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Influence of the phytochemical profile on the peel, seed and pulp of margarida, breda and geada varieties of avocado (*Persea Americana* Mill) associated with their antioxidant potential

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Abstract

Berries stand out because they present benefits to human health, however, their residues are generally discarded which contain appreciable amounts of bioactive compounds retained in the shells and seeds of these fruits. The objective of this work was to characterize the residues of berries, and compare the extraction by enzymatic treatment and by solvent, determining the bioactive compounds, antioxidant activities and individual phenolic compounds by UPLC-QDa-MS. The acerola peel extracted with the protease/peptidase enzyme showed the best result of total phenolics, equivalent to 45.46 mg GA/g DW, as well as rutin with the highest concentration identified, equivalent to 15737.13 µg/g DW. The results of antioxidant activities showed a significant increase for the FRAP assay with 120.96 µmolTE/g for the methanolic extract and 1547.00 µmolTE/g for the extract with the protease enzyme; the same occurred in the DPPH assay with 22.02 µmolTE/g to 243.93 µmolTE/g and the ABTS assay with 9.17 µmolTE/g to 211.96 µmolTE/g. The phenolic class that stood out the most was flavonol followed by flavanone, with emphasis on naringenin with the highest concentration in the methanolic extract of acerola seed, equivalent to 1347.50 µg/g DW, thus proving the importance of enzymatic extraction in agro-industrial residues and possible application in pharmaceutical and food industries.

Keywords: enzymatic treatment; chemometric analysis; phytochemical composition; mass spectrometry.

Practical Application: No research work has been published on this topic as yet focusing on enzymatically treated different berry residues, contributing significantly to the generation of new data in relation to the identification of phenolic compounds. The enzymatic treatment of agroindustrial waste is a cleaner alternative technology compared to treatments involving organic solvents, benefiting extracts with high concentrations of bioactive compounds. The enzymatic extract of acerola peel using the protease/peptidase enzyme can be viable for applications in the food industry.

1 Introduction

Avocado (*Persea americana*) belonging to the family Lauraceae is considered a fruit of great commercial importance in the world, due to its high nutritional content and beneficial effects on health, being mainly related to the source of nutrients soluble in lipids or phytochemicals (Araújo et al., 2018). Avocado is grown in almost all Brazilian states, mainly in the states of São Paulo, Minas Gerais and Paraná (Instituto Brasileiro de Geografia e Estatística, 2020). According to the Brazilian Institute of Geography and Statistics, in 2020, more than 266,784 tons of avocados were produced in Brazil (Instituto Brasileiro de Geografia e Estatística, 2020). In addition, avocado has a wide variety of species found in different parts of the national territory. In 2019, 51,109.81 tons of avocados were sold at CEAGESP and the main varieties were: Fortuna (33.46%), Quintal (22.94%), Breda (13.82%), Geada (12.63%) and Margarida (11.43%) (Companhia de Entrepostos e Armazéns Gerais de São Paulo, 2019). Avocado is widely consumed *in natura* and used in the manufacture of food and cosmetics, generating a huge amount of waste (peel, seed and pulp remains), mainly on an industrial scale, resulting in 21% to 30% of the fruit as solid waste (López-Cobo et al., 2016).

The avocado processing industries generate a significant amount of by-products, such as the seeds which constitute about 13 to 18% of the fruit part. These avocado seed residues are the main source of starch, antioxidants, natural dyes and phenolic compounds, and can be used for various applications in pharmaceutical and biomedical industries (Tesfaye et al., 2022). In addition, avocado seed residues can be used to produce biofilms from starch in order to add value and be used in low-moisture food coatings (Jiménez et al., 2022).

Avocado is a fruit cultivated worldwide and is well recognized for its nutritional and bioactive composition (Araújo et al., 2018). The nutritional composition of avocado pulp has been reported to have a moisture content varying from 67 to 78%, lipid from 12 to 24%, carbohydrate from 0.8 to 4.8%, protein from 1.0 to 3.0%, ash content from 0.8 to 1.5% and fibre from 1.4 to 3.0% (Cowan & Wolstenholme, 2016). In addition, Vinha et al. (2013) reported the percentage of chemical composition of avocado peel and seed in terms of moisture content (69.13 and 54.45%, for peel and seed, respectively), minerals (1.50 and 1.29%, for

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peel and seed), lipids (2.2 and 14.7%, for peel and seed) and proteins (1.91 and 2.19%, for peel and seed).

Previous studies have emphasized the presence of bioactive compounds in avocado pulp, seed and peel, including fiber, vitamins, minerals, carotenoids, as well as complex polyphenolic compounds such as proanthocyanidins (Araújo et al., 2018). Studies have shown that the avocado peels contains high amounts of bioactive compounds which result in several beneficial properties to human health and hence can be applied in different sectors, including the food sector (Araújo et al., 2018; Bowen et al., 2018). Several therapeutic uses of avocado have been described in folk medicine (Zafar & Sidhu, 2011), such as in treatment of cancer such as breast, skin and leukemia (Lee et al., 2008), anti-inflammatory properties, antioxidant, antidiabetic, antihypertensive (Ramos-Aguilar et al., 2019), antibacterial activity, antifungal, and antiprotozoal (Dabas et al., 2013).

