and B1. Catechin was the most abundant phenolic compound found in sapucaia shell.

Although the phenolic compounds have also been reported for other Brazilian nuts, such as Brazil, cashew, baru and pecan nuts, it has not been previously reported in the literature for sapucaia nuts (Table 3). Both sapucaia and pecan nuts present a wide variety of phenolic

Table 1
Parent and quantitative ion, retention times, limits of detection (LOD), limit of quantification (LOQ), curve equations and determination coefficients (R^2) of the phenolic analysis of sapucaia nut and shell.

Phenolic compounds	[M-H]/Quantitativeion (*m/z*)	Retention time (min.)	LOD (mg L^{-1})	LOQ (mg L^{-1})	Fitting equation	Determination coefficient (R^2)
Gallic acid	168.8/124.1	3.98	0.023	0.076	y = 52,947x − 6035	1.0000
Protocatechuic acid	153.02/109	6.65	0.006	0.021	y = 1,000,000x + 7910	1.0000
Epigallocatechin	304.82/124.90	8.15	0.062	0.205	y = 70,852x − 11,230	0.9985
Catechin	288.853/120.30	8.82	0.009	0.029	y = 13,524x + 9.562	0.9980
4 –hydroxymethylbenzoic acid	150.899/104.2	8.84	0.042	0.140	y = 40,688x + 1577	0.9999
Epicatechin	288.948/122.3	9.41	0.032	0.107	y = 14,791x + 392.3	0.9990
Vanillic acid	166.831/148.5	9.65	0.011	0.036	y = 14,256x + 4519	0.9996
Syringic acid	196.862/119.6	10.01	0.009	0.032	y = 89,117x + 796,5	1.0000
Epicatechingallate	441.60/168.90	10.13	0.038	0.126	y = 12,541x + 38.93	0.9998
Vanillin	150.892/132.8	10.42	0.014	0.048	y = 37,874x – 280.3	0.9999
p-coumaric acid	163/119	10.46	0.001	0.005	y = 3,000,000x + 2534,5	0.9987
Taxifolin	302.8/120.7	10.70	0.006	0.019	y = 48,429x – 39.69	0.9990
Ferulic acid	192.856/129.7	10.73	0.005	0.017	y = 39,798x + 4065	0.9998
Quercetin	301/149.3	10.84	0.006	0.022	y = 47,636x + 78.64	0.9968
Sinapic acid	222.885/161.6	10.87	0.006	0.021	y = 81,369x + 1409	1.0000
Salicylicacid	136.9/91.1	10.99	0.005	0.017	y = 5,000,000x + 7550	0.9987
Myricetin	316.9/150.6	11.24	0.200	0.680	y = 24,750x – 14,041	0.9997
Aromadendrin	286.8/123.9	11.29	0.002	0.007	y = 1,000,000x + 2991	0.9980
Sinapaldehyde	206.901/174.4	11.39	0.002	0.009	y = 58,654x – 925.8	0.9990
Ellagic acid	300.813/142.5	11.71	0.013	0.044	y = 19,867x + 695,4	0.9990
Kaempferol	284.8/62.6	12.34	0.013	0.446	y = 13,276x + 148.4	0.9980
Naringenin	270.847/150.2	12.37	0.001	0.003	y = 1,000,000x – 31.99	0.9970

compounds; however, the profile of phenolic compounds is different for these raw materials. Among the 22 phenolics quantified in pecan nuts, only gallic, ellagic and syringic acids, and catechin were also identified in sapucaia nut (Robbins et al., 2015). For Brazil (Gomes & Torres, 2016; John & Shahidi, 2010), cashew (Chandrasekara & Shahidi, 2011a), and baru nuts (Lemos, Siqueira, Arruda, & Zambiazi, 2012) a smaller variety of phenolic compounds was reported probably due to the differences between the sample preparation and the analytical methodology used in these studies.

