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Bioactive compounds and antioxidants activities in the agro-industrial residues of berries by solvent and enzyme assisted extraction

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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 211.96μmolTE/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

Knowing the problem of disposal of agroindustrial residue, one of the alternatives to reduce the environmental impact they cause is to use these residues and to develop new products, such as natural food additives. Thus, a viable alternative would be to identify the bioactive compounds that are present in the agroindustrial residues of fruits, which can serve as excellent substrates in bioprocesses for obtaining flavonoids, pigments and/or nutraceuticals, as they possess high nutritional values such as proteins, carbohydrates, dietary fibers, etc. (Coman et al., 2020).

Recent studies have shown that fruits are rich in many nutrients and antioxidant compounds, and that most of the time, these constituents are mostly concentrated in the peels and seeds than in the edible portions of the fruit (Bortolotti et al., 2013). Several authors have associated the beneficial effects of the regular consumption of fruits, vegetables and grains to human health, with the presence of antioxidant substances, such as phenolic compounds, ascorbic acid and carotenoids (Sousa et al., 2011). Among these fruits, acerola and strawberry could be cited which stand out as berries: small edible fruits with pleasant flavor, different colors (from red, blue to purple and black) and which have great health benefits (Li et al., 2017). Acerola (*Malpighia emarginata*), well known for its high vitamin C content and which can be properly classified as functional or nutraceutical food, for containing high content of phenolic compounds, anthocyanins, carotenoids and flavonoids (Belwal et al., 2018; Hanamura et al., 2008; Marques et al., 2016), when the fruit is processed for juice production, it generates an average of 27–41% of residue (Gualberto et al., 2022). Strawberry (*Fragaria* spp.) has a rich source of antioxidants (Biswas et al., 2019; Capocasa et al., 2008) phenolic acids and anthocyanins (Aaby et al., 2012; Kelebek & Selli, 2011; Roy et al., 2018).

One of the ways to obtain a higher yield of bioactive compounds from these fruit residues would be to use the enzyme-assisted extraction method, as this method can access compounds that are linked to the plant cell walls, which are more difficult to have access by conventional extraction (Gligor et al., 2019). Practically there is a lack of publications in the literature on the use of enzymes as a means of improving the extraction of bioactives with the residues of acerola and strawberry. Thus, the objective of this work was to compare the performance of

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enzymatic and solvents treatments so as to extract the bioactive compounds and determine phenolic and flavonoid compounds along with the antioxidant and antibacterial activities present in the extracts.

2 Materials and methods

2.1 Material and enzymes

The acerola and strawberry residues were supplied in the first quarter of 2019 by the company, Pomar Brasil, which is located at Travessa das Margaridas, 142 - Distrito Industrial de Aracaju - District Inácio Barbosa, Aracaju/SE, which have already been separated into acerola peel, acerola seed and strawberry residue. Later these residues were kept under refrigeration in the Laboratory of Flavor & Chromatographic Analysis to dry these residues, in a forced air circulation oven at 40 °C until constant weight was obtained, when crushed and the powders were stored in an amber flask at room temperature (25 °C) until analysis. The enzymes protease/peptidase (Flavourzyme TM 1000L, 1205 LPAU/g) from *Aspergillus oryzae* and α-amylase (BAN® 480L, 530 KNU-B/g) from *Bacillus amylolique faciens* were obtained from Novozymes Latin America Ltda (Barigui, Paraná, Brasil), preserved according to manufacturer.

2.2 Fourier transform infrared spectroscopy (FTIR)

The spectra of attenuated total reflection in the infrared with Fourier transform (ATR-FTIR) were obtained in a Cary 630 FTIR spectrometer (Agilent Technologies, Malaysia). The spectral range used was from 650 to 4000 cm^{-1}, with 4 cm^{-1} resolution, and 256 scans for all samples. First, the sample was spread over the surface of the ATR crystal, then the spectrum was obtained.

2.3 Bioactive compounds

Extraction of organic acids, carotenoids and tannins

The organic acids were extracted using 1 g of the powdered residue and diluted in 9 mL of monobasic phosphate (0.01M), acidified with phosphoric acid at pH 2.5, and acetonitrile (99:1, v/v), according to the method of Lee (1993) Lee (1993), with modifications. This mixture was centrifuged at 18,514 x g (12000 rpm in a F34-6-38 rotor, Model 5810R centrifuge, Eppendorf Centrifuge, Germany) for 15 min at 20°C and then filtered with a 0.45μm cellulose membrane filter (Merck Millipores, Barueri, SP, Brazil). Carotenoid extraction was performed by dissolving 1 g of the dry residue in 5 mL of acetone, and later it was placed in an ultrasound sonicator (exclusive model USC-1400A) at a frequency of 40 KHz, 25 °C, for 30 min (Gomes et al., 2018). The extract was centrifuged (Eppendorf Centrifuge, 5810 R) at 18,514 x g for 15 min at 20 °C and then filtered the supernatant using a 0.45 μm cellulose membrane filter (Merck Millipores, Barueri, SP, Brazil). For the extraction of tannins, 0.5 g of the sample was used with 20 mL of 80% methanol shaken in a shaker (SOLAB, Brazil, SL 222) at 150 rpm at 25 °C for 10 min, was done according to the methodology described by Rhazi et al. (2019).

