



Cerrado cashew (*Anacardium othonianum* Rizz) apple pomace: chemical characterization and optimization of enzyme-assisted extraction of phenolic compounds

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Abstract

Cerrado cashew (*Anacardium othonianum* Rizz) pomace is the main by-product from the processing of cashew apples. This study aimed to (i) investigate the difference between the chemical compositions of the pomaces of yellow (YCP), orange (OCP), and red (RCP) cashew apples based on their mineral, monosaccharide, and polyphenolic compositions; and (ii) optimize enzyme-assisted extraction of the phenolic compounds from Cerrado cashew apple pomace. Potassium and iron were the main macro- and microelements found in residue, and significant differences were noted in the profiles of the samples. The predominant monosaccharides in the samples included glucose (52.7–60.6 mol%) and uronic acid (12.5–14.5% mol). The complete three-level factorial design allowed the optimization of pH, extraction time, and temperature, leading to an optimal extraction of polyphenols at pH 5.5, an extraction time of 120 min, and a temperature of 55°C. Myricetin (43.03–44.26 mg/kg), vanillic acid (30.96–32.32 mg/kg), and gallic acid (11.34–29.25 mg/kg) were the major polyphenols found in the samples. These results confirmed that regardless of the color of Cerrado cashew apple pomace, this by-product is a potential source of phytochemicals for application in sustainable and functional food products.

Keywords: cashew apple; fruit by-product; enzyme-assisted extraction; central composite design; multivariate chemometric analysis.

Practical Application: The results hereby reported provide valuable information regarding cashew apple pomace as a source of nutrients and polyphenols.

1. Introduction

The Cerrado is the second-largest biome in Brazil. It occupies approximately 2,036,448 km² of the country's territory. Among the species found in the Cerrado biome that bear edible fruits, the *Anacardium othonianum* Rizzini, commonly known as the cashew tree from the Cerrado, is well known for its fruit, which consists of two parts: the cashew apple (hypocarp) and the cashew nut (Oliveira et al., 2019). The cashew apple is soft and juicy, and its skin and pulp are yellow, orange, or red. Due to the succulence and acidic taste of cashew apples from the species *A. othonianum*, they are mainly used as a raw material to prepare frozen pulps, juices, sweets, liqueurs, teas, and other beverages (Santana et al., 2020; Schweiggert et al., 2016). The processing

of frozen pulps and juices produces a large amount of solid residue that is highly perishable due to its high moisture content.

The differences regarding the colors of the *A. othonianum* cashew apples may indicate differences in their chemical compositions and nutritional properties, even though they are the same species. To the best of our knowledge, there is no report available regarding the chemical composition of the cashew apple pomace from the species *A. othonianum* according to each of its three different colors.

Although the literature shows that the extraction of phenolic compounds by organic solvents is more efficient, the use of these chemicals presents a series of disadvantages, such as toxicity to the environment and to the handlers, as well as low selectivity

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of extraction and degradation of thermolabile compounds (Gli-gor et al., 2019). Besides, diverse complex carbohydrates, such as cellulose, hemicellulose, starch, and pectin, found in plant cell wall act as barriers, thereby reducing the extraction efficiency in conventional processes (Acosta-Estrada et al., 2014). Therefore, enzyme-assisted extraction (EAE) can be used as an alternative to the conventional methods since hydrolysis of plant cell wall components by carbohydrate-hydrolyzing enzymes leads to the leaching out of intracellular components, which consequently enhances the rate of extraction, reduces time and energy consumption, and minimizes environmental hazards. In this sense, current research studies have been conducted based on green extractions using extraction aids in aqueous solvents, such as ultrasound-assisted extraction (Wen et al., 2018) and EAE (Domínguez-Rodríguez et al., 2021; González et al., 2022). These methods have been considered promising for their industrial applications because the plant extracts obtained through these methods can have a series of bioactivities and are a very profitable source for the pharmaceutical, food, flavor and perfumery, cosmetics, and textile industries in the form of dietary fibers, proteins, vegetable oils, essential oils, flavorings, pigments, and polyphenols (Das et al., 2021).

Therefore, this study aimed to investigate the difference in the chemical compositions of the pomaces from yellow (YCP), orange (OCP), and red (RCP) cashew apples based on the monosaccharide, mineral, and polyphenolic compositions of each and optimize the EAE of phenolic compounds from Cerrado cashew apple pomace.

2. Materials and methods

2.1. Juice processing and cashew apple pomace samples

Cashew apples (*A. othonianum* Rizz) of the colors yellow, orange, and red were collected separately from native trees in the Cerrado biome in the region of Montes Claros de Goiás, GO, Brazil (S16°06'20" and W51°17'11"). The fruits were collected from 33 different accessions (11 for each of the three different colors) in 2018 at the recommended harvest time (September to October). The average amount of fruit collected per accession was approximately 300 g, totaling approximately 3.3 kg for each sample. The yellow, red, and orange cashew apples were processed separately. Initially, the cashew apples were sanitized (6 mL/L of PuryVitta®, 0.96% w/w active chlorine for 15 min) and stored in an ultra-freezer (model CL580-86V, ColdLab, São Paulo, SP, Brazil) at -80°C. Then, the cashew apples were processed in an industrial blender (Model LS-08MB-N, Skymesen, Brusque, SC, Brazil) to extract the juice and thus obtain the solid residues for the pomace samples (yellow, orange, and red) (Figs. 1A–1C), and then frozen at -80°C, and lyophilized (Model Enterprise II, Terroni, São Paulo, SP, Brazil) for 24 h. The freeze-dried samples were ground in a knife mill (10 mesh) (R-TE-648, Tecnal, São Paulo, SP, Brazil) and stored at -22°C until the analysis.