Studies on the morphology and genetics of the avocado tree have pointed out some similarities and differences between different cultivars (Abasolo et al., 2022). Avocado varieties have different characteristics and chemical composition of the fruits due to different climates, producing areas, cultivation practices and the natural hybridization between avocado cultivars (Tapia-Vargas et al., 2017), which reinforces the search for their chemical characterization. However, no studies were found that evaluated the phytochemical and antioxidant activity of the different Brazilian avocado varieties Margarida, Geada and Breda. In this sense, the objective of this study was to evaluate the contents of phenolic compounds, carotenoids, chlorophyll and antioxidant activity in the different parts of the avocado fruit (peel, pulp and seed) of the different varieties (Margarida, Geada and Breda), in order to obtain better alternatives in the use of this fruit resulting in the benefits to human health.

2 Materials and methods

2.1 Analytical standards and reagents

The reagents ethanol; aluminum chloride; sodium carbonate, potassium phosphate buffer, sodium citrate, ferrous sulfate, Folin-Ciocalteau phenol reagent; FRAP reagent; fluorescein; 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS); 2,2-Azobis (2-Amidino-propane) dichloride (AAPH); 6-hydroxy-2,5,7,8-tetramethylchromo-2-carboxylic acid (Trolox); 2,2-diphenyl-1-picrylhydrazyl radical (DPPH); acetonitrile and formic acid solvents used were 98% HPLC grade purity obtained from Sigma-Aldrich and Fluka Analytica (St. Louis, MO, USA). The water used for the mobile phase was purified using a Milli-Q system (Millipore, São Paulo, Brazil; Direct-Q®3UV). Analytical standards caffeic acid ($C_9H_8O_4$), ferulic acid ($C_{10}H_{10}O_4$), catechin ($C_{15}H_{14}O_6$), epicatechin ($C_{15}H_{14}O_6$), gallic acid ($C_7H_6O_5$), campepherol ($C_{15}H_{10}O_6$), naringenin ($C_{15}H_{12}O_5$), *p*-coumaric acid ($C_9H_8O_3$), vanillin ($C_8H_8O_3$), succinic acid ($C_4H_6O_4$) and ($C_8H_8O_4$) were purchased from Sigma-Aldrich (Saint Louis, MO, USA).

2.2 Samples

Ripe avocado fruits of the Margarida, Breda and Geada varieties were collected from the State Supply Center (Latitude: -10.91610000 and Longitude: -37.06080000) in the state of Aracaju/SE, in the months between October 2017 and July 2018, according to the season of each variety. Subsequently, the fruits were washed, sanitized and manually pulped to separate the parts of interest (peel, pulp and seed). The *in natura* samples of the peels, pulp and seeds of the different varieties of avocado were sliced into small and thin pieces, placed in glass flasks and stored under refrigeration at 7 °C for further physico-chemical evaluation and extracts.

2.3 Preparation of extracts

The preparation of extracts was based on the methodology of Oliveira & Furlong (2008) with some modifications. 2 g of sample was extracted with 15 mL of 70% ethanol for 60 min in ultrasound (USC-1400[a], Unique, São Paulo, Brazil) at a frequency of 40 khz, at room temperature (25 ± 3 °C). Later, the extracts were centrifuged at 14000 rpm for 10 min using a centrifuge (Eppendorf, Centrifuge 5810R, Germany) and the supernatant was dried in an oven at 35 °C until the solvent was completely evaporated and resuspended in 70% ethanol with the necessary volume to reach an extract concentration of 100 mg/mL. The extracts obtained were collected in a glass vial and stored at -18 °C until the time of performing all the analyses.

2.4 Total carotenoids and total chlorophylls contents

The determination of total carotenoids and chlorophylls was based on the methodology presented by Lichtenthaler (1987). 2 g of the sample was transferred to a mortar, 0.2 g of calcium carbonate was added and 7 mL of 80% acetone and homogenized. The extract was filtered and transferred to a 25 mL amber volumetric flask. The filter paper residue was washed twice with 80% acetone. Later the flask was calibrated with the same solvent. Analyses were performed in triplicate. The chlorophyll content was estimated from the reading of the filtered extract in a spectrophotometer at 646.8 and 663.2 nm to determine the contents of chlorophyll a (Ca) and chlorophyll b (Cb), respectively, according to the following Equations 1-3:

$$Chlorophyll\ a\ (Ca) = 12.25\ x\ A_{663.2} - 2.79\ x\ A_{646.8} \quad (1)$$

$$Chlorophyll\ b\ (Cb) = 21.50\ x\ A_{646.8} - 5.10\ x\ A_{663.2} \quad (2)$$

$$Chlorophyll\ T\ (mg/g) = 7.15\ x\ A_{663.2} + 18.71\ x\ A_{646.8} \quad (3)$$

Where: A = absorbance.