Solvent assisted extraction

The solvent extraction was done according to the methodology reported by Rezende et al. (2017) and Araujo et al. (2019), with some modifications. Sample (2.31 g) diluted in 20 mL of the solvent (ethanol and methanol 46.49% acidified with 2N HCl at pH 2) was weighed and incubated in a shaker (SL 222 / Solab) at 50 °C for 2 h at 100 rpm. Later the mixture was centrifuged at 12,857 x g (10,000 rpm) at 4°C for 10 min.; the supernatant was filtered on Whatman No. 2 filter paper and evaporated at 40 °C on a rotary evaporator (Quimis, Q344B). The extracts were resuspended in deionized water acidified to pH 2 (HCl 2N), and kept in a freezer (-18 °C) until further analysis.

Enzyme assisted extraction

Enzymatic treatment was followed by recommendations made by Gomes et al. (2014), Miron et al. (2013), Xu et al. (2014), with modifications. Four tests were performed using the protease/peptidase and α-amylase enzymes and their combinations, as shown in Table 1. From this outside, 2.31 g of the sample diluted in 20 mL of the potassium phosphate buffer (100 μM at pH6) and the enzyme, incubated in a shaker (SL 222/Solab) for 2 h at 100 rpm and 50 °C. After incubation, the extracts were transferred to an ice bath, for rapid cooling and inactivation of the enzymes. Later these were centrifuged at 12,857 x g (10,000 rpm) at 4 °C for 10 min; the supernatant was filtered on Whatman N° 2 filter paper and stored in a freezer (-18 °C) until further analysis. Extracts with ethanolic and methanolic extraction (Section Solvent assisted extraction) were denominated as tests 5 and 6, respectively.

Determination of organic acids

The organic acids in the extracts were determined using High Performance Liquid Chromatography (Shimadzu) equipped with a diode array detector (SPD-M20A), quaternary pump (LC-20AT), auto-injector (SIL-20), column oven (CTO-20), CBM-20 system controller and were identified and quantified according to the methodology proposed by Lee (1993), with modifications. The column used was a Shimadzu VP-ODS C18 (250 cm x 4.6 mm, 5 μm) operating at a flow rate of 1 mL/min and a temperature of 40 °C. The elution was in isocratic mode, with the mobile phase as a mixture of monobasic sodium phosphate (0.01M) pH = 2.5 acidified with phosphoric acid and acetonitrile (99:1) for 30 min and the injection volume was 5μL and for quantification, a calibration curve was prepared (Table SM.1 in Supplementary Material).

Table 1. Conditions of enzyme treatment as related to assays.

Assays	Buffer (mL)	Protease/peptidase (μL)	α-Amylase (μL)
1	10	0	100
2	10	100	0
3	10	50	50
4	10	0	0

Activity: Protease/peptidase - 1205 LPAU/g and α-Amylase - 530 KNU-B/g.

Determination of carotenoids

The carotenoids in the obtained extracts were determined using an Ultra-Fast Liquid Chromatograph (Shimadzu) equipped with a diode array detector (SPD-M20A), two pumps (LC-20AT), auto-injector (SIL-20), column (CTO-20), CBM-20 system controller and were identified and quantified according to the methodology reported by Gomes et al. (2018), with modifications. The column used was a Fenomenex Kinetex C18 (250 cm × 4.6 mm, 5 µm) operating at a flow rate of 1 mL/min and a temperature of 30 °C. The elution was in isocratic mode, with the mobile phase methanol / ethyl acetate / acetonitrile (50:40:10) for 20 min and the injection volume was 10µL and a calibration curve was prepared for quantification (Table SM.1 in Supplementary Material).

Determination of condensed and hydrolysable tannins

The determination of the total content of hydrolyzable and condensed tannins was done according to the methodology described by Rhazi et al. (2019). The absorbances was read on a spectrophotometer (Molecular Devices, Sunnyvale, CA, USA; SpectraMax M2) at 550 nm and 500 nm, for hydrolyzable and condensed tannins, respectively, and the results were expressed in milligrams of tannic acid equivalent (TAE) per gram of dry sample and milligrams equivalent of quercetin (QE) per gram of dry sample, respectively.

Determination of phenolic compounds

Total phenolics content

To determine the content of total phenolic compounds by the spectrophotometric method, Folin-Ciocalteu phenol reagent was used, methodology proposed by Singleton & Rossi (1965) adapted by Rezende et al. (2017). The content was calculated using a standard curve prepared with aqueous solutions of gallic acid (0.025 – 1.100 mg/mL). The result was expressed in mg equivalent of gallic acid/100 g of dry sample.

Identification and quantification of phenolics compounds using UPLC-QDa-MS system

For the determination of total phenolics in the extracts by UPLC, chromatographic conditions validated by the Laboratory of Flavor /UFS were used, using a UPLC Acquity Class H (Waters) liquid chromatograph coupled to a PDA detector together with a simple quadrupole mass spectrometer (QDa). The mobile phase used was solution A (deionized water with 0.1% formic acid) and solution B (acetonitrile with 0.1% formic acid), with an Ascentis Phenyl column (15cm x 4.6mm, 5µm; Supelco analytical), at a flow rate of 0.35 mL/min, with a temperature of 40 °C and an injection volume of 5 µL. The method used was the gradient: 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 SIM (Selected Ion Monitoring) mode was used in the mass spectrometer, comparing the standard ion with the extract ion.Quantitation was performed from calibration curves prepared from each standard, as shown in the table (Table SM.2 in Supplementary Material), with according Andrade et al. (2017). The result was expressed in µg/g of dry sample (µg/g DW).

