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Combined methods at low temperature to minimize quality loss in acerola and jaboticaba: immersion in $CaCl_2$, freezing, and freeze-drying

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Abstract

The objective of this study was to investigate the effects of combined processes at low temperature on the quality of acerola (*Malpighia punicifolia L.*) and jaboticaba (*Myrciaria cauliflora Berg*). Acerola and jaboticaba were subjected to different combined treatments: infusion in calcium chloride, freezing at different temperatures (-20 and -80 °C) and freeze-drying. Physicochemical, colorimetric, scanning electron microscopy (SEM), and texture (firmness) analyses were conducted for acerola and jaboticaba fruits, and rheological behavior was analyzed in jaboticaba pulp. According to the results, for acerola, infusion in $CaCl_2$ interfered with microstructural analysis, freezing at -20 °C damaged the fruit, and freeze-drying with freezing at -80 °C yielded fruits with the most satisfactory quality. For jaboticaba, $CaCl_2$ infusion significantly changed the microstructure and pulp viscosity, and freezing at -20 °C further damaged the fruit; the best colorimetric results were obtained for freeze-drying with freezing at -80 °C.

Keywords: freezing; colorimetric analyses; firmness; rheological behavior; microstructure.

Practical Application: Processing fruits at low temperatures to make them available throughout the year.

1. Introduction

Among the seasonal fruits with a short shelf life of importance in Brazil are the acerola and the jaboticaba. Brazil is a great producer of these tropical fruits, highlighting the State of Goiás as the largest producer of jaboticaba (IBGE, 2017) and Pernambuco as the largest producer of acerola (IBGE, 2017). Both fruits are seasonal and are typically harvested in the months of September and October. Jaboticaba is highly perishable because of its high water and sugar contents (Sato & Cunha, 2009).

The consumption of acerola (*Malpighia* spp.) occurs in fresh or processed forms, such as juices, pulps, jams, ice creams, syrups, liqueurs, and candy syrups. For the export market, which constitutes 80% of Brazilian production, fruit pulp and fruit are exported frozen, both in the green and ripe states (Manica et al., 2003; Pereira et al., 2014). The commercial color of acerola is red. In addition to the color parameter, pH is the most viable method for determining product quality, in which fruits can be classified as not very acidic (pH>4.5), acidic (pH<4.5), or very acidic (pH<4). The acerola fruit is a very acidic fruit, with pH values ranging from 2.5 to 3.9. Soluble solid contents are relatively high in mature acerola, ranging from 3.7 to 14.1 °Brix (Manica et al., 2003). For acerola, storage of fruits for 3 days at 30 °C results in damage. At temperatures above 20 but below 30 °C, the fruits can be stored for only 3 days without damage, and the storage temperature that allows for better conservation of fresh acerola is 8 °C at humidity above 85% (Calgaro & Braga, 2012).

The jaboticaba (*Myrciaria* sp.) harvest usually occurs once per year for a period of approximately 3 months. However, jaboticaba is a highly perishable fruit and is usable for only 3 days after harvest, which hinders its commercialization. This deterioration is the result of microorganism development, enzymatic activity, and chemical reactions that interfere with the final quality of the fruit and increase postharvest losses. In jaboticaba, the values for pH range from 2.9 to 4, and the soluble solids content (°Brix) varies between 11.5 and 17.9 depending on the fruit maturation degree. It was reported that the best storage temperature for jaboticaba is 12 °C at a relative humidity of 80% (Duarte et al., 1997; Oliveira et al., 2003).

The freezing process is the main method applied for the preservation of acerola. For jaboticaba, no information from processing applications has been reported. Freezing has been an effective method in fruit storage, reducing the deteriorating effects when fruit is exposed to high temperatures, mainly due to chemical reactions, enzymatic actions, or biological degradation

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processes. Freezing and thawing in plant cells and tissues promote certain damage to cell structure and functions. One of the most frequently observed forms of injury is related to the changes in membrane permeability manifested by turgor loss and fluid loss. The formation of ice crystals in plant tissues can lead to irreversible damage to the cell wall, middle lamella, and protoplasts. With such structural damage, the texture properties can be much lower than those of the original tissue.

Fruit infusion incorporates calcium into the pore structures of tissues to increase cell wall resistance. The processes where Ca^{2+} ions are included to prevent fruit softening can easily be related to the effects of these treatments on the structure of the cell wall. It is well known that elements of the chains of peptide polysaccharides can be packed in intermolecular form in association with calcium. Calcium fixes within the cavities between the chains by binding to carboxylate groups and other electronegative atoms that are favorable ligands. The interaction with polysaccharides results in a firmer texture and increased stability of the interconnections between the cellulose/hemicellulose components and the rest of the matrix (Hall, 1979). The effect of the addition of calcium salts prior to freezing on the maintenance of the texture and cellular structure of the tissue of fruits has also been presented by Resende and Cal-Vidal (2002) and Suutarinen et al. (2000). The results showed that the presence of Ca^{2+} ions improved the characteristic texture of the fruits when compared to the untreated samples.

Freezing is also a step in the freeze-drying process. Among the several drying methods, freeze-drying is used to obtain high-quality products. This method improves the color, aroma, and flavor retention of the materials, minimizes undesirable shrinkage, improves the rehydration characteristics of the powder, and results in materials with high porosity (Oikonomopoulou & Krokida, 2012). Therefore, the objective of this work was to evaluate the effects of combined processes at low temperatures on the physical and chemical characteristics of acerola and jaboticaba using different treatments, such as infusion in calcium chloride, freezing at different temperatures, and freeze-drying, to determine the best method to prolong the shelf life of these frozen fruits with minor changes in quality.

2. Materials and methods

2.1. Sample preparation

Acerola fruits were acquired in the municipality of Goiânia, GO, Brazil, and jaboticaba fruits were acquired from producers in Hidrolândia, GO. The fruits were selected, washed in running water, and sanitized with a solution of sodium hypochlorite at 100 ppm for 15 min. The fruits were separated according to the treatments presented in Table 1.