To determine the carotenoid content, the reading of the filtered extract was performed in a spectrophotometer (Molecular Devices, USA; SpectraMax M2) at 646.8 and 663.2 and 470 nm. The concentration was estimated according to Equation 4:

$$Carotenoids\ (mg/g) = \left[1000\ x\ A_{470} - (1.82\ x\ Ca - 104.96\ x\ Cb)\right]/198 \quad (4)$$

2.5 Total phenolics content

The total phenolic content was determined according to the methodology proposed by Shori & Baba (2014). 1 mL of the extracts were transferred to test tubes, and 1 mL of 95% ethanol,

5 mL of distilled water and 0.5 mL of 1 N Folin-Ciocalteu reagent were added and homogenized. The mixtures were kept in a dark chamber for 60 min and subsequently homogenized. The absorbance reading was measured using a spectrophotometer (Molecular Devices, USA; SpectraMax M2) set at a wavelength of 725 nm and the results were expressed in terms of mg GAE/100 g of sample using a standard curve prepared by using gallic acid as standard.

2.6 Total flavonoids content

The total flavonoids content was determined using the methodology proposed by Moo-Huchin et al. (2015). 1 mL of the extract was mixed with 4 mL of deionized water and 300 µL of 5% $NaNO_2$ for 5 min. Later, 300 µL of 20 mg/mL $AlCl_3$ methanolic solution was added. The mixtures were kept in a dark chamber for 30 min. The absorbance reading was determined using the SpectraMax M2 spectrophotometer (Molecular Devices) at 415 nm and the results were expressed in mg of QE/100 g using a standard curve of quercetin.

2.7 Antioxidant activity

ABTS assay

Free radical ABTS was captured following the methodology proposed by Moo-Huchin et al. (2015). 30 µL avocado extract was mixed with 2970 µL of ABTS solution. The mixture was incubated at room temperature under a dark environment for 6 min and then homogenized. The absorbance was measured at a wavelength of 734 nm. The results were expressed in µmol TE/100 g of sample using a standard curve of Trolox.

FRAP assay

The FRAP assay was performed according to the methodology of Thaipong et al. (2006). Avocado pulp, peel and seed extracts (150 µL) were mixed with 2850 µL of FRAP solution. After 30 min of incubation in the dark, the samples were homogenized. The absorbance was measured at 593 nm using a spectrophotometer (Molecular Devices, Sunnyvale, CA, USA; SpectraMax M2). Antioxidant activity was calculated using a standard curve prepared of Trolox (20-800 µmol) and results expressed in µmol TE/g of sample.

ORAC assay

The ORAC assay was performed according to the methodology proposed by Andrade et al. (2018), with modifications. The decrease in fluorescence was kinetically monitored every 10 min for 24 h in a spectrophotometer (Molecular Devices, USA; SpectraMax M2) using an excitation filter of 485 nm and emission of 520 nm. The antioxidant activity was calculated using a standard curve prepared of Trolox (0-50 mmol.L^{-1}) and results expressed in µmol TE/g of sample.

2.8 Identification and quantification of phenolic compounds using UHPLC-MS system

The identification and quantification of phenolic compounds were performed following the methodology proposed by Andrade et al. (2017). A UPLC Acquity Class H (Waters) system was used which was coupled with PDA detector and simple quadrupole mass spectrometer (QDa). SIM (Selected Ion Monitoring) mode was used to monitor ions, with electrospray ionization in negative mode. The column used was Ascentis Phenyl (15 cm x 4.6 mm, 5 µm; Supelco analitical). The mobile phase consisted of solution A (deionized water with 0.1% formic acid) and solution B (acetonitrile with 0.1% formic acid) with a flow rate of 0.35 mL/min, at a temperature of 40 °C and injection volume was of 5 µL. The elution was performed in gradient mode, according to the following events: 0-15 min, 100% A; 15-25 min, 75% A; 25-35 min, 60% A; 35-45 min, 50% A; 45-55, 30% A; 55-60 min, 0% A. The standard calibration curve was prepared covering the concentration ranges (0.02-1 mg.mL^{-1}). All analyses were performed in triplicate.

2.9 Statistical analysis

The comparison between mean values of the analytical results was evaluated by SAS software version 9.1, through analysis of variance (ANOVA) using the Tukey test at 95%. The Multivariate statistical analysis and Pearson's correlation were performed using SAS software version 9.1.

3 Results and discussion

3.1 Carotenoids and chlorophyll

The contents of total carotenoids and total chlorophyll presents in avocado extracts of the varieties Margarida, Geada and Breda, presented in Table 1, revealed higher concentrations of carotenoids (5.24 ± 0.57 to 5.92 ± 0.21 mg/g β-carotene) and chlorophyll (9.43 ± 1.08 to 11.01 ± 0.10 mg/g) contents in the peels, with no significant difference between varieties, followed by pulps, in which the highest concentration of carotenoids (4.79 ± 0.33 mg/g β-carotene) and total chlorophyll (8.08 ± 0.32 mg/g) was observed in the Breda variety.

Wang et al. (2010) reported that among the eight avocado varieties evaluated, the total chlorophyll content was nine times higher in the peel than in the seed and pulp, as well as the total carotenoid content, which was eight times higher than in the peel, compared to the other parts of the analyzed fruit, which

Table 1. Content of carotenoids and total chlorophyll of avocado varieties Margarida, Geada and Breda.