2.2. Physicochemical analysis and color parameters

The samples were analyzed for the amounts of moisture, ash, lipid, protein, carbohydrate, pH, total soluble solids, titratable



Figure 1. Visual appearance of the samples: (A) yellow, (B) orange, (C) red cashew apples, (D) pomaces of yellow, (E) orange, and (F) red cashew apples obtained from Cerrado cashew juice processing.

acidity (TA), and vitamin C, which were determined according to AOAC methods (AOAC, 2012). The total, soluble, and insoluble dietary fiber analyses were conducted using an enzymatic kit (Megazyme International Ireland Ltd., Bray, Ireland) (AACCI, 2010). The pectin extraction procedure was based on the methodology proposed by Kliemann et al. (2009). The color (CIELab scale) was determined through a direct colorimeter assay (Delta Vista colorimeter, Delta Color 450G, São Leopoldo, RS, Brazil). The total carotenoid content was determined according to the methodology proposed by Davies (1976), and the results were expressed as $\mu\text{g } \beta\text{-carotene}$ per gram of sample on a dry basis. All analyses were performed in triplicate.

2.3. Mineral composition

The mineral composition of the samples was determined using a flame atomic absorption spectrometer (Analyst 200, PerkinElmer, Waltham, USA). The spectrometric analysis was determined according to the methodology proposed by Malavolta et al. (1997), and the elements monitored were Ca, Mg, Cu, Fe, Mn, K, S, and Zn. Boron and P were determined on a spectrophotometer Femto 600 Plus (São Paulo, SP, Brazil). All determinations were performed in triplicate, and the results were expressed on a dry basis.

2.4. Monosaccharide composition

The neutral monosaccharide composition was determined after total hydrolysis with sulfuric acid 72% (w/w) for 1 h in an ice bath, followed by dilution of this solution to 8% (w/w) with distilled water at 100°C for 8 h. The acid was neutralized with barium carbonate, and the resulting salt was removed by filtration. The filtered solution was subjected to reduction with sodium borohydride for 16 h at 4°C. Then, Lewatit® MonoPlus S 100 (Supelco, St. Louis, MO, USA) H⁺ form was added to remove Na⁺ ions. After the removal of the resin, the solvent was evaporated under a vacuum at 50°C. The sample was treated (three times) with methanol (1 mL), and the resulting methyl borate was evaporated under a vacuum at 50°C. The alditols

were acetylated using acetic anhydride (0.5 mL) and pyridine (0.5 mL) for 12 h at 25°C. The resulting alditol acetates were solubilized in chloroform, and the pyridine was removed by treatments with 5% copper sulfate and distilled water.

The alditol acetates were analyzed in a gas chromatograph model Trace GC Ultra (Thermo Fischer Scientific, Waltham, MA, USA), equipped with a flame ionization detector, a Ross injector, and a DB-225 capillary column (30 m × 0.25 mm, Agilent Technologies, Santa Clara, CA, USA), with a film thickness of 0.25 µm. The injector and detector temperatures were 250 and 300°C, respectively. The oven temperature was programmed from 100 to 220°C at a rate of 60°C/min. A mixture of helium and nitrogen (10:1 v/v) was used as the carrier gas at a flow rate of 1.0 mL/min. The alditol acetates were identified by their retention times in comparison with the commercial monosaccharide standards.

Uronic acids were quantified by the colorimetric method of m-hydroxydiphenyl after previous hydrolysis (Blumenkrantz & Asboe-Hansen, 1973; Melton & Smith, 2001). The absorbance was measured in a spectrophotometer (model SP-2000 UV, Shangai Spectrum Instruments Co. Ltd., Shangai, China) at 520 nm. The calibration curve was prepared with galacturonic acid with a linear working range from 10 to 100 µg/mL. All determinations were performed in triplicate, and the results were expressed on a dry basis.

2.5. Optimization of enzyme-assisted extraction

For the preparation of the extracts, 1 g of sample and distilled water were mixed in a proportion of 1:25 (w/v), and the mixture was subjected to a pre-treatment by ultrasound for 30 min. After the pre-treatment step, the sample was added with 20 µL of cellulose enzymatic complex (containing a wide range of carbohydrate hydrolyzing enzymes, including arabanase, cellulase, β-glucanase, hemicellulase, and xylanase) (Viscozyme® L, V2010, 100 FBGU/g – Sigma-Aldrich (St. Louis, MO, USA)). The enzymatic extraction was carried out in a water bath at 60°C for 120 min, with pH adjusted to 5.5. The temperature, time, and pH conditions described were previously optimized through a central composite design (CCD; Table 1). Finally, the sample was centrifuged (Hermle, Z200A, Berlin, BE, Germany) at 3,000×g for 5 min, and the supernatant was collected for further analysis by LC-MS/MS. All determinations were performed in triplicate, and the results were expressed on a dry basis.

