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Bioactive compounds and value-added applications of cupuassu (*Theobroma grandiflorum* Schum.) agroindustrial by-product

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Abstract

Cupuassu is a fruitful species from the Amazon basin with great economical potential, due to the multiple uses of its pulp and seeds in the food and cosmetic industries. This fruit generates large amounts of solid waste, which, despite of its high content of bioactive compounds, is discarded in the environment. Therefore, with the aim of reusing these wastes and adding economic value, we investigated the nutritional potential of the seed by-product resulting from industrial cupuassu oil extraction. The by-product was submitted to green extraction, and its contents of bioactive compounds were quantified by HPLC. The extract had a total polyphenol content (16.9 ± 1.8 mg/g_{DM}) and an antioxidant activity, ABTS + (151.0 ± 5.5 mg/100 g) and DPPH· (85.4 ± 1.7 mmol/L). HPLC analysis of the extract identified epicatechin and glycosylated quercetin as the major flavonoids. This by-product shows great potential for being used as a source of the ingredients of high nutritional value, especially dietary fiber and polyphenols.

Keywords: cupuassu; by-product; antioxidant activity; flavonoids; reuse; value-added applications.

Practical Application: The by-product feature potential for its reutilization in different biotechnological processes.

1 Introduction

The growing demand for biologically active and low-cost natural substances has stimulated the search for suitable exploitation of fruit processing by-products such as peel and seeds, which are considered cheaper sources of nutrients. Some studies did in fact report nutrient concentrations in fruit wastes even higher than in pulp (Ignat et al., 2011; Pugliese et al., 2013). By-products from processing of tropical fruits have been increasingly used in recent years as food additives and sources of bioactive compounds such as polyphenols (Ayala-Zavala et al., 2011; Pantaleón-Velasco et al., 2014). In addition, the recycling of these residues may reduce the environmental impact associated to their disposal, hence adding value to the whole production chain. Thus, the physicochemical characterization of these by-products and quantification of their bioactive compounds are of great concern to add value and enhance their commercial and industrial reuse, while preserving the biome (Ballesteros et al. 2014; Janissen & Huynh, 2018)

Cupuassu (*Theobroma grandiflorum* Willd. ex Spreng.) Schum. is a fruitful species from the Amazon basin with great economical potential, due to the multiple uses of its fruit´s pulp and seeds in the food and cosmetic industries, with high economic potential. The oil extracted from its seeds is gaining great interest because of new cosmetic applications, whereas its fruit is widely used in local cuisine due to its pleasant flavor (Carvalho et al., 2004). This exotic fruit, which is rich in polyphenolic compounds, has also been commercialized in the Brazilian and international markets because of its nutritional properties (Pugliese et al., 2013). It has been suggested that its daily consumption may contribute to prevent various diseases (Pantaleón-Velasco et al., 2014).

Based on this background, the aim of this work was to characterize the industrial by-product resulting from cupuassu oil extraction in terms of polyphenols content and antioxidant activity, nutritional composition, physicochemical properties and thermal behavior, so as to check its industrial potential in food applications, hence adding value to the production chain and saving costs.

2 Material and methods

2.1 Chemicals and reagents

To quantify phenolic compounds by colorimetric and chromatographic methods, the following standards were used: gallic acid (Fluka Chemika, Buchs, Switzerland), D(+)-anhydrous glucose (Carlo Erba, Milan Italy), D(-)-fructose (Merck, Darmstadt, Germany), sucrose (Boehringer, Mannheim, Germany), quercetin dihydrate, glycosylated quercetin, catechin hydrate, *p*-coumaric acid, (-)-epigallocatechin gallate, (-)-epicatechin,

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3,4-dihydroxybenzoic, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, (±)-6 hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, 2,2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich, Saint Louis, MO, USA). Folin-Ciocalteu phenol reagent was purchased from Sigma-Aldrich.

2.2 Preparation of the cupuassu seed by-product

Cupuassu seeds were undergone a baking process at 65 °C for 45 min, after which they were pressed to remove the oil to be industrially exploited. The resulting by-product, designated as cupuassu seed by-product (CSB), was dried in an oven with air circulation at 40 ± 2 °C up to constant weight. After dehydration, it was pulverized in a blender, model 650 W (Mondial, São Paulo, SP, Brazil), thus obtaining the so-called dry material (DM).

