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Chemistry

Enhanced antioxidant and antimicrobial properties of lyophilized vitamin c concentrates from *Malpighia emarginata* (acerola): a comparative study

Propriedades antioxidantes e antimicrobianas aprimoradas de concentrados de vitamina c liofilizados de *Malpighia emarginata* (acerola): um estudo comparativo

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ABSTRACT

Vitamin C (Vit C) plays beneficial roles in the human body and has been used as a natural preservative in foods. However, there are no technological prospecting studies aimed at producing Vit C concentrates from *Malpighia emarginata* (acerola) nor antioxidant and antimicrobial evaluations of these products. In this context, this study aimed to compare Vit C concentrates obtained from acerola through two different drying methods (spray drying and freeze-drying), evaluating these preparations' antioxidant and antimicrobial activities. The concentration of Vit C and phytochemical analyses were performed using chromatographic (HPLC) and spectroscopic (ESI-IT-MSn) methods. The antimicrobial activity was evaluated by microdilution and diffusion in a solid medium. Chemical analyses revealed the presence of six compounds, including Vit C and anthocyanins, considered markers for this species. The Vit C content was higher for freeze-drying compared to spray drying (16.30% and 10.74%, respectively). In the Minimum Inhibitory Concentration (MIC) test, the freeze-dried product also showed better activity against *Escherichia coli* and *Staphylococcus aureus* compared to the spray-dried product (0.0078 and 0.0312 g/mL, respectively). In the solid medium, both concentrates were active. The concentrates also showed antioxidant effects by the DPPH method. The results of this study provide convincing evidence that freeze-drying *M. emarginata* results in a higher concentration of Vit C and has promising antioxidant and antimicrobial effects.

Keywords: Acerola concentrate; Ascorbic acid extraction; Extract drying methods; Natural products

RESUMO

A vitamina C (Vit C) desempenha efeitos benéficos no corpo humano e tem sido usada como conservante natural em alimentos. No entanto, não existem estudos de prospecção tecnológica voltados para a produção de concentrados de Vit C a partir de *Malpighia emarginata* (acerola), bem como avaliações antioxidantes e antimicrobianas desses produtos. Neste contexto, este estudo teve como objetivo comparar concentrados em Vit C obtidos da acerola, através de dois diferentes métodos de secagem (spray dryer e liofilização), avaliando as atividades antioxidante e antimicrobiana destes preparados. A concentração de Vit C e as análises fitoquímicas foram realizadas por métodos cromatográficos (HPLC) e espectroscópicos (ESI-IT-MSn). A atividade antimicrobiana foi avaliada por microdiluição e difusão em meio sólido. As análises químicas revelaram a presença de seis compostos, incluindo Vit C e antocianinas, considerados marcadores para esta espécie. O teor de Vit C foi maior para a secagem por liofilização em comparação com o *spray dryer* (16,30 e 10,74%, respectivamente). No teste de Concentração Inibitória Mínima (CIM), o liofilizado também apresentou melhor atividade contra *Escherichia coli* e *Staphylococcus aureus* quando comparado ao *spray dryer* (0,0078 e 0,0312 g/mL, respectivamente). No meio sólido, ambos os concentrados foram ativos. Os concentrados também apresentaram efeitos antioxidantes pelo método DPPH. Os resultados deste estudo fornecem evidências convincentes de que a liofilização de *M. emarginata* apresenta maior concentração de Vit C e tem efeitos antioxidantes e antimicrobianos promissores.

Palavras-chave: Concentrado de acerola; Extração de ácido ascórbico; Métodos de secagem de extrato; Produtos naturais

1 INTRODUCTION

Fruits are foods rich in macronutrients and micronutrients such as minerals, fibers, and vitamins essential for the good metabolic performance of human beings. Vitamin C, also known as ascorbic acid, is a crucial nutrient for the human body. It is necessary for many functions in the body, including collagen formation, iron absorption, immune function, and cell repair, and it has antioxidant properties, which help protect the body's cells against damage caused by free radicals. The phytochemical complex of medicinal plants plays a fundamental role in discovering viable alternatives for the treatment of various diseases (Rossato et al., 2022a; Rossato et al., 2022b).

According to Guan et al. (2020), Vitamin C supplementation is associated with the prevention of cardiovascular diseases and can significantly reduce blood pressure in patients with hypertension. Moreover, studies carried out in 2020 showed that the administration of vitamin C, in combination with other treatments, significantly

improved immune function and reduced hospitalization time in patients with COVID-19, and was associated with immune strengthening (Hoang et al., 2020).

Vitamin C is present in several fruits, such as acerola, which has content in the range of 1500 to 4500 mg/100 g and is an excellent source of other important bioactive components, such as anthocyanins, carotenoids, flavonoids and phenolic compounds (Prakash & Baskaran, 2018; Aparício-Garcia et al., 2020; Ribeiro, Freitas, 2020). Acerola is a fruit originating in Central America, which has become popular all over the world due to its health benefits and its use in various industrial products. This fruit is considered extremely nutritious due to its high content of vitamin C, B vitamins and carotenoids, in addition to other bioactive compounds.

Functional additives have been widely used in the food industry to improve food quality and safety, as well as to provide additional health benefits to consumers. Functional additives can be defined as substances added to foods to improve their nutritional quality, sensory properties, stability and/or microbiological safety (Silva et al., 2022).

The relationship between the food market and functional additives is very close because the use of these substances is fundamental to guarantee the quality and safety of food, besides contributing to the development of innovative products with additional health benefits. The demand for functional foods has grown in recent years, boosting this market. Matos (2020) evaluated the use of *Malpighia emarginata DC*. (acerola) and *Syzygium cumini* Lamarck (jambolão) extracts in yogurts, and the results indicated that the addition of acerola extract reduced water activity, as well as increased acidity and content of total phenolic compounds, flavonoids, and anthocyanins, concluding that the fruit can be a natural alternative to improve the nutritional quality of dairy products. Silva et al. (2020) evaluated the use of acerola powder in bread, and the results indicated that adding acerola powder significantly increased the vitamin C content, improving the sensory acceptability of the products.

