

## UPLC-QTOF/MS-MS Based Profiling Coupled to Antioxidant Activity for Evaluation of Unripe Acerola Fruits from Different Clones

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The potential of four commercial clones of unripe acerola fruit was investigated regarding a source of nutraceuticals and functional foods for industrial use. The chemical profiling of the BRS 235-Apodi, BRS 236-Cereja, BRS 237-Roxinha, and BRS 238-Frutacor clones was determined by the ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF/MS-MS) technique coupled to multivariate statistical analysis, which were then correlated to ABTS<sup>•+</sup> radical capture (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and ferric reducing antioxidant power (FRAP) assays in order to measure the antioxidant capacity. A total of 24 bioactive compounds were identified, including high amounts of quercetin-*O*-hexoside, astilbin, and apigenin, which were correlated to three clones (BRS 235, BRS 237, and BRS 238). On the other hand, the BRS 236 clone presented high contents of ascorbic acid, iso-ascorbic acid, citric acid, procyanidin B trimer, rutin, phenolic compounds, and antioxidant activity. These compounds were additionally found to be strongly associated with the antioxidant capacity of unripe acerola fruits. Therefore, the present study revealed that BRS 236 is a promising clone as an antioxidant source to be used as a functional food or ingredient.

**Keywords:** *Malpighia emarginata* DC., vegetable physiology, postharvest, principal component analysis, phenolic compounds

### Introduction

The consumption of acerola fruits (*Malpighia emarginata* DC.) is of great interest to promote health and prevent diseases, which makes it a good candidate for developing new functional foods.<sup>1,2</sup> Several commercial products containing acerola are being used as dietary supplements. The global acerola consumption market is estimated to reach US\$17.5 billion by 2026 with an 8.5% compound annual growth rate.<sup>1</sup>

Acerola fruit is popular for its high vitamin C content. In addition to its antioxidant vitamin, this fruit presents satisfactory amounts of phenolics, including anthocyanins and flavonoids, and carotenoids with antioxidant, anti-inflammatory, anti-hyperglycemic, anti-tumor, anti-

genotoxic, and hepatoprotective activities.<sup>3-5</sup> However, the composition of these bioactive substances depends on several factors, such as the climatic conditions, cultural treatments, geographic location, processing, storage conditions, genetic factors, and maturation stage.<sup>6</sup> In this context, a metabolomic analysis is important to identify metabolic signatures and patterns associated with various conditions.<sup>7</sup>

The composition of bioactive compounds present in fruits can generally be disclosed through metabolomic analysis. Furthermore, the correlation between the chemical profile and bioactivity of the compounds can be found by applying multivariate statistical methods.<sup>8</sup> Therefore, the aim of the present study is to explore the potential of unripe acerola fruits as a source of nutraceuticals and functional compounds for industrial use through a correlation of the chemical composition of four different clones with their antioxidant activity by

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using ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF/MS-MS) analysis coupled to multivariate statistical analysis. The results can be useful to select and breed varieties by helping to verify the similarities and differences among the cultivars. Furthermore, knowledge of these compounds with antioxidant properties can reveal the potential bioactivity of such fruits.<sup>9,10</sup> The identified compounds can contribute as ingredients for nutraceuticals and functional products.

## Experimental

### Sampling

Four different acerola fruit clones (BRS 235-Apodi, BRS 236-Cereja, BRS 237-Roxinha, and BRS 238-Frutacor) were developed by the Genetic Breeding Program of Embrapa Agro-industry Tropical (Fortaleza-CE, Brazil). These clones were harvested at a maturation stage II (mature green with maximum size) at the Jardim Clonal Acerola Orchard, Campo Experimental da Embrapa, located in Pacajus, CE, Brazil. The fruits were processed (Phillips, R11858, Varginia, Brazil) to obtain the pulp and then frozen at  $-80\text{ }^{\circ}\text{C}$  and stored until the analyses. Before the UPLC-QTOF/MS-MS analysis, the pulps were dried by lyophilization for 48 h at  $-41\text{ }^{\circ}\text{C}$  using a bench lyophilizer (Liotop, L202, São Carlos, Brazil).

