 Phytochemical composition and antioxidant potential of different varieties viz. Flor Branca, Costa Rica and Junco of green unripe acerola (Malpighia emarginata D.C.) fruits

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Abstract

Malpighia emarginata D.C is an important crop of Brazil and significant source of antioxidant active compounds with notable health effects. The purpose of this work was to study three varieties of acerola (Malpighia emarginata D.C) at green unripe stage of maturity from the São Francisco Valley of Northeast Brazil. The fruits were evaluated for physico-chemical composition, bioactive compounds, through spectrophotometric analysis and liquid chromatography, as well as the profile of macro and micro minerals and antioxidant capacity. The fruits presented satisfactory results in physico-chemical quality parameters. HPLC-DAD phenolic profile analysis identified kaempferol-3-glucoside (32.68 mg/100 g), p-coumaric acid (28.87 mg/100 g) and isorhamnetin (11.41 mg/100 g) as the major compounds, mainly in the variety of Flor Branca (FB). The results obtained from the profiles of sugars and organic acids were similar among the varieties. The minerals were excellent sources of the elements of K, Ca, Mg and Mn. Moreover, FB variety represents higher antioxidant capacity in ABTS⁺ (8613.54 μM Trolox/g) and ORAC (2454.42 μM Trolox/g). Thus, the results show the potential to be applied for variety selection and utilization programs based on their specific nutritive characters, contributing to the development of new products.

Keywords: bioactive compounds; minerals; flavanols; antioxidant activity; chemometric evaluation.

Practical Application: The evaluation of different varieties of green unripe acerola fruits demonstrated the existence of the diversity in their chemical constituents, as well as mineral elements. Besides providing more scientific information that contribute to the knowledge of the composition and better use of various acerola cultivars.

1 Introduction

Brazil is known as one of the three main countries in the world where a large production of fruits are cultivated. The region of the São Francisco Valley (SFV), situated in the Northeast Brazil is one of the main production areas of the country (Batista et al., 2018). Scientific evidences suggest that consumption of fruits is associated with the prevention of oxidative stress related disorders since fruits are the source of important bioactive compounds (Amarowicz & Shahidi, 2017). This justifies the search and elucidation of novel plant fruits with bioactive properties, which can be used as an alternative agent for synthetic drugs, which contain health risk factors and high toxicity.

Acerola, pertaining to the family Malpighiaceae, has received considerable attention in recent years because of its excellent nutrients such as ascorbic acid, anthocyanins and other phytochemicals present both in the pulp of the fruit and in the by-products of acerola which contribute to antioxidant capacity (Rezende et al., 2017; Carneiro et al., 2020; Betta et al., 2018). In addition, in vivo biological studies report that acerola extracts have several beneficial effects (Belwal et al., 2018). Currently, Brazil has been highlighting itself as the largest producer and exporter of acerola fruits, competing mainly in the natural plant products sector due to its great relevance in the use of its fruit extracts with functional properties (Rodriguez-Amaya, 2016; Chang et al., 2018) as well as its wide use in the processing of different food products (Cappato et al., 2018a, b; Ribeiro et al., 2019). In Brazil, the Northeast region accounts for a large part of the national production, in particular, in the region of the SFV located in the Northeast of Brazil there has been an expressive and diversified production of acerola due to favorable soil and climate conditions (Souza et al., 2013).

Although it is known that acerola presents an abundant and diversified composition of bioactive substances with beneficial properties, the presence of chemical constituents varies in the fruits which are mainly linked to the conditions of origin, climate, stage of maturity, and other factors. Considering the increased interest in the search for functional foods, this study was aimed to investigate the phytochemical composition of the different functional compounds, as well as the profile of macro and micro minerals and to evaluate the antioxidant potential.

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in three Brazilian varieties (Costa Rica, Flor Branca and Junco) of green unripe acerola.

2 Materials and methods

2.1 Chemicals

All the standards reagents and analytical chemicals were purchased from Sigma-Aldrich (St. Louis, Missouri, United States) or Tedia Ltd (Fairfield, OH-45014, Unites States). Millipore water was obtained from a Milli-Q system with resistivity of 18.2 (MΩ) (Direct-Q 3UV, Millipore, Brazil).