Linked to this point, some studies evaluate the antimicrobial activity of vitamin C, since phenolic compounds represent a group of secondary metabolites that present inhibition of microbial growth and may emerge as a natural alternative for food preservation. However, these studies are scarce and the action of phenolic compounds of natural origin as preservatives is little explored, as well as their application in industry (Rocha, 2019).

The Brazilian industry has been using more and more powdered food products as ingredients in their formulations, considering that such products significantly reduce operating costs related to packaging, transport, storage, and conservation, increasing their added value. Because it is characterized as a highly perishable fruit, the dehydration of acerola is presented as an efficient alternative for its conservation and better use of its vitamin potential by the industry, and it can be an alternative for the development of food products with greater added value, which can be marketed for a longer period without compromising their quality. In this context, the development of new products based on acerola represents a viable technological alternative, considering that this fruit has been associated with a healthy diet.

The spray drying process and freeze-drying are widely used methods in the food industry. Spray drying involves atomizing the liquid product into a drying chamber, where it is exposed to a stream of hot air that quickly evaporates the water, resulting in a dry powder. This method is efficient and cost-effective but can cause the degradation of heat-sensitive nutrients, such as vitamin C. In contrast, freeze-drying, or lyophilization, freezes the product before reducing the pressure around it, allowing the water to sublimate directly from the solid to the gaseous state. This process is more time-consuming and expensive but better preserves the integrity of nutrients and vitamins, keeping their biological properties almost intact due to the absence of high temperatures (Liapis, Bruttini & Marchelli, 1996; Hagan, Kell & Mendes, 2015).

The changing eating habits of modern society and new market trends make the inclusion of new products that are inserted in the context of healthiness attractive

and profitable, a fact that can be achieved by adding nutrients to processed foods. Given the above, this work carried out the obtaining and characterization of a Vitamin C concentrate, obtained from acerola pulp by two different drying methods, spray dryer and lyophilization and evaluate the activities of antioxidant and antimicrobial of these preparations.

2 MATERIAL AND METHODS

2.1 Extract production

To obtain the extract, 600 g of frozen acerola pulp (Brand Polpa Norte) purchased from a local market was used in contact with an extracting solution containing 1% oxalic acid and 0.05% ethylenediaminetetraacetic acid (EDTA). This solution was stirred at room temperature for 5 min. Afterward, the solution was filtered.

To increase the glass transition temperature of the powder formed in the drying step, reducing its accumulation on the walls of the equipment used in drying, the whitening agent named maltodextrin (Midway) was added, using a proportion of 15% maltodextrin per liter of dry extract in spray dryer and 1% maltodextrin per liter of dry extract in a lyophilizer.

2.2 Spray drying

To obtain the powdered concentrate from the acerola pulp, a benchtop spray dryer, BUCHI brand, model B-2900, was used. The inlet temperature was set at 110 ^{o}C, with 100% sample aspiration and 20% pumping flow. With the definition of these variables, the outlet temperature was 56 ^{o}C. The sample drying time was approximately 2 hours, and the process variables were chosen based on previous studies. During this period, the solution remained under agitation. After drying, the powdered concentrate was stored at room temperature in a sealed plastic container, protected from light.

2.3 Lyophilization

Samples submitted to drying by lyophilization (freeze drying) were frozen in an ultra-freezer (Sanyo brand) at – 80 ºC for 48 hours and then lyophilized at – 51 ºC under a vacuum of 577 μHg (19.28 atm) for 40 h in a lyophilizer (Liobras brand – Brazil, Liotop L101 model). After drying, the powdered Vitamin C concentrate was stored at room temperature in a sealed plastic container protected from light.

2.4 Mass spectrometry analysis (ESI-IT-MS/MS)

The vitamin C concentrate was submitted to direct flow infusion performed on the Braker SolariX FT-ICR-MS analyzer equipped with an electrospray ionization source (ESI), in positive and negative mode, drying gas flow rate 3,0 L/min, drying gas temperature 200 ºC, spray voltage - 0.5 kV, capillary voltage –4.5 kV, ECD lens –10 V, flow sample 5 μL/h and nebulizer gas flow rate of 0.4 bar. Fragmentations (MS/MS) of the samples were performed using the collision-induced dissociation (CID) method against argon for ion activation. The first event was a full scan mass spectrum to obtain data on ions in the *m/z* range 154–2000 D. The second scan event was an MS/MS experiment performed using a data-dependent scan on molecules $[M-H]^-$ of the compounds of interest at a collision gas flow rate of 30%.

2.5 Vitamin C quantification

The concentrate samples obtained were submitted to High-Performance Liquid Chromatography (HPLC) analysis (Varian brand, Varian ProStar 230/7125 model), with a UV/visible detector, for the determination of the percentage of ascorbic acid (Vitamin C), and the device used an aqueous solution of 0.10% acetic acid, flow rate 1.0 mL/min and total analysis time of 12 min for each sample. The wavelength used was 254 nm,according to the methodology proposed by Spínola, Martínez and Castilho (2014). The calibration curve required for data conversion was previously established, obtained from the dilution of the vitamin C standard in ultrapure water at concentrations of 6,

25, 12.5, 25, 50 and 100 µg/mL. Samples of the concentrates obtained in the spray dryer and the lyophilizer were also prepared in ultrapure water, and filtered through a 0.2 µm membrane filter, which was transferred to a glass ampoule. With a 50 µL microsyringe (Hamilton), a sample was manually injected into the chromatograph, and the reading was carried out. All analyses were performed in duplicate.