After sanitization, the fruits were dried and placed in low-density polyethylene plastic bags. In the control treatment, the sample was refrigerated at a temperature of 8 °C for 24 h. In the pretreatments of immersion in a solution of calcium chloride, the fruits were immersed in a 1% calcium chloride solution for 3 min for acerola and a 4% solution for 60 min for jaboticaba (Garcia et al., 2019). In the freezing processes, the fruits packed in low-density polyethylene bags, with or without immersion in $CaCl_2$ solutions, were placed in a conventional freezer at -20 °C (Electrolux, Model F 25, Brazil — slow freezing (SF)) or in an ultralow-temperature freezer at -80 °C (ColdLab, model CL 374-80 V, Brazil — fast freezing (FF)). The temperature histories (Figure S1) during the freezing of acerola and jaboticaba fruits were obtained by acquisition of data as a function of time using T-type thermocouples (Copper-Constantan), 1.5 mm in diameter and 100 mm in length. The thermocouples were inserted into the geometric centers of the packaging. The data were recorded at 10-s intervals using a signal conditioning system (National Instruments Corporation, model SCXI-1000, Budapest, Hungary) using LabView 8.5 software (National Instruments Corporation, Newbury, Ireland). From the temperature histories, the cooling rates for slow freezing were 0.12 and 0.11 °C/min for acerola and jaboticaba, respectively. For fast freezing, the cooling rates were 0.58 and 0.60 °C/min for acerola and jaboticaba, respectively. In the freeze-drying process, all frozen samples were dried in a freeze-dryer (Thermo Electron Corporation ModulyoD, Milford, MA, USA) for 88 h. This residence time was specified after exploratory experimental tests with acerola and jaboticaba. The jaboticaba demands more time due to the pulp composition, thickness, and texture of the peel.

Table 1. Treatments and experimental planning.

Treatments	Code
Fruits at room temperature	FRT
Cooled fruits at 8 °C (control)	CF
$CaCl_2$ immersion and freezing at -20 °C	CISF
Freezing at -20 °C	SF
$CaCl_2$ immersion and freezing at -80 °C	CIFF
Freezing at -80 °C	FF
$CaCl_2$ immersion and freezing at -20 °C and freeze-drying	CISFFD
Freezing at -20 °C and freeze-drying	SFFD
$CaCl_2$ immersion and freezing at -80 °C and freeze-drying	CIFFFD
Freezing at -80 °C and freeze-drying	FFFD

2.2. Physicochemical analysis

Analyses of total soluble solids (TSS), titratable acidity (TA), and pH were performed by conventional methods from the Association of Official Analytical Chemists (AOAC, 2019).

2.3. Instrumental texture

The samples used for firmness analyses are shown in Table 1. The samples were thawed for 24 h under refrigeration at 8 °C in the treatments of CISF, SF, CIFF and FF. Firmness values were determined using a texture analyzer (TA-XT Plus, Surrey, England) with a P/2 probe. The previously fixed penetration distance was 4 mm for acerola and 8 mm for jaboticaba. Firmness was expressed in Newtons (N).

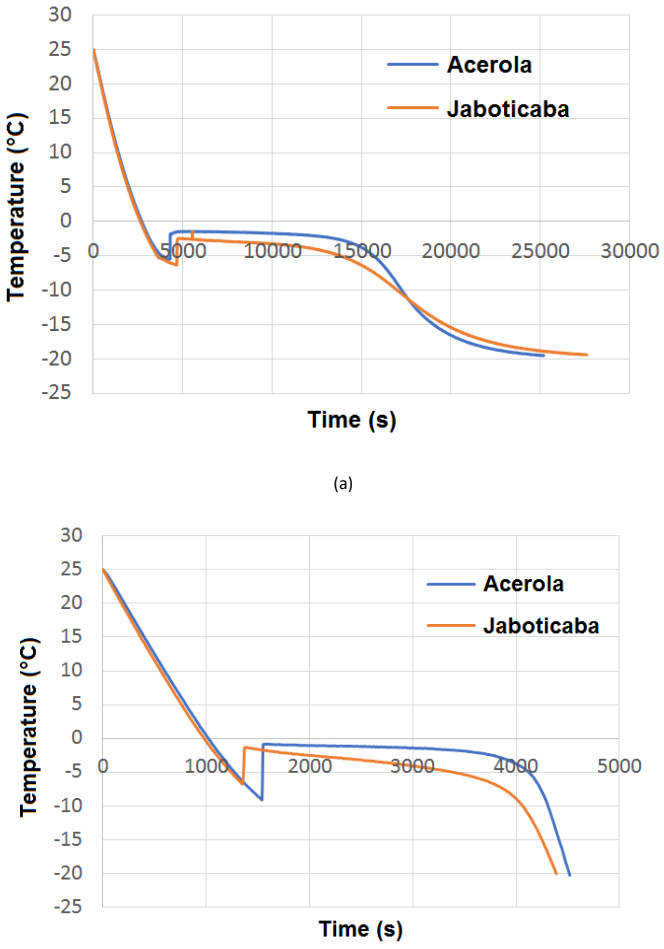

Figure S1. Temperature histories obtained during freezing process of acerola and jaboticaba fruits. (A) Slow freezing and (B) fast freezing.