### Chemicals

Gallic acid (PubChem CID: 370); Folin-Ciocalteu; ethanol (PubChem CID: 702); calcium carbonate (PubChem CID: 10112); 2,2-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (PubChem CID: 5815211); TPTZ (2,4,6-tripyridyl-*s*-triazine) (PubChem CID: 110383);  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (PubChem CID: 24380); HCl (PubChem CID: 313); and sodium acetate (PubChem CID: 16211956) were used and purchased from Sigma-Aldrich (Saint Louis, USA). The UPLC-grade methanol was purchased from Tedia (Rio de Janeiro-RJ, Brazil), and the formic acid from Fluka (Buchs-ZU, Switzerland).

### UPLC-QTOF/MS-MS analysis

The samples were initially prepared according to the method described by Guedes *et al.*<sup>11</sup> An amount of 50 mg of the lyophilized pulp were suspended in 4 mL of hexane, and the resulting mixture was sonicated for 20 min in an ultrasound bath (Eco-Sonic, Q3.8, Indaiatuba, Brazil) at 135 W and  $25\text{ }^{\circ}\text{C}$ . Then, 4 mL of an ethanol/

water solution (7:3) was added and sonicated again in an ultrasound bath under the same conditions previously mentioned in order to extract the polar components. This mixture was subsequently centrifuged (Beckman, model J2-2, California, USA) at 3,000 rpm at  $25\text{ }^{\circ}\text{C}$  for 10 min, and a 2 mL aliquot of the lower phase was filtered through polytetrafluoroethylene (PTFE) filters of  $0.22\text{ }\mu\text{m}$  (Whatman, Merck, Germany). Afterwards, 900  $\mu\text{L}$  of the filtrate containing 100  $\mu\text{L}$  of an internal standard solution (genistein  $1\text{ }\mu\text{g mL}^{-1}$ ) were added to vials and injected into the UPLC system (Waters Co., Milford, MA, USA).

The chromatographic analyses were developed using a Waters Acquity/Xevo UPLC-QTOF-MS system, which was equipped with an Acquity UPLC BEH column ( $150 \times 2.1\text{ mm}$ ,  $1.7\text{ }\mu\text{m}$ ; Waters). The binary gradient elution system consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), with a linear gradient from 2 to 95% B (0-15 min), under a flow rate of  $0.4\text{ mL min}^{-1}$ . The column temperature was adjusted to  $40\text{ }^{\circ}\text{C}$  and the injection volume was 5  $\mu\text{L}$ .

After the chromatographic separation, the compounds were detected using an electrospray ionization (ESI) interface operating in the negative ionization mode, with a range from 110 to 1180 Da and a scan time of 0.1 s. Nitrogen set at  $350\text{ }^{\circ}\text{C}$  was used as the desolvation gas under a flow rate of  $500\text{ L h}^{-1}$ . The capillary and cone voltages were adjusted to 2.6 kV and 0.5 V, respectively. The mass accuracy and reproducibility were maintained by infusing lock mass (leucine-enkephalin,  $0.2\text{ ng }\mu\text{L}^{-1}$ ;  $[\text{M} - \text{H}]^{-}$  ion at  $m/z\ 556.2771$ ). The accurate mass and molecular formula assignments were obtained by the MassLynx<sup>TM</sup> software program (version 4.1, Waters Corporation),<sup>12</sup> and the compounds were tentatively identified based on the molecular formulas (deduced from the  $m/z$  values) and MS fragmentation patterns.

### Multivariate statistical analysis of the UPLC-QTOF/MS-MS dataset

The resulting chromatograms for the numerical matrix creation were converted to American Standard Code for Information Interchange (ASCII) files, and their region between 0 and 7.0 min was selected for multivariate statistical evaluation. The unsupervised method by principal component analysis (PCA) was applied for multivariate exploratory evaluation using the PLS Toolbox<sup>TM</sup> software program (version 8.6.2, Eigenvector Research Incorporated, Manson, WA USA).<sup>13</sup>

Algorithms for baseline correction and normalization processing were applied over the chromatograms dataset (variables), and the mean-centering pretreatment was

applied over the samples for PCA evaluation. The singular value decomposition (SVD) algorithm was applied to decompose the complex matrix in scores, loadings and residues matrices; and the important information based on the study aim was retained by the first two principal components (2 PCs) under a confidence level of 95%.