2.6 Antimicrobial analysis

2.6.1 Inoculum preparation

The assays were performed using the microorganisms *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 35218) following the methodology proposed by Capelezzo (2017) and Mohr et al. (2017), with some modifications. Using a sterile inoculation loop (Cral), an aliquot of the microorganisms was transferred to tubes containing Brain Heart Infusion broth (BHI, Merck) and incubated in a bacteriological oven (Logen, LSDHG-9140A) at a temperature of 35±1 ºC for a period of 24 h. After this period, some bacterial colonies were isolated to guarantee that the absorbance read in the spectrophotometer solely and exclusively referred to the concentration of the microorganism. Using an inoculation loop, an aliquot of the bacterial suspension was removed. Subsequently, using the draining technique, some colonies were isolated in a petri dish with PCA culture medium (Plate Count Agar, Merck). The plates were incubated in a bacteriological oven at a temperature of 35±1 ºC for a period of 24 h. After this period, some bacterial colonies were removed and placed in sterile saline water (0.85%, Merck), adjusted until a concentration of 10^4 CFU/mL was obtained. For this, a spectrophotometer at a wavelength of 619 nm (Merck, Pharo 300 Spectroquant®) was used. To obtain such a concentration of microorganisms, the absorbance read on the equipment should be between 0.04 and 0.049.

2.6.2 Diffusion in solid medium from the orifice

The diffusion technique in a solid medium from an orifice was used to verify the antimicrobial activity of the Vitamin C concentrate obtained by the different drying methods. This technique was performed according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2012b), with some modifications, as suggested by Mohr et al. (2017). The microorganisms of interest at a concentration of 10^4 CFU/mL were sown in the form of streaks on PCA agar that had already solidified in a petri dish using the surface plating technique with the aid of a disposable swab, sowing in three directions, thus ensuring their complete deposition. In each plate, two equidistant holes were made, using a 1000 µL tip, with a diameter of approximately 8 mm, and approximately 0.15 g of the concentrate was deposited in each hole separately. The plates were incubated at 35±1 ^{o}C in a bacteriological oven for 24 h. After this time, the microbial growth inhibition halo formed was measured, with the aid of a millimeter ruler, and this assay was performed in triplicate. For each inhibition halo, the diameter occupied by the antimicrobial agent was discounted.

2.6.3 Broth microdilution technique for determining the Minimum Inhibitory Concentration (MIC)

The determination of MIC was carried out to calculate the minimum concentration of the compound capable of inhibiting or causing the death of the microorganisms tested. The methodology used was that described by the Clinical and Laboratory Standards Institute (CLSI, 2012a), with some modifications, as suggested by Mohr et al. (2017). A solution was prepared at a concentration of 0.5 g/mL for the lyophilized Vitamin C powder concentrates dried by spray dryer in distilled water, a concentration determined from the methodology adopted by Marques et al. (2017). Seven dilutions were performed, and the assay was carried out in sterile 96-well microplates with a "U"-shaped bottom, distributed in 8 rows named from A to H and 12 columns.

An amount of 100 µL of BHI broth was placed in each of the microwells. Then, in columns 1, 2 and 3 (characterized as the analysis triplicate) and line A, 200 µL of the most concentrated solution from the lyophilized concentrate was added; and, in columns 4, 5 and 6 and line A, 200 µL of the most concentrated solution from the concentrate dried by spray dryer was added, and the concentration of the compound is always reduced by half in the subsequent lines in descending order. After that, 5 µL of microbial suspension was added at a concentration of 104 CFU/mL, resulting in a homogeneous solution with the characteristic color of the extracts. In column 8 (positive control), 100 µL of BHI and 5 µL of bacterial suspension (10^4 CFU/mL) were inserted to attest that the broth used allowed the growth of the microorganisms tested. In column 9 (negative control), 100 µL of BHI, 100 µL of distilled water and 5 µL of bacterial suspension (10^4 CFU/mL) were added to confirm that the distilled water used as solvent was not inhibiting microbial growth. In column 10, only 100 µL of BHI was added, in order to maintain its sterility (blank). In column 7, nothing was added. The microplates were then incubated in an oven at a temperature of 35±1 ºC for 20 h. After this period, 20 µL of 2,3,5-triphenyl tetrazolium chloride (TTC) was added to each microwell, an agent that promotes staining, thus allowing visualization of bacterial growth. The microplates were taken to the oven for another 4 h and then removed to view the color, with red color indicating microbial growth and slightly yellow color indicating no growth.

2.7 Antioxidant analysis

The antioxidant activity of Vitamin C powder concentrates was determined by evaluating the reduction of the radical 2,2-diphenyl-1-picryl-hydroxyl (DPPH) by spectrophotometry according to the adapted methodology developed by Brand-Williams, Cuvelier and Berset (1995) using vitamin C (P.A) as an antioxidant standard. First, an ethanolic solution of 0.004% DPPH was prepared, with an absorbance between 0.800 and 1.000. Then, vitamin C standard solutions and concentrates were

prepared at concentrations of 2500, 625, 156.25, 39.06, 9.77, 4.88, 2.44, 1.22, 0.61 and 0.31 µg/mL. In test tubes protected from light, 1 mL of ethanol and 2 mL of 0.004% DPPH solution were added, and 1 mL of each of the prepared concentrate solutions was added to each of the tubes. After 30 minutes, the absorbance at 517 nm was read in a UV-VIS spectrophotometer. Three repetitions of this test were performed. After reading the absorbance, the antioxidant activity (AA%) of the samples and the standards were calculated according to the formula below:

$$AA\% = \frac{100 - (\text{Abs sample} - \text{Abs blank})}{\text{Abs control}} * 100 \quad (1)$$

Where: Abs sample – the absorbance of the concentrated sample;

Abs blank – absorbance of the blank (ethanol only);

Abs control – absorbance of the control (contains only ethanol and DPPH solution).

The antioxidant activity of the Vitamin C concentrates was expressed in the concentration necessary to inhibit 50% (IC_{50}) of the DPPH free radical, having ascorbic acid as a positive control.

3 RESULTS AND DISCUSSION

3.1 Obtaining the concentrates

In Figure 1, it is possible to observe the concentrates obtained by the different drying methods. It is noticeable that the samples have different colors and textures. For the sample dried in a lyophilizer, a cotton-like characteristic was observed, with agglomerated particles and with a hygroscopic character, while, for the extract dried by spray dryer, a fine, agglomerated and slightly hygroscopic powder was obtained. These differences can be explained both by the type of equipment used for drying and by the amount of maltodextrin added to each of the extracts.