2.3 Determination of bioactive compounds

2.3.1 Extract preparation

Dried and pulverized CSB was extracted with a 70% (w/v) ethanol solution in water according to the percolation process (Brasil, 1959). To obtain the crude extract (CE), the resulting solution was concentrated in a rotary evaporator, model Laborota 4000 (Heidolph, Schwabach, Germany), under low pressure and controlled temperature (40 ± 5 °C). CE obtained in this way had a dry weight content of 78.85 ± 0.50%.

2.3.2 Total polyphenols content

The solid-phase extraction (SPE) technique was applied to clean CE. For this purpose, CE was filtered through a C18-SD extraction disk cartridge (10 mm/6 mL), model 4315SD (Empore, St. Paul, MN, USA), and the retained fraction first washed with 5.0 mM sulfuric acid then eluted with methanol. All recovered fractions were separately submitted to total polyphenol (TP) quantification according to the Folin Ciocalteu method, as described by Aliakbarian et al. (2011), using a UV-Vis spectrophotometer, model Lambda 25 (Perkin Elmer, Wellesley, MA, USA), at a wavelength of 725 nm, and expressed in milligrams of gallic acid equivalent per 100 gram of dry material ($mg_{GAE}/100g_{DM}$).

2.3.3 Total flavonoids content

Total flavonoid (TF) content was determined according to the procedure of Jemai et al. (2009), with adaptations. After diluting 0.25 mL of CE with 1.25 mL of distilled water, 75 µL of a 5% $NaNO_2$ solution were added, and the resulting solution was allowed to stand for 5 min. Subsequently, 150 µL of a 10% $AlCl_3$ solution were added, and the sample was allowed to react for 6 min. Finally, 500 µL of a 1.0 M NaOH solution were added, and distilled water was added up to a final volume of 775 µL.

Samples were read at wavelength of 510 nm, using the same spectrophotometer as above. The blank consisted of all reagents without sample. A calibration curve was constructed with standard methanolic solutions of catechin at concentrations in the range 0.01-0.50 mg/mL, and the results were expressed as milligrams of catechin equivalent per 100 grams of dry material ($mg_{CA}/100\ g_{DM}$).

2.3.4 Antioxidant activity - DPPH and ABTS method

The antioxidant activity of the extract was determined according either to the assay of 2,2-diphenylpicrylhydrazyl radical (DPPH·) inhibition or that of the 2,2'-azino-bis (3-ethylbenzothiazoline) 6-sulfonic acid radical cation ($ABTS^{+\cdot}$).

To determine the antioxidant activity according to the DPPH method, 75 µL-CE aliquots were first diluted (1:150) and mixed with 2,925 µL of a 25.0 mg/L DPPH· methanolic solution, which was also used as a blank (Tepe & Sokmen, 2007). The mixture was then kept at room temperature for 30 min in the dark, and the absorbance read at 515 nm using the same UV/Vis spectrophotometer as above. The ability to capture the DPPH· radical was expressed as yield of scavenging (Y_{Sc}), according to the equation 1:

$$Y_{Sc}(\%) = \frac{A_b - A_s}{A_b} \times 100 \quad (1)$$

where A_b is the absorbance of the blank (DPPH·) at the start and A_s that of the sample (DPPH· plus the extract) after 30 min.

The antioxidant activity was finally expressed as concentration of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) taken as a reference antioxidant, through a calibration curve ($y = 0.0774x - 0.0611$) correlating the percentage inhibition (y) with Trolox concentration (x). The value of IC_{50} was defined as the final concentration, expressed in mg/mL of the extract, able to reduce the initial DPPH· concentration by 50%.

CE antioxidant activity was also determined according to the ABTS method (Re et al., 1999), with some modifications. To this purpose, 50 µL-CE aliquots were diluted and added to 1.0 mL of $ABTS^+$ solution, and the absorbance of samples was read after 2 min of reaction at 734 nm. Antioxidant activity was calculated using a standard Trolox curve expressed in µg/L. The results were finally expressed in mg of Trolox equivalent antioxidant capacity per 100 g of crude extract ($mg_{TEAC}/100g_{CE}$).