Determination of total flavonoids

To determine the content of total flavonoid compounds by the spectrophotometric method, the method used was by González-Aguilar et al. (2007). A standard quercetin curve (0.025-0.6 mg/mL) was used to calculate the total flavonoid content and the result was expressed in mg equivalent of quercetin (QE)/100 g of dry sample.

Determination of antioxidant capacity

ABTS assay

The evaluation of the ABTS radical cation elimination activity was determined using the methodology described by Miller et al. (1993). Antioxidant activity was calculated using a standard Trolox curve (0.0–0.5 mg/mL) and the result was expressed in µM equivalent Trolox (TE)/g of sample.

DPPH assay

The antioxidant activity of DPPH (2,2-diphenyl-1-picryl-hydrazyl) was calculated using a standard curve prepared from trolox (0-0.3 mg/mL) and the result was expressed in µM TE/g of dry sample, as described by Brand-Williams et al. (1995).

FRAP assay

The method used was by Thaipong et al. (2006) to test the ferric-reducing antioxidant power (FRAP). The antioxidant activity was calculated from a standard curve prepared by using trolox (0.00 - 0.16 mg/mL) and the result was expressed in µM TE/g of dry sample.

ORAC test

For the oxygen radical absorption capacity test (ORAC), the methodology applied was of Albarici et al. (2009). Antioxidant activity was calculated from a standard trolox curve (0.001-0.050 mg/mL) and expressed in µM TE/g of dry sample.

2.4 Antibacterial activity

Antibacterial activity was evaluated in each of the extracts against strains of Gram (+) bacteria: *Bacillus cereus* (CBAM 0524) and *Staphylococcus aureus* (CBAM 0629), and Gram (-): *Klebsiella pneumoniae* (CBAM 0462), *Pseudomonas aureginosa* (CBAM 0024), *Escherichia coli* (CBAM 0472), *Proteus mirabilis* (CBAM 0144), *Salmonella typhi* (CBAM 0018), *Serratia marcescens* (CBAM 0094) and *Shigella sonnei* (CBAM 0033). The bacterial suspensions met the turbidity of 0.50 McFarland standards and were inoculated on Mueller-Hinton agar. The antibiotics amoxylin (25 mg/mL) and neomycin sulfate (5 mg/g) were used as positive controls and sterile distilled water as a negative control. The method used was disk diffusion for the antibacterial activity of the samples at concentrations of 58 and 115 mg/mL, as described by Bardakci et al. (2019), with some modifications.

2.5 Statistical analysis

The results obtained were analyzed and expressed as mean ± standard deviation values, using the SAS software (SAS Institute, Cary, NC) Version 9.1.3. 2021. Analysis of variance (ANOVA) was used to determine significant differences ($p < 0.05$) between the samples and the differences between the means was detected by the Tukey. The evaluation by chemometric tools, was using the R software version 4.0.2 (R Core Team, 2020) with the FactoMineR (Lê et al., 2008) and factoextra (Kassambara & Mundt, 2020) packages. Multiple Factor Analysis (MFA) and Hierarchical Cluster Analysis (HCA) evaluated whether the test extracts had an effect on phenolic compounds and antioxidant activities. HCA was performed based on the result of MFA using Euclidean distance and Ward's linkage method. All samples were analyzed in triplicate.

3 Results and discussion

3.1 Fourier-Transform infrared spectroscopy (FTIR)

For a greater understanding of the residues studied, spectroscopy was done using a vibrational technique widely used to study the chemical composition. As can be seen in Figure 1, between the main bands, there was a similarity between the spectral profiles of the residues.

The bands located between 3000-3500 cm^{-1} (peak 3287cm^{-1} specific to the three residues and peak 3031cm^{-1} specific to the strawberry residue) are described by the elongation OH– (Sabino et al., 2020), and the absorption band that appeared in the region of 3500 cm^{-1} due to the presence of functional groups such as alcohol, carboxylic acids, nitrates and carbohydrates. The OH group plays an important role in antidiabetics, antioxidants and antibacterial activities (Patle et al., 2020). The three residuals have a band of 2923 cm^{-1}, which was observed by Sabino et al. (2020) at peak 2930 cm^{-1}, which would be CH extension, typical for carotenoids (Ursache et al., 2018). The stretching vibration

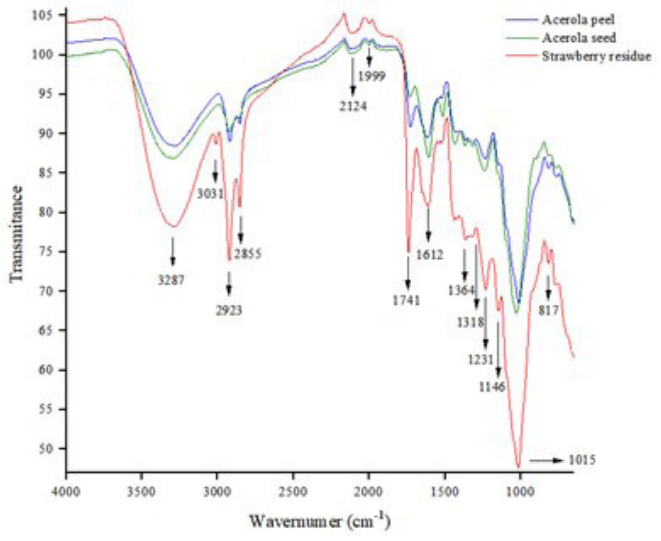

Figure 1. Infrared spectra of strawberry residue, acerola peel and acerola seed.