#### Determination of the bioactive compounds and antioxidant activity

The vitamin C concentrations in the acerola pulps were determined based on the method proposed by Sánchez-Mata *et al.*<sup>14</sup> The total experimental procedure was carried out in the absence of light. A total of 5 g of the fruit pulp were initially solubilized in 20 mL of acetic acid (PA 8% v/v) and metaphosphoric acid (PA 3% m/v), as well as 25 mL of distilled water. After 5 min, 3 mL were filtered (PTFE, 0.45  $\mu\text{m}$ , Jet biofilm™) and introduced into vials for high-performance liquid chromatography (HPLC-UV, Waters Co., Milford, MA, USA) injection. The results were determined by comparing the retention time and the absorption area of the chromatographic signal at 245 nm based on a standard curve of ascorbic acid (100 to 500 mg L<sup>-1</sup>) and dehydroascorbic acid (25 to 1000 mg L<sup>-1</sup>). The results are presented in mg of ascorbic acid 100 g<sup>-1</sup> fresh weight (FW).

The total extractable polyphenols (PET) were determined by the Folin-Ciocalteu method based on Obanda *et al.*<sup>15</sup> with modifications. The samples were initially prepared using a total of 0.5 g of pulp with 4 mL of 50% methanol, which was placed in the dark for 60 min. Then, this material was centrifuged at 3000 rpm at 25 °C for 15 min, the supernatant was filtered into a 10 mL flask, and the precipitate was suspended in 4 mL of 70% acetone. The resulting material was centrifuged again at the same conditions (described above), the supernatant was mixed with the previous supernatant, and then the flask volume was completed with distilled water.<sup>16</sup>

Next, a total of 20  $\mu\text{L}$  of the extract, 980  $\mu\text{L}$  of distilled water, 1000  $\mu\text{L}$  of the Folin-Ciocalteu reagent (1:3), 2000  $\mu\text{L}$  of anhydrous sodium carbonate (20%), and 2000  $\mu\text{L}$  of distilled water were mixed in a test tube used for the Folin-Ciocalteu test. The spectrophotometer was read at 700 nm after a period of 30 min at room temperature (25 °C) and in the absence of light. The PET content was calculated using the standard curve of gallic acid (0-50  $\mu\text{g}$ ), and results were presented in mg of gallic acid 100 g<sup>-1</sup> FW.

The total antioxidant activity (TAA) was determined using the ABTS<sup>•+</sup> radical method (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) based on the method by Re *et al.*,<sup>17</sup> and adapted in 2007 by Rufino *et al.*<sup>18</sup>

A total of 30  $\mu\text{L}$  of the extracts from the acerola fruits were mixed with 3000  $\mu\text{L}$  of the ABTS<sup>•+</sup> radical in ethanol (with previously adjusted absorbance of  $0.700 \pm 0.02$ ). Then, after 6 min of reaction in the dark, the absorbance was read at 734 nm using a spectrophotometer (Agilent Technologies Cary 60, Santa Clara, CA, USA). The TAA ABTS<sup>•+</sup> was obtained from the standard Trolox curve (100 to 2000  $\mu\text{M}$ ), and the results are presented in  $\mu\text{M}$  of trolox g<sup>-1</sup> FW.