2.3.5 Quantification of single polyphenols content

The contents of the main polyphenols contained in the CE were also quantified by HPLC. For this purpose, standard solutions of gallic acid, protocatechuic acid, epigallocatechin, epicatechin, *p*-coumaric acid and quercetin were prepared at concentration of 0.5 mg/mL, while that of glycosylated quercetin at 0.1 mg/mL. Before the analyses, standard solutions were diluted (1:2) with methanol, while the CE, whose concentration was 100 mg/mL, was filtered through membranes with 0.22 µm-pore diameter (Millipore, Bedford, MA, USA). Analyses were performed using a HPLC system, model 1100 (Agilent, Palo Alto, CA, USA), coupled with a photodiode array detector (PAD), model 1260 Infinity (Agilent), and equipped with a C18 reverse phase Eclipse plus column (4.5 x 250 mm) packed with 5 µm diameter particles (Agilent). Samples (20 µL) were analyzed at a constant flow rate of 1.0 mL/min and a column temperature of 30 °C. The mobile phase was a gradient system, with mobile phase A (methanol/acetonitrile, 1:1) and B (1% acetic acid in water), varying the mobile phase B, for 0-5 min to 100%, 10 min to 95%, 30 min to 70%, 40 min to 60%, 45 min to 52%, 55 min to 30%,

60-65 min to 0%. Chromatographic peaks of these analytes were detected at 280 nm for all phenolics. Then they were confirmed by comparing their retention time and DAD-UV spectra with those of reference standards. Quantification was carried out by integration of peaks using the external standard method.

2.4 Quantification of non-hydrolysable sugars

For quantification of non-hydrolysable sugars, the extract (62.5 mg/mL) was filtered before the analyses through membranes with 0.22 μm-pore diameter (Millipore). Analyses were done by the same HPLC system equipped with a refractive index detector, model HP 1047A (Hewlett Packard, Valley Forge, PA, USA), and a column HI – Plex H (7.7 x 300 mm) (Agilent). Samples (20 μL) were analyzed at a constant flow rate of 0.5 mL/min and a column temperature of 50 °C. The mobile phase was an isocratic system with 0.01 N sulfuric acid as the eluent.

2.5 Physicochemical characterization

2.5.1 Nutritional composition and energetic value

CSB was also analyzed for protein, lipids, carbohydrate, crude fiber, ash and moisture contents according to the Association of Official Analytical Chemists (Horwitz & Latimer, 2005). The crude protein content was determined by the macro-Kjeldahl method (AOAC 991.02) using N x 6.25 as the nitrogen-to-protein conversion factor, the lipids (AOAC 989.05) by extracting a known mass of powdered sample with petroleum ether in a Soxhlet apparatus, the ash content by incineration at 550 ± 20 °C (AOAC 935.42) and the moisture content gravimetrically, while total carbohydrates (including fiber) were calculated by difference. Total energy referred to a mass of 100 g was calculated according to the equation 2 and the results were presented in g per 100 g of dry material (DM).

$$\text{Energy}(\text{kcal}) = 4(\text{kcal/g})\,m_P + 4(\text{kcal/g})\,m_C + 9(\text{kcal/g})\,m_F \quad (2)$$

where: m_P, m_C and m_F are the masses of proteins, carbohydrates and lipids (g), respectively.

2.6 Mineral composition

After digestion of DM and CE samples in 5% (v/v) HNO_3 (Horwitz & Latimer, 2005), mineral composition was determined in terms of calcium, sodium, potassium, magnesium, manganese, iron, copper, zinc and lead contents, by a fast sequential flame atomic absorption spectrometer, model AA240FS (Varian, Milan, Italy).

2.7 Total solid content

The solids content was determined by the gravimetric method using a scale analyzer and infrared heating system, model IV 2000 (Gehaka, São Paulo, Brazil). The results were expressed as weight percent, and all analyses were performed in triplicate.

2.8 Thermogravimetric behavior

Thermal Analysis (TGA) and Differential Thermal Analysis (DTA) of CSB and CE were performed by means of a thermal analyzer, model DTG-60 (Shimadzu, Japan), using an aluminum crucible containing approximately 9.0 mg samples, under nitrogen atmosphere (N_2) and flow rate of 50 mL/min. Experiments were carried out in the temperature range between 25 and 600 °C, at a heating rate of 10 °C/min (Sampaio et al., 2016).

2.9 Statistical analyses

Experimental data were subjected to one-way analysis of variance (ANOVA) and Tukey's multiple-range test, using the Statistica version 7.0 software (StatSoft Inc., Tulsa, OK, USA). Differences were considered to be significant at $P < 0.05$.

