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Research Article

Production and Characterization of Dehydrated Acerola Pulp: A Comparative Study of Freeze and Refractance Window Drying

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Abstract: Acerola is a perishable fruit with high vitamin C levels and an attractive nutrition property. The goal of the present work was to produce dehydrated acerola pulp with preserved vitamin C, selecting freeze-drying and refractance window (considered a novel and promising drying method) as suitable technologies. The final properties of dehydrated pulps using the drying technologies freeze-drying and refractance window were compared: i) water activity, ii) moisture content, iii) hygroscopicity, iv) color parameters, v) microstructure, vi) antioxidant activity, and vii) ascorbic acid stability (30 °C, 75% RH). The dried acerola pulps exhibited low moisture, water activity, and hygroscopicity regardless of the drying technology. However, the pulp dehydrated by refractance window presented significantly higher antioxidant activity than the freeze-dried pulp, with higher $ABTS^{\bullet+}$ (1,838.60 μM TE/g dehydrated pulp) and FRAP (1,290.00 μM TE/g dehydrated pulp) values. The ascorbic acid stability values were also higher for the pulp dried by the refractance window, which showed a final content of 98.03 mg/100 g dehydrated pulp after ten days of storage. The refractance window is a more appropriate technology to dehydrate acerola pulp with high vitamin C content, antioxidant activity, and ascorbic acid stability than freeze-drying.

Keywords: *Malpighia emarginata*, pulps, drying, vitamin C, antioxidant, stability

1. Introduction

Vitamin C is an important physiological antioxidant and an essential micronutrient in human metabolism [1]. Vitamin C could prevent several diseases, which has always attracted great interest in industrial applications. Additionally, it is water-soluble and has high antioxidant potential [2]. According to the literature reports, vitamin C has been associated with more than 65% of the antioxidant and antiradical activity in fruits and beverages [3]. Moreover, vitamin C can be used as a postharvest quality parameter [4]. Several factors influence the concentration of vitamin C in fruits, such as harvest time (ripening stage), storage conditions, and mechanical damages [5].

Pineapple, soursop, pinecone, jackfruit, papaya, mangaba, umbu, and tamarind are excellent sources of vitamin C

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[6]. A Brazilian fruit that stands out is camu-camu (*Myrciaria dubia*), but its production is limited to specific regions. Garcia et al. [7] reported that the pulp of camu-camu processed by spray drying presented a vitamin C concentration of 6,754.7 mg/100 g dry basis. Acerola fruit also has a high vitamin C concentration and is produced in many regions of Brazil. In the industry, acerola fruit is commonly used in pulp, juice, jelly, and jams process [8]. Frozen acerola pulp purchased in retail stores showed a concentration of 685.00 mg of vitamin C/100 g [9]. Spray-dried acerola pulp presented a vitamin C concentration of 2,063.8 mg/100 g dry basis [7].

Acerola fruit is abundantly available but is highly perishable, which is necessary special attention in commercialization, storage, consumption, and out-of-season availability. The acerola preservation could be improved by applying drying processes [10], trying to maintain the vitamin C present in the fresh fruit.

The literature has numerous techniques for drying acerola pulps. The freeze-drying preserved the pulp ascorbic acid presented in an acerola pulp [11]. The effect of hot air, vacuum drying, and freeze-drying was investigated on acerola pulp quality attributes, including its active compounds. Freeze-drying was more effective in maintaining and preserving ascorbic acid when compared to hot air and vacuum drying [10]. There are several reports in the literature about freeze-drying for dehydrating fruit pulps [12-14]. The freeze-drying process increases the shelf-life of fruit pulps, which is widely used to develop high-quality food products [14]. On the other hand, this technique requires a long drying period, increasing the process costs that tend to be much higher than most drying techniques [15].

The Refractance Window (RW) drying can be used to dry pulps, juices, and thin fruit pieces [16], with a reduced cost as compared to freeze-drying process [16]. Nindo & Tang [17] reported that the RW requires 50 to 70% less cost and 50% less energy than the freeze dryer. Hot water is used to heat a thin layer of fruit pulp applied onto a permeable membrane (permeable tape or conveyor belt) [17]. The heat transfer occurs by conduction, convection, and, to a lesser degree, thermal radiation [18]. Asiimwe et al. [19] investigated the drying conditions of passion fruit puree by RW, while Padhi et al. [20] investigated the use of RW to dry green banana flours and the physicochemical, structural, and functional properties of the resulting powder. Despite the commonly used freeze-drying and refractance window, it is still incipient in the literature for the evaluation of parameters in the drying of acerola pulp, mainly in relation to the concentration of vitamin C.