C=O, acquired in 1748 cm^{-1}, is due to the extraction efficiency of different phytochemical species, such as gallic acid, quercetin, rutin and tannic acid (Patle et al., 2020).

3.2 Bioactive compounds

The bioactive compounds are widely studied in fruits, due to their biological effects after ingestion, since they associate the reduction of cancer risk and degenerative diseases (Coman et al., 2020; Saura-Calixto, 2011; Shahidi & Ambigaipalan, 2015). Table 2 presents the results of bioactive compounds: carotenoids, organic acids and condensed and hydrolysable tannins. Among the injected carotenoids, only β-carotene was identified in the three residues, with higher concentration in the acerola peel (5.12mg of β-carotene/g of dry sample), followed by the acerola seed (3.24mg/g) and finally the strawberry residue with traces of β-carotene, equivalent to 0.15 mg/g. The organic acids identified were tartaric, quinic, citric, L-ascorbic and 3,4-dihydroxybenzoic acids. Among these, the main bioactive organic acids that predominate in the fruits are the quinic, citric and ascorbic acid (Nemzer et al., 2018). L-ascorbic acid was identified in the three residues, with higher concentration in acerola seed, which was equivalent to 26.83 mg/g DW, acerola is well known for its high content of ascorbic acid which acts against free radicals and peroxidative damage (Nogueira et al., 2019). The strawberry residue was the one that presented the main acids identified, with concentrations equivalent to 10.33 mg/g of tartaric acid, 10.57 mg/g of quinic acid, 3.16 mg/g of citric acid and 0.86 mg/g of L-ascorbic acid.

Tannins are polyphenol compounds divided into hydrolysable and condensed tannins. In the residues, hydrolysable tannins were found in higher concentrations and consist of esters of gallic acid and glycosides of ellagic acid (Grasel et al., 2016). The acerola peel stood out with higher concentration in both, equivalent to 28.21 mg/g of hydrolysable tannins and 3.86 mg/g of condensed tannins.

The phenolic compounds can replace preservatives and be considered as functional food ingredients due to their antioxidant and antibacterial activities and present several health benefits as anti-inflammatory activities (Gligor et al., 2019), thus, this subject of the study brings enrichment to the residues, which a priori are discarded in the environment. Firstly, effects bioactive compounds and antioxidant activities of the 6 assays in the 3 fruit residues (Strawberry Residue - SR, Acerola Peel - PA and Acerola Seed - SA) are presented in Figure 2A (detailed Table SM.3 in Supplementary Material). Enzymatic treatment showed an increase in all samples in antioxidant activity except for ORAC and phenolic and flavonoid compounds. The control (test 4), equivalent to the extract with buffer without the enzymes, had results, much lower than the results with the enzymes, showing the better yield of the enzymatic treatment.

The phenolic compounds that had the highest concentration were the acerola peel of test 2, with the enzyme protease/peptidase, equivalent to 45.46mg of gallic acid/g DW, later the samples such as acerola peel in tests 1 and 5. The AS from test 5 showed the higher concentration of total flavonoids. Among all the antioxidant assays tested, FRAP was the one with the highest

Table 2. Bioactive Compounds (Carotenoids, organic acids and tannins) present in the residues of Berries.

Compounds	Contents ($mg.g^{-1}$ of sample dried)		
	Strawberry residue (SR)	Acerola peel (AP)	Acerola seed (AS)
Carotenoids			
Canthaxanthin	N.D.	N.D.	N.D.
β-carotene	0.15 ± 0.01^{c}	5.12 ± 0.06^{a}	3.24 ± 0.02^{b}
Lycopene	N.D.	N.D.	N.D.
Organics Acids			
Tartaric Acid	10.33 ± 0.27	N.D.	N.D.
Malic Acid	N.D.	N.D.	N.D.
Succinic Acid	N.D.	N.D.	N.D.
Quinic Acid	10.57 ± 0.12	N.D.	N.D.
Citric Acid	3.16 ± 0.02	N.D.	N.D.
L-ascorbic Acid	0.86 ± 0.03^{c}	3.06 ± 0.04^{b}	26.83 ± 0.18^{a}
3,4-Dihydroxybenzoic Acid	N.D.	N.D.	0.23 ± 0.01
Fumaric Acid	N.D.	N.D.	N.D.
Tannins			
Condensed	2.12 ± 0.01^{c}	3.86 ± 0.05^{a}	2.90 ± 0.05^{b}
Hydrolyzable	23.55 ± 0.10^{b}	28.21 ± 0.66^{a}	19.76 ± 0.45^{c}

N.D. (Not Detected). Means followed by the same letter in lines, do not differ significantly by Tukey's test at 5% probability.