Sample/Method	Pulp	Seed	Peel
Total carotenoids (mg/g β-carotene)			
Margarida	4.02 ± 0.03^{bAB}	2.95 ± 0.06^{cB}	5.98 ± 0.05^{aA}
Geada	3.07 ± 0.67^{bB}	2.55 ± 0.32^{bB}	5.24 ± 0.57^{aA}
Breda	4.79 ± 0.33^{bA}	3.96 ± 0.52^{bA}	5.92 ± 0.21^{aA}
Total chlorophyll (mg/g)			
Margarida	6.09 ± 0.14^{bB}	2.92 ± 0.07^{cB}	11.01 ± 0.10^{aA}
Geada	5.38 ± 1.05^{bB}	1.50 ± 0.23^{cB}	9.43 ± 1.08^{aA}
Breda	8.08 ± 0.32^{bA}	6.34 ± 1.30^{bA}	10.74 ± 0.46^{aA}

Means followed by the same lowercase letter on the same line do not differ statistically from each other ($p < 0.05$), applying Tukey's test; means followed by the same capital letter in the same column do not differ statistically from each other for each analysis ($p < 0.05$), applying the Tukey test.

confirms that the peel is the part that had the highest levels of these phytoconstituents.

Santana et al. (2019) demonstrated that avocado peels are rich in carotenoids and chlorophyll and can be used as a source of these compounds in food and other sectors. In addition, carotenoids can act as protectors against photosensitive oxidation (Zegane et al., 2015) and chlorophyll has anticancer, anti-inflammatory, cardioprotective, antimutagenic, antioxidant and antigenotoxic effects (Ramos-Aguilar et al., 2019).

3.2 Total phenolics and flavonoids

The contents of total phenolics present in the samples of peel, pulp and seed of Margarida, Geada and Breda varieties of avocado, presented in Table 2, show the seed is the part of the fruit with the highest content of total phenolics (33.03 to 83.38 mg GAE/g) in relation to peels (23.06 to 55.57 mg GAE/g) and pulps (0.22 to 0.40 mg GAE/g), regardless of the avocado variety, with a significant difference ($p < 0.05$). Wang et al. (2010) studied eight avocado varieties and reported that all varieties had a higher total phenolic content in the seed, confirming the results obtained in this work.

The analysis by the Folin-Ciocalteu method of the total phenolic content was higher in the seed of the Margarida variety (83.38 mg GAE/g); this value being higher than that of the seed of the Hass (60.82 mg GAE/g) and Fuerte varieties (69.12 mg GAE/g), found by Rodríguez-Carpena et al. (2011), which further instigates interest in the Margarida variety, as it is a good source of phenolic compounds compared to the main avocados used for commercialization. Previous studies reported that Hass (51.60 mg GAE/g), Algarvia (704.0 mg/100g) and Hass Brasileira (57.30 mg GAE/g) varieties had a higher content of total phenolics in relation to part of the pulp (Wang et al., 2010; Vinha et al., 2013; Daiuto et al., 2014). Contreras et al. (2021) reported that epicarp avocado extracts had phenolic compounds content of 44.00 mg GAE g^{-1}.

This may be related to the genetic content of the fruit variety that directly influences the presence of these metabolites, as well as temperature, seasonality, water availability, ultraviolet radiation, addition of nutrients, among others (Gobbo-Neto & Lopes, 2007).

The total levels of flavonoids present in avocado extracts ranged from 0.15 ± 0.04 to 6.90 ± 0.23 mg QE/g). It is observed that, among all avocado parts and varieties, the highest levels were found in the peels of the Geada (6.90 mg QE/g) and Breda (4.57 ± 0.85 mg QE/g) varieties. In addition, the seed of the Breda variety (3.38 ± 0.03 mg QE/g) and pulp of the Margarida variety (1.03 ± 0.18 QE/g) also presented better contents, in relation to the other samples. Vinha et al. (2013) reported that the Algarvia variety peel of avocado had a value of 0.44 mg QE/g of flavonoid content.

In general, avocado varieties reveal a phenolic composition significantly richer in simple phenolic compounds than in flavonoids. Moreover, the peel and seed showed a higher content of phenolics and flavonoids in relation to the pulp, which is in agreement with the data described in the literature.

Table 2. Total phenolics, flavonoids contents and antioxidant capacities (ABTS, FRAP and ORAC) of extracts of the avocado varieties Margarida, Geada and Breda.