2.4. Colorimetry

The samples were previously thawed at room temperature. The analyses of the color were performed by readings in triplicate of the parameters L*, a*, and b* obtained by the colorimeter (Hunterlab, ColorQuest XE). The chroma index (Cr), which is considered a quantitative attribute of color, and hue, which is considered the qualitative attribute of color, were calculated by Equations 1 and 2:

$$Cr = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$^{\circ}h = tan^{-1}\frac{b^*}{a^*} \quad (2)$$

2.5. Rheological behavior

For rheological analysis, the frozen samples were previously thawed at room temperature, and freeze-dried samples were reconstituted in water at room temperature for 1 h. The jaboticaba juice was extracted without any dilution and with the minimum number of particles possible to avoid interference. Rheological analyses for acerola were not carried out because it is not possible to extract acerola juice without dilution with water. The rheological behavior of jaboticaba juice was carried out at 20 °C using 15 mL of sample in a computerized rheometer (Anton Paar, Physica MCR 101 Ostfildern, Germany). The experimental data of the flow curves (shear stress vs. shear rate) were fitted to Newton's law (Equation 3), the law of power (Equation 4), and the *Herschell–Buckley* model (Equation 5).

$$\sigma = \mu\dot{\gamma} \quad (3)$$

$$\sigma = k\dot{\gamma}^n \quad (4)$$

$$\sigma = \sigma_o + k_H\dot{\gamma}^{n_H} \quad (5)$$

2.6. Scanning electron microscopy

The microstructures of freeze-dried samples of acerola and jaboticaba were analyzed using high-resolution microscopy (SEM) with different magnifications (40×, 200×, and 1000×) on a Jeol instrument (model JSM-6610) equipped with EDS and Thermo Scientific NSS Spectral Imaging, and an electron beam voltage of 8 kV was used.

2.7. Reconstitution of the fruits

The freeze-dried fruits were immersed in water for 60 min. All pH, TSS, texture, and rheological analyses were performed with reconstituted samples, except for color analyses, which were performed with freeze-dried fruit before reconstitution.

2.8. Statistical analyses

The results of the physicochemical, texture, color, and reconstitution analyses were subjected to analysis of variance (ANOVA) and Tukey's test ($p<0.05$) to compare the means and determine significant differences between treatments. All analyses were performed in triplicate. The SAS University Edition statistical package (SAS Institute Inc., Cary, NC, USA) was used in these analyses. SAS University Edition (SAS Institute Inc., Cary, NC, USA) statistical software was also used in the adjustment of flow curves for the determination of the rheological properties. The graphs were generated using the SigmaPlot software version 11 (Systat Software, Inc., San Jose, USA).

3. Results and discussion

3.1. Physicochemical analyses

The physicochemical analyses of the fruits are shown in Table 2. The pH values obtained indicate that acerola is classified as an acidic fruit with a pH range of 3.74–3.92, above the minimum pH value (2.8) established by the Identity and Quality Standards (Brasil, 2018) for frozen pulp. The highest pH value of 3.91 was obtained for FF and CIFF, and the lowest pH value of 3.74 was obtained for CISF (Table 2). According to Do Nascimento et al. (2018), in a study on acerola pulp in industrial and artisanal frozen foods, the pH values ranged from 3.2 to

Table 2. The total soluble solids (TSS) contents, pH, titratable acidity and colorimetric parameters of acerola and jaboticaba subjected to combined treatments.

Treatments	Physicochemical properties			Color parameters				
	TSS (°Brix)	pH	Acidity (g/100 mL)	L	a*	b*	Cr*	Hue (°)
Acerola								
CF	7.00±0.01^{a}	3.92±0.03^{c}	0.59±0.02^{a}	55.45±0.30^{d}	31.11±1.17^{ef}	34.88±1.06^{f}	46.75 ±1.18^{g}	48.29±1.29^{c}
CISF	7.00±0.02^{a}	3.74±0.04^{a}	0.67±0.03^{c}	48.93±0.93^{b}	28.44±1.51^{d}	28.60±2.29^{d}	40.40±1.35^{c}	45.11±3.44^{b}
SF	7.00±0.01^{a}	3.81±0.12^{ab}	0.65±0.02^{b}	50.53±0.29^{c}	30.48±0.28^{e}	30.04±0.19^{de}	42.80±0.23^{de}	44.58±0.34^{b}
CIFF	8.00±0.01^{b}	3.88±0.06^{bc}	0.73±0.02^{e}	40.47±0.33^{a}	35.35±1.06^{g}	25.35±2.50^{b}	43.54±1.56^{ef}	35.34±2.89^{a}
FF	7.01±0.01^{a}	3.91±0.12^{c}	0.76±0.01^{g}	40.48±0.28^{a}	31.84±2.6^{f}	30.38±0.28^{e}	44.03±1.95^{f}	43.65±2.18^{b}
CISFFD	8.50±0.48^{d}	3.85±0.06^{bc}	0.70±0.01^{d}	40.06±0.77^{a}	15.09±0.21^{a}	20.67±0.82^{a}	25.59±0.64^{a}	53.82±0.10^{d}
SFFD	8.08±0.19^{bc}	3.81±0.03^{ab}	0.70±0.01^{d}	56.94±1.11^{e}	20.22±0.37^{b}	27.12±0.31^{c}	33.83±0.29^{b}	53.28±0.10^{d}
CIFFFD	8.58±0.51^{d}	3.84±0.05^{bc}	0.72±0.02^{e}	61.39±1.58^{f}	20.00±0.10^{b}	34.87±1.60^{f}	40.20±1.38^{c}	60.12±0.10^{f}
FFFD	8.42±0.51^{cd}	3.79±0.05^{ab}	0.71±0.02^{de}	61.98±2.49^{f}	23.13±2.23^{c}	34.90±0.59^{f}	41.90±1.26^{d}	56.52±0.09^{e}
Jaboticaba								
CF	14.92±0.03^{b}	4.06±0.04^{a}	0.62±0.02^{bc}	24.97±1.08^{c}	3.83±1.51^{c}	-1.01±0.16^{a}	3.96±0.41^{cd}	-14.79±5.53^{a}
CISF	14.50±0.52^{ab}	4.22±0.13^{b}	0.60±0.01^{a}	22.19±1.63^{b}	3.20±0.49^{a}	-0.76±0.45^{c}	3.29±0.51^{a}	-13.37±2.01^{b}
SF	14.42±0.51^{ab}	4.25±0.11^{b}	0.62±0.01^{bc}	22.45±1.09^{b}	3.56±1.04^{b}	-0.78±0.04^{c}	3.64±0.99^{b}	-12.37±2.87^{c}
CIFF	14.25±0.29^{a}	4.26±0.12^{b}	0.61±0.01^{ab}	21.09±1.14^{a}	3.57±0.94^{b}	-0.94±0.14^{b}	3.69±0.79^{b}	-14.75±1.67^{a}
FF	14.08±0.29^{a}	4.19±0.11^{b}	0.63±0.01^{c}	22.93±1.64^{b}	3.91±1.47^{cd}	-0.91±0.15^{b}	4.01±1.37^{d}	-13.05±4.22^{bc}
CISFFD	14.50±0.52^{ab}	4.17±0.09^{ab}	0.63±0.01^{c}	24.77±4.81^{c}	3.79±1.24^{c}	-0.41±0.15^{d}	3.80±1.24^{bc}	-6.24±1.49^{d}
SFFD	14.25±0.45^{a}	4.18±0.06^{ab}	0.63±0.01^{c}	25.36±2.61^{c}	4.03±1.79^{d}	-0.32±0.12^{e}	4.04±1.79^{d}	-4.56±1.02^{e}
CIFFFD	14.42±0.51^{ab}	4.17±0.07^{ab}	0.63±0.01^{c}	25.48±1.67^{c}	4.44±1.37^{e}	-0.41±0.19^{d}	4.46±1.35^{e}	-5.27±1.21^{e}
FFFD	14.50±0.52^{ab}	4.21±0.05^{b}	0.63±0.01^{c}	27.62±2.82^{d}	3.89±1.30^{cd}	-0.44±0.13^{d}	3.92±1.29^{cd}	-6.46±5.03^{d}

