Domestic processing and storage on the physical-chemical characteristics of acerola juice (*Malpighia glabra* L.).

Processamento doméstico e armazenamento nas características físico-químicas de suco de acerola (*Malpighia glabra* L.).

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**ABSTRACT**

Acerola is a tropical fruit that stands out for its high content of ascorbic acid and phenolic compounds. However, there is currently a lack of information on the effects of the preparation and domestic storage of fruit juices, especially that of acerola. The objective of this work was to evaluate the effect of different liquefying times (10, 30 and 50 s) and cold storage at 4 °C for 0, 24, 48 and 72 h in domestic acerola juice. In relation to such, physicochemical determinations were performed, including pH, total titratable acidity, antioxidant potential, contents of phenolic compounds and total flavonoids in juices produced from the whole fruit, the pulp and acerola seed extracts. Whole fruit juice, liquefied for 10 s, had the highest pH and lowest acidity averages ($P \leq 0.05$). The contents of phenolic and flavonoid compounds ranged from 26.06±1.18 to 168.34±24.63 mg of gallic acid equivalents (GAE)/100 mL and 11.17±0.96 to 49.45±1.43 mg of catechin equivalents (CE)/100 mL, respectively. Total phenolics were higher in whole acerola juices and total flavonoids in seed extracts, both when liquefied for 50 s. The antioxidant potential ranged from 0.12±0.01 to 4.26±0.78 mmol of Trolox equivalents (TE)/100 mL, and was also higher in whole acerola juice, liquefied for 50 s ($P \leq 0.05$). The results showed that acerola pulp has higher phenolic content and antioxidant potential, while seeds have more flavonoids. The liquefying time of the whole fruit for 50 s and consumption during the first 24 h, were the best conditions tested in this study, for obtaining a juice with a high content of phenolic and antioxidant activity.

**Index terms:** Antioxidant potential; phenolic compounds; flavonoids.

**RESUMO**

A acerola é uma fruta tropical que se destaca pelo alto teor de ácido ascórbico e compostos fenólicos. No entanto, atualmente há carência de informações sobre os efeitos do preparo e armazenamento doméstico de sucos de frutas, em especial de acerola. O presente trabalho teve por objetivo avaliar no suco de acerola doméstico, o efeito de diferentes tempos de liquidificação (10, 30 e 50 s) e do armazenamento refrigerado a 4 °C, por 0, 24, 48 e 72 h. Relacionado a isto, foram executadas determinações físico-químicas, comprendendo, pH, acidez titulável total, potencial antioxidante, teores de compostos fenólicos e flavonoides totais em sucos produzidos com a fruta inteira, somente com a polpa e em extratos de sementes de acerola. Os sucos das frutas inteiras, liqueficados por 10 s, apresentaram as maiores médias de pH e menores de acidez ($P \leq 0.05$). Os teores de compostos fenólicos e flavonoides variaram de 26,06±1,18 a 168,34±24,63 mg Eq. A.G./100 mL e de 11,17±0,96 a 49,45±1,43 mg Eq. Cat./100 mL, respectivamente. Os fenólicos totais foram superiores nos sucos de acerolas inteiras e os flavonoides totais nos extratos de sementes, ambos liqueficados por 50 s. O potencial antioxidante variou de 0,12±0,01 a 4,26±0,78 mmol de trolox/100 mL, sendo também superior nos sucos de acerolas inteiras, liqueficadas por 50 s ($P \leq 0.05$). Os resultados demonstraram que a polpa de acerola possui maior teor de fenólicos e potencial antioxidante, enquanto as sementes, flavonoides. O tempo de liqueficação da fruta inteira por 50 s e o consumo imediato ou durante as primeiras 24 h foram as melhores, dentre as condições testadas neste estudo, a fim de se obter um suco com elevado conteúdo de compostos fenólicos e atividade antioxidante.