The TAA for the FRAP method was determined based on the method by Pulido *et al.*<sup>19</sup> and was adapted in 2007 by Rufino *et al.*<sup>18</sup> Therefore, a total of 90  $\mu\text{L}$  of the extracts were mixed with 270  $\mu\text{L}$  of distilled water and 2700  $\mu\text{L}$  of the FRAP reagent (from a mixture of 25 mL of 0.3 M acetate buffer solution and 2.5 mL of 10 mM TPTZ solution (salt 2,4,6-tri-(2-pyridyl)-1,3,5-triazine) and 2.5 mL of 20 mM ferric chloride solution). The reaction mixture occurred at 37 °C in a water bath for 30 min. Next, the absorbance was read at 595 nm on a spectrophotometer (Agilent Technologies Cary 60, Santa Clara, CA, USA). The TAA was achieved by the standard ferrous sulfate curve (0 to 2000  $\mu\text{M}$ ), and the results are presented in  $\mu\text{mol}$  ferrous sulfate g<sup>-1</sup> FW.

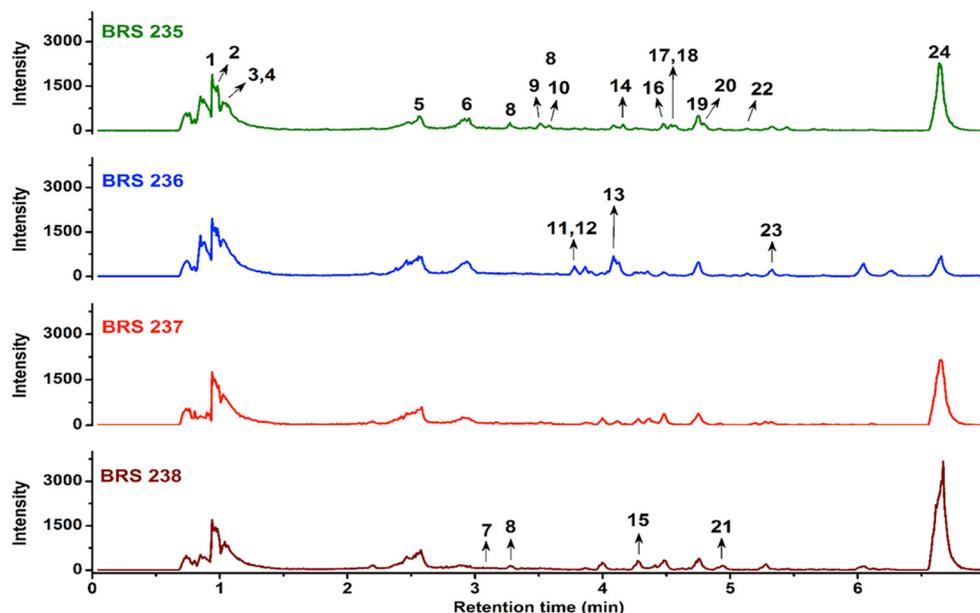
#### Univariate statistical analysis

The quantitative results from the bioactive compounds and antioxidant activity analyses were evaluated using the analysis of variance (ANOVA) single factor analysis. The following parameters were applied to statistically certify the differences among the mean values: significance level of 0.05; means comparison using the Tukey's test; and the Levene's test for the homogeneity of variance.

## Results and Discussion

Figure 1 illustrates a comparison between the chromatograms from the UPLC-QTOF/MS-MS of the unripe acerola fruits (BRS 235, BRS 236, BRS 237, and BRS 238). The signals were numbered according to compounds described in Table 1, which exhibits their respective characterization parameters: retention time (min); [M - H]<sup>-</sup> ion; product ions (MS/MS); empirical formula; and respective error.

A total of 24 organic compounds were identified in the acerola pulps considering the four acerola clones. Unripe acerola pulps were generally composed by short chain organic acids, flavonoid glycosides, proanthocyanidins, and flavonoids, independent of the clone. Due to the complexity of the chromatogram's dataset, an unsupervised chemometric method by PCA was developed in order to explore the variability of the fruits related to the respective



**Figure 1.** Comparison between the UPLC-QTOF/MS-MS chromatograms presented in the same intensity from four unripe acerola fruit clones (BRS235, BRS 236, BRS 237, and BRS 238), with the signals numbered according to compounds described in Table 1.

clone. Figure 2 presents the PC1 × PC2 scores coordinate system (Figure 2a) and the relevant loadings for sample discriminations plotted in line form (Figure 2b).