2.6. Determination of total phenolic content and individual polyphenols of the Cerrado cashew apple pomace

Total phenolic content was determined using the Folin-Ciocalteu assay, according to Singleton and Rossi (1965), and the results were expressed as milligram gallic acid equivalent (GAE) per gram of sample on a dry basis. The individual polyphenols were analyzed in a liquid chromatograph (1200 Series, Agilent Technologies, Santa Clara, CA, USA) coupled to a triple quadrupole mass spectrometer (G6420A, Agilent Technologies) equipped with an electrospray ionization interface. The source was operated in negative ion mode. The separation of the polyphenols was carried out on a C18 column (2.1×50 mm, 1.8 µm) model Zorbax (Agilent Technologies, Santa Clara, CA, USA).

Table 1. Planning matrix of CCD for optimization of pH, temperature, and time in the proposed extraction of polyphenols from Cerrado cashew apple pomace.

Experiment	pH	Temperature (°C)	Time (min)
1	3.5	40.0	60
2	3.5	40.0	120
3	3.5	60.0	60
4	3.5	60.0	120
5	5.5	40.0	60
6	5.5	40.0	120
7	5.5	60.0	60
8	5.5	60.0	120
9	2.8	50.0	90
10	6.2	50.0	90
11	4.5	33.2	90
12	4.5	66.8	90
13	4.5	50.0	40
14	4.5	50.0	140
15	4.5	50.0	90
16	4.5	50.0	90
17	4.5	50.0	90

An elution gradient using 0.350 mL/min of mobile phase (water and methanol) was employed with chromatographic analysis starting at 2:98 v/v methanol:water for the first 0.5 min; from 0.5 to 5 min, the methanol was increased to 85% and maintained at this proportion until the end of the analysis (9.5 min). The injection volume was 5 µL, and the column temperature was maintained at 30°C. The quantification of the 15 analytes obtained in this study was performed using a calibration curve. The linear working range of gallic acid, protocatechuic acid, catechin, epicatechin, chlorogenic acid, epigallocatechin, syringic acid, p-coumaric acid, ferulic acid, and myricetin was 0.01–15 mg/L, and vanillic acid, rutin, ellagic acid, myricetin, quercetin, and kaempferol was 0.01–10 mg/L.

2.7. Statistical analysis

Statistical analysis was performed using the STATISTICA version 13.0 software (StatSoft Inc., Tulsa, OK, USA). One-way analysis of variance and Tukey's HSD test were used to compare the chemical data and assess statistical differences ($p < 0.05$) among the pomaces from cashew apples of the three different colors. The results were expressed as mean ± standard deviation. Principal component analysis (PCA) was used to show the grouping of the samples as well as their main chemical characteristics associated with the cashew apple pomace of different colors. The PCA was performed with the STATISTICA version 13.0 software (StatSoft Inc., Tulsa, OK, USA).

3. Results and discussion

3.1. Physicochemical analysis and color parameters

The results obtained from the physicochemical analysis and the color parameters of the cashew apple pomace samples are shown in Table 2. Overall, among the investigated chemical

Table 2. Chemical composition and color parameters of the pomace from yellow, orange, and red cashew apple obtained from Cerrado cashew apple juice processing.