2.2 Sample collection and preparation

Fresh fruits of three varieties, namely CR, FB and JC of commercially cultivated acerola in its green unripe physiological maturity (>75% green skin color) were obtained. These were procured from the Niagro company - Nichirei do Brasil Agricola Ltd. located in the SFV region, Petrolina, Pernambuco, Brazil. The three different varieties of acerola were harvested at the beginning of August 2017, according to records of meteorological observations of the region during the harvest season when the average temperature varied from 29.8 to 28.6 °C, relative humidity from 45.4 to 41.5% and the precipitation of 1.0 mm measured in the Bebedouro experimental field (Petrolina, Brazil, 09°09'S, 40°22'W). Initially, the acerola was analyzed for the firmness and color attributes of the epicarp fruit. Later, the seedless fruits were placed in trays and dehydrated at 55 °C until constant mass and then the dried material was triturated and sieved to obtain particles of 0.5 mm. The samples were packed and stored away from the light before the analysis.

2.3 Determination of quality parameters and physicochemical characterization

Firmness was evaluated separately of 10 randomly selected fruits of each variety using a digital texture analyzer (CT3, Brookfield Engineering Laboratories, USA). The fruits were individually placed on a central platform equipped with a 25 kg load cell and a cylindrical probe (TA3/100) to simulate the force exerted, which was programmed to penetrate 5 mm into the sample at a speed of 1.5 mm/s. The Texture Profile Analysis test was selected, where it was possible to measure the resistance of the pulp to penetration with rupture and the mean values were expressed in Newton (N). The color measurements of the acerola samples were determined by a colorimeter (CHROMA METER, CR 400, Konica Minolta Inseng, Japan). The CIELAB system was used to define the color in a three-dimensional space and data were then reported as average values L, a*, b*, Chroma and Hue.

For the physicochemical characterization the official methods recommended by the AOAC - Association of Official Analytical Chemists (2000) were followed. The determined analyses were: moisture, total soluble solids, the titratable acidity, pH and water activity obtained through a direct reading on an Aqua Lab electronic meter (4 TEV, Aqua Lab, USA).

2.4 Extraction procedure

In order to obtain the extracts, ethanol was used at 46.5% (v/v), acidified to pH 2 using hydrochloric acid (2N) and solid:solvent ratio in the ratio of 1:8.7 g/mL (Rezende et al., 2017). The equipment Ultrasonic Bath (USC-1400A, Unique, São Paulo, Brazil) with an ultrasonic frequency of 40 kHz, at 30 °C for 30 min was used. After extractions, samples from each variety were centrifuged (5810R, Epperdorf AG, Hamburg, Germany) at 6000 x g at 10 °C for 10 min and the supernatant (extract) was collected and stored in a freezer at -18 °C.

2.5 Phytochemical analyses

For the spectrophotometric analysis, the extracts were diluted according to the analyte range of each analysis. The readings were performed on a 96-well microplate spectrophotometer (SpectraMax M2 Molecular Devices, Sunnyvale, CA, USA). All determinations were performed in triplicate.

Total phenolic and total flavonoid contents

The total phenolic content (TPC) was determined using the methodology described by Singleton & Rossi (1965). Absorbance measurement was performed at 765 nm after reacting with the Folin-Ciocalteu phenolic reagent. The TPC in the samples was quantified and expressed in mg gallic acid equivalents (GAE)/g of dry weight (dw). The concentration of flavonoids was determined according to the methodology of Gonzalez-Aguilar et al. (2007). The measurements were done at 415 nm and for the construction of the calibration curve, quercetin was used as the standard and the results were expressed as mg quercetin equivalents (QE)/g of dw.

Carotenoids, total chlorophyll contents and total ascorbic acid content

The determination of the carotenoid and chlorophyll contents was done by using the method of Lichtenthaler (1987). The results were expressed as μg/g of samples fresh weight (fw). The method for the determination of ascorbic acid was according to the methodology proposed by Benassi & Antunes (1988), using 2% of oxalic acid and titrated with a DCPIP and the results were expressed as mg of ascorbic acid (AA)/100g fw.