The objective of this work was to compare two widely used methods of food drying in acerola pulp, freeze-drying, and refraction window, evaluating the antioxidant activity, ascorbic acid concentration, and stability for 10 days.

2. Material and methods

2.1 *Material*

Frozen acerola pulp was purchased from De Marchi Fruit Industry and Commerce Ltda. (Campinas, SP, Brazil). The carrying agent, maltodextrin, was purchased from Corn Products Ingredientes Industriais Ltda. (Mogi Guaçu, SP). The antioxidant activity determination used 2,2-diphenyl-1-picrilhidrazil radical, 2,2′-and-bis (3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt, 2,4,6-Tris(2-pyridyl)-s-triazine and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, purchased from Sigma Aldrich (St. Louis, MO, USA) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, purchased from Sigma Aldrich (St. Louis, MO, USA). Ascorbic acid was used as a standard and purchased from Synth (São Paulo, Brazil).

2.2 *Drying methods*

A freeze-dryer L101 (Liobrás, São Carlos, Brazil) with a vacuum system with a speed of 10.2 m^3/h was used to obtain freeze-dried acerola; the thawed samples (50 g in each beaker) were distributed in stainless steel trays and frozen at -18 °C for 24 h. After this period, they were dehydrated in the freeze-dryer under vacuum, at -55 °C, for 54 h.

RW-dried pulp was obtained using the experimental apparatus consisting of a reservoir with circulating hot water, over which the polyester tape (Mylar film, Dupont) was attached. A thermostatic bath (DIST, model DI-921, Florianópolis, Brazil) kept the circulating hot water at 65 ± 2 °C. Then, the acerola pulp (200 g) was applied manually to the Mylar film top by a spreader (doctor blade), adjusting the pulp thickness to 1 mm (determined by preliminary tests, not shown), and the time of drying was 15 min.

The pulp temperature was monitored with T-type thermocouples (IOPE, model A-TX-TF-R-30AWG, Brazil) connected to a data acquisition system (Agilent, model 34970A, Malaysia). The pulp temperature was around 55 °C for most of the 16 min of drying. After drying, the pulps were stored in amber glasses at room temperature.

2.3 *Characterization of dehydrated acerola pulp*

2.3.1 *Water activity, moisture content, and hygroscopicity*

The water activity of the dehydrated pulps was determined using an Aqualab 3 analyzer (Decagon Devices, USA) at 25 °C. Moisture content was determined by gravimetric method at 105 °C for 24 h [21].

To determine hygroscopicity, samples (1 g) were stored on plates inside hermetically sealed desiccators with saturated Na_2SO_4 solution (81% relative humidity) at 25 °C. After 7 days, the mass was determined, and hygroscopicity was expressed as g water absorbed/100 g dry solids [22].

2.3.2 *Color parameters*

The freeze-dried and refractance window dried acerola pulps were characterized for color parameters L* (white - black), chroma a* (blue-yellow), and chroma b* (green - red), using a Miniscan XE collimeter (HunterLab, Reston, USA, illuminant D65). The determination of color parameters was performed using the CIE Lab system (Commission Internationale de Eclairage). The analysis was performed in triplicate. The parameter angle hue (h*) was calculated using Eq. 1 [23].

$$h^* = tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (1)$$

2.3.3 *Scanning Electron Microscopy (SEM)*

The dehydrated powders' microstructure was evaluated to estimate the effect of different drying methods. A scanning electron microscope model LEO 440i (Electron Microscopy, Cambridge, England) was used at 100× magnification. Before analysis, the powders were stored in desiccators with silica for 10 days.

2.4 *Determination of vitamin C*

Samples (0.5 g of freeze-dried pulps and dried by refractance window) were dispersed in acidified ultrapure water (pH 2.5, pH adjusted with phosphoric acid - 50 mM) and kept in an ultrasound bath (Ultra Clear, 1,400 A, Unique) for 10 min. The samples were then centrifuged (Centrifuge 5,430 R) at 6,000 rpm for 5 min, and the supernatant was filtered (0.45 µM Nylon filter, Millex). The analysis was performed using a Shimadzu - Prominence chromatograph with an LC 20 AD pump (automatic injector: SIL10AF, detector: SPD-M20A, and Res Elut C18 VP-ODS column). Acidified ultrapure water (pH 2.5, pH adjusted with phosphoric acid - 50 mM) was used as the mobile phase, with a flow rate of 1mL/min, 20 µL injection volume, and 245 nm wavelength [7]. The results were expressed in g/100 g dehydrated fruits.