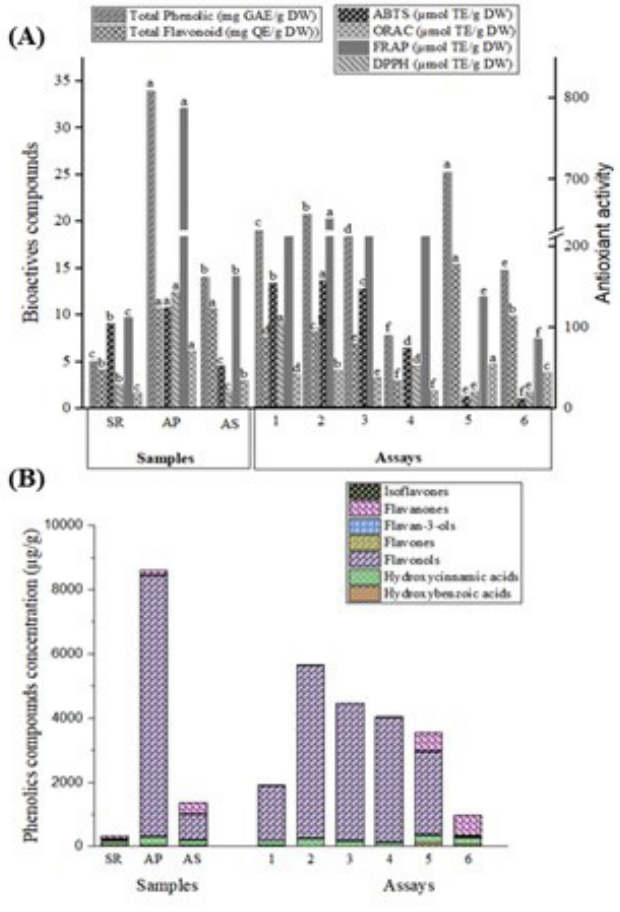

Figure 2. (A) Effect of different enzymatic and solvent treatments on concentrations of bioactive compounds (Total Phenolics and Flavonoids) and antioxidant activities (ABTS, DPPH, FRAP and ORAC); (B) Effect of different enzymes and solvents treatments on concentrations of phenolic compounds identified by UPLC, as classified by their subclasses.

concentrations of the samples, with emphasis on the extract of the acerola peel from test 3 (combination of the two enzymes) that obtained the highest concentration, equivalent to 1547 μmol of Trolox/g DW. DPPH and ABTS assays resulted in activities with greater expression in the PA extract of trial 2, equivalent to 243.93 and 211.96 μmol of Trolox/DW, respectively. The capture of the ORAC radical, behaved differently from other antioxidant activities, showing greater activity for alcoholic extracts (5 and 6) than for the enzymatic extracts (1, 2 and 3).

In Figure 2A, we can observe that among all the samples, independent of the assays, the AP obtained significant results ($p < 0.05$) in relation to the samples of AS and SR. The enzymatic assays showed higher activities in relation to the control (assay 4) and the extracts with solvents, proved to be a promising technique to capture some free radicals. Figure 2B shows the effect of phenolic classes, the AP sample and assay 2, had higher concentrations of phenolic compounds identified, the class of flavonols had higher participation among the others, due to the compound rutin which obtained concentration of 15737.13 μg/g DW (see Table SM.5 in Supplementary Material). Nogueira et al., (2019) identified rutin in the acerola residue, with the highest concentration equivalent to 1.43 mg Rutin/g DW, i.e., much lower than this experiment (15.737mg Rutin/g DW).

3.3 Effects of various treatments evaluated by chemometrics tools

Figures 3, 4, 5 and 6 show chemometric tools, called MFA and HCA, to assess the effects of treatments with visualization of the heat map. The number of significant dimensions was selected by the Kaiser criterion (eigenvalues greater than 1), so that the first three dimensions explained 67.9 and 87.6% of the total variance for acerola and strawberry residues, respectively. The HCA technique organized the extracts of the three solvents (methanol, ethanol and buffer) and the four enzymes (alpha-