The PC1 was the main axis for the acerola samples discrimination, which retained 79.54% of the total variance. Acerola fruits from BRS235 (green), BRS237 (light red), and BRS 238 (dark red) clones generally clustered to positive scores of PC1 by elevated amounts of quercetin-*O*-hexoside isomer (15), astilbin (16), and mainly apigenin (26), according to the loading intensities. On the other hand, the acerola fruits from the BRS 236 clone (blue) clustered at negative PC1 and PC2 scores due to the high amounts of ascorbic acid (3), citric acid (4), ascorbic acid isomer (6), procyanidin B trimer (12), and rutin (13).

After the multivariate exploratory evaluation of the organic composition variability of unripe acerola fruits according to the clone, the single factor analysis of variance (one-way ANOVA) was developed in order to corroborate and complement the chemical variability results, statistically certifying the differences and equality among variation of the means related to the acerola clones. Figure 3 describes the quantitative results from the total phenolics, vitamin C (ascorbic acid), and the antioxidant activities determined by the ABTS and FRAP methods.

Figure 3a demonstrates significant differences in the phenolic compound content in the studied fruits, except between BRS 235 and BRS 238. Acerola fruits from the BRS 236 clone were highlighted by an average value of 1790.97 mg gallic acid equivalent. The vitamin C content (Figure 3b) presented a significant difference between the BRS 236 clone (3013.53 mg of ascorbic acid 100 g<sup>-1</sup> FW)

and all the others evaluated, which had an average content of 2198.67 mg.

The ABTS method (Figure 3c) showed that the BRS 236 clone had the highest TAA (232.82 μmol trolox g<sup>-1</sup> FW), while the BRS 237 clone had the lowest (104.90 μmol trolox g<sup>-1</sup> FW), and there was no difference between the BRS 235 and BRS 238 clones. The quantification of the antioxidant capacity by the FRAP method also showed that the highest was the BRS 236 variety (439.05 μM ferrous sulfate g<sup>-1</sup> FW), and there was no significant difference between the values found for the BRS 235 and BRS 237 clones. The higher results found for the antioxidant activity of the BRS 236 clone were already expected, since it presented higher vitamin C and phenolic compound concentrations which can act as antioxidants. Therefore, the higher amounts of total phenolics, vitamin C, and antioxidant activities were directly correlated to the higher amounts of ascorbic acid and its isomer, citric acid, procyanidin B trimer, and rutin, since the BRS 236 clone presented the highest values compared to the other clones.

Similar phenolic content was found by Oliveira *et al.*<sup>24</sup> who studied predominantly unripe acerola fruits from BRS 236 (1641 mg gallic acid equivalent 100 g<sup>-1</sup> FW) and BRS 237 clones (949 mg gallic acid equivalent 100 g<sup>-1</sup> FW). Although the BRS 237 clone presented the lowest amount of these compounds (996.73 mg gallic acid equivalent 100 g<sup>-1</sup> FW), the phenolic content detected by the present study was higher compared with other fruits at the same maturation stage (immature), such as white guava (350 mg gallic acid equivalent 100 g<sup>-1</sup> FW), and blueberry (cv. Northblue, 324 mg gallic acid equivalent 100 g<sup>-1</sup> FW).<sup>25,26</sup>

**Table 1.** Chemical characterization of unripe acerola (*Malpighia emarginata* DC.) fruits from different commercial clones based on UPLC-QTOF/MS analysis, showing the retention time ( $t_R$ ), the observed and calculated  $[M - H]^-$  ions with their product ions (MS/MS), empirical formula, respective error, and reference for each compound