2.6 Chromatographic analysis by HPLC

The determination of the sugars content was performed as described by Gomes et al. (2018), using a Shimadzu LC (Shimadzu Corporation, Japan) chromatograph coupled to a refractive index detector (RID-10A) containing a pump system (LC-20AT). The column used was a Supelcogel Ca (300 x 7.8 mm; Supelco Inc., USA) with the oven temperature maintained at 80 °C. The mobile phase consisted of ultrapure water and the injection volume of the samples was 10 μL with the flow rate of 0.5mL/min with total running time of 40 min.

For the identification and quantification of organic acids, 0.5 g of the dried and triturated samples were weighed and diluted in 9 mL of a mixture of sodium phosphate monobasic (0.01 M)/
acetonitrile in the ratio of 99:1 (v/v), acidified to pH 2.5 by using phosphoric acid. Subsequently, the extracts obtained were centrifuged and filtered through 0.45 μm cellulose membrane (Merck Millipore, Brazil).

The organic acids content was determined using HPLC Prominence instrument (Shimadzu Corporation, Kyoto, Japan) with diode array detector (DAD) system (SPD-20A) equipped with a pump (LC-20AT) and degasser system (DGU-20A5), an automatic injector (SIL-20A HT) coupled to a CBM-20A controller system. A VP-ODS Shimadzu C18 column (250 x 4.6 mm, 5 μm) was used. The mobile phase was in an isocratic mode, being the same mixture for the extraction of the organic acids. The analysis conditions were as follows: flow rate of 1 mL/min, injection volume 5 μL, column temperature maintained at 40 °C during the run time of 30 min.

The identification and quantification of the individual phenolic compounds was done following the method described by Rajan et al. (2019) using a liquid chromatograph (UFLC-DAD, Shimadzu, Japan), equipped with a DGU-20A degasser, consisting of two quaternary LC-20AD pumps, SIL-20AHT autoinjector, CBM -20A, connected to a SPD-M20A diode array detector and Ascentis’ Express F5 (15 x 2.1 mm, 2.7 μm) column (Sigma Aldrich, USA). The sample injection volume was 10 μL with mobile phase flow rate of 0.2 mL/min and the oven temperature was set at 40 °C.

### 2.7 Determination of mineral elements

A flame atomization atomic absorption spectrometer (Perkin Elmer, model Analyst ™ 400 Norwalk, USA) was used to quantify the calcium (Ca), magnesium (Mg), copper (Cu), manganese (Mn), iron (Fe) and zinc (Zn). Hollow cathode lamps of these elements were used with respective wavelengths: 422.67, 524.75, 248.33, 213.86, 285.21 and 279.48 nm and lamps of these elements were used with respective wavelengths: 407.76, 428.74, 852.12, 213.86 and 285.21 nm for calcium, respectively. While sodium (Na) and Potassium (K) were analyzed using a flame photometer (Micronal, Model B-462) in dehydrated samples and submitted to nitro-perchloric digestion (Parimelazhagan, 2016). The analyses were performed in triplicate and the contents for all elements were expressed as mg/100 g dw.

### 2.8 Antioxidant capacity determinations

For the accomplishment of the tests of antioxidant capacity, four different tests ORAC (Oxidative Radical Absorbance Capacity), FRAP (Ferric Reducing Antioxidant Power), DPPH (1,1-diphenyl-2-picrylhydrazyl radical) and ABTS+ (2,2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid radical cation) were used following the methods described by Albarici et al. (2013), Thaipong et al. (2006), Kim et al. (2002) and Re et al. (1999), respectively. The analytical standard, Trolox was used to construct the calibration curves and all results were expressed as μM equivalent to Trolox/g of dw.

### 2.9 Statistical analysis

All results were expressed as mean ± standard deviation and obtained variables were submitted to analysis of variance (one-way ANOVA) followed by Tukey’s post-hoc comparison test. The multivariate statistics approach was used, applying the Biplot type principal component analysis (PCA) test with pre-treatments of the data. Statistical analyses were performed using SPSS version 20 software.

### 3 Results and discussion

#### 3.1 Physiochemical and phytochemical characterization

The fruit maturity characteristics were determined basically by the firmness and color analysis. The results of the studied parameters in the different varieties of unripe acerola are presented in Table 1.