Brazil nut skin and cashew nut skin show a smaller variety of phenolic compounds identified, most of which were also present in sapucaia nuts (Chandrasekara & Shahidi, 2011a; John & Shahidi, 2010) (Table 3). In addition, from the 29 phenolic compounds identified reported in pecan nut shell, 16 were identified in sapucaia shell. Among them, epigallocatechin, epicatechin, vanillin, taxifolin, and ferulic acid presented higher content for sapucaia shell (Hilbig, Policarpi, et al., 2018).

Polyphenol-containing food, including nuts, can bring several antioxidant-related health benefits. The potential mechanism of the protective effects of phenolic compounds has been extensively reported in the literature (Croft et al., 2018; Lorenzo & Munekata, 2016; Shahidi & Ambigaipalan, 2015; Suzuki-Sugihara et al., 2016). An important finding of this study was the presence in high concentrations of phenolic acids in samples of sapucaia nut. It has been reported that the oral administration of ellagic acid, one of the phenolic acids found in the sapucaia nut, showed anti-inflammatory (Rosillo et al., 2012), neuroprotective (Busto et al., 2018), and anti-cancer (Larrosa, Tomas-Barberan, & Espin, 2006) effects. On the other hand, e experimental studies with mice and rats showed evidence of effectiveness of vanillic acid in the treatment of liver (Itoh et al., 2009) and cardiovascular (Prince, Rajakumar, & Dhanasekar, 2011) diseases, and beneficial effect on ulcerative colitis (Kim, Kim, Um, & Hong, 2010). Antihyperglycemic (Bettaieb et al., 2014), anti-atherosclerotic and anti-inflammatory effects (Norata et al., 2007), were also reported in experimental models with rats and mice. The consumption of flavonoids, through the consumption of cocoa, was associated with the decrease of blood pressure and improving of insulin sensitivity in healthy people (Grassi, Lippi, Necozione, Desideri, & Ferri, 2005) and in hypertensive patients with impaired glucose tolerance (Grassi et al., 2008). The presence of different phenolic compounds in sapucaia highlights the importance of incorporating this kind of nut in the diet as it may bring several health benefits as indicated in several studies.

3.2. Total phenolic content, condensed tannins and antioxidant capacity

Table 4 shows the TFC, CT and antioxidant capacity (FRAP, ABTS, and DPPH) of sapucaia nut and shell extracts. The TFC of sapucaia nut extract was significantly ($p < 0.05$) higher in the sample B1. This difference can be related to environmental and agronomic factors that play important roles in the phenolic composition (John & Shahidi, 2010). Studies about phenolic compounds in sapucaia nut are still sparse. Teixeira, Ávila, Silveira, Ribani, and Ribani (2018) have reported TPC values of 1.59 and 1.25 mg GAE100 g^{-1} for sapucaia nut oil extracted by Bligh & Dyer and by Soxhlet, respectively. In sapucaia shell extract, the TFC was significantly ($p < 0.05$) higher in the sample A1. These results were higher than those reported by Ferreira et al. (2014) for ethanol extract of sapucaia shell (0.88 mg GAE g^{-1}).

Different methods have been used to assess antioxidant capacity in foods and the assay principles and experimental conditions differ among published studies (Floegel, Kim, Chung, Koo, & Chun, 2011; Ma et al., 2011). On the other hand, antioxidants may present different mechanisms of action. Therefore, more than one assay is usually required for accurately determining the antioxidant capacity in a complex matrix or system. The ability of the sapucaia nut extracts to reduce Fe^{3+} to Fe^{2+} in the FRAP assay was significantly ($p < 0.05$) higher in the samples A1 and B1. The highest antioxidant capacity ($p < 0.05$) for the sapucaia nut extract was observed in the ABTS assay for the sample A1.

In sapucaia shell extract, the antioxidant capacity observed in the FRAP, ABTS and DPPH assays and it was significantly higher ($p < 0.05$) in samples A1, A2, and A3 when compared to the sample B1. The higher antioxidant activity of the shell can be associated with a higher content of phenolic compounds in the outermost parts of the sapucaia nut.

