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Acerola (*Malpighia emarginata***) pulp: characterization and stability of anthocyanins under different conditions**

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Abstract

Tropical fruits provide a high economic potential and their inclusion in the diet can provide health benefits. In this sense, this study aimed to evaluate the proximal composition, bioactive compounds, antioxidant activity, and stability of anthocyanins of acerola pulp in the absence and presence of light at temperatures of 7 and 21.7°C. The results for the proximal composition (g/100 g) were 93.49 moisture, 0.04 protein, 0.09 lipid, 0.60 ash, and 6.28 carbohydrates, totaling a 28.29 kcal/100 g caloric value. For total bioactive compounds, the results were 3.22 *μ*g/g chlorophyll, 29.71 *μ*g/g carotenoids, 3.74 g/100 g flavonoids, and 6.12 mg/100 g phenolic compounds. Acerola pulp showed high antioxidant activity by DPPH and ABTS methods with values of 6.17 *μ*mol/g and 3.19 mM TEAC/g, respectively. Regarding the stability of anthocyanins, in acerola pulp stored away from light and at a temperature of 7°C, these pigments remained more stable than when subjected to light and a temperature of 21°C. Thus, it was possible to perceive that, in addition to the acerola being important for the food industry, storage under low temperatures and the absence of light can contribute to greater stability of anthocyanin pigments.

Keywords: pigment stability; acerola pulp; bioactive compounds; food composition.

Practical Application: Anthocyanin stability of acerola pulp is important to determine better storage conditions.

1 INTRODUCTION

Malpighiaceae is a family that consists of herbs, shrubs, trees, or lianas comprising about 75 genera, with approximately 1,300 species (Quaresma et al., 2020). Plants belonging to the Malpighiaceae family are rich in antioxidant substances such as tannins and flavonoids (Sacramento et al., 2022). The genus *Malpighia* has approximately 40 species of shrubs and small trees, most of which are found in their native state in the Antilles (da Silva et al., 2020).

The species *Malpighia emarginata* (Malpighaceae) is popularly known as acerola and has fruits with a smooth surface or divided into three buds, with a size that can vary from 3 to 6 cm and with an orange to intense red color. The fruits have a fleshy, juicy pulp and have a high content of vitamin C, carotenoids, anthocyanins, as well as iron and calcium (Costa et al., 2022; Souza et al., 2022). Due to their high nutritional value and attractive sensory characteristics, the small fruits, which are often marketed for fresh consumption, are also processed in an artisanal way, resulting in products such as liqueurs, popsicles, ice cream, and jams, which are sold in local shops and informally in rural properties (Santos, D., et al., 2022).

Tropical fruits are conquering more space in the diet of the population. This fact is a result of the increased interest of the population itself, which is currently more aware of the nutritional value of food. Compounds such as vitamins, minerals, and anthocyanins have received attention due to their ability to protect the human body against oxidative stress and help prevent chronic degenerative diseases (Akbari et al., 2022; Maia et al., 2019).

Anthocyanins are secondary metabolites and water-soluble pigments belonging to the phenolic group, which is responsible for the red color of acerola. These natural pigments are used as natural dyes in the food industry, due to the wide range of colors they can produce, from red to blue and purple (Liu et al., 2020). Furthermore, anthocyanins also have several biological properties such as anti-inflammatory, chemoprotective, vasoprotective activities, inhibition of LDL oxidation, and reduced risk of cardiovascular diseases (Mattioli et al., 2020).

The main limitation associated with anthocyanins lies in their notable instability, which can be easily influenced by a wide variety of factors, such as relative humidity, exposure to light, pH, temperature, presence of sugars (both acylated and non-acylated), vitamin content C, oxygen levels, presence of sulfur dioxide or sulfites, enzyme activity, and interaction with co-pigments and the presence of metal ions (Sharma et al., 2016). Consequently, these elements and processes have the potential to induce changes in the concentration and bioactivity of anthocyanins, which, in turn, can affect consumer acceptance

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of the product (Slavu et al., 2020). The objective of this study was to evaluate the chemical composition, bioactive compounds, and antioxidant activity of acerola pulp and to analyze the stability of the anthocyanin extract regarding temperatures and in the presence and absence of light.