From the data (Table 1) it could be observed that the fruits of the FB variety possessed higher firmness (28.12 N), in comparison to the other varieties such as CR (16.49 N) and JC (14.77 N). This fact may be related to the size of the fruit, considering that the acerola FB had smaller sizes and hence it could have influenced the hardness of the penetration. Figueiredo et al. (2014) analyzing the firmness in different varieties of acerola and at different stages of maturation observed that the firmness of the fruits reduced during maturation, with values varying from 22.33 N to 38.36 N for immature acerola and 8.54 N to 10.49 N for ripe fruits.

The mean values for the colorimetric analyses of the epicarp (peel) of the different varieties did not have any statistical significant difference (p ≤ 0.05) although, results for L* value indicated a maximum coloration 45.67 and 44.38 for acerola CR and JC, respectively, when compared to the variety FB with 40.70. For the color coordinate a*, the results obtained were in negative scale indicating the intensity of the green color. The values for the b* coordinate indicated a tendency to yellowish color, especially in the varieties FB and JC. Chromaticity was lower (22.93) for the FB variety. For the value of the angle h° of the acerola fruits, it was observed that the average values were 91° for all the varieties. In general, the results indicated that the surfaces of the fruits were greener and brighter, when compared to the values reported by Vendramini & Trugo (2000), who reporting lower values for immature acerola for the coordinates L (27.92) and b* (11.37).

The different varieties of acerola at green unripe stage of maturation were significantly different (p ≤ 0.05) with respect to the moisture content that varied between 90.53 to 92.36%, where the variety CR presented slightly the highest value. In relation to the water activity of the fruits, all samples presented higher values (0.99), which indicate that the fruits were of high perishability, since there is an environment conducive to the promotion of biochemical and microbial reactions. The soluble solids content varied from 4.03 to 6.01 °Brix, with emphasis on the FB variety, which presented the highest result (6.01 °Brix), while CR contained the lowest mean (4.03 °Brix). According to Figueiredo et al. (2014), the soluble solids content of the FB variety was 9.11 °Brix, higher than the result obtained in this study.

Regarding titratable acidity and pH values, significant differences (p ≤ 0.05) were observed among all varieties (Table 1). The highest values were found for JC fruits with 1.20% in citric.
Table 1. Phytochemical composition and antioxidant potential of green unripe acerola fruit obtained from the São Francisco Valley, Northeast Brazil.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fluor Branca</th>
<th>Costa Rica</th>
<th>Junco</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ac. ascorbic (mg AA/100 g fw)</strong></td>
<td>2216.62 ± 28.61a</td>
<td>1562.20 ± 18.59b</td>
<td>1673.33 ± 10.81b</td>
</tr>
<tr>
<td><strong>Total Phenolic (mg GAE/g dw)</strong></td>
<td>169.62 ± 7.28a</td>
<td>210.80 ± 4.58b</td>
<td>196.44 ± 4.29a</td>
</tr>
<tr>
<td><strong>Total Flavonoid (mg QE/g dw)</strong></td>
<td>26.76 ± 2.83a</td>
<td>23.71 ± 1.95a</td>
<td>27.50 ± 1.81a</td>
</tr>
<tr>
<td><strong>Chlorophyll (μg/g fw)</strong></td>
<td>36.97 ± 3.96a</td>
<td>21.54 ± 1.41c</td>
<td>50.34 ± 1.72c</td>
</tr>
<tr>
<td><strong>Carotenoids (μg β-CE/g fw)</strong></td>
<td>21.18 ± 2.03b</td>
<td>11.15 ± 0.77c</td>
<td>27.75 ± 0.64a</td>
</tr>
</tbody>
</table>

The above results are expressed as the mean values ± standard deviation of triplicate determinations (n = 3); *a > b > c* values followed by the same superscript letter in each row are not significantly different (Tukey’s test, P ≤ 0.05).

acid, while the lowest content was observed in the FB variety with 0.70% in citric acid. The pH ranged from 3.50 to 3.74. Higher pH relates to lower acidity, which is consistent with the values observed in these results. It is worth mentioning that the acerola pH remains stable even in different maturation stages when compared to other varieties grown in Brazil (Ribeiro et al., 2019; Carmo et al., 2018; Mariano-Nasser et al., 2017). In this study, the SS/TA ratio presented variation between the studied varieties, (Table 1) demonstrating that these varieties are recommended for technological applications such as usage as a gelling agent, which corroborates the suggestion of Assis et al. (2001), who proposed that acerola fruits in the green ripening stage can be an important source of pectin for confectionery industries.