Components (g/100 g)	Samples		
	YCP	OCP	RCP
Moisture	7.22±0.11 ^a	7.05±0.19 ^a	7.04±0.06 ^a
Ash	1.20±0.04 ^b	1.28±0.08 ^{ab}	1.37±0.03 ^a
Protein	10.69±0.05 ^{ab}	11.56±0.47 ^a	9.92±0.73 ^b
Carbohydrates	33.83±0.21 ^c	35.37±0.52 ^b	41.47±0.78 ^a
Lipids	7.44±0.20 ^a	5.79±0.04 ^b	4.96±0.01 ^c
Total dietary fiber	46.85±0.84 ^a	46.01±1.61 ^a	42.27±1.38 ^a
Insoluble fiber	38.40±0.72 ^a	35.14±1.34 ^a	35.07±1.16 ^a
Soluble fiber	8.45±0.12 ^b	10.87±0.27 ^a	7.21±0.22 ^c
Pectin	14.13±1.48 ^{ab}	16.47±1.69 ^a	9.45±1.13 ^b
Titrate acidity	0.61±0.01 ^a	0.56±0.02 ^b	0.58±0.01 ^b
Soluble solids	5.07±0.21 ^b	6.40±0.20 ^a	5.13±0.35 ^b
Vitamin C (mg/100 g)	87.44±4.77 ^a	72.62±4.71 ^b	78.65±5.05 ^{ab}
pH	3.63±0.01 ^b	3.67±0.01 ^a	3.68±0.02 ^a
Total phenolic content (mg of GAE/g)	8.14±0.02 ^c	8.38±0.03 ^b	10.29±0.03 ^a
Total carotenoids (µg/g)	63.80±3.09 ^a	48.90±3.82 ^b	36.64±3.77 ^c
<i>Macroelements (mg/g)</i>			
P	1.60±0.10 ^a	1.60±0.10 ^a	1.70±0.01 ^a
K	4.50±0.44 ^b	5.67±0.47 ^a	5.33±0.23 ^{ab}
Ca	0.63±0.15 ^a	1.17±0.55 ^a	3.37±2.55 ^a
Mg	0.40±0.10 ^a	0.67±0.29 ^a	0.70±0.26 ^a
S	0.27±0.06 ^a	0.43±0.15 ^a	0.27±0.06 ^a
<i>Microelements (µg/g)</i>			
B	4.00±1.00 ^a	4.67±0.58 ^a	4.33±1.15 ^a
Cu	21.67±1.53 ^{ab}	19.33±1.53 ^b	23.67±1.53 ^a
Fe	146.67±4.16 ^b	143.00±4.58 ^b	191.33±3.21 ^a
Mn	33.33±2.89 ^a	33.00±0.01 ^a	29.33±1.15 ^a
Zn	12.00±2.65 ^a	11.67±0.58 ^a	11.00±0.01 ^a
<i>Monosaccharide mol %</i>			
Rhamnose	0.7±0.18 ^a	0.9±0.12 ^a	0.9±0.12 ^a
Fucose	0.4±0.06 ^a	0.6±0.12 ^a	0.4±0.06 ^a
Arabinose	10.7±0.89 ^a	11.0±0.35 ^a	8.4±0.91 ^a
Xylose	4.0±0.26 ^a	5.3±0.31 ^a	4.2±0.30 ^a
Mannose	3.7±0.36 ^a	3.8±0.12 ^a	3.5±0.18 ^a
Galactose	8.6±0.22 ^b	11.2±0.86 ^a	9.6±0.14 ^{ab}
Glucose	58.9±1.73 ^a	52.7±0.86 ^b	60.6±1.58 ^a
Uronic acid	13.0±0.28 ^b	14.5±0.07 ^a	12.5±0.14 ^b
<i>Color parameters</i>			
L*	65.11±0.28 ^a	52.73±0.09 ^b	48.14±0.18 ^c
a*	3.82±0.03 ^c	8.23±0.02 ^b	20.28±0.14 ^a
b*	37.00±0.20 ^a	25.91±0.20 ^b	13.99±0.11 ^c

*Results expressed as mean±SD on a dry basis (n=3). Different letters in the same line indicate statistical difference between samples (p<0.05); OCP: orange cashew apple pomace; RCP: red cashew apple pomace; YCP: yellow cashew apple pomace.

compositions of the pomaces from cashew apples according to each different color, significant differences were noted for ash, protein, carbohydrates, lipids, TA contents, and vitamin C. Cerrado cashew apple pomace is an excellent source of vitamin C, and the YCP sample showed the highest concentration of this vitamin (87.44 mg/100 g), followed by the RCP sample (78.65 mg/100 g) and the OCP sample (72.72 mg/100 g). Preethi et al.

(2021) reported lower vitamin C content (65.25 mg/100 g) in cashew apple pomace of the species *Anacardium occidentale*. These encouraging data suggest the potential use of this by-product as a dietary supplement. Regular consumption of vitamin C has been associated with the prevention of many human health disorders, such as diabetes, atherosclerosis, and heart disease (Chambial et al., 2013).

The OCP sample showed slightly higher values (p<0.05) compared to the YCP and the RCP samples regarding pectin, soluble solids, and soluble fiber content. None of the samples showed any significant difference in moisture content, total fiber, or insoluble fiber, and all of them contained high amounts of dietary fiber. Regardless of its color, Cerrado cashew apple pomace has the potential to be used in the food industry as an important source of dietary fiber. The World Health Organization (WHO) recommends a daily intake of at least 25 g of dietary fiber. The use of cashew apple pomace as a food ingredient can contribute to a higher consumption of dietary fiber, thus lowering the risk of various coronary artery diseases and cancer (Gill et al., 2021; Cheong et al., 2022).

Overall, the carotenoid content was higher (p<0.05) in the YCP sample, followed by the OCP and RCP samples. The carotenoid content found in the samples of Cerrado cashew apple pomace is higher than those reported in the literature for pequi by-product flours (21.16–34.99 µg/g) (Leão et al., 2017), flour made with green kiwi fruit skin (24.69 µg/g) (Soquetta et al., 2016), and buriti by-product flours (1.50–11.87 µg/g) (Resende et al., 2019). Carotenoid pigments, particularly β-carotene and lycopene, play a vital role in maintaining human health. β-Carotene is known to quench singlet oxygen and may have strong antioxidant activity (Black et al., 2020).