2 MATERIALS AND METHODS

2.1 Raw materials

The fruits that had more than 80% of the red fruit of *M. emarginata* were collected in the region of Rio Verde-GO in May 2015. The plant material was identified and deposited in the Herbário Jataiense Professor Germano Guarin Neto under registration number HJ 428. This study was carried out as shown in Figure 1.

2.2 Physical, physicochemical, and chemical characterization

The color of the acerola pulp was determined in a COLOR-FLEX EZ colorimeter (Hunterlab, Reston, Brazil) in 10 repetitions to determine the values $L^*(0 - \text{black}, 100 - \text{white})$, a^{*} (negative — green, positive — red), and b^* (negative — blue, positive — yellow) (Lima et al., 2011). The pH, titratable acidity in citric acid, and soluble solids expressed in °Brix were determined as described by Instituto Adolfo Lutz (2005).

The proximal composition was carried out according to the official methods described by the AOAC (1994): moisture (method 934.06), proteins (method 960.52), ash (method 940.26), and carbohydrates (100–moisture–lipids–proteins– ash). For the determination of total lipids, the method of Bligh and Dyer (1959) was used. Using the Atwater conversion factors (Merrill & Watt, 1973) and based on the centesimal composition, the total caloric value was calculated.

The total carotenoid content was determined by the method of Talcott and Howard (1999) and calculated using Equation 1 (Gross, 1991):

Total carotenoid content
$$
\left(\frac{\mu g}{g\ fresh\ matter}\right) = \frac{A_{470}xVx10^6}{A^{1\%}x100xg}
$$
 (1)

Where:

 A_{470} : the absorbance measured at 470 nm;

V: the total extract volume (100 mL);

 $A_{1,0}$: the extinction coefficient for the solvent mixture (2,500);

g: the weight of the sample in grams.

The total chlorophyll content was determined by the method described by Bruuinsma (1963), and the result was calculated using Equation 2:

Total chlorophyll content
$$
\left(\frac{\mu g}{mL}\right) = 20.2 x A_{645} + 8.02 x A_{663}
$$
 (2)

Where:

 $A₆₄₅$: the absorbance measured at 645 nm;

 A_{663} : the absorbance measured at 663 nm.

The ascorbic acid content was determined according to method 43.064 of the AOAC (2005) modified by Benassi and Antunes (1988).

The crude extract (a combination of 50% methanolic and 70% acetone extract) was prepared according to the method by Larrauri et al. (1997) with modifications as described by Almeida et al. (2019) and used to determine the content of total phenolic content and total flavonoid content as well as antioxidant activity by the DPPH and ABTS methods.

The total flavonoid content was determined according to Subhasree et al. (2009) using the crude extract, and the absorbance was measured in a spectrophotometer at 510 nm against the blank (extract solution). The results were expressed as g quercetin equivalents per 100 g fruit.

The content of total phenolic compounds (TPC) was determined by the Folin-Ciocalteau method (Singleton & Rossi, 1965) using the crude extract. After 120 min of reaction in the absence of light and at room temperature, the absorbance was measured at 725 nm and gallic acid was used as a standard, and the results were expressed as g of gallic acid equivalents per 100 g of pulp.

The ABTS and DPPH methods were used to determine the antioxidant activity of the samples. The determination of antioxidant activity by the ABTS method (2, 2-azino-bis (3 ethylbenzothiazolin-6-sulfonic acid)) was performed according to Miller et al. (1993). The antioxidant activity by the DPPH method was performed according to Brand-**Figure 1**. Flowchart for acerola fruit processing. Williams et al. (1995), where 100 *μ*L of crude extract was added

to 3.9 mL of methanolic solution of DPPH radical 60 *μ*M. After 30 min of incubation in the dark, at room temperature, the absorbance was measured at 515 nm. The results were expressed as mmol of Trolox equivalent (TE) per 100 g of fruit and with percentage of discoloration.

2.3 Determination of the stability of anthocyanins

The extract for the determination of total anthocyanins was performed according to Stringheta (1991) with modifications. Briefly, acerola pulp was homogenized in an HCl:methanol solution (85:15) at a ratio of 1:20 (g/mL), and 12 h after protection from light and under refrigeration, the solution was filtered and the extract was used for the determination of total anthocyanins and in the stability study.