* Means followed by the same letter in the columns do not differ according to Tukey's post hoc test (p<0.05).

3.5, with an average of 3.3, showing no significant differences when compared with artisanal pulps.

The acidity values of all treatments showed significant differences, except for the CISFFD, SFFD and FFFD treatments, with acidity values of 0.70 and 0.71, respectively, which did not show differences among themselves but differed from the others. All other samples showed significant differences, with the highest acidity value of 0.76 observed for FF. Faraoni et al. (2012) obtained a titratable acidity value of 0.90 g/100 g for acerola, which exhibited the highest variation in acidity from 0.70 to 1.38 in this study. Acidity is a relevant parameter in assessing the conservation status of a product.

The soluble solids values ranged from 7.00 to 8.58 °Brix, which are within the identity and quality standards, whose minimum value is 5.0 °Brix for fruit juice (Brasil, 2018). The CF, SF, FF, and CISF samples showed no significant differences at a level of 5% of significance, but they differed from the others by presenting the lowest TSS value of 7.0 °Brix. The freeze-dried samples, CIFFFD and CISFFD (8.58 and 8.50° Brix, respectively), did not exhibit significantly different TSS values, but their TSS values did differ from those of the other samples, with the highest TSS values observed with freeze-drying, as this process removes the water and concentrates the sugars. Nasser et al. (2018) studied the composition of acerola of different genotypes at two harvest times and obtained total soluble solids values between 6.5 and 8.3 °Brix, which are values close to those obtained in this study. The treatments in which the acerola was freeze-dried showed the highest values of TSS (°Brix) (Table 2). The freeze-dried fruit pulps exhibit an increase in the sugar contents of almost five times when compared to the natural form. The CIFFFD treatment showed better conservation of the studied parameters, including pH value (3.84) and relatively high TSS content (8.58). The combination of rapid freezing, infusion of calcium chloride, and freeze-drying showed satisfactory results.

The pH values of jaboticaba were acidic (4.06–4.26) and above the minimum limit (2.9) established by the identity and quality standards (PIQ) (Brasil, 2018). The titratable acidity ranged from 0.60 to 0.63 g/100 g in all treatments evaluated (Table 2) and statistically did not differ from the control sample CF. CF presented the highest value of TSS (14.92 °Brix), the TSS values ranged from 14.08 to 14.92 °Brix, and the samples did not show significant differences. Brunini et al. (2004) obtained values ranging from 12.0 to 15.5 °Brix and found variations between 9.0 and l4.0 °Brix for jaboticaba pulps. Contents above 15.0 °Brix may suggest lower postharvest conservation for jaboticaba (Barros et al., 1996). The lowest TSS value (14.08 °Brix) was observed in the FF sample. The CIFF sample showed the highest pH value (4.26) and lowest acidity value (0.61 g/100 g), and the CF sample showed the lowest pH (4.06) and a high acidity value (0.62 g/100 g), confirming that the physicochemical characteristics were similar between all treatments.

3.2. Color analyses

Table 2 shows the results of the colorimetric parameters studied in the different treatments of frozen and freeze-dried acerola. In Table 2, the highest values of luminosity were for the CIFFFD (61.39) and FFFD (61.98) treatments, which were both different from the other treatments. The lowest value of L*

was observed in the CISFFD treatment (40.06). Statistically, the CISFFD (40.06), CIFF, and FF (40.48) treatments did not differ significantly but differed from the other samples, showing the lowest luminosity values. Several studies have shown that during freezing, there is color degradation. The treatment with fast freezing, combined with freeze-drying, maintained greater luminosity in acerolas. Fast freezing, by degrading the pigments, and freeze-drying, by concentrating the components, showed satisfactory results when the samples were rehydrated.

Table 2 also shows that for parameter a*, all treatments showed positive values and colors closer to red. The highest value of a* was for the sample CIFF (35.35), evidencing that this sample presented the reddest color among the studied samples, and the treatment CISFFD (15.09) presented the lowest value of a*; that is, this sample exhibited the least red color. The CIFFFD (20.00) and SFFD (20.22) treatments showed no significant differences between themselves, but they differed from the other samples. The low a* values show that the treatments subjected to freeze-drying degraded red anthocyanins, which are very unstable pigments. The control sample did not differ from the SF or from the FF sample; in this case, the type of freezing and the infusion or not of calcium chloride had no influence on a* values. The control sample (CF) (b*=34.88) was the most yellow, and the least yellow was the CISFFD (20.67) sample, as shown in Table 2. The type of freezing and calcium chloride also did not influence this color parameter.