As expected, the color parameters of all the samples showed significant differences in their L*, a*, and b* values. These results showed greater luminosity for the YCP sample, followed by the OCP and RCP samples. As expected, the RCP sample displayed the highest value for the a* parameter (related to the color red). Likewise, the highest values for the b* parameter (related to the color yellow) were obtained for the YCP sample, which showed the highest amount of carotenoids.

3.2. Mineral profiling of the Cerrado cashew apple pomace

The mineral analysis revealed the presence of many macro- and microelements (Table 2), and no significant differences were noted for most of the minerals investigated. In general, the results obtained in this present study suggest that regardless of its color, the daily consumption of Cerrado cashew apple pomace as a food supplement may contribute to the intake of the recommended daily quantities of some nutritionally important minerals, especially K, P, Fe, Cu, and Mg.

The mineral composition showed that the Cerrado cashew apple pomace represented a source of K, followed by Ca. However, potassium levels in the samples (Table 2) are lower than those reported for feijoa peel flours (8.0 mg/g) (De Almeida et al., 2020) and *Anacardium occidentale* L. pomace powder (15.60 mg/g) (Preethi et al., 2021). Potassium and calcium are macroelements that are absorbed from the soil by plants

and are present in high concentrations in fruit by-products. Potassium intake is fundamental to human health, as it plays an important role in controlling blood pressure and promoting muscle growth (Gharibzadeh & Jafari, 2017).

Microelements are present in foods at low concentrations. Levels of Fe and Cu were higher in the RCP sample than in the YCP and OCP samples. RCP and YCP samples presented higher copper contents in comparison to cantaloupe waste (4.56 $\mu\text{g/g}$) (Benmeziane et al., 2018) and similar copper contents in comparison to *Anacardium occidentale* cashew bagasse from Colombian varieties (26.1 $\mu\text{g/g}$) (Reina et al., 2022). Copper is an essential trace element and is needed only in small amounts in the human diet. It acts as a cofactor for many redox enzymes and plays an important role in iron metabolism since ceruloplasmin is a ferroxidase that converts Fe^{2+} to Fe^{3+} . In addition, Cu intake promotes other health benefits, such as antioxidant defense, neuropeptide synthesis, and immune function (Bost et al., 2016).

The levels of Fe in the RCP sample were 30 and 33% higher than those found in the YCP and OCP samples, respectively. It is important to note that among the samples analyzed, the RCP sample can contribute more significantly to the intake of the recommended daily levels of Fe in human diet. The recommended daily intake of Fe for adults in Brazil is 14 mg (Brasil, 2005), and for adults in the USA, it is 18 mg (FDA, 2020). Similar amounts of iron were found in other fruits, such as citrus (residue) (Silva et al., 2017) (116.4 $\mu\text{g/g}$), cagaita (*Eugenia dysenterica*) (115.3 $\mu\text{g/g}$), coquinho azedo (*Butia capitata*) (114.7 $\mu\text{g/g}$) of the Cerrado of northern Minas Gerais (Nascimento et al., 2020), and hydroponic strawberry (*Fragaria ananassa* L.) (140.9 $\mu\text{g/g}$) (Jeon et al., 2019). Iron consumption is important because this mineral participates in a wide variety of metabolic processes in the human body, including oxygen transport, deoxyribonucleic acid (DNA) synthesis, and electron transport (Abbaspour et al., 2014).

3.3. Monosaccharide composition of the Cerrado cashew apple pomace

Table 2 shows the monosaccharide composition of the samples. Predominant monosaccharides in the samples included glucose, uronic acids, galactose, arabinose, and xylose. The YCP and RCP samples showed higher glucose content

than the OCP sample. Similarly, glucose was also reported as the major monosaccharide in the flours obtained from pequi peel (34–43%) (Leão et al., 2017) and feijoa peel (34–43%) (De Almeida et al., 2020).

Different amounts of uronic acid were noted among the samples. The uronic acid content was greater in the following order: OCP>YCP>RCP. This result is in close agreement with that obtained for pectin content, which follows the same tendency as that obtained for soluble fiber content. The uronic acid contents found in this study were higher than those reported for feijoa peel (9–12%) (De Almeida et al., 2020) and cacao pod husk flour (6.7%) (Vriesmann et al., 2012). High concentrations of arabinose and galactose were found in Cerrado cashew apple pomace. However, there were no significant differences in the concentration of arabinose among the samples of the three different colors.

3.4. Effect of enzyme-assisted extraction on the phenolic yield

The response surface was obtained through a quadratic mathematical model and showed the predicted and adjusted R^2 values of 0.8834 and 0.9490, respectively. The significance of the factors was confirmed by the ANOVA test, which showed that all the investigated factors were significant ($p < 0.05$). Figure 2 presents the response surfaces obtained for the CCD. When evaluating pH and temperature (Figure 2A), it was possible to note an increase in the analytical response at pH greater than 5.5 and temperature greater than 55°C. When evaluating the temperature and extraction time (Figure 2B), it was possible to note an increase in the analytical response at a temperature close to 55°C and a time greater than 120 min. Regarding the pH and the extraction time (Figure 2C), there was an increase in the analytical response at pH greater than 5.5 and extraction time greater than 120 min. According to the recommendations of the manufacturer for using the cellulose enzymatic complex, it is desirable to apply pH values between 4 and 6 and temperatures between 40 and 55°C. Due to these factors, the compromise conditions for the extraction method were fixed at pH 5.5 and a temperature of 55°C. The extraction time chosen was 120 min in order not to impair the analytical frequency. These conditions were considered optimal for the