2.5 *Determination of antioxidant activity*

The freeze-dried and refractance window dried acerola pulps were dispersed in distilled water and kept in an ultrasound bath (Ultra Clear, 1,400 A, Unique) for 10 min at room temperature. Then, the samples were centrifuged (Eppendorf 5,430 R) at 6,000 rpm for 5 min to extract the active compounds for the analysis of DPPH• and FRAP. For $ABTS^{\bullet+}$ analysis, ethanol (Synth) was used as a solvent, and the extraction was carried out as described above. The antioxidant potential by the $ABTS^{\bullet+}$ method was determined according to Rufino et al. [24]. The ABTS radical was produced by reacting the $ABTS^{\bullet+}$ solution (7 mM) with potassium persulfate (145 mM) and kept at room temperature for 16 h in the absence of light. After forming the radical ($ABTS^{\bullet+}$), the solution was diluted in ethanol until it presented an absorbance of 0.7 ± 0.02 at 734 nm. Then, 30 µL of the dehydrated pulp diluted in water was added to 3 mL of ABTS radical. The absorbances were determined after 6 min in a spectrophotometer (PerkinElmer, Lambda 35 UV-Vis,

USA) at 734 nm. The calibration curve was performed using Trolox, and the results were expressed in μM trolox/g of dehydrated pulp.

The antioxidant activity by the DPPH• method was determined according to Brand-Willians et al. [25]. DPPH• solution (0.6 Mm, 3.9 mL) was added to 100 μl of the dehydrated pulps (freeze-dried and dried by reflectance window) diluted in water. The readings were monitored in a spectrophotometer (PerkinElmer, Lambda 35 UV-Vis, USA) at 517 nm after 65 min of reaction. Antioxidant activity was expressed as the concentration of extract required to reduce the original amount of free radicals by 50% (EC_{50}).

For the FRAP method, 150 μL of dehydrated pulps (freeze-dried and dried by reflectance window) diluted in water were added to 2850 μL of the FRAP solution prepared according to Benzie et al. [26]. The solutions were kept for 30 min without light; afterward, the reading was performed in a spectrophotometer (PerkinElmer, Lambda 35 UV-Vis, USA) at 593 nm. The calibration curve was prepared using Trolox as a standard, and the results were expressed in μM Trolox equivalent/g dehydrated pulp.

2.6 *Vitamin C stability*

The vitamin C stability was evaluated by storing the freeze-dried, and refractance window dried acerola pulps in desiccators (saturated sodium chloride solution, 75% relative humidity), kept at 30 ± 2 °C (BOD incubator, Marconi MA415) under controlled conditions for 10 days. Ascorbic acid levels were determined in quadruplicate at 0, 1, 7, and 10 days.

2.7 *Statistical analysis*

Analyses were performed in triplicate, and the standard deviation was determined. The experimental results were analyzed using the SAS program (Statistic Analysis System), version 9.3. Duncan's test determined the difference between the means (95% confidence interval).

3 Results and discussion

3.1 *Characterization of dehydrated acerola pulp*

3.1.1 *Water activity, moisture content, and hygroscopicity*

The characteristics of water activity, moisture content, and hygroscopicity are important for the stability of the dry extract. Table 1 shows that the water activity values of the samples dehydrated by the two methods did not differ significantly, indicating that they are independent of the method used. According to Singh et al. [27], water activity values between 0.2 and 0.4 ensure greater stability, and the dehydrated pulps may present microbiological stability. The values were higher than spray-drying acerola pulp, with water activity values of 0.25 [7].

Table 1. Effect of the drying process (freeze-drying and refractance window) on water activity, moisture content, and hygroscopicity of acerola pulp

Drying	Water activity	Moisture content (%)	Hygroscopicity (%)
Freeze-drying	0.35 ± 0.01^{a}	9.60 ± 0.34^{a}	5.58 ± 0.19^{a}
Refractance window	0.36 ± 0.01^{a}	8.55 ± 0.21^{b}	5.18 ± 0.07^{b}

Different lowercase letters in the same column indicate a significant difference ($p < 0.05$)

The moisture content of freeze-dried acerola pulp was significantly higher than that observed for the pulp dried by refractance window (Table 1). The literature has reported some works which describe that freeze-dried pulp of different fruits has higher moisture when compared to other drying methods such as spray-dryer [28, 29]. Soares et al. [30] dried acerola pulp using the foam-mat drying with air circulation at 60 to 70 °C for 90 min. They reported that the dehydrated

powder had a moisture content of 7.24%.