Fast freezing combined with freeze-drying preserved more pigments. The acerola sample that showed the highest saturation was the control (CF) (Cr=46.75), and the lowest saturation value was with the CISFFD sample (25.59) (Table 2). The type of freezing, slow or fast, and the use or absence of infusion in calcium chloride did not significantly interfere with the results. The hue angle was also calculated; smaller values close to zero represent a reddish color, and all values in this study were close to this range. The hue angle closest to zero was observed in the CIFF treatment (Table 2), and the highest value was observed in the CIFFFD treatment.

Fast freezing degraded the color of the acerola less than slow freezing, and therefore the chromatic characteristics of the pulp of acerola were located within the first quadrant, presenting positive values of a* and b*, representing red and yellow, respectively. These results are related to the present pigments, such as anthocyanins and carotenoids. Freezing is one of the most suitable processes for preserving the chemical, nutritional, and sensory properties of fruit pulps. However, enzyme activity is an issue, as it can cause significant color changes in the pulps of frozen fruits.

Table 2 also presents the results of the colorimetric parameters studied in the different treatments of frozen jaboticaba. Comparing treatments, the lowest luminosity was observed for CIFF (21.09), and the highest luminosity was observed for FFFD (27.62). The values of the coordinate a* were measured and are presented in Table 2. All values were positive, and the least red sample was CISF (3.20), which differed significantly from all other samples. The highest a* value was for the CIFFFD (4.44) treatment, which showed statistically significant differences when compared to that of all other studied samples. The combination of fast freezing and freeze-drying was able to sufficiently reduce the degradation of the pigments. All values of b* in Table 2 were negative because the jaboticaba peel does not have a yellow color. Negative b* values indicate blue color, so the sample with the highest blue color value was the control sample (CF) (-1.01), and the lowest was SFFD (-0.32). Fast freezing showed higher values for b* than slow freezing.

Chromaticity was also calculated, and the results are also presented in Table 2. The sample that presented the highest value was CIFFFD (4.46), and the lowest saturation value was observed for the CISF sample (3.29). The type of freezing (slow or fast) and the absence or presence of calcium chloride infusion did not interfere with the results obtained. The hue angle was also calculated, and the values obtained are represented in Table 2. The hue angle values were located in the third quadrant, close to the 270° angle; in this case, they were represented as the mirror of the first quadrant, therefore presenting negative values. Statistically, there was no significant difference in hue angles among CF (-14.79) and CIFF (-14.75) samples, but the values of these samples differed from those of the other samples. The hue angles of freeze-dried samples CIFFFD (-5.27) and SFFD (-4.56) did not differ significantly from each other but showed differences when compared with those of the other samples, exhibiting the lowest values. Garcia et al. (2019) showed that variations in a* values were observed throughout the period of storage of cooled jaboticaba; however, despite the variations, the skin color tended more toward red. This result was similar to that found for jaboticaba in this study, where freeze-drying preserved the color of jaboticaba fruits.

The treatment that presented the highest luminosity was FFFD (27.62), and the highest values of a* (4.44) and Cr (4.46) were observed in the CIFFFD sample, so fast freezing, combined with freeze-drying, is a good process to preserve the color of jaboticaba. The values of b* (-1.01) and °h (-14.79) were higher for the control sample (trending to blue) than the other samples. On the other hand, the lowest values of L* (21.09), a* (3.20), and Cr (3.29) were observed for the CISF sample, which indicated that slow freezing caused greater losses of color, and the least blue sample was the SFFD, presenting lower values of b* (-0.32) and °h (-4.56). It was observed that the infusion of calcium chloride did not interfere with the results obtained. The type of freezing had the greatest impact on fruit preservation for all the parameters analyzed, with the most satisfactory results observed in the samples subjected to fast freezing and the lowest values observed for samples subjected to slow freezing.

3.3. Instrumental texture

Figure 1A shows the results of the firmness of acerola, and Figure 1B of jaboticaba fruits in their different treatments. The highest values of acerola firmness were, in descending order, for treatments CF (control) and FF (1.9 N), FRT (1.8 N), CIFF (1.7 N), CISF (1.3 N), and SF (1.0 N). Figure 1A shows that pretreatments with calcium have been effective in preserving the firmness loss in acerola fruits after freeze-thawing. Various works also prove this statement, such as with guava (Werner et al., 2009), apple (Hussain et al., 2012), and strawberry (Reno et al, 2011; Van Buggenhout et al., 2006). The elasticity, strength,