**Termos para indexação:** Potencial antioxidante; compostos fenólicos; flavonoides.
INTRODUCTION

Acerola is a tropical fruit native to Central America and northern South America, belonging to the family Malpighiaceae Juss. Among the species of this fruit, two (Malpighia glabra L. and Malpighia emarginata Sessé & Moc. ex. DC.) have been commonly cultivated for consumption and marketing, especially in Brazil, Mexico and certain parts of South East Asia and India (Belwal et al., 2018). The cultivation of acerola trees in Brazil began with the importing of seeds from Puerto Rico in the 1950s. The Antillean fruit has already been considered as a hybrid of the species M. glabra L. and M. punicifolia, the former coming from a small tree and the latter from a bush (Simão, 1971).

In a study published by Gomes et al. (2001), the authors report that the names M. glabra L. and M. punicifolia are synonyms applied to a different species of acerola and point out that the proper use of the nomenclature to designate acerola should be M. emarginata. However, the Integrated Taxonomic Information System shows the current taxonomic status of M. glabra L. and M. emarginata Sessé & Moc. ex. DC. as accepted, but M. punicifolia as misapplied, with a current status of unaccepted (Itis, 2019).

With the increasing interest in healthier life habits, the consumption of fruits with functional properties has become increasingly popular, besides being a fundamental component of a nutritionally balanced diet aimed at the prevention of chronic diseases. Acerola (Malpighia glabra) is a tropical fruit that stands out for its high content of ascorbic acid and phenolic compounds, in particular the flavonoids: rutin (Caetano; Daiuto; Vieites, 2012; Leffa et al., 2015), quercetin glycosides and kaempferol, the latter being the main flavonol present in ripe acerola (Vasavilbazo-Saucedo et al., 2018).

Phenolic compounds have known antioxidant activity related to their ability to chelate metals, inhibit lipoxygenase enzymes and capture and neutralize free radicals from hydrogen donation. The antioxidant potential of phenolic compounds is directly linked to the number and arrangement of hydroxyl groups, the extent of structural conjugation, as well as the electronic ring resonance (Ho; Rafi; Ghai, 2010; Gómez-Mejía et al., 2019). Due to these characteristics, several commercial products containing acerola have been used as a dietary supplement in the optimization of immune response, as a source of antioxidant compounds and to meet nutritional needs (Pereira et al., 2013; Belwal et al., 2018).

Brazil is the world’s largest producer and exporter of acerola in natura or its pulp, a product developed in response to short fruit shelf life. In addition to this application, the fruit is widely used to produce acerola juice, a slightly acidic flavored beverage, usually consumed either pure or mixed with other fruits (Belwal et al., 2018). Acerola should be harvested when it is intensely red in color, yet still firm in consistency, in order to avoid losses during transport and handling. If the fruit is not frozen, it should be consumed as soon as possible because, after 24 hours, the fruit considerably loses its characteristics of freshness and quality (Corrêa et al., 2017).

In addition to the nutritional and sensory aspects, practicality remains an important factor of consumer desire and intention to purchase. Convenience, when attributed to fruit, is related to the ease of storage and preparation for the consumption of fresh fruit or juice. The processing of acerola affects its physicochemical, functional and even its nutritional characteristics. Maia et al. (2007) demonstrated that the industrial processing of juice increased the pH and carotenoid content and reduced the vitamin C and anthocyanin contents, keeping other evaluated parameters unchanged (total soluble solids, reducing and total sugars). In contrast, the processing, freezing and storage of fruit pulp, according to commercial practice for 11 months, did not significantly affect carotenoid contents, thus proving to be efficient for preservation (Agostini-Costa; Abreu; Rossetti, 2003).

The literature includes numerous scientific publications regarding the effects of different types of industrial processing on the physicochemical, nutritional and sensory characteristics of acerola (Chaves et al., 2004; Maia et al., 2007; Caetano; Daiuto; Vieites, 2012; Corrêa et al., 2017; Nascimento et al., 2018). However, there is currently a lack of information on the effects of the preparation and domestic storage of fruit juices, especially that of acerola. Thus, the present study aimed to evaluate the physicochemical characteristics of acerola juice through domestic preparation at different times of blending and throughout storage.