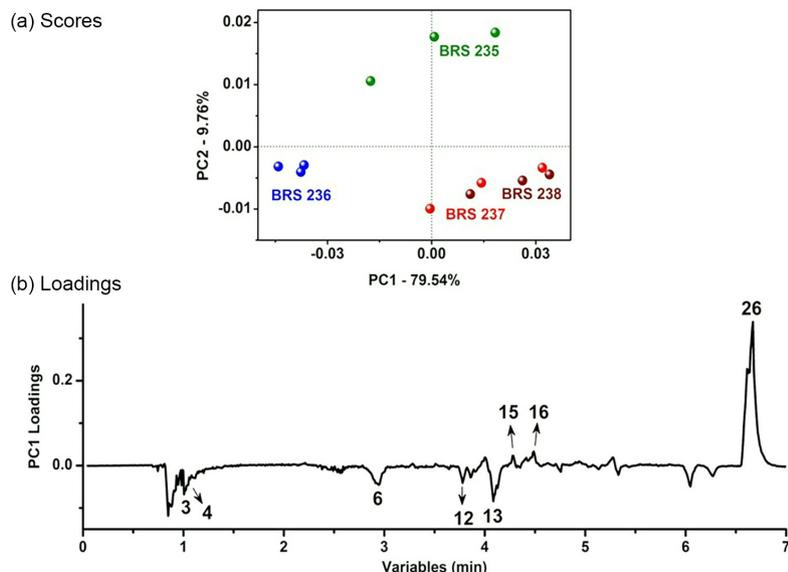
Peak	$t_R$ / min	$[M - H]^-$ obs.	$[M - H]^-$ calcd.	Product ions (MS/MS)	Empirical formula	Error / ppm	Putative name	Reference
1	0.94	133.0134	133.0137	–	$C_4H_5O_5$	–2.3	malic acid	–
2	0.98	175.0247	175.0243	115.0016, 87.0086	$C_6H_7O_6$	2.3	ascorbic acid isomer	–
3	1.01	175.0238	175.0243	115.0026, 87.0082	$C_6H_7O_6$	–2.9	ascorbic acid isomer	–
4	1.04	191.0191	191.0192	111.0095	$C_6H_7O_7$	–0.5	citric acid	–
5	2.58	175.0241	175.0243	115.0017	$C_6H_7O_6$	–1.1	ascorbic acid isomer	–
6	2.93	175.0245	175.0243	115.0053	$C_6H_7O_6$	1.1	ascorbic acid isomer	–
7	3.08	289.0724	289.0712	–	$C_{15}H_{13}O_6$	4.2	catechin	–
8	3.28	577.1335	577.1346	451.1105, 425.0934, 289.0698	$C_{30}H_{25}O_{12}$	–1.1	procyanidin B dimer	Silva <i>et al.</i> <sup>8</sup>
9	3.51	289.0713	289.0712	–	$C_{15}H_{13}O_6$	0.3	epicatechin	–
10	3.58	865.1971	865.1980	739.1781, 577.1403, 425.0906, 407.0788, 289.0686	$C_{45}H_{37}O_{18}$	–1.0	procyanidin B trimer isomer	Silva <i>et al.</i> <sup>8</sup>
11	3.65	–	–	1153.2620	–	–	procyanidin B tetramer	Silva <i>et al.</i> <sup>8</sup>
12	3.78	865.1979	865.1980	577.1294, 425.0888, 407.0755, 289.0690	$C_{45}H_{37}O_{18}$	–0.1	procyanidin B trimer isomer	Silva <i>et al.</i> <sup>8</sup>
13	4.08	609.1456	609.1456	301.0377, 300.0269	$C_{27}H_{29}O_{16}$	0.0	rutin	–
14	4.16	755.2052	755.2035	609.1409, 301.0314, 300.0263	$C_{33}H_{39}O_{20}$	2.3	quercetin- <i>O</i> -rhamnoside isomer	Chen <i>et al.</i> <sup>20</sup>
15	4.25	463.0891	463.0877	301.0324, 300.0263	$C_{21}H_{19}O_{12}$	3.0	quercetin- <i>O</i> -hexoside isomer	Pereira <i>et al.</i> <sup>21</sup>
16	4.48	449.1099	449.1084	303.0580, 285.0462	$C_{21}H_{21}O_{11}$	3.3	astilbin	–
17	4.54	623.1635	623.1612	315.0508, 285.0331	$C_{28}H_{31}O_{16}$	3.7	isorhamnetin- <i>O</i> - neohesperidoside	Chen <i>et al.</i> <sup>20</sup>
18	4.56	623.1617	623.1612	315.0430, 271.0495	$C_{28}H_{31}O_{16}$	0.8	isorhamnetin- <i>O</i> -rutinoside	Chen <i>et al.</i> <sup>20</sup>
19	4.75	447.0924	447.0927	301.0346, 300.0237	$C_{21}H_{19}O_{11}$	–0.7	quercetin- <i>O</i> -rhamnoside isomer	Pereira <i>et al.</i> <sup>21</sup>
20	4.79	447.0923	447.0927	301.0343, 300.0228	$C_{21}H_{19}O_{11}$	–0.9	quercetin- <i>O</i> -rhamnoside isomer	Pereira <i>et al.</i> <sup>21</sup>
21	4.91	433.1151	433.1135	271.0556	$C_{21}H_{21}O_{10}$	3.7	naringenin- <i>O</i> -glucoside	Tauchen <i>et al.</i> <sup>22</sup>
22	5.14	307.0812	307.0818	161.0473, 143.0298	$C_{15}H_{15}O_7$	–2.0	unknown	–
23	5.33	677.2866	677.2868	645.2515, 489.1866	$C_{27}H_{49}O_{19}$	–0.3	unknown	–
24	6.64	269.0453	269.0450	–	$C_{15}H_9O_5$	1.1	apigenin	Motta <i>et al.</i> <sup>23</sup>