3 Results and discussion

3.1 Bioactive compounds content and antioxidant activity

Table 1 shows the total polyphenol (TP) content and antioxidant activity of cupuassu seed by-product (CSB) crude extract (CE). TP content of methanolic fraction of dry material extract was more than thrice (16.9 ± 1.8 mg/g_{DM}) that reported for cupuassu pulp (5.4 mg/g_{DM}) (Pugliese et al., 2013), while those of the first two fractions resulting from washing with sulfuric acid (SPE) were negligible. These results are consistent with the observations of Pugliese et al. (2013), who found that seeds and peel had by far higher TP contents than other fruit parts and detected a TP content of seeds 4-5 times higher than that of pulp.

TP content of CE was also significantly higher ($P < 0.05$) than those of some by-products from other tropical fruits such as mango (3.76 mg/g_{DM}), passion fruit (4.51 mg/g_{DM}), papaya (7.83 mg/g_{DM}) and sapotilla (10.53 mg/g_{DM}) (Ribeiro da Silva et al., 2014), as well as those of grape pomace of different Sicilian cultivars such as Nuello Mascalese (12.36 mg/g_{DM}), Frappato (6.91 mg/g_{DM}) and Cabernet Sauvignon (10.65 mg/g_{DM})

Table 1. Functional properties and pysicochemical characterization of cupuassu seed by-product.

Parameter	Cupuassu seed by-product (dry material)
Moisture content (% *w*/w)	7.6 ± 0.4
Non-hydrolyzable sugars (g/100 g_{DM})	2.0 ± 0.2
Ash content (% *w/w*)	5.2 ± 0.1
Solid content (% *w/w*)	49.9 ± 9.2
Proteins (% *w/w*)	14.2 ± 0.2
Lipids (% *w/w*)	24.4 ± 0.8
Carbohydrates (% *w/w*)	26.4 ± 1.2
Crude fiber (% *w/w*)	22.2 ± 0.3
Energetic value (kcal/100g)	382.0 ± 2.0
Total phenolic ($mg_{GAE}.g_{DM}^{-1}$)	16.9 ± 1.8
Total flavonoids ($mg_{CA}.g_{DM}^{-1}$)	5.92 ± 3.4
AA_{ABTS} ($mg_{TEAC}.100g_{CE}^{-1}$)	151.0 ± 5.5
AA_{DPPH} (mM Trolox eq.L^{-1})	85.4 ± 1.7
IC_{50} ($mg.mL^{-1}$)	44.5 ± 1.5

Results are expressed as mean of triplicates ± standard deviation; GAE = gallic acid equivalent; DM = dry material; CE = crude extract; CA = catechin equivalent; IC_{50} = extract concentration able to reduce the initial $DPPH^{\cdot}$ concentration by 50%; $AA_{DPPH^{\cdot}}$ = antioxidant activity determined according to the $DPPH^{\cdot}$ radical assay; AA_{ABTS} = antioxidant activity determined according to the $ABTS^{+\cdot}$ radical cation assay.

(Ruberto et al., 2007). It is noteworthy that most of these authors considered as by-products all the non-edible portion of fruits, while in the present study we consider such only the pressed seeds after oil extraction.

The antioxidant activity of CE was determined by both methods of DPPH· ($AA_{DPPH·}$ = 85.4 ± 1.7 mmol/L) and ABTS+· ($AA_{ABTS+·}$ = 151.0 ± 5.7 $mg_{TEAC}/100g_{CE}$) (Table 1). The relatively low IC_{50} value (44.5 ± 1.5 mg/mL) indicates that a small amount of extract was able to inhibit DPPH· oxidation by 50% (Schinella et al., 2010). Fresh cupuassu seeds exhibited lower antioxidant activity than CE either by the DPPH· (32.5-38.3 mmol/L of fresh weight) (Pugliese et al., 2013). For the ABTS+· method (36.3 mg Trolox/100g of fresh weight), likely due to their lower TP content (Contreras-Calderón et al., 2011). In addition, the above antioxidant activity by the DPPH method of CE was more than twice that of spent coffee grounds (Ballesteros et al., 2014).

Antioxidant compounds have numerous applications in food, cosmetic, and pharmaceutical areas, because they can protect against chronic and degenerative diseases such as cancer and diabetes mellitus, and decrease the risk factors of cardiovascular diseases, among others (Ao et al., 2011). These results suggest the possibility of reusing cupuassu residues to obtain such compounds.