The concentration of ascorbic acid varied from 1562.20 to 2216.62 mg/100 g fw, with significant differences (p ≤ 0.05) among the various varieties in this study. According to the USDA, the average content of AA in raw mature acerola is 1677 mg AA/100 g (U.S. Department of Agriculture, Agricultural Research Service, 2016). The Brazilian Table of Centesimal Composition of Foods (Universidade Estadual de Campinas, 2011), established concentration for acerola fruits to be about 941.90 mg AA/100 g. However, higher values were found in this study, which can be attributed to the green maturation stage of the fruits in addition to the edaphoclimatic conditions of the cultivation region.

Analyzing the obtained flavonoid results, it was verified that the pulps of different varieties of acerola did not have any significant difference (p ≤ 0.05) and their values varied from 23.71 to 27.50 mg QE/g dw. The total phenolic contents presented higher amounts. Although their values were not significantly different (p ≤ 0.05) among the studied varieties ranged from 169.62 to 210.80 mg GAE/g of dw. These values are higher than those found in other studies (Mariano-Nasser et al., 2017; Oliveira et al., 2012). It is known that some factors including polarity of the solvent may influence the efficacy of the extraction of compounds, as combinations of ethanol and water, which are efficient in the extraction of polyphenols due to the polarity of these mixtures being similar to those of phenolic compounds which may have provided greater solubilization of the solute and consequently, a higher extraction of these compounds (Machado et al., 2015).

The total chlorophyll content varied from 21.54 to 50.34 μg/g and the JC variety had the highest average value. The total carotenoid content was about 11.15 μg β-CE/g dw for the CR variety and 27.75 μg β-CE/g dw for JC variety fruits. The content of this compound in the acerola fruits is much higher than those found in other studies (Mariano-Nasser et al., 2017; Oliveira et al., 2012). It is known that some factors including polarity of the solvent may influence the efficacy of the extraction of compounds, as combinations of ethanol and water, which are efficient in the extraction of polyphenols due to the polarity of these mixtures being similar to those of phenolic compounds which may have provided greater solubilization of the solute and consequently, a higher extraction of these compounds (Machado et al., 2015).

3.2 Chromatographic analysis

Table 2 lists the contents of sugars, organic acids and phenolic composition in the different samples analyzed.

For the total content of quantified sugars, values ranged from 0.49 to 0.23 g/100 g dw. Regarding individual sugars, sucrose...
was the main sugar found in acerola FB and JC fruits, different from the CR variety which presented higher fructose content. There is currently little information on the relative content of sugars, mainly in the different stages of development of acerola, however, reducing sugars, fructose and glucose are known to be present in larger amounts in ripe fruit juices (Righetto et al., 2005).

The contents of the organic acids detected in the different varieties of acerola in the green stage of maturity is shown in Table 2, among which four types of acids were identified, namely citric, succinic, fumaric and ascorbic acids. The values for total organic acids ranged from 72.44 mg/100 g dw to 1219.93 mg/100 g dw. Acerola fruits are world renowned for being excellent sources of ascorbic acid and their content was high in varieties FB and JC with 1091.87 and 1219.93 mg/100 g dw respectively, however for the FB variety the major organic acid was succinic with 1167.98 mg/100 g dw. When comparing the different varieties of unripe acerola, fruits FB and JC contained the highest amount of organic acids and sugars, while CR presented the lowest levels. Thus, it is verified that the acerola studied here presented variation in the concentration of the compounds depending on the varieties of the fruits.

From HPLC-DAD analysis, nine phenolic compounds were identified in the three varieties of acerola produced in SFV region (Table 2, Figure 1).