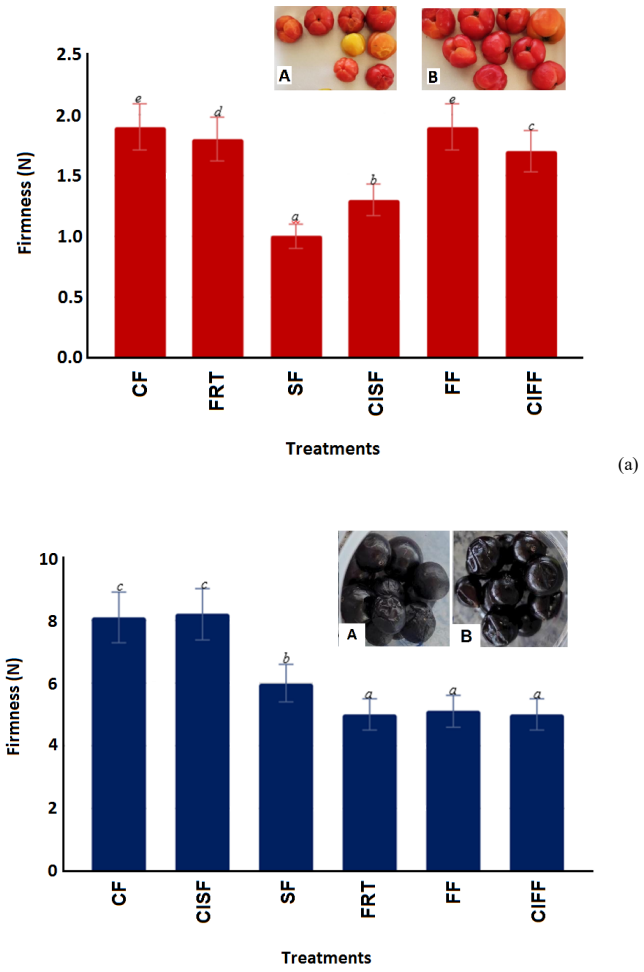

Figure 1. Firmness (N) of (A) acerola and (B) jaboticaba fruits subjected to different treatments. (A) Slow freezing and (B) fast freezing. Means followed by the same letter in the bars do not differ according to Tukey's post hoc test ($p<0.05$).

and stiffness of fruit tissues are attributed to the properties and interactions of cell wall components. The polyhydroxyl nature of the main wall polymers indicates that a large amount of hydrogen bridges will probably form on the cell wall, which is structurally significant when a substantial number of bonds form between two macromolecules (Brett & Waldron, 1990). Ionic bonds also occur between two nearby galacturonic acid residues, with calcium ions acting as ionic bridges between negatively charged galacturonate ions. The processes where Ca^{2+} ions are included to prevent fruit softening can easily be related to the effects of these treatments on the structure of the cell wall. Calcium fixes within the cavities between the chains by binding to carboxylate groups and other electronegative atoms that are favorable ligands. The formation of polysaccharide gels results in a firmer texture and increased stability of interconnections between the cellulose/hemicellulose components and the rest of the matrix (Hall, 1979).

It was observed in this work that, in general, immersion in calcium chloride was not effective for the acerola, and what truly differentiated the firmness values were the freezing temperatures. Figure 1A also shows photographs of the two types of freezing. Slow freezing (A) degraded the cells and showed less firmness than fast freezing. Comparing SF (1.0 N) and CISF (1.3 N) treatments, both were slowly frozen, but the sample that received the treatment by infusion of calcium chloride was firmer than the sample under the same temperature conditions without the calcium chloride infusion.

Statistically, all treatments differed from each other except for the control and FF. The FF presented texture characteristics closer to those of the original fruit. In this case, the preservation of the texture is attributed to the size and location of the ice crystals formed during the freezing process, which affect the final texture of the thawed fruit. Figure 1B shows that for jaboticaba, the treatments that presented the highest firmness values, in descending order, were CF (8.2 N), CISF (8.1 N), SF (6.0 N), FF (5.1 N), FRT (5.0 N), and CIFF (5.0). In this case, fast freezing showed greater degradation/damage to the fruit peel than slow freezing. Some jaboticaba fruits burst due to low temperatures for long periods of time and expansion of the pulp volume, impairing the quality, and the peel became wilted and less resistant (Figure 1B).

Figures 1BA and 1BB compare samples subjected to slow freezing and fast freezing and show that the most satisfactory treatments are control and slow freezing. Freezing damages fruits due to the breakdown of the membrane and the release of cellular fluid. Internal pressure occurs because the outer layers of the food freeze before the inner layers, forming a film that is frozen on the surface of the product. With freezing, there is an increase in the volume of the frozen water, increasing the internal pressure due to the resistance found in the surface barrier and causing tissue rupture. The treatments using slow freezing showed satisfactory results compared with fast freezing. The combination with the infusion of calcium chloride resulted in greater firmness than samples without calcium chloride. The firmness of samples subjected to slow freezing with immersion in calcium chloride (CISF) was not significantly different from that of the control sample (CF), but both differed from the other samples and showed greater firmness and better overall visual characteristics.

3.4. Scanning electron microscopy

Figures 2A and 2B show the microstructures of acerolas, and Figures 2C and 2D show the microstructures of jaboticabas subjected to slow and fast freezing with and without infusion in calcium chloride solution. Figure 2 shows that the treatments of acerola without calcium chloride (Figures 2A1 and 2B1), subjected to slow and fast freezing, respectively, showed cavities, greater damage, and a high degree of cell wall rupture when compared to the infusion treatment with calcium chloride. Calcium chloride infusion resulted in more intact structures and strengthened the acerola cell wall, both in the slow and fast freezing processes, as shown in Figures 2A2 and 2B2. Several studies (Falade & Igbeka, 2007; Neri et al., 2020; Resende & Cal-Vidal, 2002; Suutarinen et al., 2000) indicate that pretreatment with calcium has been effective in delaying firmness loss in fruits. Pretreatment of vegetables intended for freezing with calcium salts is particularly relevant, as it results in effective preservation of the product's texture. The interaction of

calcium ions with polygalacturonic acid molecules leads to the formation of calcium pectate, which confers firmness to vegetal tissue cells. Figure 2 shows that the best treatment for acerola was the combination of freezing with an infusion of calcium chloride, which provided less damage to the acerola cell wall.

The changes in the microstructures of jaboticabas submitted to slow and fast freezing and pretreatments with and without $CaCl_2$ infusion are shown in Figures 2C and 2D. Figures 2C1 and 2D1 show that the treatment without calcium chloride showed a smaller number of cavities left after the freeze-drying process when compared to treatments with $CaCl_2$. These cavities were formed after the sublimation of ice, which indicated a greater amount of water in systems treated with $CaCl_2$. Calcium competed with structural water hydrogen bonds by releasing more water into systems that was then available for the growth of ice crystals. Considering the freezing type, the treatment that maintained the best structure and quality of jaboticaba was the combination of fast freezing with calcium chloride infusion. Biological products have high water content, and the size and shape of ice crystals are related to freezing rates. Van Buggenhout et al. (2006), in their study of strawberries subjected to slow and fast freezing, observed less damage in fast freezing than in slow freezing. This result again elucidates the effect of the type of freezing that better preserved the cellular microstructure over the course of storage. These authors concluded that the cells remained more intact with faster freezing, while slow freezing caused greater intensity of damage to the cellular microstructure.