Despite the significantly different hygroscopicity values ($p > 0.05$) of the dehydrated pulps, these values were lower (< 6%). According to Rezende et al. [28], low hygroscopicity values facilitate the conservation and prevention of color and bioactive compounds. Thus, it can be stated that the pulps presented high stability due to the low value of water activity and hygroscopicity.

3.1.2 *Color parameters*

The color parameters, L*, chroma a*, and chroma b*, showed no significant differences in freeze-dried or refractance window acerola pulp. This may be associated with no other ingredient used during the drying process and the relatively low temperatures of both methods. The results obtained (Figure 1) showed that all color parameters are in the first quadrant (+ a* and + b*), indicating a tendency towards yellowish red, which was expected, due to the characteristic color of nature pulp (L * 34.6 ± 0.8, chroma a* 10.9 ± 0.6 and chroma b* 21.5 ± 0.7).

The angle hue (h*) values were 197.53 ± 0.86 and 196.76 ± 0.82 for the pulp dried by freeze dryer and refractance window, respectively. The h* data did not differ significantly, indicating that the different drying techniques do not show the color saturation of the dried pulps.

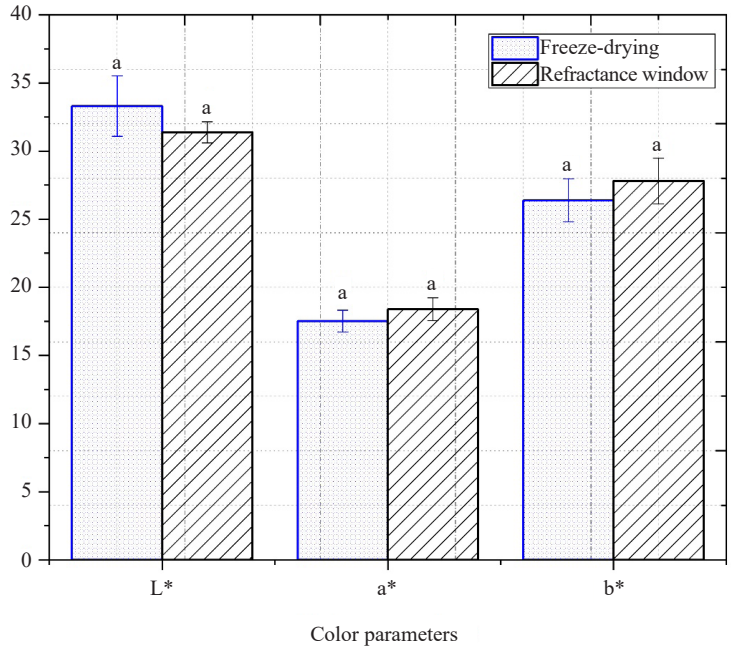

Figure 1. Effect of the drying process (freeze-drying and refractance window) on the color parameters of acerola pulp. Different lowercase letters in the same parameter indicate a significant difference ($p < 0.05$)

3.1.3 *Scanning Electron Microscopy (SEM)*

The freeze-dried and refractance window acerola pulps generally showed similar microstructures (Figure 2). After drying, the pulps presented irregular surfaces and different dimensions. It could be associated with the non-use of carrier agents, which provides the opening of pores and the formation of irregular particles. According to Aguilera and Stanley [31], the main quality of freeze-dried food products was the porous structure without shrinkage caused by the structural rigidity provided by the frozen surface where sublimation occurs and the lack of water in the liquid state.

The freeze-dried acerola pulp presented samples similar to a broken glass structure of varying sizes. Yamashita et al. [32] observed the same results. The literature reports several studies on forming irregular surfaces for freeze-dried fruit pulps [29, 32]. Caparino et al. [33] evaluated the microstructure of the mango pulp dried by refractance window

and reported the presence of almost identical thick, smooth flakes. They attributed these characteristics to the efficient spreading of the mango pulp in the plastic film of the refractance window. Shende et al. [34] reported the formation of irregular particles in the drying of mango pulp by refractance window, as observed in this work.