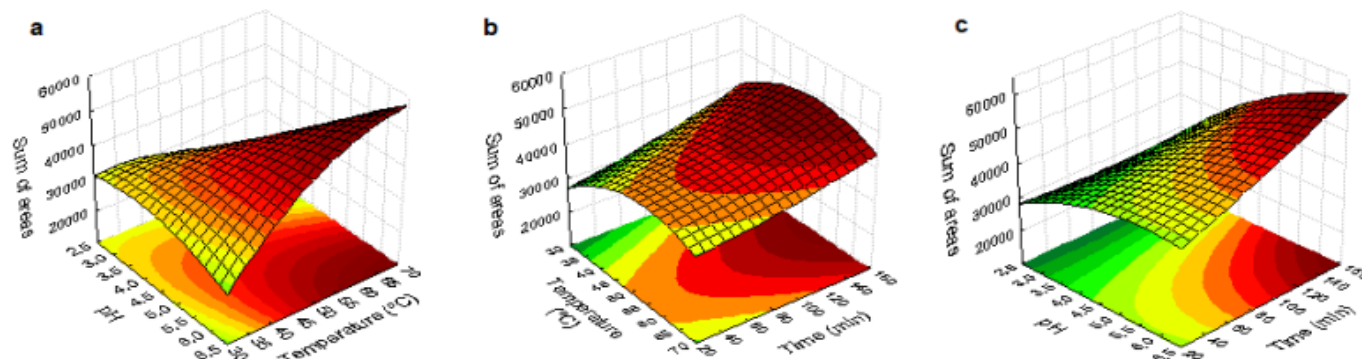


Figure 2. Response surface plots of the effect of pH, temperature, and time on the extraction of individual polyphenols (sum of peak areas). (A) pH versus temperature (°C). (B) Temperature (°C) versus time (min). (C) pH versus time (min).

enzymatic extraction of polyphenols from Cerrado cashew apple pomace.

3.5. Total phenolic content and phenolic profile of the Cerrado cashew apple pomace

It was observed that the RCP sample presented a higher ($p < 0.05$) polyphenol content in comparison with the OCP and YCP samples (Table 2). The literature presents great variability in the concentration of phenolics in the cashew fruit due to differences in soil, climate conditions, growing season, post-harvest storage, extraction methods, the methodology used to identify compounds, and the choice of solvent. Moo-Huchin et al. (2015), for example, reported a concentration of 633.2 mg of GAE/100 g and 1,316.8 mg of GAE/100 g for freeze-dried peels of yellow and red cashew (*Anacardium occidentale*) from Yucatan (Mexico), respectively. Mendes et al. (2019) reported a concentration of 78.46 mg of GAE/100 g in a raw extract obtained from the cashew apple (*Anacardium occidentale*). Polyphenols have received considerable attention as bioactive compounds because of their capacity to replace synthetic preservatives due to their ability to scavenge free radicals and prevent oxidation reactions in food (Shahidi & Ambigaipalan, 2015).

Among all the compounds included in the group of prebiotics, polyphenols are probably the most important secondary metabolites produced by the plant kingdom. Polyphenols are the new class of prebiotics that meet the criteria to be categorized as prebiotics (resistance to the host's digestion, the capacity to be fermented by intestinal microorganisms, and the stimulation of the intestinal bacteria) (Gibson et al., 2017; Moorthy et al., 2020; Nazzaro et al., 2020). Regarding their role as a prebiotic substrate, the advantages of polyphenols are attributed to the ability of the intestinal microbiota to metabolize phenolic compounds. According to Morais et al. (2016), the beneficial

prebiotic effects of polyphenols can result not only in the enhancement of growth and establishment of probiotic bacterial families, such as *Bifidobacteriaceae* and *Lactobacillaceae*, but also in the inhibition of pathogenic bacteria, such as *Escherichia coli*, *Clostridium perfringens*, and *Helicobacter pylori*.

The phenolic profile of the pomaces of YCP, OCP, and RCP is shown in Table 3. To the best of our knowledge, this is the first report on the phenolic profile of cashew apple pomace from the species *A. othonianum* Rizz. Altogether, 15 phenolic compounds were identified in the samples. Phenolic acids (gallic acid, ferulic acid, protocatechuic acid, syringic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, and vanillic acid), flavonols (catechin, epicatechin and epigallocatechin), and flavan-3-ols (quercetin, rutin, and kaempferol) were found in the samples at a wide range of concentrations. In all, 6 of the 15 phenolic compounds (chlorogenic acid, epicatechin, myricetin, quercetin, rutin, and kaempferol) that are reported in this work were also noted by Moo-Huchin et al. (2015) and Oliveira et al. (2019) in cashew apples and in their peels.