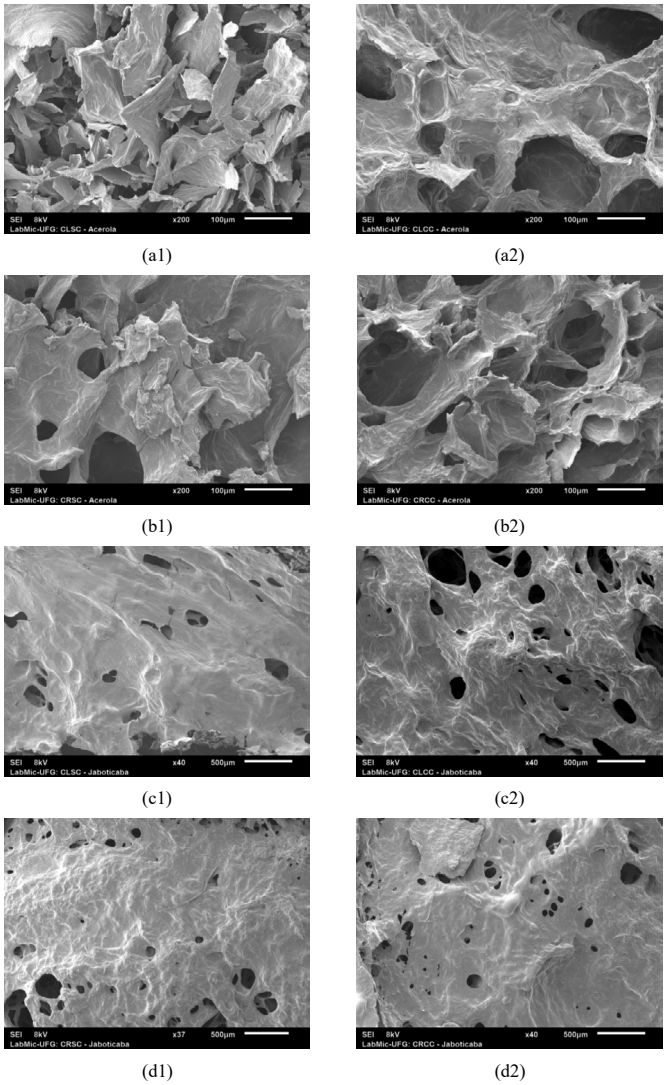

Figure 2. Electromicrographs obtained by scanning electron microscopy of acerola subjected to (A) slow freezing and (B) fast freezing and jaboticaba subjected to (C) slow freezing and (D) fast freezing, (1) without and (2) with infusion in calcium chloride solution.

3.5. Rheological behavior of the jaboticaba pulp

Figure 3A shows the relationship between shear stress and shear rate for all treatments. The nonlinearity between the shear stress and the shear rate indicates that the jaboticaba pulp has non-Newtonian behavior. The Newtonian model (Equation 3) yielded lower correlation coefficients (R^2<0.89), and the Herschell–Buckley model (Equation 5) presented negative yield stress values (Table 3).

Therefore, the power law (Equation 4) was the rheological model that best fit the rheological data with shear rates ranging from 0 to 500 s^{-1}. The model yielded high correlation coefficients (R^2>0.99) and a low mean square of the residue value (SQMR<0.12). The higher the SQMR value is, the greater the

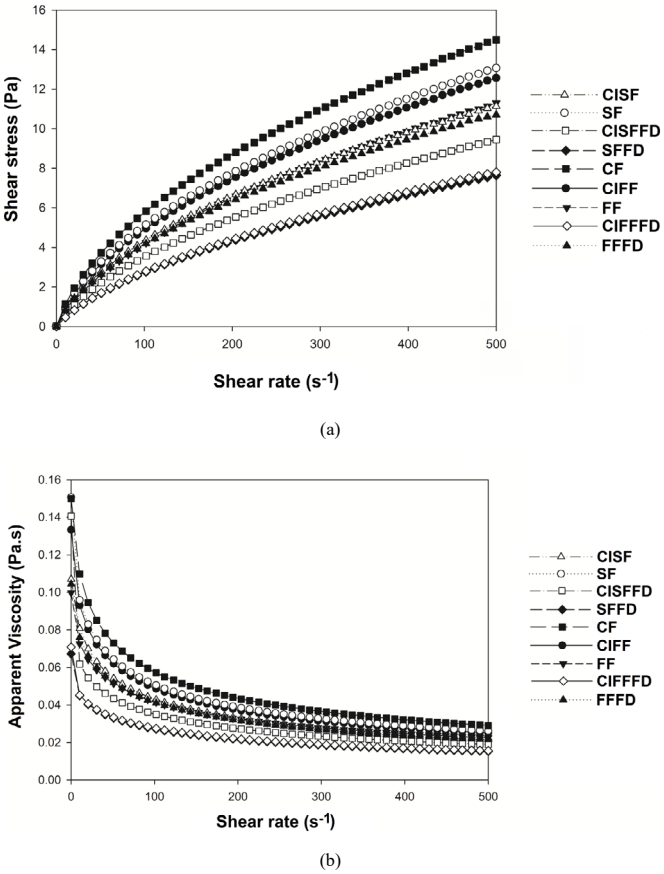

Figure 3. Flow curves of the jaboticaba pulps. (A) Shear stress as a function of shear rate. (B) Apparent viscosity as a function of shear rate.