3.2 Quantification of phenolic compounds in the by-product

The main phenolics detected in CE by HPLC were gallic, protocatechuic and *p*-coumaric acids, epigallocatechin gallate, epicatechin, glycosylated quercetin and quercetin (Table 2). Other signals with different intensities were observed at different retention times, which can be ascribed to the presence of other flavonoids and/or glycosylated compounds.

Protocatechuic acid was the most abundant phenolic compound of the extract (33.36 mg/100 g_{DM}), followed by glycosylated quercetin (28.01 mg/100 g_{DM}) and epicatechin (20.74 mg/100 g_{DM}). The amounts of quercetin, either glycosylated or not (5.79 mg/100 g_{DM}), are promising taking in mind that these flavonoids exert a protective effect against cardiovascular diseases when assimilated through food in rates 16-24 mg/day (Kris-Etherton et al., 2002). The same applies to epicatechin that was shown to exert a protective effect against isoproterenol-induced oxidative stress thereby reducing cardiac tissue damage (Prince, 2011).

Table 2. Polyphenolic compounds detected at 280 nm by HPLC in the cupuassu seed by-product and related retention times (*Rt*).

Compound	*Rt* (min)	Concentration ($mg/100g_{DM}$)
Gallic acid	8.8	5.68
Protocatechuic acid	17.32	33.36
Epigallocatechin gallate	25.45	6.81
Epicatechin	25.9	20.74
p-Coumaric acid	29.5	1.26
Glycosylated quercetin	35.6	28.01
Quercetin	40.78	5.79

DM = dry material

The above glycosylated quercetin content is consistent with the high levels of glycosylated flavones (mainly quercetin) detected in cupuassu, especially in its unfermented seeds (Pugliese et al., 2013), while that of epicatechin corroborates the findings of Pugliese et al. (2013) and Barros et al. (2016), who identified it in cupuassu seeds either as terminal unit of proanthocyanidin oligomers (4.09 and 29.13 mg/g, respectively) or as extension unit (18.33 and 31.23 mg/g, respectively). On the other hand, unlike these authors, catechin was not detected in our by-product, likely because it was mainly present in the oil fraction.

3.3 Physicochemical characterization

Consistently with the high solids content (49.9 ± 9.2% *w/w*) and estimated carbohydrate content (26.4 ± 1.2% *w/w*) of CE, a significant amount of total simple sugars was detected (2.0 $g/100g_{DM}$), being fructose the most abundant of them (1.3 $g/100g_{DM}$), followed by sucrose (0.5 $g/100g_{DM}$) and glucose (0.2 $g/100g_{DM}$) (Table 1). These relatively high free sugar contents may have been the result of fruit ripening (Dessimoni-Pinto et al., 2010), during which polysaccharides are partially hydrolyzed to simple sugars.

3.4 Nutritional composition and energetic value

Nutritional composition of CSB revealed that carbohydrates (26.4 ± 1.2% *w/w*) and lipids (24.4 ± 0.8% *w/w*) were the most abundant macronutrients, followed by fiber (22.2 ± 0.3% *w/w*) and proteins (14.2 ± 0.2% *w/w*) (Table 1). These contents, which are even better than those reported for the cupuassu pulp (Souza et al., 2011), correspond to an energy value as high as 382.0 ± 2.0 kcal/100g.

Protein content was 42% higher than those of fermented or toasted seeds, 56% higher than that of cocoa seeds (Pugliese et al., 2013), but only half that of roasted and degreased cupuassu seeds (Lopes et al., 2008). Compared to CSB, cocoa seed by-product had higher contents of proteins (17.30 ± 0.37% *w/w*), sugars (42.28% *w/w*) and lipids (33.50 ± 0.53% *w/w*), but total fiber content was lower (15.03 ± 0.39% *w/w*) (Gabbay-Alves et al., 2017). So, the higher fat content of this by-product can be ascribed to oil pressing. CSB moisture content was only 7.6 ± 0.4% *w/w*, while that of ash was high (5.2 ± 0.1% *w/w*) due to the abundance of inorganic compounds.

These results suggest that CSB may be used as an alternative fiber-rich material able to reduce serum levels of triglycerides and glucose (Delcour et al., 2016) and to accelerate the transit of stool through the intestine, thereby contributing to prevent constipation and even colorectal cancer.