Figure 1 – Acerola powder concentrates (a) with freeze-drying and (b) with spray dryer

Source: Authors' private collection (2023)

According to Engel et al. (2017), spray drying makes it possible to obtain a more uniform sample, with a spherical shape and good retention, since it is a fast drying process. In Figure 1(b), this parameter can be seen because the extract obtained is a fine and loose powder. In addition, the more intense color of the lyophilized extract can be related to the greater amount of anthocyanins, a subclass of flavonoids, which are natural pigments responsible for the red, purple, and blue colors in many fruits, including acerola. Carneiro (2020) identified levels of anthocyanins, flavonoids, and polyphenols in samples of the pulp and by-product of lyophilized (freeze-dried) acerola, emphasizing the importance of measuring these compounds because of the commercial importance given the aspects of appearance and color of the fruits by the consumer.

Moreover, in this extract, reduced amounts of the carrier were used, which consequently dilutes the extract in lower proportions. When a matrix is dried using the lyophilizer, the water is sublimed and the physical and chemical properties of this matrix are preserved.

3.2 Mass spectrometry analysis (ESI-IT-MS)

The vitamin C concentrate obtained in the present study was analyzed by Tandem mass spectrometry using an electrospray ionization source coupled to an ion trap mass

spectrometer analyzer based on the direct infusion technique. The structures of the constituent compounds were determined based on their MS2 and MS3 fragmentation patterns and compared with literature data. The analysis of negative and positive modes of the concentrate showed the presence of six compounds. Among them, one can find the phytoconstituents of the antioxidant class, such as ascorbic acid and quercetin (Table 1; Figure 2).

Table 1 – Phytochemical analysis of vitamin C concentrate produced from *Malpighia glabra* L. fruits by ESI-IT-MS/MS, in negative and positive modes

Compound	$[M-H]^-$	MS_2	Reference
Ascorbic acid	175[a]	115, 87	(Mesquita et al., 2022)
Delphinidin	303[b]	285	(Takeoka et al., 2005)
Malvidin	331[b]	313, 299, 287, 270	(Hayasaka & Asenstorfer, 2002)
Caffeoylquinic acid	353[a]	191	(Dou et al., 2007)
Quercetrin	447[a]	301, 179, 151	(Poletto et al., 2021)
Kaempferol-3,7-di-*O*-diglucoside	653[a]	447, 285	(Lorach et al., 2003)

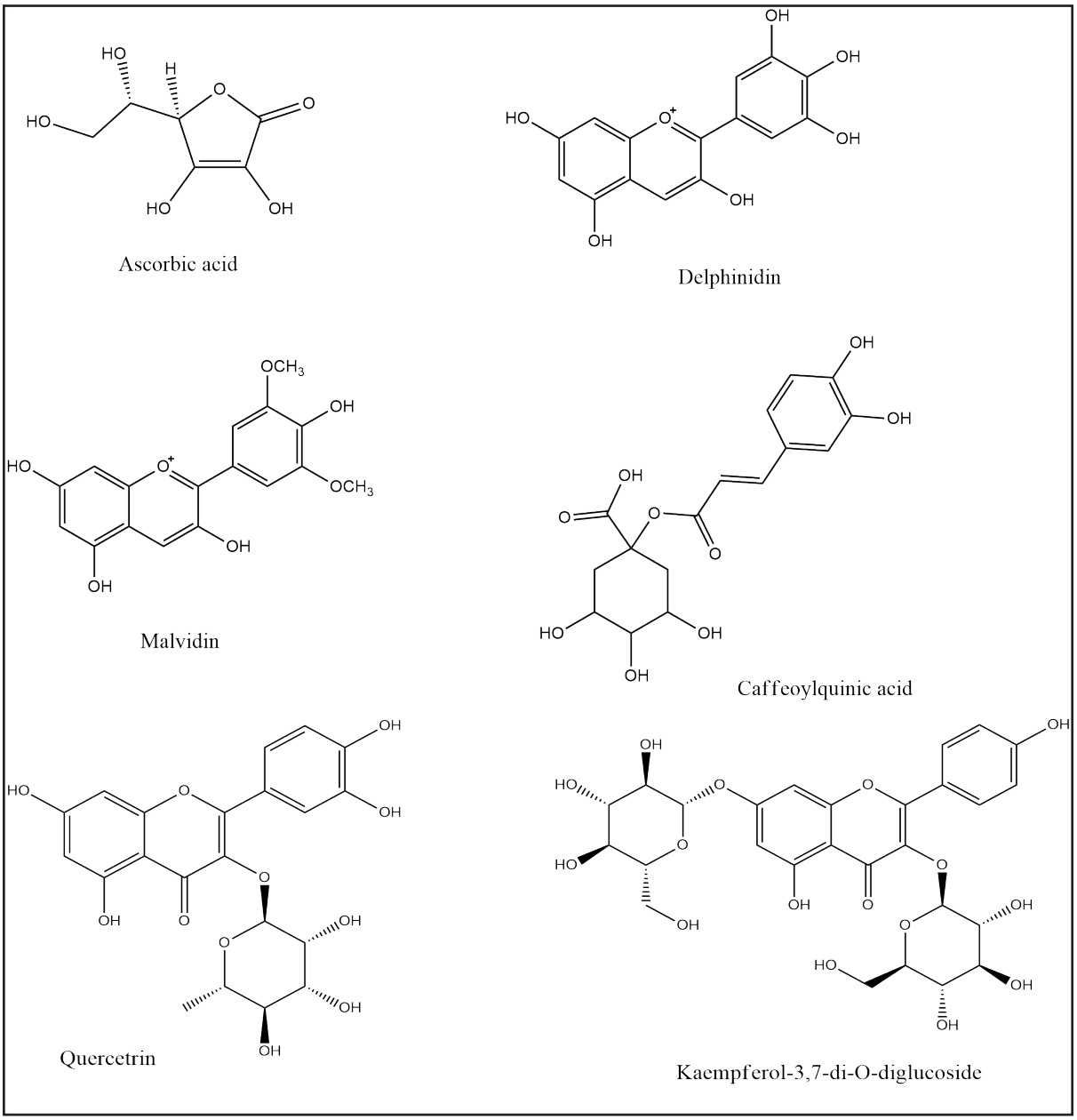
[a] Spectra recorded in negative mode

[b] Spectra recorded in positive m ode

Source: Authors (2023)

The potentiality of plants and their fruits, perpetuated over generations, is justified by the presence of bioactive components that have many proven biological activities (Cragg & Newman, 2016). The phytochemical investigation aims to verify the presence of groups of secondary metabolites, study the structural characterization, evaluate the properties, and investigate the biosynthesis of natural compounds (Twaij & Hasan, 2022).