Sample/Method	Pulp	Seed	Peel
Total phenolics (mg GAE/g)			
Margarida	0.40 ± 0.05^{cA}	83.38 ± 2.46^{aA}	42.84 ± 1.76^{bB}
Geada	0.22 ± 0.01^{cB}	74.04 ± 1.48^{aB}	55.57 ± 1.23^{bA}
Breda	0.39 ± 0.03^{cA}	33.03 ± 0.08^{aC}	23.06 ± 0.77^{bC}
Total flavonoids (mg QE/g)			
Margarida	1.03 ± 0.18^{bA}	2.71 ± 0.08^{aA}	1.31 ± 0.13^{bC}
Geada	0.15 ± 0.04^{cB}	1.46 ± 0.12^{bB}	6.90 ± 0.23^{aA}
Breda	0.23 ± 0.01^{bB}	3.38 ± 0.03^{aA}	4.57 ± 0.85^{aB}
Antioxidant capacities (μmol TE/g of sample)			
ABTS			
Margarida	0.33 ± 0.04^{bB}	4.07 ± 0.21^{aC}	4.45 ± 0.34^{aC}
Geada	0.12 ± 0.00^{cC}	14.90 ± 0.16^{aA}	6.27 ± 0.12^{bB}
Breda	0.62 ± 0.03^{cA}	6.83 ± 0.14^{bB}	11.19 ± 1.72^{aA}
FRAP			
Margarida	21.21 ± 0.69^{cB}	401.45 ± 39.31^{aB}	276.20 ± 12.75^{bB}
Geada	13.28 ± 1.85^{cB}	859.27 ± 5.71^{aA}	545.62 ± 7.10^{bA}
Breda	148.56 ± 7.85^{cA}	322.11 ± 35.55^{aB}	263.45 ± 1.55^{bB}
ORAC			
Margarida	505.59 ± 20.67^{cA}	1739.42 ± 17.58^{aA}	1022.53 ± 32.76^{bB}
Geada	156.58 ± 23.11^{cB}	1768.26 ± 12.31^{aA}	1582.98 ± 17.45^{bA}
Breda	166.29 ± 25.18^{cB}	486.98 ± 8.81^{aB}	353.86 ± 5.93^{bC}

Means followed by the same lowercase letter on the same line do not differ statistically from each other ($p < 0.05$), applying Tukey's test; means followed by the same capital letter in the same column do not differ statistically from each other for each analysis ($p < 0.05$), applying the Tukey test.

3.3 Antioxidant capacity

The antioxidant activities of pulp, seed and peel of different fruit varieties were evaluated by different non-biological assays such as ABTS, FRAP and ORAC (Table 2). The results regarding the antioxidant activity through the radical ABTS, showed that the seeds and peels present greater antioxidant activity in relation to the pulp, with emphasis on the seed of the Geada variety (14.90 µmol TE/g) and peel of the Breda variety (11.19 ± 1.72 µmol TE/g), which had a significant difference ($p < 0.05$). According to Moo-Huchin et al. (2015) evaluating several tropical fruits, the authors revealed that the antioxidant capacity measured through the capture of the free radical ABTS showed that the seeds had the highest values for antioxidant activity. Rodríguez-Carpena et al. (2011) reported that the Hass variety avocado seed has greater antioxidant activity (7.89 µmol TE/g) than the peel (7.40 µmol TE/g) and that in the Fuerte variety, the peel showed the highest activity (18.58 µmol TE/g) in relation to the seed (12.16 µmol TE/g) and pulp (0.78 µmol TE/g).

Regarding the FRAP method, the seeds of the Geada variety (859.28 µmol TE/g) showed superior antioxidant activity, followed by the peel of the Geada variety (545.62 ± 7.10) and the Breda pulp (148.56 µmol TE/g). Soong & Barlow (2004), when carrying out a study with several fruits, emphasize that among the pulp and unconventional parts of consumption, the seed obtained the fourth highest value in their FRAP value (1484.01 µmol TE/g) with values being lower for the pulp while being the lowest reported value (9.6 µmol TE/g) among the analyzed fruits.

The ORAC method presents the seed as the part of the avocado that has the highest antioxidant activity, being the Geada variety (1768.26 µmol TE/g) with the highest potential above the Margarida (1739.42 µmol TE/g) and Breda (486.98 µmol TE/g). Kosińska et al. (2012) reported ORAC values obtained in the seed of 2101.15 µmol TE/g and 3500.20 µmol TE/g in Hass and Shepard varieties, respectively. The peels show expressive values of antioxidant capacity, being the varieties Geada (1582.98 µmol TE/g) and Margarida (1022.53 µmol TE/g) that showed higher activities. Thus, it is evident that the ORAC method showed higher antioxidant activity in relation to the ABTS and FRAP methods. It is worth noting that bioactive compounds from avocado seeds act as hydrogen or electron donors and reducing agents and can play a significant role against oxidative stress, preventing lipid oxidation through a chain-breaking reaction.

3.4 Identification and quantification of bioactive compounds by LC-MS

The contents of individual bioactive compounds present in the peels, pulps and seeds of the avocado varieties (Margarida, Geada and Breda) are shown in Table 3. It was possible to identify twelve compounds in total, which showed variations in quantitative contents, according to the varieties and the parts of the fruit studied, providing important information in the characterization of the phenolic profile, relating to the presence of compounds of interest for application in different areas with a focus on beneficial health effects.