The stability of anthocyanins was determined in four treatments as follows:

- T1: at $7^{\circ}C \ (\pm 0.2)$ in the presence of light;
- T2: at 7°C in the presence of light;
- T3: room temperature in the presence of light;
- T4: ambient temperature in the absence of light.

The extracts at the lowest temperature were placed in BOD TE-371 (Tecnal, Piracicaba, Brazil), and the ambient temperature was controlled by a thermometer at 21.7° C (\pm 0.4). In the absence of light, the tubes were protected from light with aluminum foil, and at room temperature, the samples were 150 cm from the light source. The determination of anthocyanins was performed every 24 h until the 8th day and every 48 h until the 28th day.

2.4 Statistical analysis

Data are expressed as mean ± standard deviation. Analysis of variance and Tukey's test were loaded at a significance level of 5%.

3 RESULTS AND DISCUSSION

3.1 Physical, physicochemical, and chemical characterization of acerola pulp

Table 1 presents the physicochemical analyses of the acerola pulp. The color found for acerola fruit pulp was $L^* = 28.28 \pm 10^{-10}$ 0.30, $a^* = 34.32 \pm 0.60$, and $b^* = 31.77 \pm 0.65$. The values for parameters L^* , a^* , and b^* obtained in this study are close to those reported by Adriano et al. (2011) (a^* of 37.16 and b^* of 21.70) and by Brunini et al. (2004) (L* between 22.12 and 43.27) for acerola fruits. Furthermore, it is known that the planting regions, harvest time, cultural practices, and genetic characteristics play a significant role in the expression of color (Farinelli et al., 2021).

Many authors report that, for red fruits, the most important parameter for color determination is the positive a* (red); however, in this study, the a^* and b^* parameters showed high averages. This shows that both are important for the acerola pulp, as reported by Lima et al. (2011). The measurement of color is essential because color is a quality attribute that directly influences consumption and acceptability, and acerola fruits can present

Table 1. Physical, physicochemical, and chemical characterization of acerola (*Malpighia emarginata*) pulp

Analysis	Mean \pm standard deviation
L^*	28.28 ± 0.30
a^*	34.32 ± 0.60
b^*	31.77 ± 0.65
Soluble solids (Brix ^o)	5.23 ± 0.03
Titratable acidity in citric acid $(g/100 g)$	1.59 ± 0.02
pH	3.17 ± 0.01
Moisture $(g/100 g)$	93.49 ± 0.93
Protein $(g/100 g)$	0.04 ± 0.00
Lipids $(g/100g)$	0.09 ± 0.00
Ash $(g/100g)$	0.60 ± 0.15
Carbohydrates $(g/100 g)$	6.28 ± 0.99
Calorific value (kcal/100 g)	28.39 ± 0.10

great differences. In addition, color is related to the degree of fruit maturity and color can become a limiting factor when these fruits are used in processing (Egea & Pereira-Netto, 2019).

The soluble solids content in the acerola pulp in this study (5.23 ºBrix) corroborates the results reported by Aquino et al. (2011) (5–12ºBrix) and indicates the most advanced degree of maturity of the selected fruits as averages close to 5.7 ºBrix were reported by Righetto et al. (2005) for acerola juices from ripe fruits.

The pH of the pulp was 3.16 and a similar value had already been reported by other authors in which the pH ranged from 2.39 to 4.00 (Aquino et al., 2011; Brunini et al., 2004; Faraoni et al., 2013). The titratable pulp acidity was higher than that reported by Lima et al. (2011) (1.20%) for acerola fruits. This analysis is an indication that the presence of organic acids in foods influences flavor, odor, color, stability, and quality maintenance (Kaddumukasa et al., 2017).

The moisture content found in this study for acerola fruit pulp was 93.49 ± 0.93 g/100 g, which is close to that reported by other authors (between 89.82 and 92.88 g/100 g) (França & Narain, 2003; Soares et al., 2001). Moisture determination is related to its stability, quality, and composition and can affect storage, packaging, and processing (Costa et al., 2022).