Figure 2 – Chemical structures of the compounds identified in the vitamin C concentrate by ESI-IT-MS/MS, in negative and positive modes



Source: Authors (2023)

The species *M. emarginata* is a tropical fruit originating in Central America, which has great economic importance due to its high content of ascorbic acid, considered one of the main responsible for the various biological activities attributed to the fruit (Cefali et al., 2018; Pires et al., 2022). The various properties are also associated with the presence of phenolic substances such as flavonoids, phenolic acids and

carotenoids, which have nutraceutical properties (Carmo, Nazareno, & Rufino, 2018; Chang, Alasalvar, & Shahidi, 2018; Xu et al, 2020).

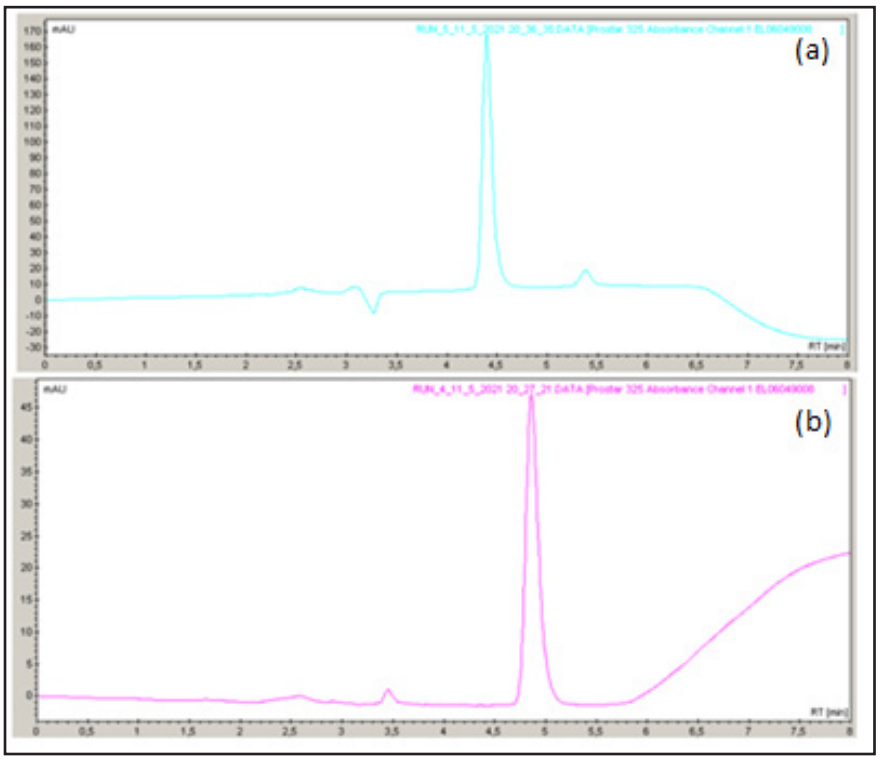
In this study, based on a vitamin C concentrate obtained from the fruits of this plant, phytochemical tests were carried out to characterize the major compounds of this substance. The chemical prospection of the vitamin C concentrate was performed by spectroscopic analysis (ESI-IT-MS), revealing the presence of six compounds, including the anthocyanins named malvidin and delphinidin, recognized in the pigmentation of the species and as important antioxidants (Xu et al, 2020), flavonoids such as quercetin and kaempferol (Lima et al, 2000; Ribani & Amaya, 2008; Macedo et al, 2022); phenolic acids such as caffeoylquinic acid (Almeida et al, 2006; Fereira et al, 2021) and ascorbic acid, an important antioxidant present in large amounts in the fruit (Matta et al, 2004; Mezadri et al, 2006; Cefali et al, 2018; Pires et al, 2022).

All compounds present in the vitamin C concentrate have anti-inflammatory potential and high antioxidant capacity, corroborating the main characteristics found in the fresh fruit (Silva et al., 2022; Silva et al., 2020). These findings are also in line with the literature, which points to the class of flavonoids, anthocyanins, phenolic compounds, and antioxidants as active compounds present in *M. emarginata* (Freire et al, 2013; Seraglio et al, 2018).

3.3 Vitamin C quantification (Vit C)

The calibration curve, determined from the areas of the Vit C peaks, showed linearity in the concentration range of 5 to 100 μg/mL. The linear regression equation was y = 2.5354 – 15.588, where y is the Vit C peak area and x is its concentration. The correlation coefficient (R^2) obtained was 0.9663. In Figure 3, the chromatograms of the acerola extracts dried in a lyophilizer (a) and spray dryer (b) from the HPLC readings, respectively, are presented.

Figure 3 – Chromatograms of freeze-dried concentrate (a) and spray dryer concentrate (b)



Source: Authors (2023)

It is possible to see from the figures that the peak referring to ascorbic acid has a retention time of approximately 5 min, and that the peak area varies according to each sample under evaluation. In addition, the chromatograms show some unidentified peaks such as ascorbic acid, thus indicating the presence of possible impurities characterized as compounds that have not been determined and quantified so far. Through the area of the largest peak identified and through the equation obtained through the calibration curve, the concentration of Vit C in each sample was determined, as shown in Table 2.