According to our results, the acerola fruits presented high content of the studied bioactive compounds, suggesting that these fruits may be exploited by the food and pharmaceutical industries to take advantage of the functional properties. Studies<sup>27–29</sup> have shown that phenolics help in the prevention and control of pathologies, such as cardiovascular and renal diseases, cancer and type 2 diabetes, which is attributed to their ability to sequester free radicals, chelate metal cations and donate hydrogen atoms or electrons and thereby, reducing the oxidative effects caused by active oxygen species.

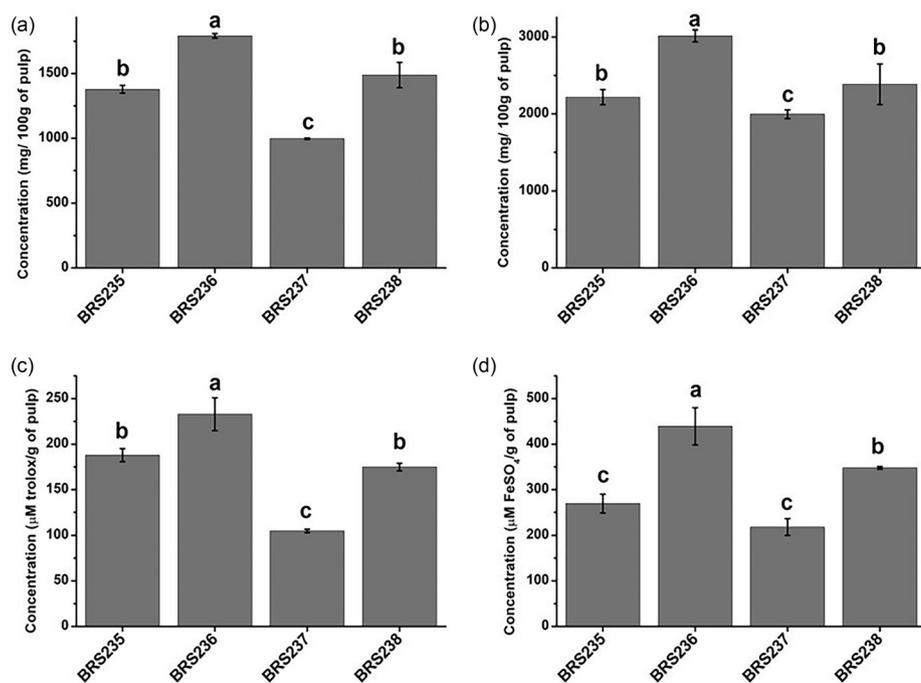
In a study carried out by Mariano-Nasser *et al.*<sup>30</sup> that evaluated the same clones of the present study but at a mature stage, the acerola fruit from the BRS 236 clone also statistically differed from the other clones, and presented