The ash content, normally associated with the presence of minerals, found in this study was 0.60 ± 0.15 g/100 g, which is close to that reported by Sousa et al. (2011). The lipid content found (0.09 g/100 g) was lower than that reported by Sousa et al. (2011) (3.59 g/100 g).

In addition, the chemical composition and color of the acerola pulp evaluated in this study were in accordance with current Brazilian legislation (Brasil, 2016), which establishes acceptable pH limits of 2.8, 5.5 ºBrix, 0.80% acidity, total solids 6.50 g/100 g, and color ranging from yellow to red.

Table 2 presents the results obtained for the content of total chlorophyll, total carotenoids, total flavonoids, ascorbic acid, TPC, and total acid ascorbic content for acerola pulp.

The total chlorophyll content in the acerola pulp was 3.22 μg/g, which is consistent with the final stage of ripening, corroborating with its intense red color. At this stage of ripening,

Table 2. Bioactive compounds of acerola (*Malpighia emarginata*) pulp.

Analysis	Mean \pm standard deviation
Total chlorophyll $(\mu g/g)$	3.22 ± 0.65
Total carotenoids $(\mu g/g)$	29.71 ± 1.72
Ascorbic acid	7.94 ± 1.49
Total phenolic compounds (TPC) (mg gallic acid equivalent/100 g)	6.12 ± 0.36
Total flavonoids $(g/100 g)$	3.74 ± 0.27

the fruits have a low chlorophyll content, mainly due to the degradation of chlorophyll and the synthesis of anthocyanins and/or carotenoids (Yu et al., 2020).

The carotenoid content found in this study for acerola fruit pulp (29.71 *μ*g/g) was higher than that reported by Freitas et al. (2006) for tropical acerola juice sweetened and bottled during shelf life. However, although Aquino et al. (2011) reported higher carotenoid contents for frozen acerola (960 *μ*g/100 g), the acerola pulp of this study presented approximately half of the carotenoid content when compared with the potential sources of carotenoids such as corn (880 *μ*g/100 g), papaya (859 *μ*g/100 g), and peach (651 *μ*g/100 g) (Sousa et al., 2011).

The ascorbic acid content found in acerola pulp (7.94 mg of vitamin C per 100 g of sample) was higher than that observed by Yamashita et al. (2003) for fresh frozen acerolas (1,360 \pm 26 mg of vitamin C per 100 g of sample) and pasteurized frozen pulp $(1,360 \pm 26 \text{ mg of vitamin C per 100 g of sample})$ and lower than that reported by Uchôa et al. (2017) for commercially available acerola pulp in supermarkets, which ranged from 610.0 to 111.0 mg of vitamin C per 100 g of sample. These differences may be primarily related to the variety and ripeness stage of the fruit, the type of processing, and the date of the analyses, as the determinations were not made immediately after harvesting or processing.

Phenolic compounds have several beneficial health effects, such as antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic activities (Nishimoto-Sauceda et al., 2022). The total phenol content found in this study (6.12 mg GAE/100 g of pulp) was similar to that reported by Rufino et al. (2010) (6.24 mg GAE/100 g of fruit).

Acerola pulp showed a high content of flavonoids (3.74 g/100 g) when compared with other fruits such as pineapple (0.09 g/100 g), cupuaçu (0.11 g/100 g), orange (0.9 g/100 g), and cashew (1.3 g/100 g) (Sousa et al., 2011). The levels of flavonoids, which are responsible for the red color in fruits, are influenced genetically and by factors such as season of the year, climate, soil composition, maturation stage, preparation, processing, and storage of food (Newerli-Guz et al., 2023).

3.2 Antioxidant activity

Figure 2 shows the antioxidant activity of acerola pulp quantified by the ABTS and DPPH methods. The value found for radical scavenging capacity by the DPPH method in this study for acerola fruit pulp was 6.17 μmol/g. The aqueous extract of acerola has a strong antioxidant capacity, which is most effective in scavenging free radicals, compared with other aqueous extracts of different fruits (Castro et al., 2022).

Figure 2. Antioxidant activity of acerola (*Malpighia emarginata*) pulp by the ABTS and DPPH methods.