It was noted that the Vit C content identified in the sample submitted to the spray dryer drying process is lower compared to the lyophilized sample, as confirmed by statistical analysis using the Tukey test ($p>0.05$). This possibly occurs due to the significantly greater presence of the drying agent required for the spray dryer process, which can affect the amount of ascorbic acid. Furthermore, it is known that Vit C is highly susceptible to oxidation by exposure to light and oxygen, and the samples were

submitted to chromatographic analysis a few days after obtaining the extracts, that is, they were naturally exposed to environmental conditions. The peak area values for each duplicate revealed a significant difference, as shown by the standard deviations. The equation used to quantify the Vit C content was the result of the calibration curve performed before the development of this study. Therefore, some differences may have occurred.

Table 2 – Vit C concentration in each sample

Sample	Area (mAU.Min)	Vit C concentration (µg/mL)
Spray-dried concentrate	11.65 ± 4.25[a]	10.74
Freeze-dried concentrate	25.75 ± 3.85[b]	16.30

Means followed by different letters in the same column indicate significant differences from each other using the Tukey test ($p > 0.05$). Source: Authors (2023)

It was noted that the Vit C content identified in the sample submitted to the spray dryer drying process is lower compared to the lyophilized sample, as confirmed by statistical analysis using the Tukey test ($p>0.05$). This possibly occurs due to the significantly greater presence of the drying agent required for the spray dryer process, which can affect the amount of ascorbic acid. Furthermore, it is known that Vit C is highly susceptible to oxidation by exposure to light and oxygen, and the samples were submitted to chromatographic analysis a few days after obtaining the extracts, that is, they were naturally exposed to environmental conditions. The peak area values for each duplicate revealed a significant difference, as shown by the standard deviations. The equation used to quantify the Vit C content was the result of the calibration curve performed before the development of this study. Therefore, some differences may have occurred.

Moraes (2018) evaluated the ascorbic acid content in freeze-dried mature acerola extract with and without the addition of a drying agent (3%). The authors

obtained a content of 16594.42 ± 34.32 mg/100 g without the addition of Gum Arabic and 15522.66 ± 21.82 mg/100g for the extract with a carrier agent.

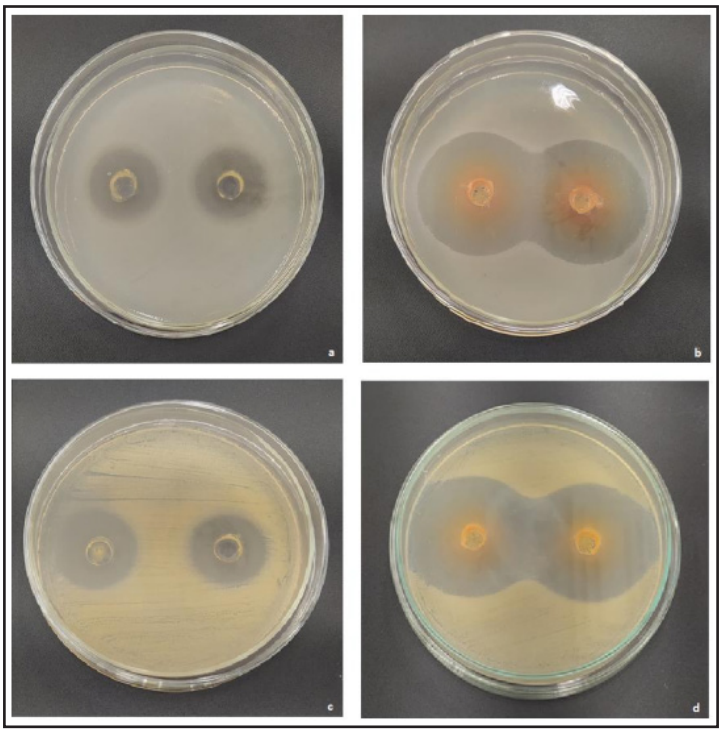
The Vit C content found is in line with expectations, since it was desired that the results were more expressive for the lyophilized extract. This is because it has a lower amount of whitening agent, indicating an extract richer in phenolic compounds and ascorbic acid. Comparing the concentration of Vit C found in the present work with Rocha (2019), the values are higher, and the author obtained 0.003 µg/mL of Vit C in concentrated acerola juice and 0.0004 µg/mL for non-concentrated juice.

3.4 Diffusion in solid medium from the orifice

In Figure 4, the average inhibition halo can be seen for powdered extracts obtained from frozen acerola pulp, obtained by different drying methods against *E. coli* and *S. aureus* bacteria. The values are presented in Table 3.

The statistical analysis shows that there is a significant difference in antimicrobial activity between the drying methods for both bacteria. For both *Escherichia coli* and *Staphylococcus aureus*, the concentrate obtained by the lyophilization method showed a larger inhibition zone compared to the concentrate processed by the spray dryer. It appears that the antimicrobial activity was slightly higher against the gram-positive bacterium *S. aureus*. According to Albano (2016), the distinction in the action of antimicrobial compounds is due to the structure of the cell wall of bacteria. Gram-positive bacterial cells, such as *S. aureus*, have a cell wall made up of a layer with about 90% peptidoglycan and cover the entire cell membrane, which facilitates the penetration of external molecules, thus promoting interaction with the cytoplasmic membrane and making them more fragile when compared to gram-negative bacterial cells. These have only about 10% peptidoglycan, and therefore have an additional membrane with a bilayer structure, rich in lipids, generating greater resistance to this class, such as *E. coli*. Other authors also suggest that this difference may be related to the polarity of the cell membrane, since the membrane of *E. coli* has a greater negative charge compared to *S. aureus* (Sonohara et al, 1995; Gordon et al, 2011).