MATERIAL AND METHODS

The raw material used was acerola (Malpighia glabra L.) harvested in Alfenas-MG, Brazil (latitude: -21.43333 and longitude: -45.950000). Approximately 4 kg of acerola were harvested from different trees at maturity stages identified by the firmness of the pulp, through manual palpation, and completely red-colored bark, mixed and packed in polyethylene packaging. After
harvesting, the fruits were transported at room temperature to the Experimental Nutrition Laboratory of the College of Nutrition of the Federal University of Alfenas - UNIFAL-MG, Brazil.

The fruits were sanitized in chlorinated water (200 ppm), then packaged in polyethylene containers, and then stored frozen at -18 ºC until use. For seed and pulp analysis, the fruits were manually pulped, with their seeds separated and washed in running water. Whole fruits and their parts (seeds and pulp) were separately liquefied with 30% (w/v) distilled water, with 60 g of sample and 200 mL of water using an 800 w Philco household blender, 60 Hz, at power 1 (China). Extracts of whole fruits and their parts were prepared in triplicate for the different liquefying times (10, 30 and 50 s).

The juices were passed through a 20 mesh, 850 µm mesh opening sieve for domestic use and packed in 500 ml clear glass containers with a lid. The juices obtained from whole fruits and pulp, as well as the seed extracts, were immediately analyzed (time 0 h). The juices of whole acerola were then stored under refrigeration at 4 ºC for 72 h while being analyzed again after 24, 48 and 72 h of its preparation.

The pH determinations were performed in a Lucas-210 (Lucadema) potentiometer and the total titratable acidity was performed with 0.1 M factorized NaOH titrating solution, according to the methodology published by AOAC (1995).

Total flavonoid analyses were performed according to the methodology proposed by Gómez-Mejía et al. (2019) with modifications. A 250 µL aliquot of the filtered samples was added to 1.0 mL distilled water and 75 µL NaNO₂ solution (5% w/v). After homogenization and 5 min incubation at room temperature, 75 µL AlCl₃ (10% w/v) was added, repeating the shaking and incubation process. Finally, 0.5 mL NaOH (1 mol/L) was added, with the mixture homogenized and incubated again for 5 min. The mixtures were analyzed by BelPhotonics Ultraviolet/Visible-M51 (Bel) spectrophotometer at 510 nm and the flavonoid contents were obtained from a 6-point catechin standard curve.

The contents of total phenolic compounds were obtained according to the methodology described by Untea et al. (2018), with modifications. A 50 µL aliquot of the samples was added to 250 µL Folin-Ciocalteu reagent and 2.5 mL distilled water. The mixture was homogenized for 1 min and then added to 1.0 mL Na₂CO₃ (7% w/v) and incubated for 1 h at room temperature. The samples were analyzed by spectrophotometer at 750 nm.

The antioxidant potential of the samples was verified by the cation free radical discoloration method 2,2’-azinobis (3-ethylbenzothiazoline-6-sulfonic) (ABTS), as a result of neutralization with the antioxidant compounds present in the samples, according to the method performed by Untea et al. (2018). The antioxidant potential of the sample was calculated from a standard curve of the synthetic antioxidant, Trolox, in the concentration range of 0 to 7 nmols.

The blank solutions were prepared for each analysis, consisting of the same reagents specified in each methodology with distilled water replacing the aqueous extract of the acerola or its parts. Statistical analyses were performed by analysis of variance (ANOVA), followed by the Tukey test with $P \leq 0.05$ and were correlated by the Pearson Method with $P \leq 0.01$.

**RESULTS AND DISCUSSION**

The average pH values obtained from whole acerola juices, obtained from different liquefying times and during 72 h of storage, ranged from 3.41 to 3.57 (Figure 1), while pulp extracts ranged from 3.47 to 3.48, and seeds from 4.44 to 4.45 (Table 1).

The pH range of the aqueous extracts of whole fruits and pulp was similar to those obtained by Nascimento et al. (2018), with a pH of 3.35 for industrially obtained acerola pulp and 3.63 for artisanal pulp, and by Caetano et al. (2012), with a pH of 3.47 for juice and 3.44 for pulp. Seed and pulp extracts showed no significant difference in the pH value regarding the different liquefying times (Table 1).