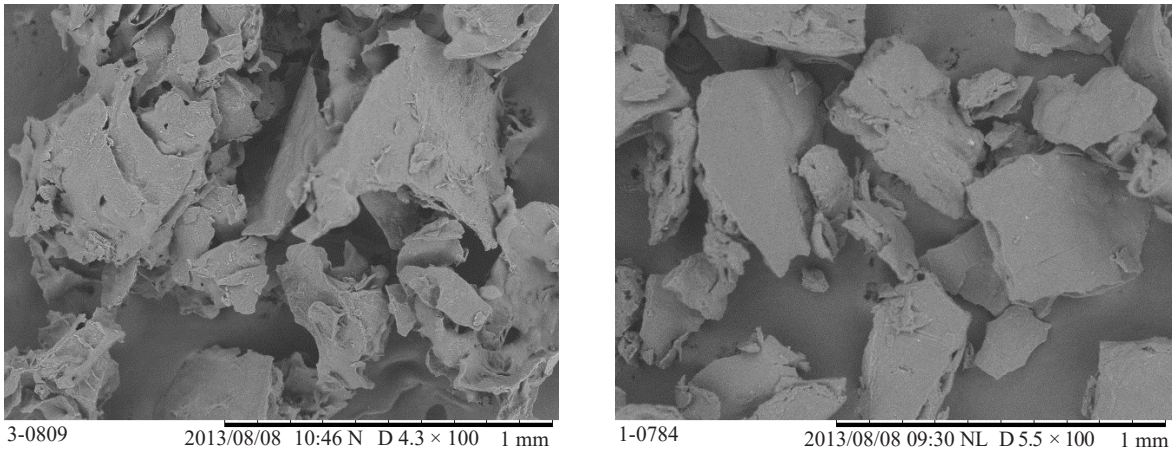

Figure 2. Micrographs of freeze-dried acerola pulp (a) and obtained by refractance window (b) at 100× magnification

3.2 *Ascorbic acid concentration and stability*

The ascorbic acid concentration in the dehydrated acerola pulp obtained by the refractance window was higher than the freeze-dried pulp (Figure 3). The shorter drying time obtained by the refractance window may have preserved the vitamin C. Rezende et al. [28] evaluated the drying process of acerola residue and pulp by spray-dryer and freeze-drying and reported percentages of vitamin C of 334.96 mg/100 g. Garcia et al. [7] performed the drying process of acerola pulp by spray-dryer and found a concentration of 1,593.2 ± 64.3 mg/100 g dry basis. Teixeira et al. [35] evaluated the process of encapsulation of acerola from green acerola pulp using lyophilization and spray dryer methods and reported that the concentration of vitamin C in the powders obtained were 39.26 and 32.52 mg/100 mg of ascorbic acid, respectively.

The Recommended Daily Intake (RDI) of vitamin C, according to FAO/WHO [36], is 45 mg/day for adults. Based on the results obtained, it can be suggested that 3.0% and 2.3% of freeze-dried and refractance window acerola pulp, respectively, will provide 100% of the RDI of vitamin C for an adult, which could be an excellent source of ascorbic acid.

There was a significant reduction in the concentration of ascorbic acid in the dehydrated pulps, by freeze-drying and refractance window, after 10 days of storage (30 °C, 75% RH). Furthermore, the freeze-dried acerola pulp showed a reduction of ~90% after 7 days of storage, and the ascorbic acid could not be quantified after 10 days of storage (Figure 3). The refractance window acerola pulp presented a reduction of ~77%, possibly due to higher initial vitamin concentration. Additionally, the lower moisture content (Table 1) observed for the pulp obtained by RW may have resulted in a lower loss of ascorbic acid in relation to storage time. According to Anandharamakrishnan et al. [37], the lower moisture content results in a reduced tendency for agglomeration, thus reducing the exposure of the dehydrated pulp to oxygen and conserving the compounds present, such as vitamin C.

Junior et al. [38] evaluated different drying methods of mixed acerola-ceriguela pulp. They reported that the pulp obtained by freeze-drying resulted in greater ascorbic acid retention about the techniques used, such as spouted bed drying.