Acerola FB and CR were the varieties with the highest content of individual quantified phenolic compounds, with predominance of the flavanols compound class with 61.21 and 39.78 mg/100 g dw, respectively, followed by the presence of

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**Table 2. Sugars, organic acids and phenolic composition of different varieties of green unripe acerola fruit obtained from the São Francisco Valley, Northeast Brazil.**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Costa Rica</th>
<th>Flor Branca</th>
<th>Junco</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugars (g/100 g dw)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>0.04 ± 0.00(^a)</td>
<td>0.04 ± 0.00(^a)</td>
<td>0.03 ± 0.00(^a)</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.16 ± 0.00(^a)</td>
<td>0.15 ± 0.00(^b)</td>
<td>0.14 ± 0.00(^a)</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.03 ± 0.00(^a)</td>
<td>0.30 ± 0.01(^b)</td>
<td>0.32 ± 0.01(^b)</td>
</tr>
<tr>
<td>(\Sigma) Sugars*</td>
<td>0.23</td>
<td>0.49</td>
<td>0.49</td>
</tr>
<tr>
<td>Organic acids (mg/100 g dw)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citric</td>
<td>440.74 ± 4.72(^b)</td>
<td>450.84 ± 5.80(^b)</td>
<td>477.45 ± 11.84(^c)</td>
</tr>
<tr>
<td>Succinic</td>
<td>559.95 ± 2.24(^a)</td>
<td>1167.98 ± 23.58(^a)</td>
<td>836.12 ± 11.43(^b)</td>
</tr>
<tr>
<td>Fumaric</td>
<td>72.59 ± 0.61(^a)</td>
<td>72.44 ± 0.73(^b)</td>
<td>72.81 ± 0.12(^a)</td>
</tr>
<tr>
<td>Ascorbic</td>
<td>804.79 ± 11.54(^a)</td>
<td>1091.87 ± 0.94(^b)</td>
<td>1219.93 ± 4.31(^a)</td>
</tr>
<tr>
<td>(\Sigma) Organic acids(^*)</td>
<td>1035.96</td>
<td>1432.58</td>
<td>1508.15</td>
</tr>
</tbody>
</table>

| Phenolic compounds (mg/100 g dw) |            |             |         |
| Flavonols                |            |             |         |
| Coumarin                 | 1.76 ± 0.38\(^b\) | 4.06 ± 0.05\(^a\) | 1.69 ± 0.41\(^b\) |
| Rutin                    | 2.35 ± 0.01\(^a\) | ND          | ND      |
| Quercetin                | 8.13 ± 0.12\(^a\) | 6.77 ± 0.02\(^b\) | 6.63 ± 0.07\(^a\) |
| Kaempferol 3- glucoside  | 11.23 ± 0.37\(^b\) | 32.68 ± 1.29\(^a\) | 8.50 ± 0.34\(^a\) |
| Isorhamnetin             | 10.13 ± 0.02\(^a\) | 11.41 ± 0.14\(^a\) | 10.68 ± 0.56\(^a\) |
| Kaempferol               | 6.18 ± 0.01\(^b\) | 6.29 ± 0.01\(^a\) | 6.30 ± 0.10\(^b\) |
| \(\Sigma\) Flavonols*    | 39.78      | 61.21       | 33.80   |
| Flavanols               |            |             |         |
| Epigallocatechin gallate | 5.83 ± 0.21\(^a\) | ND          | ND      |
| \(\Sigma\) Flavanols*    | 5.83       |             |         |
| Hydroxycinnamic acids    |            |             |         |
| Ferulic acid             | ND         | 0.34 ± 0.11\(^b\) | 1.63 ± 0.12\(^a\) |
| \(p\)-coumaric acid      | ND         | 28.87 ± 2.97\(^a\) | ND      |
| \(\Sigma\) Hydroxycinnamic acids\(^*\) | 29.21   | 1.63       |
| \(\Sigma\) Phenolic compounds | 45.61  | 90.42      | 35.43   |

The above results are expressed as the mean values ± standard deviation of triplicate determinations (n = 3); \(^a\ > \(^b\ > \(^c\); Mean values followed by same letters in the same line do not differ by Tukey test at 5% probability of error; \(^*\): Sum of all the individual compounds for each variety analyzed by HPLC. ND: not detected.
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hydroxycinnamic acids which was 1.63 mg/100 g dw in FB fruits and 29.21 mg/100 g dw in JC fruits. Thus the main phenolic compounds of the pulp of acerola fruit at green stage of maturation in the tested varieties are flavonols. According to the sum of all the individual phenolic compounds obtained, the variety FB was distinguished with higher content of phenolic compounds (90.42 mg/100 g dw), followed by the CR and JC variety, being 45.61 and 35.43 mg/100 g dw of sample, respectively (Table 2).