3.5 Mineral elements

Mineral compositions of dry material (DM) and CE listed Table 3 show statistically significant differences ($P < 0.05$) for almost all the mineral components analyzed. Despite the low Ca content of CE (7.2 ± 0.4 mg/100g), the one of DM (206.6 ± 19.6 mg/100g) was more than 5-fold that of cupuassu seeds (36.9 mg/100g) (Naozuka et al., 2011), which suggests the nutritional potential of this by-product. Whereas Na

contents of DM and CE were within the limits established by regulatory agencies for healthy diet (Agência Nacional de Vigilância Sanitária, 2005; Institute of Medicine, 2006), those of Mg and Zn were close to the limits and almost 3.0 times higher than those of cupuassu seeds (80.2 and 0.73 mg/100g, respectively) (Naozuka et al., 2011). On the other hand, copper content was higher than those reported for seeds of other Amazonian fruits such as nuts of sapucaia (*Lecythis pisonis*) (1.15 mg/100g), Brazil nut (*Bertholletia excelsa*) (2.18 mg/100g) (Naozuka et al., 2011) and of "umari" variety Y (*Poraqueiba sericea* Tul.) (3.24 mg/100 g) (Berto et al., 2015), even though these values may have been influenced by soil features. Mn content of DM (4.4 mg/100g) was almost twice the Recommended Daily Intake (RDIs) limits of both Agência Nacional de Vigilância Sanitária (2005) and Institute of Medicine (2006) (Table 3), while that of CE (1.1 mg/100g) complied with both, and an analogous situation occurred for iron, whose content in CE corresponded to 6% of Fe RDI. Moreover, Mg, Fe, Zn and Mn contents of CE were 32-120% higher than those of piquiá (*Caryocar villosum*) seeds and 34-162% higher than those of biribá ones (*Rollinia mucosa*) (Berto et al., 2015).

On the other hand, Pb levels were undetectable in both. Therefore, the most important minerals present in CSB are considered macro and micronutrients essential for the human health.

3.6 Thermal Behavior

The TGA and DTA curves (Figure 1) shows the thermal events of the samples between 25 and 600 °C obtained at a heating rate of 10 °C/min under a constant nitrogen atmosphere. DTA is a technique used in conjunction with TGA to obtain information on a sample through the change in temperature. The main function of the TGA-DTA coupling is to provide higher resolution at specific temperatures in which the mass

Table 3. Mineral composition of dry material (DM) and crude extract (CE) of cupuassu seed by-product.

Element	DM(mg/100g_{DM})	CE (mg/100g_{DM})	RDI (mg/day)
	Macrominerals		
Ca	206.6 ± 19.6[a]	7.2 ± 0.4[b]	1000/700*
Na	62.8 ± 3.9[a]	61.9 ± 8.5[a]	1500 - 2300**
K	3463 ± 87[a]	1648 ± 75[b]	4700**
Mg	370.4 ± 4.4[a]	224.7 ± 9.7[b]	260/100*/320 - 420**
	Microminerals		
Cu	3.9 ± 0.1[a]	3.9 ± 0.1[a]	0.900/0.440* - 0.900**
Fe	16.3 ± 0.2[a]	1.2 ± 0.1[b]	14/9.0* - 8-18**
Zn	7.4 ± 0.2[a]	2.2 ± 0.1[b]	7.0/5.6* - 8-11**
Mn	4.4 ± 0. 1[a]	1.1 ± 0.1[b]	2.3/1.5* - 1.8-2.3**
Pb	ND	ND	-

DM = dry material; CE = crude extract; RDI = Recommended Daily Intake for adults/children up to 10 years according to *Agência Nacional de Vigilância Sanitária (2005) or **Institute of Medicine (2006). ND = not detectable. Experimental values obtained in this work are expressed as means of triplicates (n = 3) ± standard deviation. Mean values in the same line with the same letters do not differ significantly at 5% confidence level according to the Tukey's test.