The concentrations of the polyphenols are in the following order: myricetin > vanillic acid > gallic acid in the samples. Myricetin was also the major flavonol in the yellow cashew peels (125.72 mg/100 g) and in the pomace (91.80 mg/100 g) from the processing of yellow cashew apples of the species *A. occidentale* (Batista et al., 2018; Moo-Huchin et al., 2015). However, the amount of myricetin found in the present study was lower than the values reported by the abovementioned authors, who used organic solvents in the extraction process. The literature provides strong evidence of the nutraceutical and antioxidant properties of myricetin. The significant antioxidant activity of myricetin is attributed to the presence of three hydroxyl groups in the B ring compared to other flavonoids. There are many health benefits attributed to myricetin, such as

Table 3. LC-MS/MS parameters for analysis of polyphenols and polyphenolic composition of yellow, red, and orange cashew apple pomace obtained from Cerrado cashew juice processing.

Analyte	Retention time (min)	Working range (mg/L)	M (g/mol)	Precursor ion [M-H ⁺] (m/z)	Products ions (m/z)	Concentration (mg/kg)		
						YCP	RCP	OCP
Gallic acid	1.277	0.01–15	170	169	125; 79	29.25±0.54 ^a	11.34±0.03 ^c	16.67±0.14 ^b
Ferulic acid	2.339	0.01–15	194	193	178; 149; 134	3.58±0.96 ^a	3.70±0.51 ^a	3.50±0.39 ^a
Protocatechuic acid	2.819	0.01–15	154	153	109; 108	3.26±0.43 ^c	8.09±1.65 ^a	4.50±0.59 ^b
Kaempferol	2.884	0.01–10	286	285	239; 229; 187; 185	5.58±1.10 ^a	5.75±1.05 ^a	5.53±1.16 ^a
Syringic acid	3.777	0.01–15	198	197	182	5.02±0.65 ^a	4.92±0.80 ^b	4.52±0.66 ^c
Catechin	5.112	0.01–15	290	289	203; 123; 109	5.46±0.54 ^b	6.75±0.56 ^a	5.83±0.18 ^a
Epicatechin	5.113	0.01–15	290	289	203; 123; 109	5.71±0.72 ^c	6.88±0.34 ^a	6.27±0.13 ^b
Chlorogenic acid	5.396	0.01–15	354	353	191; 85	4.82±0.84 ^a	4.83±0.75 ^a	4.89±1.31 ^a
Epigallocatechin	6.071	0.01–15	458	457	169; 125	5.02±0.77 ^a	5.03±0.72 ^a	5.01±1.57 ^a
<i>p</i> -Coumaric acid	6.141	0.01–15	164	163	119; 93	12.27±0.52 ^a	7.96±1.52 ^c	11.21±0.96 ^b
Rutin	6.703	0.01–10	610	609	301; 300	13.55±2.03 ^a	6.66±0.04 ^c	12.88±0.48 ^b
Ellagic acid	6.850	0.01–10	302	301	284	5.52±0.75 ^a	5.27±0.91 ^a	4.69±1.02 ^b
Vanillic acid	6.931	0.01–10	168	167	152; 123; 108	32.32±2.59 ^a	30.96±2.54 ^c	31.16±6.90 ^b
Myricetin	6.936	0.01–15	318	317	151; 137	44.26±2.44 ^a	43.12±1.45 ^a	43.03±8.68 ^a
Quercetin	7.368	0.01–10	302	301	151; 121	5.30±0.60 ^a	5.24±0.40 ^a	5.21±1.41 ^a

*Results are expressed as mean ± SD on a dry basis. Different letters on the same line indicate a significant difference ($p < 0.05$) between the samples; OCP: orange cashew apple pomace; RCP: red cashew apple pomace; YCP: yellow cashew apple pomace.

hepatoprotective, antitumor, anti-inflammatory, analgesic, and antidiabetic actions (Imran et al., 2021). Surprisingly, the YCP sample showed a higher concentration of gallic acid compared to the concentrations noted in the RCP and OCP samples, 43 and 61%, respectively.