Among all the flavonols analyzed, kaempferol-3-glucoside was found to be present in higher concentration. The second compound was isorhamnetin with average values ranging from 10.13 to 11.41 mg/100 g dw, however it was the main flavonol found in the JC cultivar with 10.68 mg/100 g dw. Other compounds such as quercetin and kaempferol were also identified in the studied varieties. Hoffmann-Ribani et al. (2009) reported in different varieties of acerola fruits that quercetin was the main flavonol in relation to kaempferol, which corroborates the results obtained in the present study, when compared with the concentration of these compounds in the varieties tested. Flavonol coumarin was also identified in the different varieties of acerola in unripe stage, however, lower value of 1.69 and 4.06 mg/100 g dw were found in the JC and FB varieties, respectively, while rutin (2.35 mg/100 g dw) and flavanol epigallocatechin gallate (5.83 mg/100 g dw) were found only in acerola of the CR variety. Hydroxycinnamic acids such as ferulic acid and p-coumaric acid, were found in the FB variety with values of 0.34 and 28.87 mg/100 g dw, respectively. In the cultivar JC, only ferulic acid with 1.63 mg/100 g dw was found.

Based on the HPLC data, it was confirmed that the identified compounds are in agreement with the fruits and by-products of acerola (Betta et al., 2018). In general, these data ensure that acerola varieties are sources of important phytochemicals which can be considered as health promoters due to the presence of several compounds, which are related to the risk of reduction of various degenerative diseases.

### 3.3 Mineral elements

Mineral profiles in unripe acerola fruits of different varieties are shown in Table 3. Among the total elements determined from the microminerals Mn, Cu, Fe and Zn, the values varied from 0.21 to 4.15 mg/100 g dw. Fe is present in higher concentration in

![Figure 1. HPLC chromatograms profiles detected at 370 nm for phenolic compounds.](image)

Table 3. Mineral content of different varieties of green unripe acerola fruit obtained from the São Francisco Valley, Northeast Brazil.

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Costa Rica</th>
<th>Flor Branca</th>
<th>Junco</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (g/100 g)</td>
<td>% DV</td>
<td>% DRI</td>
</tr>
<tr>
<td>Mn</td>
<td>0.81 ± 0.05a</td>
<td>40.50</td>
<td>35.22</td>
</tr>
<tr>
<td>Cu</td>
<td>0.21 ± 0.03c</td>
<td>10.50</td>
<td>23.33</td>
</tr>
<tr>
<td>Fe</td>
<td>4.09 ± 0.35c</td>
<td>22.72</td>
<td>29.21</td>
</tr>
<tr>
<td>Zn</td>
<td>0.81 ± 0.06c</td>
<td>7.36</td>
<td>11.57</td>
</tr>
<tr>
<td>Ca</td>
<td>512.77 ± 19.02a</td>
<td>39.44</td>
<td>39.44</td>
</tr>
<tr>
<td>K</td>
<td>1930.28 ± 0.01b</td>
<td>41.07</td>
<td>41.07</td>
</tr>
<tr>
<td>Mg</td>
<td>120.91 ± 13.38b</td>
<td>28.79</td>
<td>46.50</td>
</tr>
<tr>
<td>Na</td>
<td>40.64 ± 0.01b</td>
<td>1.69</td>
<td>1.69</td>
</tr>
</tbody>
</table>