Chlorogenic acid was the principal compound, especially in the avocado peels of the Geada (19.47 ± 0.02 mg/g) and Breda (12.87 ± 0.05 mg/g) varieties. López-Cobo et al. (2016) identified the presence of chlorogenic acid in avocado seeds and peels. The presence of this compound was identified only in the peels and seeds of the analyzed samples while this compound was absent in all the pulps. *In-vivo* and *in-vitro* studies have associated protective actions of chlorogenic acid for several diseases, such as metabolic syndrome, obesity, hyperlipidemia, NAFLD and atherosclerosis, diabetes, hypertension and neuropathic pain (Nwafor et al., 2022).

Table 3. Content of bioactive compounds in the peel, pulp and seed of different avocado varieties by UHPLC-MS.

Phenolics compounds	Margarida (mg/g)			Geada (mg/g)			Breda (mg/g)		
	Pulp	Seed	Peel	Pulp	Seed	Peel	Pulp	Seed	Peel
(+)-catechin	NQ	1.45 ± 0.01^{d}	NQ	NQ	14.31 ± 0.02^{a}	7.12 ± 0.01^{b}	NQ	3.34 ± 0.00^{c}	NQ
Epicatechin	NQ	0.13 ± 0.00^{f}	2.04 ± 0.00^{d}	NQ	0.24 ± 0.00^{e}	11.86 ± 0.00^{a}	NQ	2.29 ± 0.01^{c}	3.43 ± 0.01^{b}
Kaempferol	0.10 ± 0.00^{a}	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Narigenin	NI	NI	NQ	NI	NI	NI	NI	NI	NQ
Succinic acid	8.76 ± 0.00^{e}	0.68 ± 0.00^{h}	17.10 ± 0.01^{a}	11.03 ± 0.01^{d}	6.91 ± 0.02^{f}	13.08 ± 0.01^{c}	0.82 ± 0.01^{h}	2.04 ± 0.00^{g}	13.54 ± 0.01^{b}
Ferulic acid	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	5.62 ± 0.01^{a}
Gallic acid	NI	NI	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Chrologenic acid	NI	1.32 ± 0.01^{a}	3.71 ± 0.00^{c}	NI	0.66 ± 0.00^{e}	19.47 ± 0.02^{a}	NI	12.85 ± 0.03^{b}	12.87 ± 0.05^{b}
Caffeic acid	NQ	NI	NQ	NI	NI	NQ	NQ	NI	NQ
Vanillic acid	NI	0.58 ± 0.00^{d}	0.65 ± 0.00^{c}	NI	1.20 ± 0.00^{b}	0.50 ± 0.00^{e}	NI	1.37 ± 0.00^{a}	0.48 ± 0.00^{e}
p-coumaric acid	0.46 ± 0.00^{a}	NI	NQ	NQ	NQ	0.09 ± 0.00^{d}	0.27 ± 0.00^{b}	NQ	0.18 ± 0.00^{c}
Vanilin	0.27 ± 0.00^{a}	NI	NI	NI	NI	NI	NI	NI	NI
∑ *Phenolics compounds*	9.59 ± 0.00	4.16 ± 0.02	23.50 ± 0.01	11.03 ± 0.01	23.32 ± 0.04	52.12 ± 0.04	1.09 ± 0.01	21.89 ± 0.04	36.12 ± 0.08

Mean values followed by different letters on the same line, indicate significant difference ($p<0.05$) by Tukey's test; NI = Unidentified; NQ = Not Quantified (< Quantification Limit 0.009 ± 0.002 mg/mL).

Succinic acid stands out for being one of the compounds with one of the highest concentrations in analytes (17.10 ± 0.01 mg/g) and for being present in all avocado varieties analyzed, especially in peels. Hurtado-Fernández et al. (2011), when analyzing 13 avocado varieties in Spain, observed that succinic acid had a higher content in the different fruit cultivars compared to other compounds, with the Colin V33 variety being the one with the highest content (171.09 mg/kg of dry sample). Succinic acid is an organic dicarboxylic acid that participates in energy metabolism in all animal and plant cells as an intermediate in the Krebs cycle (Weger et al., 2016). It is a natural antioxidant used in the food industry for the production of beverages such as soft drinks and beer, serving as a flavoring and neutralizing agent, as well as a nutritional supplement (Matrka et al., 2017). In the pharmeutical industry, it is used in the preparation of ulcer-fighting agents and radiation protectors. In addition, they suggested that stimulation of the nervous system occurs which improves the immune system and has a positive impact on the heart (Nagy-Szakal et al., 2018; Kerins et al., 2017).