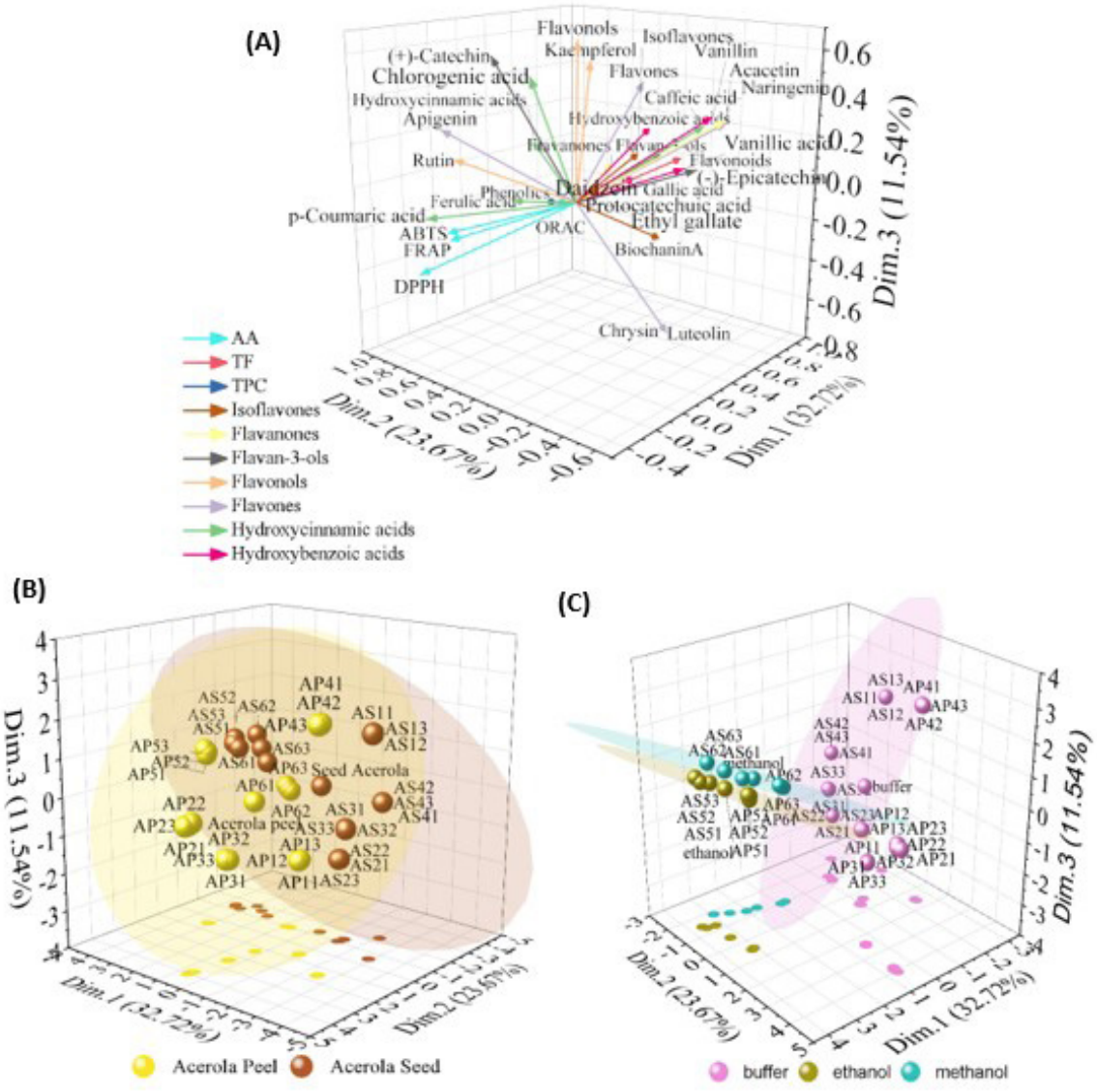

Figure 3. Evaluation by MFA of the enzymatic and non-enzymatic extracts of acerola residues: (A) MFA 3D plot of correlation between the phenolic compounds found and antioxidant activities of peel and seed extracts from acerola; (B) scatter plot of peel and seed extracts from acerola by origin; (C) scatter plot of peel and seed extracts from acerola by extracting solvent.

amylase, protease, alpha-amylase + protease and the control) from acerola residues (Figure 4) and from the strawberry residue (Figure 6) in bunches according to the dissimilarities of the contents of phenolic compounds and antioxidant activities.

According to the MFA shown in Figure 3B, there is a separation between acerola peel (positive second dimension) and acerola seed (negative second dimension), with distinct groups of extraction assays, except the methanol extract of acerola peel (AP6) which was in negative first dimension. A separation was observed in MFA 3D graph for the extracts according to solvent used: the samples extracted with buffer were placed on negative first dimension, while the samples extracted with ethanol and methanol were placed on the positive side (Figure 3C). Samples AP2 (Acerola Peel of Assay 2) and AP3 (Acerola Peel of Assay 3) were in the positive first dimension. Thus, the first dimension was able to explain the variation between samples by origin (peel and seed), and the second dimension distinguished them by the solvent used in the extraction. The contribution of the variables to the discrimination between the samples can be seen in Figure 3A. Total phenolic compounds, individual flavonoids (rutin, apigenin, and (+)-catechin), hydroxycinnamic acids (ferulic acid and *p*-coumaric acid), and antioxidants activities had high correlation with the second dimension. In contrast, total flavonoids, phenolic acids (caffeic acid and vanillin), and individual flavonoids (acacetin and naringenin) were best explained by the first dimension. Observing the Figures 3A and 3B, we can highlight sample AP2, which has a different profile from the others, with greater intensity for the rutin compound, proving what was mentioned above. In HCA (Figure4), all extracts were