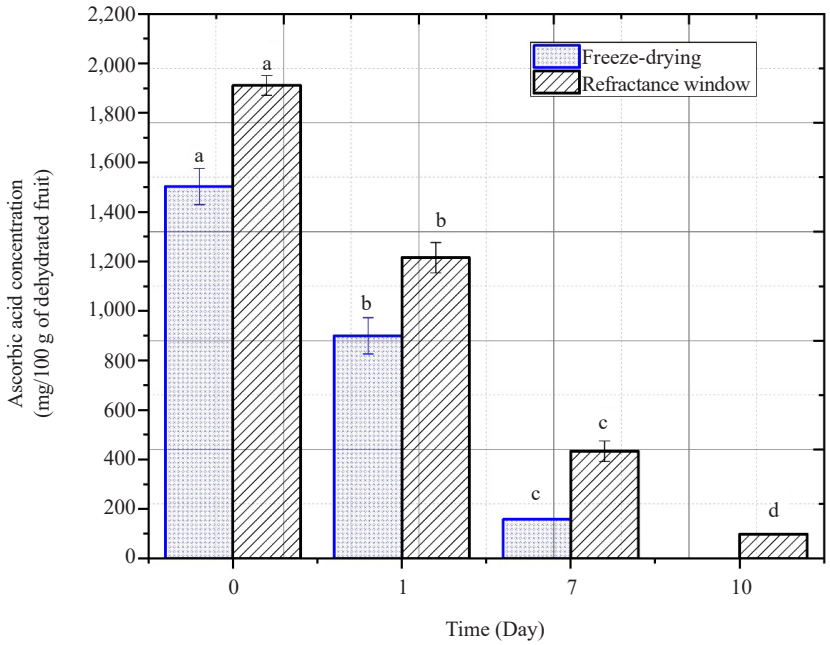

Figure 3. Effect of storage time under controlled conditions (30 °C and 75% relative humidity) on ascorbic acid concentration in acerola pulps obtained by freeze-drying and refractance window processes

3.3 *Antioxidant activity*

The pulp obtained by the refractance window (Table 2) showed significantly higher antioxidant activity than the freeze-dried pulp. This result may be related to the higher concentration of vitamin C (Figure 3) and the lower drying time observed in the refractance window method. The higher antioxidant activity observed could be related to the shorter exposure time of the sample during the drying process. Rezende et al. [28] found a higher concentration of bioactive compounds in acerola powder dried by spray-dryer in relation to production by freeze-drying. Among the various treatments performed, the content of $ABTS^{\bullet+}$ ranged from 13.97 to 15.12 µM TEAC.100 mg.

Table 2. Effect of the drying process (freeze-drying and refractance window) on the antioxidant activity of acerola pulps

Antioxidant activity	Drying	
	Freeze-drying	Refractance window
$ABTS^{\bullet+}$ [A]	1,452.50 ± 8.30[b]	1,838.60 ± 12.70[a]
DPPH[B]	2.05 ± 0.06[a]	2.00 ± 0.12[a]
FRAP[C]	1,258.33 ± 36.17[b]	1,290.00 ± 15.00[a]

Different lowercase letters in the same column indicate a significant difference ($p < 0.05$)
[A] µM Trolox/g dehydrated pulp, [B] EC_{50} (mg/mL), [C] µM TE/g dehydrated pulp

According to Cruz et al. [39], acerola is rich in phenolic compounds and vitamin C, and its antioxidant activity can be compared to synthetic antioxidants. Teixeira et al. [35] reported that the bioactive compounds present in acerola include vitamins, amino acids, and phenolic compounds.

The antioxidant activity of acerola pulp obtained at different stages of maturation was evaluated, in the immature stage present, 180.52 ± 15.41 µM TE/g DW (DPPH•) and 141.26 ± 10.12 µM TE/g DW ($ABTS^{\bullet+}$). It was attributed

to phenolic compounds and vitamin C in the pulp, which is related to the antioxidant activity of acerola pulp [40]. According to Teixeira et al. [35], the green acerola extracts ranged from 165.15 and 111.22 μM TEAC. 100 mg^{-1} ABTS radical in the lyophilized and microencapsulated, respectively.

4. Conclusion

The use of freeze-drying and refractance window processes for the dehydrated acerola pulp produces materials with similar microstructure characteristics. The refractance window dehydrated pulps showed a higher concentration of ascorbic acid and stability during storage time (10 days). The results may be related to the shorter drying time. The dehydrated acerola pulp can be used in the food industry for different applications, mainly due to its high antioxidant activity. For future work, it is recommended to evaluate the effect of the benefits of RW drying on the nutritional characteristics of acerola pulp, as well as other drying conditions.

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Conflicts of interest

The authors declare no competing financial interest.

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