The flavonoids catechin and epicatechin were present in higher concentrations in the seed (14.31 ± 0.02 mg/g) and peel (7.12 ± 0.01 mg/g) of the Geada variety. The spectrophotometric analysis also revealed that the Geada peel has higher total flavonoids content. Similarly, catechin and epicatechin compounds were abundantly found in different types of avocado varieties from Spain (Hurtado-Fernández et al., 2011). Recent studies have suggested that epicatechin can prevent oxidative damage and endothelial dysfunction, cardiovascular and neuropsychological disease, imparting benefits in human health and longevity (Bernatova, 2018) and catechin has beneficial health effects such as anticancer, anti-inflammatory, antioxidant and antiapoptotic (Zhao et al., 2022).

p-Coumaric acid (0.46 ± 0.00 mg/g) was obtained from fruit pulp of the Margarida variety. López-Cobo et al. (2016) reported higher *p*-coumaric acid content in Hass avocado pulp. Vanillic acid was found in considerable amounts (1.37 ± 0.00 mg/g) in Breda seeds. The presence of vanillic acid was also reported in different varieties such as in Hass avocado seed (6.74 mg/100 g of dry matter) (López-Cobo et al., 2016) and in Sir Prize pulp (6.80 mg/kg dry sample) (Hurtado-Fernández et al., 2011). The compounds vanillin (0.27 ± 0.00 mg/g) and kaempferol (0.10 ± 0.00 mg/g) were identified in the pulp of the Margarida variety. A similar result was reported by Hurtado-Fernández et al. (2011) when analyzing avocado varieties found vanillin only in the pulps of two varieties, namely Colin V33 (0.021 mg/mL) and Sir Prize (0.062 mg/mL); these values being closer to those found in this study. Ferulic acid was identified in the peel of the Breda fruit (5.62 ± 0.01 mg/g).

Vanillin was identified only in the pulp of the Margarida variety, which suggests that this compound may be a phenolic marker, which could be used to differentiate from other varieties. In addition, some compounds such as kaempeferol in the pulp of the Margarida variety and ferulic acid in the peel of the Breda variety had higher concentrations than the other parts of the fruit, which had not quantified it (NQ: < Quantification Limit 0.009 ± 0.002 mg/mL). This difference in the chemical composition of avocado varieties may be related to geographic location, cultivation method, climate, and the natural hybridization between avocado cultivars (Tapia-Vargas et al., 2017).

The compounds naringenin, gallic acid and caffeic acid, identified in parts of the fruit, were below the limit of quantification (< 0.009 mg.mL^{-1}). A similar result was reported by Hurtado-Fernández et al. (2011) not detecting gallic acid in 12 avocado cultivars from Spain. In addition, very low concentrations were observed in the pulp of all the varieties under study, indicating that the most consumed part of this fruit does not have a very strong presence of phenolic compounds which suggests that avocado peel and seed could be used in the development of new food and nutraceutical products, due to their phenolic compounds contents and antioxidant activity that contribute significantly to prevent damage to human health.

3.5 Multivariate statistical analysis

In the principal component analysis (PCA), the avocado extracts of the varieties Breda, Geada and Margarida, allowed to explain 60.38% of the total variation of the data for phenolic characterization (n = 17) and antioxidant activity by the ABTS, FRAP and ORAC methods, (Figures 1A-1B). Phenolic compounds and antioxidant activity are represented by the vectors (Figure 1A) and the sample score chart shows the difference and similarity between the avocado varieties (Figure 1B). In this sense, the closer the regions of the samples, the more similar they are and

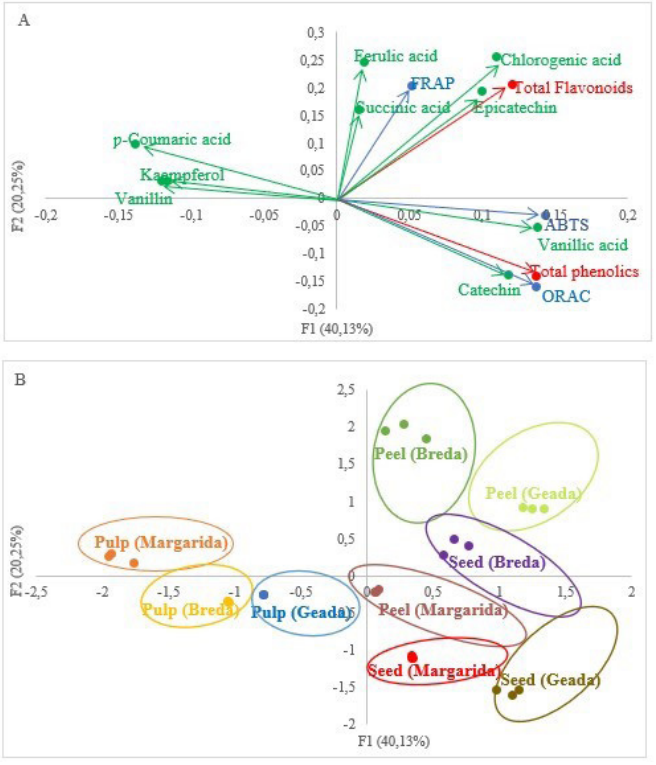

Figure 1. Multiple factors analysis in avocado pulp, peel and seed extracts of Breda, Geada and Margarida varieties. *Phenolic compounds and antioxidant activity are represented by the vectors (Figure 1A) and the sample score chart shows the difference and similarity between the avocado varieties (Figure 1B).

well correlated. The levels of total flavonoids, epicatechin and chlorogenic acid allocated in the first quadrant are represented in greater proportion in the samples of seeds (Breda), peel (Breda) and peel (Geada) and the antioxidant activity by the FRAP method in the samples of seeds (Breda) and peel (Breda). In addition, Peels of Breda, Geada and Margarida varieties stand out for the concentrations of succinic acid while peel of Breda variety was the only one that has ferulic acid in its composition.