higher vitamin C content (2331, 6 mg 100 g<sup>–1</sup> FW). The result obtained by Oliveira *et al.*<sup>24</sup> for the BRS 236 clone was similar (2719 mg 100 g<sup>–1</sup> FW) to that found in the present study with fruits at the same maturation stage (unripe). Although acerola fruits are well known for having high vitamin C content, they can undergo modifications during ripening. The highest concentration is found in unripe or immature fruits. Xu *et al.*<sup>31</sup> reported that mature fruits contained approximately half the vitamin C content (1225 mg of ascorbic acid 100 g<sup>–1</sup> FW) found in unripe fruits (2386 mg of ascorbic acid 100 g<sup>–1</sup> FW).

Reduced vitamin C content during the maturation process was attributed to the action of the enzyme called ascorbic acid oxidase (ascorbate oxidase), as well as the action of the ascorbate peroxidase enzyme.<sup>32,33</sup> In a study



**Figure 2.** PCA results of the unripe acerola fruits from four different clones: (a) PC1 × PC2 score coordinate system, with clone BRS 235 illustrated in green, BRS 236 in blue, BRS 237 in light red, and BRS 238 in dark red; (b) relevant loadings plotted in lines.



**Figure 3.** Analysis of variance (ANOVA) of the quantitative results from four unripe acerola fruit clones (BRS235, BRS 236, BRS 237, and BRS 238): total phenolics (a); vitamin C (b); and antioxidant capacities based on ABTS (c) and FRAP (d). The same overwritten letters at the top of the bars represent irrelevant statistical differences between the sample's values.

by dos Santos *et al.*,<sup>34</sup> it was verified that the enzymatic activity of the ascorbate peroxidase in ripe acerolas was greater than in unripe ones. It is noteworthy that not only metabolism directly influences vitamin C, but factors such as species, variety and growing and harvesting conditions can also alter its content.<sup>35</sup>

Ascorbic acid is particularly known for its roles in photosynthetic functions in stress tolerance, in addition to having other important functions in growth and metabolism,

such as being an enzymatic cofactor and being involved in the mechanisms of cell growth and division. This is largely due to its ability to directly counteract oxidative stress produced by normal or stressed cellular metabolism as a scavenger of reactive oxygen species, being a potent antioxidant.<sup>36,37</sup> The human body does not produce vitamin C, so it must be acquired through dietary ingestion or supplementation, as it performs several functions such as collagen production and maintenance, wound healing,

reduced susceptibility to infections, in addition to its antioxidant action.<sup>24-38</sup> Considering the antioxidant activity, the values found herein were higher than those shown by Xu *et al.*<sup>31</sup> using the ABTS method. The authors reported mean concentrations of 130 and 90  $\mu\text{mol trolox g}^{-1}$  FW for green and ripe acerola, respectively.

## Conclusions

The present study showed high ascorbic acid content and its isomer, in addition to citric acid, procyanidin B trimer and rutin which contributed to the high antioxidant capacity of unripe acerola fruits. The highest content of the bioactive compounds among the studied acerola clones was found in fruits from the BRS 236 clone. Therefore, this clone is considered a promising crop for its use as a functional food and ingredient which can be explored in the extraction of these phytochemicals.

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## Author Contributions

Amanda G. Silveira was responsible for investigation, methodologies, conceptualization, formal analysis and writing; Lorena M. F. Sampaio for writing-review and editing of the manuscript; Elenilson G. Alves Filho for methodologies and writing-review and editing; Laíza R. Brito for formal analysis; Amélia R. N. Lima for writing-review and editing; Paulo R. V. Brito for formal analysis and investigation; Kirley M. Canuto for writing-review and editing; Luciana S. Oliveira for funding acquisition, investigation, methodologies, conceptualization, writing-review and editing; Carlos F. H. Moura for writing-review, editing and funding acquisition; Jesus F. Ayala-Zavala for writing-review and editing.

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