Figure 4 – Diffusion in the solid medium of powdered extracts from frozen acerola pulp (a) Spray-dried concentrate for *Escherichia coli* (b) for *Staphylococcus aureus* and (c) Freeze-dried concentrate for *Escherichia coli* (d) Freeze-dried concentrate for *Staphylococcus aureus*



Source: Authors (2023)

Table 3 – Average inhibition halo

Microorganism	Spray-dried concentrate	Freeze-dried concentrate
	Average inhibition halo (mm)	
Escherichia coli	17.5 ± 0.05[a]	20.0 ± 0.17[b]
Staphylococcus aureus	33.2 ± 0,09[a]	37.0 ± 0,00[b]

Means followed by the same letter in the same row do not differ significantly from each other using the Tukey test ($p > 0.05$). Source: Authors (2023)

The statistical analysis shows that there is a significant difference in antimicrobial activity between the drying methods for both bacteria. For both *Escherichia coli* and *Staphylococcus aureus*, the concentrate obtained by the lyophilization method showed

a larger inhibition zone compared to the concentrate processed by the spray dryer. It appears that the antimicrobial activity was slightly higher against the gram-positive bacterium *S. aureus*. According to Albano (2016), the distinction in the action of antimicrobial compounds is due to the structure of the cell wall of bacteria. Gram-positive bacterial cells, such as *S. aureus*, have a cell wall made up of a layer with about 90% peptidoglycan and cover the entire cell membrane, which facilitates the penetration of external molecules, thus promoting interaction with the cytoplasmic membrane and making them more fragile when compared to gram-negative bacterial cells. These have only about 10% peptidoglycan, and therefore have an additional membrane with a bilayer structure, rich in lipids, generating greater resistance to this class, such as *E. coli*. Other authors also suggest that this difference may be related to the polarity of the cell membrane, since the membrane of *E. coli* has a greater negative charge compared to *S. aureus* (Sonohara et al, 1995; Gordon et al, 2011).

In addition, comparing Figures 4(a) and 4(b) with Figures 4(c) and 4(d), it is observed the difference in the size of the halo formed by both drying methods against the different microorganisms. As previously mentioned, the lyophilized extract of acerola pulp has greater purity, that is, a greater amount of phenolic compounds, since the amount of drying agent added to this sample was 15 times lower compared to that which was dried in a spray dryer. Silva et al. (2022) evaluated the average inhibition halo of green and mature acerola extracts against *S. aureus* and *E. coli*, at concentrations of 2491.30 mg/100g and 3406.34 mg/100g. The authors obtained values of 9.67±1.15 mm for *S. aureus* and 9.50±0.71 mm for *E. coli* with ripe fruit and 11.15±1.20 mm and 10.67±1, 53 mm with the unripe fruit against the same microorganisms, respectively.

Rocha (2019) studied the antimicrobial action of the concentrated and diluted extract (1:5) of acerola against *S. aureus*, finding an inhibition halo of 15 mm for the concentrated extract and 10 mm for the diluted one. The divergence of the values found indicates that the extraction of phenolic compounds and ascorbic acid, as well as the method of obtaining the extract in lyophilized powder, is directly related

to a sample richer in compounds with antimicrobial potential. Marques et al. (2017) evaluated the antibacterial activity through the agar diffusion technique of the acerola bagasse flour extract with a concentration of $5x10^{-4}$ g/mL against *E. coli* and the results showed that the average inhibition halo was 8.5±0.86 mm. If these results are compared with those of the present study, it appears that the concentration of extract used by the authors was lower and, consequently, obtaining smaller inhibition halos against the gram-negative bacterium *E. coli*. It is also noticed that the inhibition halos formed by the lyophilized extract, for both bacteria under study, came together, indicating that analyses should be redone in a larger petri dish, in order to obtain more accurate results.

3.5 Minimum Inhibitory Concentration (MIC)

Table 4 shows the results obtained for the MIC test for freeze-dried acerola concentrate powder and spray-dried acerola concentrate against *E. coli* and *S.aureus.*

Table 04 – MIC found in the two types of Vit C concentrate

Microorganism	Freeze-dried concentrate	Spray-dried concentrate
	CIM (g/mL)	
Escherichia coli	0.0156 – 0.0078	0.062 – 0.031
Staphylococcus aureus	0.0156 – 0.0078	0.062 – 0.031

Source: Authors (2023)

For the gram-negative bacterium *E. coli*, the Minimum Inhibitory Concentration obtained from the lyophilized acerola extract is between 0.0156 and 0.0078 g/mL, while for the acerola extract dried in a spray dryer, the Minimum Inhibitory Concentration of acerola is between 0.062 and 0.031 g/ml. Again, this corroborates the fact that the dry extract applied to the lyophilizer has a lower amount of carrier and, due to the milder process conditions, maintains a greater amount of compounds present in the extract that promote antimicrobial activity. When observing the Minimum Inhibitory

Concentration against the gram-positive bacterium *S. aureus*, a similar behavior to that seen for *E. coli* was observed, where the same values were found as those previously observed. If gram-negative and gram-positive bacteria are compared, the latter has only one outer layer as a plasma membrane, which facilitates the penetration of antimicrobial compounds and interaction with the bacterial cytoplasm, making gram-positive bacteria more susceptible when compared to gram-positive bacteria. When evaluating the structure of gram-negative bacteria, they have an additional membrane, forming a more resistant phospholipid bilayer structure that increases the cytoplasmic protection of antimicrobial agents through additional protection (SANTOS, 2022). However, in the present study, no difference was observed in the Minimum Inhibitory Concentration for both bacterial strains. Lima et al. (2022) evaluated the Minimum Inhibitory Concentration of acerola extract against *E. coli*, with a result of 31.25 mg/mL, concluding that acerola extract is effective and acts through a multi-target mode with disturbances in different psychological functions in the microorganism. Some studies have reported the antibacterial effects of catechin (Sinsiwar, Valdivel, 2020) and p-coumaric acid (Bag & Chattopadhyay, 2017; Ojha & Patil, 2019) on some pathogenic bacteria, indicating the association of antimicrobial potential with phenolic compounds.