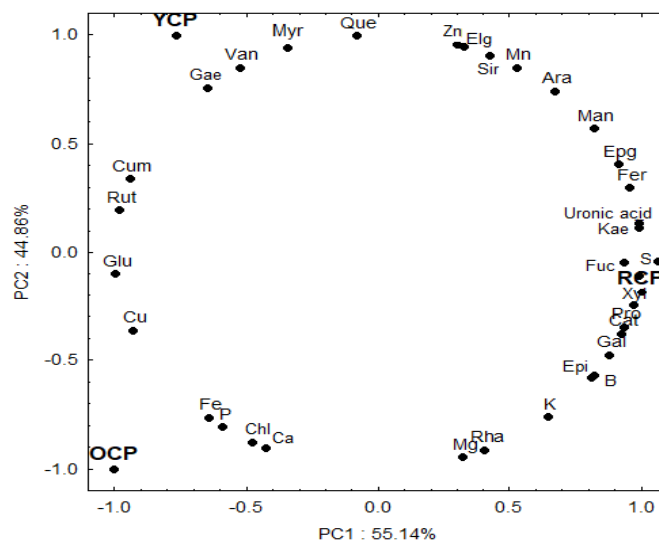
The YCP sample also showed a high concentration of *p*-coumaric acid and rutin, which were 8.63 and 35.13% higher than the concentrations obtained for the OCP sample and 4.94 and 50.85% higher than those obtained for the RCP sample, respectively. Remarkably, the RCP sample showed a higher concentration of protocatechuic acid than those obtained for the OCP and the YCP samples. It is noteworthy that these polyphenols were not found in cashew apple peels of the species *A. occidentale* (Moo-Huchin et al., 2015), except for rutin, which was found in Cerrado cashew apples (hypocarp) (*A. othonianum*) (Oliveira et al., 2019). These polyphenols play an important role as nutraceuticals and exhibit important biological activities. Protocatechuic acid is used as an active component of many herbal medicines because of its pharmacological properties, including antibacterial, anti-inflammatory, and antioxidant activity (Kakkar & Bais, 2014). It has been reported that *p*-coumaric acid lowers total cholesterol and atherosclerosis index and increases serum catalase, antioxidant capacity, and glutathione peroxidase (Shen et al., 2019).

The concentrations of ferulic acid, kaempferol, chlorogenic acid, epigallocatechin, and quercetin showed no significant differences between the samples. Moreover, there were significant differences in the concentrations of syringic acid, catechin, epicatechin, and ellagic acid. These findings support the authors' hypothesis that the different colors of the pomaces from the Cerrado cashew apple show differences in their polyphenolic compositions.

It is important to emphasize that there is a variation in the phenolic profile noted in the present study in relation to some of those found in the literature (Batista et al., 2018; Moo-Huchin et al., 2015; Reina et al., 2022). This can be explained by methodological differences in the procedures used during extraction and quantification. Furthermore, intrinsic and extrinsic factors, such as genetic variety, stage of maturation, type of cultivar, weather and culture conditions, and harvesting and post-harvesting conditions, along with other variables, can contribute to the difference in the quantities of the extracted compounds. Besides, the extraction protocols found in the literature include no-GRAS (not Generally Recognized As Safe) solvents, which are contaminant and biologically aggressive and thus inadequate for the food, cosmetics, or pharmaceutical industries, differently from the EAE process used in the present study.

3.6. Correlation between polyphenolic profile, monosaccharide composition, and mineral profile of YCP, OCP, and RCP

There was a clear separation between the Cerrado cashew apple pomace of different colors. The data set of the samples revealed that 100% of total variability was depicted in two main principal components (PC1×PC2) (Figure 3). PC1 explained 55.14% of the data variability, while PC2 explained 44.86%.



Gae: gallic acid; Fer: ferulic acid; Pro: protocatechuic acid; Kae: kaempferol; Syr: syringic acid; Cat: catechin; Epi: epicatechin; Chl: chlorogenic acid; Epi: epigallocatechin; Cou: *p*-coumaric acid; Elg: ellagic acid; Van: vanillic acid; Myr: myricetin; Que: quercetin; Rha: rhamnose; Fuc: fucose; Ara: arabinose; Xyl: xylose; Man: mannose; Gal: galactose; Uro: uronic acid.

Figure 3. Principal components analysis of the polyphenolic profile, monosaccharide composition, and mineral profile of the pomaces obtained from YCP, OCP, and RCP.

The multivariate analysis revealed that most individual phenolic compounds, monosaccharides, and minerals separated the RCP sample in PC1. It was noted that the RCP sample showed a strong association with ellagic acid, syringic acid, epigallocatechin, kaempferol, protocatechuic acid, gallic acid, catechin, and epicatechin. In addition, the RCP sample showed a strong association with several monosaccharides (rhamnose, galactose, xylose, fucose, uronic acid, mannose, and arabinose) and microelements (Zn, Mn, S, B, K, and Mg). However, the OCP sample was associated only with Ca, P, Fe, Cu, glucose, and uronic acid. On the contrary, the YCP sample was associated only with the polyphenols quercetin, myricetin, vanillic acid, gallic acid, *p*-coumaric acid, and rutin. The multivariate approach revealed that the pomaces from Cerrado cashew apples of different colors show considerable differences in their chemical compositions.

4. Conclusion

Regardless of its color, Cerrado cashew apple pomace has the potential to be used as an important source of dietary fiber and minerals, such as K, P, Fe, Cu, and Mg, which are essential and physiologically important for human health. Optimal extraction conditions to extract polyphenols by EAE from cashew apple pomace were obtained at pH 5.5, an extraction time of 120 min, and a temperature of 55°C. Myricetin, vanillic acid, and gallic acid were the predominant polyphenols in the samples, where YCP stood out. PCA showed that the samples of cashew apple pomace were grouped according to their colors, considering their potential polyphenolic and monosaccharide profiles. The results obtained in this study provide valuable information regarding the bioactive potential and nutritional properties of Cerrado cashew apple pomace and thus encourage its sustainable use in the fields of food science and technology.

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