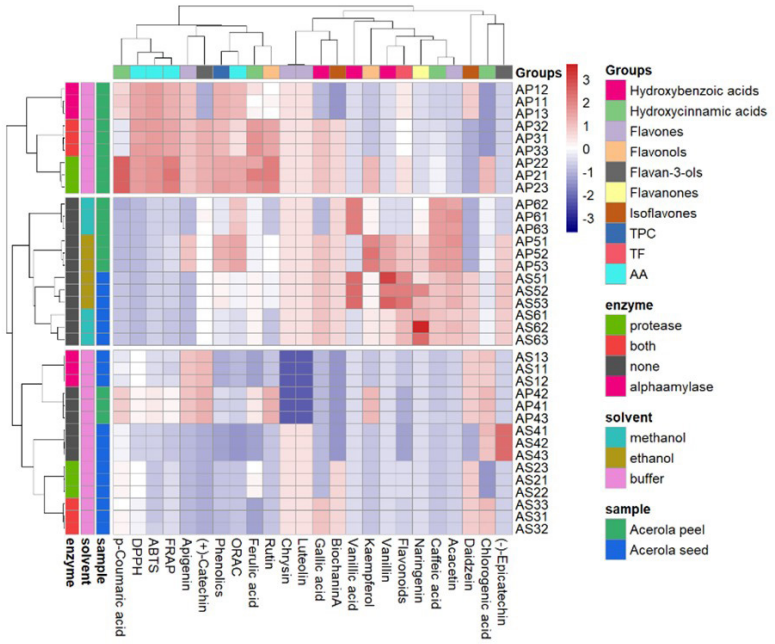

Figure 4. Evaluation by HCA of the enzymatic and non-enzymatic extracts and heat map visualization of dataset of peel and seed extracts from acerola.

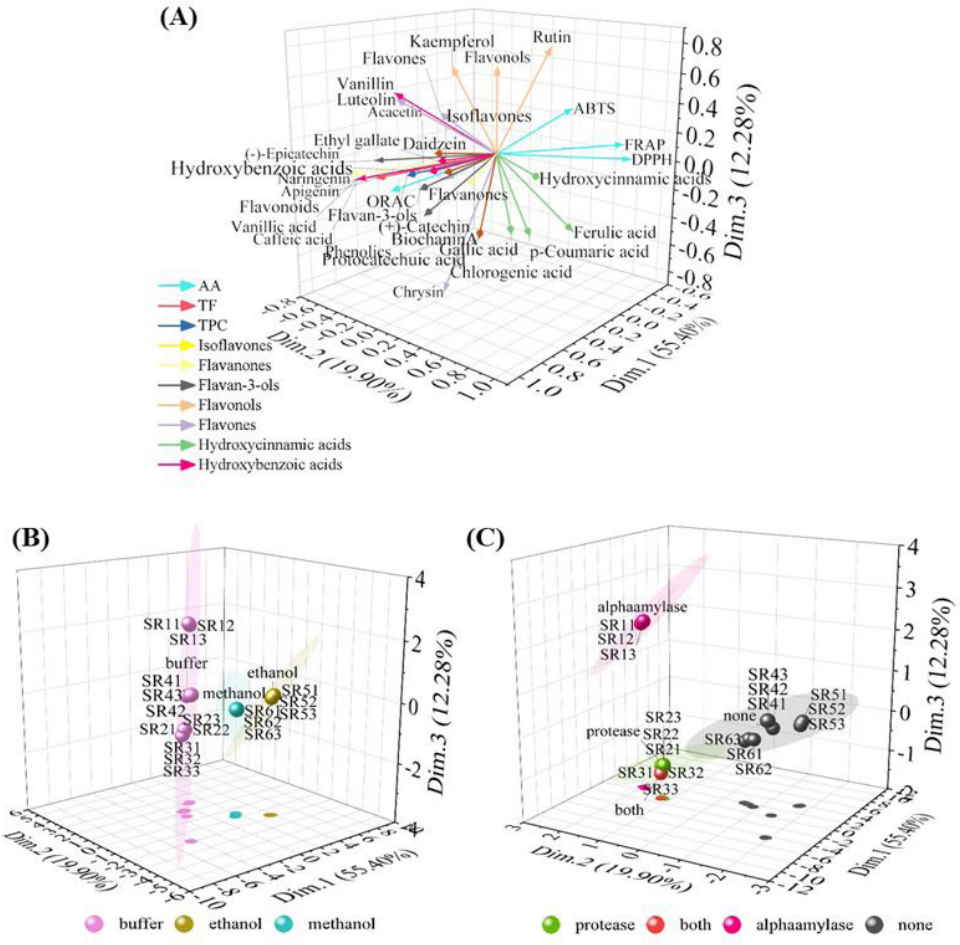

Figure 5. Evaluation by MFA of the enzymatic and non-enzymatic extracts of strawberry residues (A) MFA 3D plot; (B) scatter plot of strawberry residues by extraction solvent, (C) scatter plot of strawberry residues by treatments.