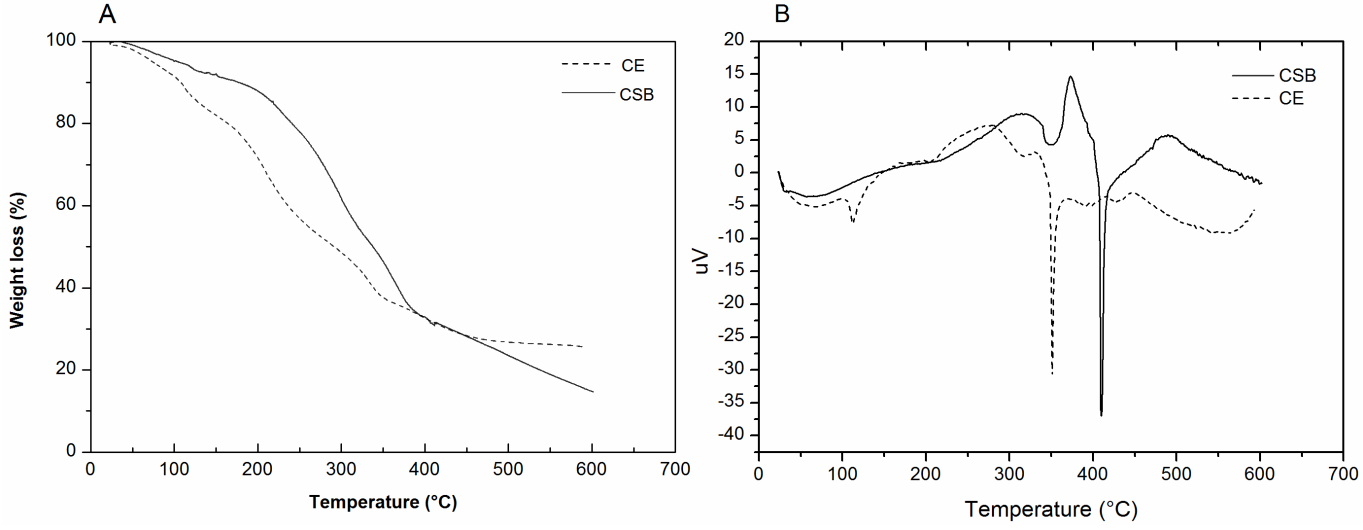

Figure 1. TGA (A) and DTA (B) curves obtained for cupuassu seeds by-product (CSB) and their crude extract (CE), at 10 °C/min, under N_2 atmosphere and flow rate of 50 mL/min.

variation is small and successive to other mass losses, leaving the visualization only by the graph of mass vs. temperature (typical of TGA) (Ionashito et al., 2004).

The thermograms obtained for CSB and CE exhibited three and two steps of mass decomposition, respectively. The first event evidenced by TGA curves (Figure 1A) at 150 °C corresponds to weight losses of about 8.6% and 12.9%, respectively, as a result of the water evaporation (dehydration of the samples), while the DTA curves (Figure 1B) highlighted a small endothermic event in a close temperature range (115.86-128.66 °C). The greatest transformation and mass losses occurred during the second thermal step, evidenced by the TGA curve in the temperature range 200-400 °C, may be related to the onset of thermal decomposition of carbohydrates, fibers, proteins and 8-25% of vegetable oil residue due to pyrolysis of cupuassu fat. It implied the largest mass loss (57.4% and 60.6%, respectively) and resulted in a large endothermic peak (407.88-413.58 °C and 339.24-365.23 °C, respectively). CSB also presented a third thermal event of mass loss (18.8%), from 400 to 600 °C, related to thermal degradation of these compounds present in the complex by-product mixture.

4 Conclusion

Full utilization of the agroindustrial by-product of cupuassu seeds, which is routinely wasted, has never been obtained until today, even though there is evidence to support its potential. The results of this study did in fact demonstrate that this by-product still possesses significant amounts of bioactive compounds with antioxidant activity and nutritional potential, which opens up possibilities for its reutilization in different biotechnological processes. It showed significant concentrations of macronutrients such as fibers, crude fat and carbohydrates as ingredients that may be exploited industrially. In addition, CSB extract exhibited interesting contents of minerals, phenolic compounds and flavonoids. These results suggest that cupuassu by-product may be used as preservative in food formulations, as natural antioxidant source in food and pharmaceutical products, or as raw material to obtain new functional ingredients for food industry, thus acquiring economic value and reducing the production of fruit processing waste. CSB is also physical thermal resistance in a large range of temperature, hence being suitable for the manufacture and encapsulation of biomaterials for several industrial purposes. Despite some efforts have recently been made to find possible alternatives to reuse this residue, the implementation of industrial processes using it as raw material is still a challenge to be faced. This study gives support to address further research and developments in this area.

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