The mean titratable acidity values ranged from 0.27 to 0.32 g of citric acid/100 mL of whole acerola extract (Figure 2). The liquefying time significantly interfered, with the lowest values observed in the extracts with shorter liquefying time obtained from the whole acerola and its parts: seeds (0.08 to 0.10 g citric acid/100 mL) and pulp (0.32 to 0.34 g citric acid/100 mL) (Table 1). The refrigerated storage of the whole acerola extracts did not significantly alter the titratable acidity (Figure 2).

The mean titratable acidity values obtained in the present study were lower than those reported by Freitas et al. (2006), which ranged from 0.53 to 2.27 g citric acid/100 g fruit, and by Caetano et al. (2012) who analyzed whole juice and fruit pulp, obtaining average results of 0.84 and 0.94 g citric acid/100 g, respectively. It is important to highlight that in both works the results were expressed by 100 g of pulp or whole fruit juice, which differs from those recorded in the present work.
Figure 1: pH values of extracts of whole acerola obtained at different times of liquidization and stored at 4 °C for 72 h.

Table 1: pH values, total titratable acidity, contents of phenolic compounds, flavonoids and antioxidant potential in acerola seed extracts and pulp juices obtained from different liquefying times.

| Part  | Liquefying time | pH       | TTA (g citric acid/100 mL) | Phenolic compounds (mg GAE/100 mL) | Flavonoids (mg CE/100mL) | Antioxidant potential (mmol TE/100 mL) |
|-------|-----------------|----------|-----------------------------|-------------------------------------|--------------------------|----------------------------------------|
| Seed  | 10              | 4.44±0.01a | 0.08±0.01d                 | 26.06±1.18c                         | 11.17±0.96e              | 0.12±0.01b                              |
|       | 30              | 4.45±0.01a | 0.09±0.01c,d               | 34.03±5.89b,c                       | 28.46±1.33b              | 0.15±0.01b                              |
|       | 50              | 4.45±0.01a | 0.10±0.01c                 | 44.12±1.73b                         | 49.45±1.43a              | 0.18±0.01b                              |
| Pulp  | 10              | 3.48±0.01b | 0.32±0.01b                 | 159.84±9.81a                        | 20.19±2.18d              | 3.56±0.40a                              |
|       | 30              | 3.47±0.01b | 0.34±0.01c,b               | 156.17±4.69a                        | 19.28±1.14d              | 3.57±0.26a                              |
|       | 50              | 3.48±0.01b | 0.33±0.01a,b               | 148.49±3.64a                        | 24.30±1.31c              | 3.44±0.12a                              |

Leg.: TTA: total titratable acidity; GAE.: gallic acid equivalents; CE.: catechin equivalents; TE.: Trolox equivalents. Values from the same column with the same letter are not significantly by ANOVA and Tukey Test (P≤0.05).

Figure 2: Total titratable acidity expressed in g of citric acid/100mL of whole acerola extracts obtained at different liquefying times and stored over 72 h.
The total phenolic content ranged from 103.22 to 168.34 mg GAE/100 mL aqueous extract of whole acerola obtained at different liquefying times over the 72 h of refrigerated storage (Figure 3).

The different liquefying times did not significantly affect the content of total phenolic compounds when analyzed immediately after obtaining the whole fruit extracts. However, throughout the 24, 48 and 72 h of storage, a significant difference was observed, with the lowest contents linked to the shorter liquefying times.

After 72 h of storage, phenolic compounds reduced by 26.8%, 18.6% and 7.1% in the juices liquefied at 10, 30 and 50 s, respectively. The lower impact of storage on the total phenolic contents observed in the juices obtained with longer liquefying times is possibly associated with the higher acidity and lower pH of these juices. Cunha et al. (2014) observed the same trend in the stability of ascorbic acid in the juices of various fresh fruits, with the lowest losses in juices that presented higher initial acidity.

The decrease of phenolic compound content can be explained by oxidation reactions influenced by external factors, such as light and average temperature (Righetto; Netto; Carraro, 2005), although biochemical reactions are catalyzed by phenol oxidases. Liquefying acerolas into its juice causes the descompartmentalization of enzymes and its substrates which, after the disruption of plant tissues, come into direct contact, thereby facilitating and increasing enzymatic activity (Ruiz-Rodrigues et al., 2008; Parkin, 2010).