A similar result was obtained by Kuskoski et al. (2006) who demonstrated that the frozen acerola pulp stood out for having shown the highest radical scavenging capacity by the DPPH method, in which the average of the highest values of TEAC were 126, 21, 68, and 13.7 μmol/g corresponding to the extracts of baguaçu, jambolan, acerola pulp, and mango pulps, respectively.

The percentage of discoloration by the DPPH method found in this study was 89.36% which is close (90%) to that reported by de Almeida et al. (2006). These authors emphasized that this high percentage of discoloration is due to the high content of ascorbic acid and phenolic compounds present in the fruit, which may also be the case in our study.

According to the ABTS method, the antioxidant activity of the acerola fruit pulp was defined based on the concentration of TE (TEAC), that is, it was observed that the amount of acerola pulp had the same percentage of inhibition as a concentration of 1 mM of the Trolox reference compound. Acerola pulp showed high antioxidant capacity, with TEAC values of $3.19 \pm$ 0.34 mM/g. According to the literature, the higher the TEAC value, the stronger the antioxidant potential, so it can be stated that acerola has an important antioxidant activity through the capture of free radicals. Vieira et al. (2011) found high antioxidant capacity with TEAC values of 1.6 and 3.69 mM Trolox/g aqueous and hydroalcoholic extracts of the pulp, respectively.

3.3 Anthocyanins stability

Anthocyanins are very unstable pigments, being degraded during food processing and storage, resulting in color change, followed by a yellow coloration and the formation of insoluble products (Santos & Martins, 2022). In this context, this study analyzed the stability of anthocyanins at two different temperatures (21 and 7°C), in the presence and absence of light.

The incidence of light on anthocyanins favors their biosynthesis and subsequently accelerates their degradation along with the association of light and oxygen (Mattioli et al., 2020; Oliveira Filho et al., 2021). Even in the absence of light and at all pH values, degradation of this compound occurs through the mechanism of direct or indirect oxidation of the constituents of the medium, directly affecting the anthocyanin molecules (Neuenfeldt et al., 2022).

Figure 3 shows the stability of the anthocyanin extract subjected to temperatures of 7 and 21°C in the absence and presence of

Figure 3. (a) Stability of anthocyanins in acerola (*Malpighia emarginata*) extract in the presence and absence of light at 7°C (± 0.2) and (b) in the presence and absence of light at 21.7°C (± 0.4). Means followed by distinct letters differ statistically from each other according to Tukey's test at a 5% probability level.

light. In this study, it was possible to verify a decrease of 73% and 60% in the anthocyanin content in the presence and absence of light, respectively, at 21°C. Meanwhile, for a temperature of 7°C, a decrease of 65% and 46% was observed in the anthocyanin content in the presence and absence of light, respectively. Regarding the storage time, there was a decrease over the days for the two temperature variations in both the presence and the absence of light. These data prove the instability of this class of compound when associated with oxygen and light, which are the factors that can cause the degradation of these pigments (Groeneveld et al., 2022).

The behavior of anthocyanins was different at the two evaluated temperatures. At temperatures of 7 and 21°C, there was a decrease of 19 and 13%, respectively, in the anthocyanin content. However, when comparing the type of storage for the two temperatures, it is observed that, in the presence of light, there is a smaller reduction (8%) than in the absence of light (14%) in the anthocyanin content.

Anthocyanins are responsible for the red color of acerola, and it is important to measure them due to the commercial interest being focused on appearance (Ferreira et al., 2022). Thus, yellow-colored acerola pulp will probably be rejected by consumers. Thus, these findings emphasize the significance of regulating light exposure and storage conditions to preserve the stability of anthocyanins in food products.

4 CONCLUSION

The chemical composition and antioxidant activity of acerola showed that, in addition to being an important raw material for the food industry, its consumption would be very beneficial, aiming at good nutritional and functional quality.

According to the results obtained, it was possible to conclude that the extract of anthocyanin pigments from acerola pulp stored away from light at a temperature of 7°C remained more stable than when subjected to light and a temperature of 21°C. This method may be an alternative for maintaining the quality of this product, reducing alterations.

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