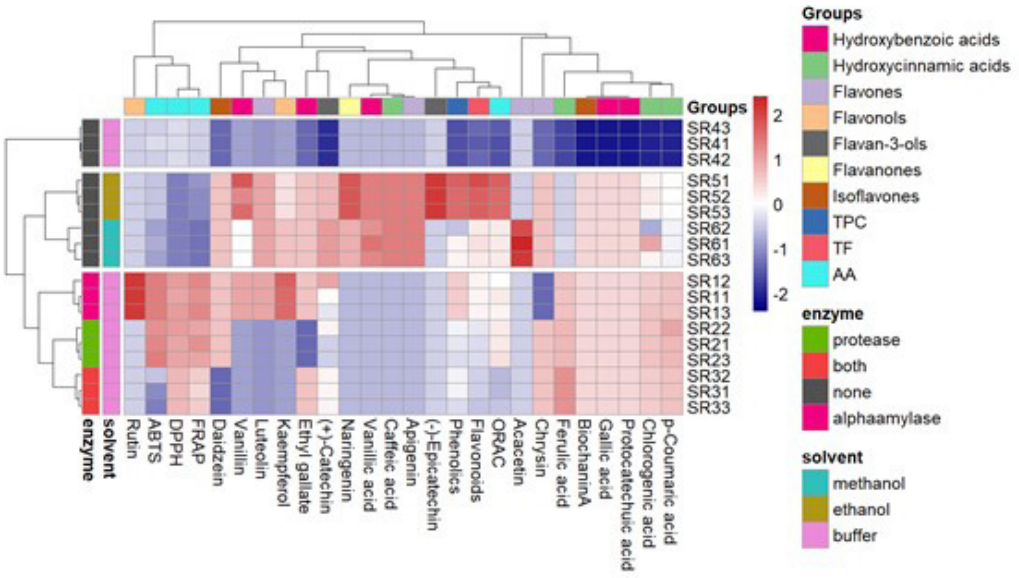

Figure 6. Evaluation by HCA of the enzymatic and non-enzymatic extracts and heat map visualization of dataset of strawberry residue.

grouped into two clusters: the first cluster, the acerola extracts, included the enzymatic extracts of the acerola peel, while the second cluster, with the other extracts, was subdivided into two clusters: one containing alcoholic extracts (methanolic and ethanolic) of the peel and seed acerola, and another for the enzyme extracts from acerola seed and the controls (Assay 4) of the samples.

In MFA 3D scatter plot (Figure 5C), the samples of enzymatic treatments from strawberry residues (SR1, SR2 and SR3) were grouped in the positive side of second dimension, whereas the non-enzymatic treatments samples (SR4, SR5 and SR6) were in the negative side of second dimension. In Figure 5B the first dimension separated the samples from the non-enzymatic treatments into two groups: one group of treatments with solvents (SR5 and SR6) in positive side of first dimension; and another group with only the buffer (SR4) in negative side. In Figure 5A, the first dimension had high correlation with total flavonoids, total phenolic compounds, caffeic acid, vanillic acid, apigenin, naringenin, luteolin, vanillin, gallic acid, biochanin-A, and ORAC. The phenolic acids, chlorogenic, and *p*-coumaric acids, stand out, present in all treatments, except in the control treatment, as shown in Table SM.4 in Supplementary Material. The HCA results of the strawberry residue extracts (Figure 6) were also grouped into two clusters, one with samples from the control treatment, and the second cluster divided into two subclusters: one for the ethanolic and methanolic extracts, and other for the enzymatic extracts, referring to treatments 1, 2 and 3. The SR1 samples showed a high concentration of rutin, kaempferol, luteolin, and vanillin, which contributed to distinguish them from other enzymatic treatments. Finally reviewing all the data reported, we can conclude that the acerola peel was very promising for the extraction of phenolic compounds, both by enzymatic extraction and by extraction with methanol or ethanol.

3.4 Antibacterial activity

As observed in Table SM.6 in Supplementary Material, the most promising activity for the strawberry residue was observed against *Proteus mirabilis*, which presented in all assays, and with the highest inhibition zone in assay 1, of approximately 11 mm (+++), equivalent to the antibiotic amoxiline. The alcoholic extracts showed strong activity against the bacteria *S. Sonnei* and moderate activity against the bacteria *B. cereus, S. aureus, K. pneumoniae, P. aeruginosa* and *S. typhi*. Acerola peel showed stronger activity against *S. aureus* equivalent to 24.2 mm inhibition zone, from test 1, as well as activity against *K. pneumoniae, P. aeruginosa, P. mirabilis* and *S. sonnei* bacteria. The acerola seed assay 6 obtained stronger antibacterial activity against *S. aureus*, with an inhibition zone of 33.2 mm, being the largest inhibition zone found in these experiments, and for this bacterium, all the trials showed inhibition.

4 Conclusion

The enzymatic extraction showed greater bioactivity among the extracts while alcoholic solvents extraction showed also very expressive comparative results. The acerola peel residue was the one that presented the highest phenolics when compared with the strawberry and acerola seed residues. The UPLC data showed that Assay 2, with the enzyme protease/peptidase, presented a higher concentration of phenolic compounds, equivalent to 15737.13 µg/g for acerola peel. The extracts from the six assays confirmed their property to inhibit pathogenic bacteria, mainly *B. cereus, S. aureus, K. pneumoniae, P. aeruginosa, P. mirabilis, S. sonnei* and *S. typhi*.

Conflict of interest

The authors declare no potential conflicts of interest.

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Supplementary Material

Supplementary material accompanies this paper.

Table SM.1 Retention times, calibration equations, correlation coefficients and linear limits for carotenoids (UPLC) and organic acids (HPLC) identified in the strawberry residue, acerola peel and acerola seed.

Table SM.2 UPLC parameters, concentration, retention time, calibration equation and correlation coefficient for phenolic compounds identified in the extracts.

Table SM.3 Phenolic compounds, flavonoids and antioxidant activities of fruit residues and in various antioxidant assays.

Table SM.4 Phenolic compounds, flavonoids and antioxidant activities of residues and assays.

Table SM.5 Phenolic compounds quantitation in extracts of acerola peel, acerola seed and strawberry residue obtained from different tests by the LC-MS system.

Table SM.6 Antimicrobial activity of enzymatic extracts (Assays 1, 2, 3 and 4), Ethanolic (Assay 5) and Methanolic (Assay 6) extracts against pathogenic bacteria.

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