In addition, Stafussa et al. (2021) evaluated the effect of acerola extracts on pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella Enteritidis*. The results revealed that the acerola extracts showed antimicrobial activity against all strains tested, with minimum inhibitory concentration values ranging from 6.25 to 25 mg/mL. The results obtained were satisfactory, since, for both evaluated extracts, it was possible to determine the minimum concentration capable of causing inhibition of the growth or cell death of the bacterium under study, but, when compared to other authors, the concentration of extract necessary to prevent the microbial growth was higher.

In addition, the antimicrobial activity of the solvents used for the extraction of ascorbic acid in acerola pulp, oxalic acid and EDTA was not evaluated. These were

added in low concentrations in the development of the present work, and it is believed that the use of heat and low pressures used in the drying processes are sufficient to volatilize these compounds. Anyway, this is a point that should be considered in future works. Kwak et al. (2016) studied different concentrations of oxalic acid extracted from the Shiitake mushroom against different pathogenic bacterial strains. The authors verified that, for example, for *Bacillus subtilis*, the highest concentration of oxalic acid tested (300 mg/L) was not able to inhibit the growth of this bacterium, on the contrary, the presence of this acid potentiated the growth of this bacterium, which went from an initial of viable cells from $3.2x10^3$ to $1.2x10^6$ (CFU/mL).

3.6 Antioxidant activity

Regarding the antioxidant activity by the DPPH free radical scavenging method, the vitamin C concentrates for both drying methods were compared with the pure ascorbic acid standard, and the antioxidant activity (%AA) with the respective standard deviation is shown in Table 5.

It can be noted that the Vit C concentrate obtained via drying in a lyophilizer showed higher antioxidant activity (%AA) when compared to the concentrate obtained in the spray dryer, being 96.77% ± 0.05 and 54.22% ± 1.89, respectively, for a product concentration of 625.00 µg/ml. When comparing with the ascorbic acid standard at this same concentration, it was noticed that the Vit C concentrate obtained in the lyophilizer came very close to 100% antioxidant activity. Accordingly, one can observe the comparison of obtaining 50% antioxidant activity (IC50). Moreover, it is noted that the concentrated extract of Vit C obtained in the lyophilizer needs a lower concentration than the concentrate obtained in the spray dryer, 60.89 ± 3.40 and 508.77 ± 1.03, respectively, to reach 50% antimicrobial activity. Compared to the pure ascorbic acid standard, it needs 1.88 µg/ml to reach 50% antioxidant activity. It can be seen that from a concentration of 156.25 µg/mL there is a significant difference between the

results obtained for both methodologies for obtaining the compound. However, from a concentration of 1.22 µg/mL the results obtained are statistically equal.

Table 5 – Antioxidant Activity (AA%) of the concentrates compared to the Vit C standard

Concentration (µg/mL)	(AA%) Spray-dried concentrate	(AA%) Freeze-dried concentrate	(AA%) Ascorbic acid standard
2500.00	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
625.00	54.22 ± 1.89^{b}	96.77 ± 0.05^{a}	100.00 ± 0.00^{a}
156.25	37.20 ± 0.17^{c}	88.86 ± 2.61^{b}	99.77 ± 0.08^{a}
39.06	33.66 ± 0.61^{c}	41.10 ± 4.20^{b}	97.98 ± 0.96^{a}
9.77	10.91 ± 0.13^{b}	15.61 ± 4.35^{b}	92.66 ± 0.27^{a}
4.88	3.13 ± 0.16^{c}	9.35 ± 0.77^{b}	78.74 ± 0.65^{a}
2.44	2.73 ± 0.81^{c}	6.53 ± 2.61^{b}	62.56 ± 1.39^{a}
1.22	0.73 ± 0.25^{b}	1.48 ± 3.86^{b}	35.18 ± 0.58^{a}
0.61	0.55 ± 0.54^{b}	0.40 ± 0.90^{b}	25.59 ± 0.54^{a}
0.31	0.33 ± 0.90^{b}	0.20 ± 0.65^{b}	15.83 ± 1.12^{a}

Means followed by the same letter in the same row do not differ significantly from each other using the Tukey test ($p > 0.05$). Source: Authors (2023)

Rezende et al. (2018) evaluated the antioxidant activity of acerola pulp and waste submitted to lyophilization and drying in a spray dryer. The authors determined that the antioxidant activity of the microencapsulated powders ranged from 139.69 to 756.96 µM TE/g. For the DPPH assay, the antioxidant activity was independent from the raw materials and methods used. When comparing the four microencapsulated extracts, regardless of the type of drying, the antioxidant activity was higher in the acerola pulp extracts, being related to the presence of phenolic compounds, together with the higher concentration of AA, which protects against uncontrolled oxidation in the aqueous medium of the cell, due to its high reducing power. These results suggest that PC is one of the main contributors with a positive correlation with the antioxidant

activity of these foods, as the increased concentration of these compounds increases the antioxidant activity.

Carvalho et al. (2021) suggest phenolic compounds are the main contributors with a positive correlation with Vit C in the extracts of this research, since an increase in the concentration of these compounds will increase ascorbic acid when evaluating industrial waste from acerola. Moraes (2018) compared the antioxidant activity of fresh and freeze-dried acerola fruits. The author concluded that the antioxidant activity of freeze-dried acerola pulp was maintained high and constant throughout the storage period (120 days), and that the addition of the microencapsulating agent influenced the retention of antioxidant activity measured by the DPPH method ($p< 0.05$).

4 CONCLUSIONS

When evaluating the concentrates obtained through freeze-drying and spray-drying methods, it was observed that both were effective in producing the powder extract. However, the extract subjected to spray-drying showed a more uniform appearance, indicating particles of similar sizes and lower moisture content than the freeze-dried extract.

Analyzing the presence of Vitamin C in the powdered acerola pulp concentrate obtained by different drying methods, it was found that the freeze-dried concentrate had a significantly higher percentage of Vitamin C. The best results for antimicrobial and antioxidant activity were also obtained with the freeze-dried concentrate, demonstrating greater efficacy against *S. aureus* and *E. coli* and more potent antioxidant activity compared to the spray-dried concentrate. Therefore, the freeze-drying method was concluded to be the most effective.

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