Pulp of the Margarida variety was placed in the second quadrant which allows to distinguish it from the other groups, being the only sample that has the presence of vanillin and kaempferol in the composition and high content of p-coumaric acid. The samples seeds of Geada and Margarida varieties stand out for the concentration of total phenolics, catechin and vanillic acid. In addition, they had a greater influence on the antioxidant activity by the ORAC method, standing out from the other samples. The antioxidant activity by the ABTS method stands out in the seeds of Breda variety and of Geada varieties. The main phenolic compounds present in avocado extracts that correlate with antioxidant activity are shown in Figure 2.

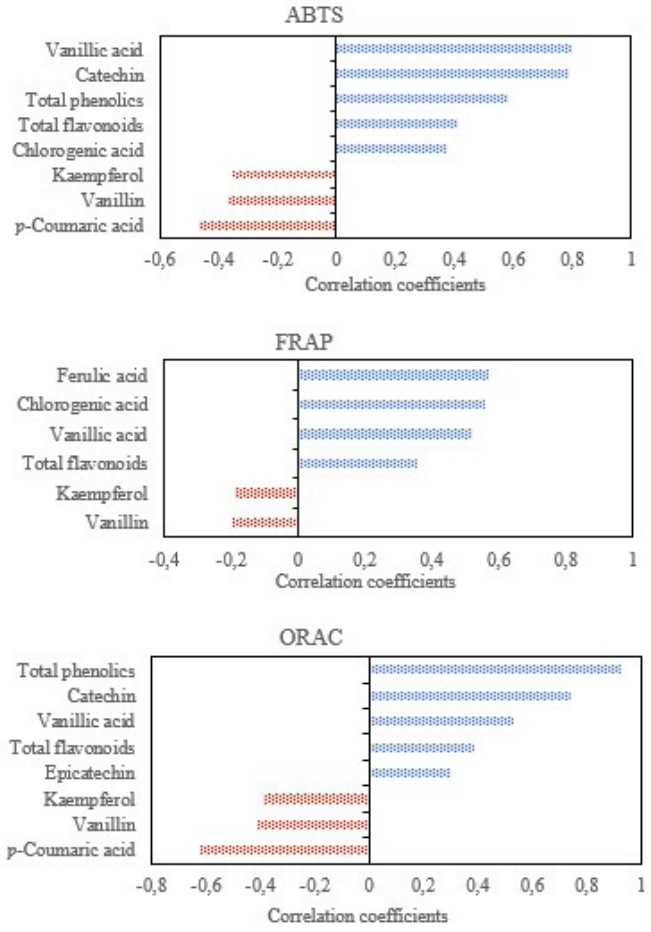

Figure 2. Main compounds identified in avocado pulp, peel and seed extracts of Breda, Geada and Margarida varieties that correlated with antioxidant capacity (ABTS, FRAP and ORAC). Values of Pearson's Correlations coefficients are different with a significance level alpha = 0.05. The blue bars show those compounds showing a positive correlation and the red bars show those with a negative correlation.

In general higher the Pearson correlation coefficient and closer to 1, the greater the potential contribution of phenolics to antioxidant activity, at the 5% significance level. The total phenolics showed a very strong and positive correlation with the antioxidant method ORAC (r = 0.9278) and positive with ABTS (r = 0.5811) while the total flavonoids had a positive correlation with ABTS, FRAP and ORAC values. The main individual phenolic compounds that showed a strong and positive correlation with antioxidant capacity (ABTS) were vanillic acid (r = 0.8030) and catechin (r = 0.7886), ($p \leq 0.05$). The FRAP method has a positive correlation with ferulic acid, chlorogenic acid and vanillic acid and the ORAC method has a strong and positive correlation with catechin (r = 0.7412) and a positive correlation with vanillic acid and epicatechin. Thus, these results suggest a relationship between antioxidant activity which is influenced by phenolic compounds and flavonoids.

4 Conclusion

Avocado seeds showed high levels of phenolics, highlighting the varieties Margarida and Geada (83.38 mg GAE/g and 74.04 mg GAE/g, respectively). The avocado peels of the Breda and Geada varieties obtained the best total flavonoid results. The variety Geada showed higher antioxidant activity by the ABTS, FRAP and ORAC methods. In the analysis of individual phenolic compounds in the LC-MS, it was possible to identify twelve phenolic compounds, where the presence of chlorogenic acid, succinic acid, catechin, epicatechin and vanillic acid were in higher concentrations. In addition, the flavonoids vanillin and kaempferol were reported only in the pulp of the Margarida variety and ferulic acid was found only in the peel of the Breda variety, which could be indicators of compounds only in these varieties. In this way, the influence of avocado varieties on their phenolic composition and their impact on antioxidant activity is well established. From this study it can be said that avocado has high nutritional value with important characteristics being a good source of natural antioxidants and thus has great potential for using both its pulp, peel and seed residues, adding value and contributing to the benefits of human health.

Conflict of interest

All authors declare that they have no conflict of interest.

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