Results showed high condensed tannin contents in the sapucaia shell. Tannins, on the other hand, are mainly found in the skin and shell of fruits and seeds, where they play an important role in the defense system of the nut, protecting it from oxidative damages (Shahidi & Ambigaipalan, 2015).

Moreover, the correlation between TFC, TC, FRAP, ABTS, DPPH and individual phenolic compounds in sapucaia nut and shell extracts are

Table 2
Phenolic compounds (mg g^{-1} in dry matter) of sapucaia nut and shell extracts.

Phenolic compounds	Samples (mg g^{-1})							
	A1		A2		A3		B1	
	Nut	Shell	Nut	Shell	Nut	Shell	Nut	Shell
Phenolic acids								
Gallic acid	< LOQ	0.453 ± 0.040^{A}	< LOQ	0.338 ± 0.004^{A}	< LOQ	0.149 ± 0.007^{B}	< LOQ	0.366 ± 0.006^{A}
Protocatechuic acid	–	0.040 ± 0.006^{A}	–	0.032 ± 0.003^{A}	–	0.020 ± 0.003^{A}	–	0.048 ± 0.014^{A}
4-hydroxymethylbenzoic acid	–	< LOQ	–	< LOQ	–	< LOQ	–	< LOQ
Vanillic acid	0.032 ± 0.001^{a}	0.443 ± 0.042^{A}	0.037 ± 0.006^{a}	0.326 ± 0.040^{A}	0.039 ± 0.002^{a}	0.454 ± 0.026^{A}	0.045 ± 0.014^{a}	0.075 ± 0.021^{B}
Syringic acid	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
p-coumaric acid	–	< LOQ	–	< LOQ	–	< LOQ	–	< LOQ
Ferulic acid	0.014 ± 0.013^{a}	0.043 ± 0.010^{A}	0.011 ± 0.001^{a}	0.025 ± 0.010^{A}	0.023 ± 0.006^{a}	0.038 ± 0.002^{A}	0.018 ± 0.006^{a}	0.027 ± 0.001^{A}
Sinapic acid	< LOQ	< LOQ	–	< LOQ	< LOQ	< LOQ	< LOQ	–
Salicylic acid	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Ellagic acid	0.065 ± 0.001^{a}	0.386 ± 0.047^{A}	0.024 ± 0.003^{b}	0.180 ± 0.031^{B}	0.021 ± 0.003^{b}	0.350 ± 0.056^{A}	0.021 ± 0.002^{b}	0.410 ± 0.023^{A}
Flavonoids								
Epigallocatechin	< LOQ	0.169 ± 0.036^{A}	< LOQ	0.031 ± 0.001^{B}	< LOQ	0.027 ± 0.001^{B}	< LOQ	0.061 ± 0.007^{B}
Catechin	0.144 ± 0.023^{a}	3.995 ± 0.294^{AB}	< LOQ	2.572 ± 0.410^{B}	< LOQ	4.692 ± 0.637^{A}	0.023 ± 0.011^{b}	3.606 ± 0.488^{AB}
Epicatechin	0.022 ± 0.005	1.338 ± 0.142^{A}	< LOD	0.611 ± 0.193^{B}	< LOD	1.010 ± 0.051^{AB}	< LOQ	0.795 ± 0.213^{AB}
Epicatechingallate	–	< LOQ	–	< LOQ	–	< LOQ	–	< LOQ
Taxifolin	< LOQ	0.191 ± 0.003^{A}	–	0.088 ± 0.043^{AB}	–	0.043 ± 0.001^{B}	< LOQ	0.171 ± 0.028^{A}
Quercetin	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Myricetin	0.047 ± 0.005^{a}	0.045 ± 0.001^{A}	0.019 ± 0.001^{b}	0.031 ± 0.005^{A}	0.021 ± 0.002^{b}	0.041 ± 0.010^{A}	0.032 ± 0.002^{b}	0.074 ± 0.020^{A}
Aromadendrin	–	0.013 ± 0.002^{A}	–	< LOQ	–	< LOQ	–	0.014 ± 0.001^{A}
Kaempferol	–	0.039 ± 0.002^{A}	–	< LOQ	–	0.010 ± 0.005^{B}	–	0.036 ± 0.006^{A}
Naringenin	–	< LOQ	–	< LOQ	–	< LOQ	–	< LOQ
Phenol aldehyde								
Vanillin	–	0.015 ± 0.003^{A}	–	0.025 ± 0.001^{A}	–	0.032 ± 0.016^{A}	–	0.010 ± 0.001^{A}
Sinapaldehyde	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Total	0.324	7.170	0.091	4.259	0.104	6.866	0.139	5.693