The above results are expressed as the mean ± standard deviation values of triplicate determinations (n = 3); values followed by the same superscript letter in each row are not significantly different (Tukey’s test, p ≤ 0.05); DRI: Daily Recommended Intake established by ANVISA (Brasil, 2003); DV: Daily value fixed by FDA.
CR and FB varieties being 3.34 to 4.15 mg/100 g dw, respectively. Regarding the concentration of macrominerals Ca, K, Mg and Na, their values varied from 0.86 to 2414.20 mg/100 g. Among these, the content of K and Ca were predominant among all varieties analyzed. Comparing these results with the values defined by the Food and Drug Administration (Food and Drug Administration, 2019) and the Agência Nacional de Vigilância Sanitária (Brasil, 2003), which assigns as a source of minerals any food with at least 15% of the Daily Recommended Intake (DRI) and as a high content food containing at least 30% of the reference DRI per serving (100 g) (Brasil, 2012), the values present in Table 3 on acerola in the unripe stage are excellent sources of important minerals, with higher mineral content in the order of Cu > K > Ca (JC variety), Mg > K > Ca > Mn (CR variety) and Cu > Ca > K > Mg > Mn > Fe (FB variety).

### 3.4 Antioxidant capacity

Due to the great interest on the role of antioxidants in plant extracts, the measurement of the antioxidant capacity was performed by DPPH\(^{•}\), ABTS\(^{•}\), FRAP and ORAC assays (Figure 2).

The JC variety was highlighted as possessing the highest DPPH\(^{•}\) scavenging potential with 2154.93 μM Trolox/g, followed by the FB sample having 1910.87 μM Trolox/g. The extract of FB variety exhibited the greatest free radical scavenging (ABTS\(^{•}\)), with value of 8613.54 μM Trolox/g as compared to 7526.80 μM Trolox/g of CR and 7475.86 μM Trolox/g for JC variety. From the results on FRAP assay, the JC variety showed higher antioxidant capacity (1447.97 μM Trolox/g), followed by FB (1166.09 μM Trolox/g) and CR (824.23 μM Trolox/g) varieties. The FB variety presented the highest oxygen radical absorbance capacity (ORAC assay) value of 2454.42 μM Trolox/g and it was followed by CR (2172.53 μM Trolox/g) and JC (1950.10 μM Trolox/g) varieties. Regarding the results obtained, it was verified that all varieties of green unripe acerola presented different values in all the four tests used and it was expected as the mechanism of action varies between different methods (Capanoglu et al., 2017). It is possible to verify that the antioxidant capacity in acerola is high, fact related to the maturation stage, corroborating the findings in other studies, which verified the tendency of decreased antioxidant capacity in relation to the stage of development of acerola (Oliveira et al., 2012). Thus, the results are directly related to the substances and the different concentrations present in the samples.

### 3.5 Chemometric evaluation

In Figure 3, it is possible to check the results of the PCA analysis, where the plotting of two-dimensional graph shows the load values (> 0.7) by correlation between the contents of phenolic, organic acids, sugars and antioxidant activity as a function of the three commercial varieties of green unripe acerola, resulting in 25 principal components.

The two main components (PC1 and PC2) presented a total cumulative variation of 90.12% which explains the data obtained from the phytochemical composition analysis of the different varieties of unripe acerola. PC1 explained 57.69%, mainly distinguishing the CR variety from the other samples, revealing that the variables that favored the major contribution to the separation of acerola varieties were carotenoids, chlorophyll, isorhamnetin, kaempferol, ferulic acid, FRAP assay, ascorbic acid and sucrose positively correlating in PC1 with the JC and FB varieties, while the CR variety was negatively correlated in PC1 by high levels of rutin, quercetin, epigallocatechin, citric acid and fructose. The FB variety positively correlated in PC2 with 32.43% of the total variability of the dataset, which was mainly influenced by ascorbic acid, kaempferol-3-glucoside, p-coumaric acid, ABTS\(^{•}\) and ORAC and succinic acid, and negative correlation with total phenolic compounds. In summary, the results of the PCA analysis demonstrated in a simple way the distribution between the phytochemical composition in the CR, FB and JC varieties.
4 Conclusions

This study indicated that the three varieties of acerola at green unripe stage of maturation to be a source of important chemical constituents, as well as mineral elements, mainly for acerola FB. Thus, that acerola variety is superior to others and the most promising for the applications associated with the development of new products, with an emphasis on the exploration of extracts of unripe acerola in food or pharmaceutical matrices.

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References


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