Maia et al. (2007) verified significant losses of anthocyanins during the industrial processing of acerola juice. The authors state that these compounds are considerably unstable and can become degraded by the action of light, temperature, oxygen, metal ions and enzymes. Rosso and Mercadante (2007) observed that high ascorbic acid content is the main cause of the low stability of anthocyanin in acerola, causing condensation of ascorbic acid with anthocyanin carbon 4, resulting in losses of both bioactive compounds.

The extracts obtained from acerola seeds and pulp presented average contents in the range of 26.06 to 44.12 and 148.49 to 159.84 mg GAE/100 mL, respectively. The extracts of seeds liquefied for 10 s and 50 s showed a significant difference regarding the average levels of total phenolics, which was not observed in the pulp extracts obtained from different liquefying times (Table 1). These results demonstrate the importance of increasing the fruit liquefying stage at home in order to obtain fruit juice with high content of bioactive compounds.

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Figure 4 expresses the flavonoid contents ranging from 22.43 to 35.22 mg CE/100 mL of whole acerola aqueous extract obtained at different blending times and over 72 h of storage. The domestic processing significantly interfered with the flavonoid contents, with the highest contents observed with the longest fruit liquefying time. At the end of refrigerated storage of 72 h, it was possible to observe a significant reduction in flavonoid contents only in the extracts obtained from 30 s of liquefying (Figure 4).

The same trend can be observed for seed and pulp extracts, which increased the flavonoid contents with the increase of the liquefying period. Seed extracts processed at 10 and 50 s and pulp extracts obtained under the same conditions were 11.17 and 49.45; 20.19 and 24.30 mg CE/100 mL, respectively (Table 1).

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The antioxidant potential of aqueous extracts of whole acerola ranged from 1.77 to 4.26 mmol TE/100 mL of the sample. Fruit processing time as well as storage significantly changed this parameter. The longer the liquefying time, the higher the antioxidant potential and the longer the storage period, the lowest activity (Figure 5).

The juices obtained by longer liquefying showed less reduction of antioxidant activity at the end of 72 h of storage, corresponding to 17.6%, while the juices liquefied for 10 and 30 s showed loss of 44.2% and 44.2%, respectively. The antioxidant activity in acerola juices is directly related to the content of phenolic compounds and ascorbic acid (Belwal et al., 2018).

In the extracts of acerola seeds and pulp, the antioxidant potential ranged from 0.12 to 0.18 and from 3.45 to 3.57 mmol TE/100 mL, however, the liquefying time did not significantly change this parameter (Table 1).

In Pearson regression and correlation analysis, positive correlations were demonstrated, between antioxidant potential and total phenolic compound contents in whole acerolas (r = 0.76, P ≤ 0.01) and seeds (r = 0.88, P ≤ 0.01), for antioxidant potential and total flavonoid contents in extracts of whole acerolas (r = 0.43, P ≤ 0.01) and seeds (r = 0.99, P ≤ 0.01) and for the contents of phenolic compounds and total flavonoids in whole fruit (r = 0.61, P ≤ 0.01) and seed extracts (r = 0.92, P ≤ 0.01). Acerola pulp extracts did not show significant correlations between the evaluated parameters.

**Figure 4:** Flavonoid contents, expressed as mg of catechin equivalents/100 mL of whole acerola extracts obtained at different liquefying times and stored over 72 h.

**Figure 5:** Antioxidant potential, expressed in mmol trolox equivalents/100 mL whole acerola extracts obtained at different blending times and stored over 72 h.
CONCLUSIONS

Liquefying time and storage are determinant variables in the intensity of the functional properties of acerola juice. The liquefying time of the whole fruit for 50 s and the immediate consumption, or during the first 24 h, were the best, out of the conditions tested in the present study, for obtaining a juice with a high content of phenolic compounds and antioxidant activity. The acerola seed presents different physicochemical parameters than those of the pulp and the extracts obtained with 50 s of liquefying time presented higher flavonoid contents, even when compared to fruit pulp extracts, thus reinforcing the potential of using acerola seeds in the preparation of food products claiming functionality.

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