A1, A2, A3: sapucaia nuts from Piauí; B1: sapucaia nut from Minas Gerais. Mean ± S.D. ($n = 3$). $^{a\text{-}d}$The same lowercase letters in the same row do not differ significantly (Tukey test, $p < 0.05$) between the sapucaia nut extracts. $^{A\text{-}D}$The same uppercase letters in the same row do not differ significantly (Tukey test, $p < 0.05$) between the sapucaia shell extracts. -: < LOD (limit of detection). LOQ: Limit of quantitation. Data relating to LOD and LOQ of each phenolic compound are showed in Table 1.

Table 3
Phenolic compounds profile of the nuts and skin/hard shells of Brazil, cashew, baru and pecan nuts.

	Phenolic compound	Unit	Nut	By-product (skin/hard shell)	References
Brazil nut	Gallic acid	mg Kg^{-1}	70.0		Gomes and Torres (2016)
	Protocatechuic acid		207		
	Catechin		421		
	p-Hydroxybenzoic acid		151		
	2,4-Dihydroxybenzoic acid		168		
	p-Coumaric acid		136		
	Sinapinic acid		124		
	Gallic acid	μg g^{-1}	82.0[a], 52.0[b]	294[a], 1639[b]	John and Shahidi (2010)
	Protocatechuic acid		120[a], 33.0[b]	884[a], 1320[b]	
	Catechin		25.0[b]	2875[b]	
	Vanillic acid		35.0[a], 9.0[b]	58.0[a], 285[b]	
	Ellagic acid		11.0[a], 15.0[b]	130[a], 77.0[b]	
	Taxifolin		–	123[a], 333[b]	
	Quercetin		–	28.0[b]	
Cashew nut	Gallic acid	mg g^{-1}	0.11	0.36	Chandrasekara and Shahidi (2011a)
	Syringic		0.61	2.51	
	p-coumaric		0.10	0.25	
	Catechin		11.7	47.3	
	Epicatechin		7.43	28.3	
	Epigallocatechin		4.46	2.01	
Baru nut	*p*-Coumaric acid	mg 100 g^{-1}	14.3[e], 2.60[f]		Lemos et al. (2012)
	Ellagic acid		8.50[e], 2.80[f]		
	Caffeic acid		6.30[e], 1.80[f]		
	Gallic acid		224[e], 133[f]		
	Hydroxybenzoic acid		2.30[e], 0.30[f]		
	Catechin		87.2[e], 20.3[f]		
	Ferulic acid		45.4[e],9.60[f]		
	Epicatechin		23.9[e], 8.0[f]		
Pecan nut	Caffeic acid hexoside	μg g^{-1}	6.74[a], 6.70[d]		Robbins et al. (2015)
	Gallic acid derivative		13.6[a], 79.0[c], 45.4[d]		
	Brevifolin carboxylic acid		5.20[a]		
	Valoneic acid dilactone		9.45[a], 109[c], 262[d]		
	Ellagic acid pentose		9.30[a]		
	Ellagic acid		132[a], 119[c], 103[d]		
	Methyl ellagic acid		7.40[a]		
	Ellagic acid galloyl pentose		4.40[a]		
	Methyl ellagic acid pentose		8.50[a]		
	Ellagic acid galloyl pentose		4.20[a]		
	Dimethyl ellagic acid		3.10[a]		
	Protocatechuic acid		21.0[c]		
	p-hydroxybenzoic acid		48.0[c], 30.9[d]		
	Sinapoylquinic acid		14.0[c]		
	Methylellagic acid		7.0[c]		
	Ellagic acid derivative		7.0[c]		
	Gallic acid		86.2[d]		
	Catechin		82.3[d]		
	Syringic acid derivative		24.0[d]		
	Ellagitannin derivative		8.0[d]		