Table 3. Rheological parameters of jaboticaba pulps at 20 °C after different treatments obtained by power law and Hershell–Bulkley models.

Treatments	Power law				Hershell–Buckley				
	R^2	SQMR	k	n	R^2	SQMR	k_H	n_H	σ_o
CF	0.9990	0.1245	0.3960^{a} (±0.0535)	0.5817^{d} (±0.0105)	0.9995	0.0903	0.5034^{a} (±0.0690)	0.5479^{c} (±0.0107)	-0.4949^{d} (±0.0490)
CISF	0.9992	0.0859	0.2769^{bc} (±0.0049)	0.5960^{bcd} (±0.0019)	0.9996	0.0613	0.3459^{bc} (±0.0062)	0.5645^{bc} (±0.0019)	-0.3398^{bc} (±0.0079)
SF	0.9992	0.1013	0.3327^{ab} (±0.0072)	0.5922^{bcd} (±0.0011)	0.9996	0.0724	0.4156^{ab} (±0.0061)	0.5606^{bc} (±0.0004)	-0.4021^{c} (±0.0077)
CIFF	0.9992	0.0965	0.3230^{ab} (±0.0050)	0.5905^{cd} (±0.0011)	0.9996	0.0689	0.4028^{b} (±0.0030)	0.5592^{bc} (±0.0003)	-0.3843^{c} (±0.0105)
FF	0.9992	0.0863	0.2375^{c} (±0.0234)	0.6231^{ab} (±0.0064)	0.9996	0.0617	0.2971^{c} (±0.0292)	0.5911^{ab} (±0.0064)	-0.3293^{bc} (±0.0235)
CISFFD	0.9992	0.0713	0.2038^{cd} (±0.0154)	0.6188^{abc} (±0.0061)	0.9996	0.0513	0.2539^{cd} (±0.0212)	0.5875^{ab} (±0.0073)	-0.2720^{ab} (±0.0263)
SFFD	0.9993	0.0576	0.1418^{d} (±0.0529)	0.6486^{a} (±0.0305)	0.9996	0.0724	0.1779^{d} (±0.0674)	0.6161^{a} (±0.0311)	-0.2167^{a} (±0.0586)
CIFFFD	0.9993	0.0579	0.1396^{d} (±0.0129)	0.6493^{a} (±0.0062)	0.9996	0.0413	0.1742^{d} (±0.0164)	0.6174^{a} (±0.0064)	-0.2138^{a} (±0.0158)
FFFD	0.9991	0.0896	0.2633^{bc} (±0.0050)	0.5982^{bcd} (±0.0016)	0.9995	0.0649	0.3333^{bc} (±0.0060)	0.5648^{bc} (±0.0014)	-0.3474^{bc} (±0.0046)

R^2: the coefficients of determination; SQMR: the square roots of the mean errors; σ_o: the yield stress (Pa); k: the consistency index (Pa s^n); k_H: the consistency index of Herschel-Bulkley model (Pa s^n); n: the flow index (-); n_H: the flow index of the Herschel–Bulkley model (-). Values correspond to the mean and standard deviation. Means followed by the same letter in the column do not differ according to Tukey's post-hoc test (p<0.05).

discrepancy between the frequencies observed and expected. The smaller the value of R^2, the greater the distance of the data point to the fitted model.

The flow index values (N) ranged from 0.58 to 0.65, demonstrating that jaboticaba pulp was a pseudoplastic fluid (n<1) under all evaluated conditions, showing that shear thinning behavior was not affected by the addition of $CaCl_2$, the freezing method, freeze-drying, or reconstitution and that the viscosity decreased with an increase in the shear rate. The consistency index (k) is indicative of the viscosity of the system. Table 3 and Figure 3 show that freezing and $CaCl_2$ infusion led to pulp exhibiting Newtonian behavior (higher n values) and lower viscosity (lower k values) than CF samples, and the $CaCl_2$ infusion further decreased the viscosity of these systems. The behavior of pseudoplastic fluid is typical of fruit juices and pulps and has been previously observed in jaboticaba pulp (Sato & Cunha, 2007).

4. Conclusion

Considering the firmness of acerolas, the control and fast-frozen samples without and with calcium immersion did not differ significantly from each other. The acidity values of all treatments showed significant differences. The TSS of CF, SF, FF, and CISF samples showed no significant differences at a level of 5% significance. The treatment with fast freezing, combined with freeze-drying, maintained the greater luminosity of acerolas and preserved more pigments. Calcium chloride infusion resulted in more intact structures and strengthened the acerola cell wall, both in the slow and fast freezing processes. The best treatment for acerola was the combination of freezing with an infusion of calcium chloride, which provided less damage to the acerola cell wall.

The pH values of jaboticaba were acidic, and in all treatments evaluated statistically, they did not differ from the control sample CF. Statistically, the CISFFD, CIFF, and FF treatments did not differ significantly but differed from the other samples, showing the lowest luminosity values. The highest a* value was for the CIFFFD treatment, which showed statistically significant differences when compared to all other studied samples. The combination of fast freezing and freeze-drying was able to reduce the degradation of the pigments. Fast freezing of jaboticaba showed greater degradation/damage in the fruit peel than slow freezing. Some jaboticaba fruits burst due to low temperatures and expansion of the pulp volume, and the peel became wilted and less resistant. The combination with the infusion of calcium chloride resulted in greater firmness than samples without calcium chloride. The firmness of samples subjected to slow freezing with immersion in calcium chloride (CISF) was not significantly different from that of the control sample (CF), but both differed from the other samples and showed greater firmness and better overall visual characteristics. Calcium chloride infusion significantly influenced the microstructure and pulp viscosity. The power law and the Herschell–Buckley models best fit the data, characterizing frozen jaboticaba pulp and reconstituted freeze-dried pulp as shear thinning fluids. Colorimetry shows that the best treatment for color parameters is fast freezing combined with freeze-drying.

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