(*continued on next page*)

Table 3 (*continued*)

	Phenolic compound	Unit	Nut	By-product (skin/hard shell)	References
	Gallic acid	mg g^{-1}		128[g], 138[h]	Hilbig, Alves, et al. (2018)
	Protocatechuic acid			2.27[g], 3.30[h]	
	Mandelic acid			0.62[g]	
	Epigallocatechin			2.38[g], 4.73[h]	
	(+) Catechin			260[g], 352[h]	
	Chlorogenic acid			0.15[g], 0.15[h]	
	(−) Epicatechin			24.9[g], 24.4[h]	
	Vanillic acid			6.92[g], 9.01[h]	
	Siryngic acid			0.59[g], 0.90[h]	
	Epicatechingallate			0.29[g], 0.73[h]	
	Fustin			0.02[g], 0.10[h]	
	Vanillin			0.14[g], 0.10[h]	
	p-coumaric			0.08[g], 0.14[h]	
	Taxifolin			3.99[g], 5.60[h]	
	Ferulicacid			0.16[g], 0.31[h]	
	Rosmarinicacid			0.01[g], 0.01[h]	
	Quercetin			0.26[g], 0.28[h]	
	Salicylicacid			0.05[g], 0.08[h]	
	Myricetin			12.3[g], 58,3[h]	
	Ellagicacid			11.6[g], 36.3[h]	
	Eriodictyol			0.12[g], 0.12[h]	
	Naringenin			0.05[g], 0.08[h]	

–: not detected.
[a] Free phenolics.
[b] Bound phenolics.
[c] Ester-linked.
[d] Glycoside-linked.
[e] Baru nut with peel.
[f] Baru nut peeled.
[g] Pecan nut shell aqueous extract.
[h] Pecan nut shell hydroalcoholic extract.

Table 4
Total phenolic content, antioxidant capacity and condensed tannins in sapucaia nut extracts and sapucaia shell extracts (dry weight).

Samples	Antioxidant capacity				CT (mg CE g^{-1})
	TFC (mg GAE g^{-1})	FRAP (μmol TEAC g^{-1})	ABTS (μmol TEAC g^{-1})	DPPH (μmol TEAC g^{-1})	
Nuts					
A1	34.02 ± 0.37[b]	19.38 ± 1.04[a]	48.13 ± 0.27[a]	Nd	–
A2	29.03 ± 0.19[b]	15.82 ± 0.78[b]	34.15 ± 0.18[d]	Nd	–
A3	34.52 ± 0.76[b]	15.82 ± 1.76[b]	39.28 ± 0.43[c]	Nd	–
B1	41.34 ± 0.25[a]	18.35 ± 0.58[ab]	41.34 ± 0.25[b]	Nd	–
Shell					
A1	377.64 ± 1.32[a]	1365.2 ± 3.35[a]	196.91 ± 0.17[a]	140.88 ± 1.19[a]	123.81 ± 1.74[a]
A2	347.89 ± 1.91[b]	1369.8 ± 2.09[a]	196.12 ± 0.78[a]	139.11 ± 2.32[a]	91.99 ± 1.59[d]
A3	349.86 ± 1.56[b]	1362.5 ± 3.19[a]	197.70 ± 0.17[a]	140.03 ± 1.01[a]	105.28 ± 0.48[c]
B1	350.35 ± 0.53[b]	975.9 ± 2.53[b]	193.45 ± 0.89[b]	135.14 ± 0.83[b]	115.66 ± 0.18[b]

A1, A2, A3: sapucaia nuts from Piauí; B1: sapucaia nut from Minas Gerais. Mean ± S.D. (n = 3). Nd: not determined. -: not detected. [a-d] The same letters in the same column do not differ significantly (Tukey test, $p < 0.05$) for each extracts. CT: condensed tannins. TFC: Total phenolic content. GAE: gallic acid equivalent. TEAC: Trolox equivalent antioxidant capacity. CE: Cathechin equivalent.

shown in Table 5. A positive and significant correlation among individual phenolic compounds and antioxidant capacity suggest that these phenolic compounds were associated with the antioxidant capacity assessed by these methods (FRAP, ABTS and DPPH). Phenolic compounds are known to have strong *in vitro* and *in vivo* antioxidant effects as they are reducing agents and to be able to donate hydrogen or electrons to free radicals (Granato, Santos, Maciel, & Nunes, 2016). The activity is also related with the number of hydroxyl groups present, the greater the number the greater the activity (Granato, Katayama, & Castro, 2011).

4. Conclusions

A large number of phenolic compounds were identified in the nut and shell of sapucaia (14 and 22, respectively). Catechin, epicatechin, myricetin, ellagic acid, and ferulic acid of the sapucaia nut presented significant correlation to the antioxidant activity. Overall, the sapucaia shell extracts presented higher total phenolic compounds and antioxidant activity when compared to the nut extracts. The results indicated that the high antioxidant capacities found in the sapucaia nut and shell are based on its high phenolic compounds content. The

Table 5
Correlation between antioxidant capacity and phenolic compounds in sapucaia nut and shell.

Parameters	TFC	FRAP	ABTS	DPPH
Nuts				
FRAP	0.261	–	–	–
ABTS	−0.185	0.766*	–	–
Catechin	−0.084	0.746*	0.896*	–
Ellagic acid	−0.246	0.632**	0.854*	–
Epicatechin	−0.178	0.665**	0.846*	–
Ferulic acid	0.628**	0.173	−0.136	–
Myricetin	0.178	0.828*	0.891*	–
Vanillic acid	0.544	−0.229	−0.359	–
Shell				
FRAP	0.764*	–	–	–
ABTS	0.886*	0.912*	–	–
DPPH	0.482	0.225	0.229	–
CT	0.376	0.132	−0.151	0.123
Aromadendrin	0.660**	−0.358	−0.557	0.110
Catechin	−0.014	0.609**	0.323	−0.265
Ellagic acid	0.559	−0.246	−0.479	−0.226
Epicatechin	−0.081	0.315	0.243	−0.091
Epigallocatechin	−0.056	0.266	0.087	0.635**
Ferulic acid	−0.391	0.753*	0.603**	0.068
Gallicacid	0.207	−0.348	−0.398	0.653**
Kaempferol	0.565	−0.142	−0.369	0.086
Myricetin	0.877*	−0.557	−0.655**	−0.376
Protocatechuicacid	0.683**	−0.652**	−0.819*	0.117
Taxifolin	0.407	−0.432	−0.484	0.479
Vanillic acid	−0.184	0.293	0.239	−0.045
Vanillin	−0.652**	0.543	0.654**	−0.104

TFC: Total phenolic content. CT: condensed tannins.
* $p < 0.01$.
** $p < 0.05$.

phenolic profile and concentration in sapucaia nut and shell indicate that this underutilized raw material may be used as a good source of dietary phenolic compounds, as an ingredient in the formulation of different healthy foods and as an ingredient in food matrices. Future work should be performed to determine the bioactivity of the extracts as well as their use in food, cosmetics, and medicines.

Conflict of interest

The authors confirm that there are no known conflicts of interest associated with this publication.

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Appendix A. Supplementary data

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