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EFFECT OF BLOCK FREEZE CONCENTRATION PROCESS ON ACEROLA JUICE (*MALPIGHIA EMARGINATA*)

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ABSTRACT

Acerola is a fruit rich in vitamin C, in addition to having high levels of anthocyanins and carotenoids, which are antioxidant pigments that give the fruit its red color. Thus, the objective of this work was to concentrate bioactive compounds, by the method of block freeze concentration, using acerola juice in natura. Fresh juice and freeze concentration fractions (concentrate and ice) were evaluated for pH, acidity, soluble solids, total solids, quantification of total phenolic compounds and antioxidant activity. From the results obtained, it was observed that the method of freeze block concentration in blocks resulted in the concentration of soluble and total solids and acidity. In addition, it significantly concentrated the phenolic compounds, keeping the process efficiency above 39%. Regarding the antioxidant activity, the values were significantly higher in the obtained concentrates than in the initial juice. The highest antioxidant potential found was for the juice retained in the last step of the process, with activity about 1.7 times greater than the initial juice. As the freeze concentration stages progressed, an increase in the concentration factors in the total solids content was observed, with an average increase of approximately 131% in the third stage. Thus, the results obtained in this work suggest that the method of block freeze concentration applied to acerola juice, provided a product with greater antioxidant activity and concentration of phenolic compounds, which shows that this is a viable method for the concentration of bioactive compounds. from acerola.

1.Introduction

Acerola (*Malpighia emarginata*) is native to the Antilles and is cultivated in tropical and subtropical climate regions, like northern South America and Central America, and mainly including Brazil, which has the greatest plantations in the world (Barros *et al.*, 2020). Is a fruit known for its high content of ascorbic acid (in the range of 1500–4500 mg/100 g) and other important bioactive compounds, like phenolic compounds, including benzoic acid derivatives, phenylpropanoids, flavonoids and anthocyanins, and of total carotenoids (Nascimento *et al.*, 2018). The fruit is used in the industry for the production of pulp, juices, and jellies and also has a great potential for use in the production of food supplements that enhance the immune response of the body due to the presence of antioxidant compounds and high vitamin C content (Belwal *et al.*, 2018).

The antioxidant activity of red fruits, such as acerola, is due to the chemical structure of anthocyanins. Anthocyanins are glycosylated polyhydroxy or polymethoxy derivatives of 2-phenylbenzopyrylium, containing two benzoyl rings separated by a heterocyclic ring. Different anthocyanins are characterized by differences in: number and degree of methylation of hydroxyl groups; nature, number and position of sugar moieties attached to the phenolic molecule

(aglycone); nature and number of aliphatic or aromatic acids attached to the sugars (Santini and Huyke, 1993). Besides the colorant property, the anthocyanins have been found to exhibit potential therapeutic effect as antiinflammatory, radiation-protective, chemoprotective, vasoprotective, inhibition of LDL oxidation and decrease the risks of cardiovascular diseases (Wang *et al.*, 1997; Seeram and Nair, 2002).

In the food industry, thermal processing is a widely used method to extend the shelf life of foods. However, it has some drawbacks, such as the degradation of heat-sensitive nutrients and changes in food color (Patras *et al.*, 2010; Van Boekel *et al.*, 2010; Sui *et al.*, 2016).

Non-thermal technologies, like freeze concentrantion are widely used in the food industry for their ability to preserve product quality by preventing the thermal degradation of temperature-sensitive compounds (Chemat *et al*., 2017). The block freeze concentration (BFC) consists of crystallizing part of the water from a liquid to separate it from the concentrated solution. Cryoconcentration can be understood as dehydration, allowing to preserve to the maximum the organoleptic and nutritional characteristics of the liquid food (Safiei and Shaikh Alaudin, 2021; Wu *et al.*, 2017).

This technology uses low temperatures during the process, avoiding undesirable chemical, physical and biological changes that occur in other types of processing (Gunathilake *et al*., 2014).

Block freeze concentration method is reported as promising and effective in obtaining food products with high nutritional value and preservation of sensorial properties, such as fruit juices, coffee and whey (Meneses *et al.*, 2021; Haas *et al.*, 2022; Almeida *et al.*, 2023; Benedetti *et al.*, 2015).

Based on these aspects, this study seeks to evaluate the concentration of the bioactive compounds in the acerola juice, obtained from block cryoconcentration by gravitational method.

2. Materials and methods

2.1. Materials

Fresh acerolas were purchased from a local supplier (Mato Grosso do Sul, Brazil), crushed in a fruit processor and filtered to separate out the seeds and skin from the juice, avoid the presence of solids in the juice that might interfere with the freeze concentration process. Acerola juice was kept in a freezer at -18 ± 2 ºC (Biplex CRM45 Consul, Brazil) until the cryoconcentration process.

2.2. Block freeze concentration process

The freezing conditions were performed by the method of Aider and Ounis (2012), with modifications. The method is based on total freezing of the solution followed by partial thawing through simple gravitational separation, it is possible to obtain two fractions: concentrate (C) and ice (G). A schematic representation of the three process stages, sample division, and sample masses are shown in Fig. 1. The initial weight of 2 L of acerola juice was frozen in polypropylene pots at -18 ± 2 ºC in static freezer.

The cryoconcentration apparatus consisted of a mesh stainless steel screen, a plastic funnel, a tripod stand, a 500 mL beaker flask, and a semi-analytical balance (AUX 320 Shimadzu) (Fig. 1). At each stage, the frozen samples were removed from the pots and placed on top of the stainless-steel screen maintained in contact with the funnel, and partial defrosting was allowed using only gravitational force.

Thawing was conducted inside of a refrigerator, with a controlled temperature of 10±2 ºC, until the fraction thawed collected in the beaker reached 50% of the initial frozen sample. The liquid that was thawed consisted of the concentrate of the first step C1. The frozen sample that remained in the stainless-steel screen was taken as the ice fraction I1. The C1 concentrate was again frozen at -18 ± 2 ºC for 24h, used as a feeding solution for the second stage. In the second stage, 50% of the frozen solution was thawed, collected and refrozen as a feeding solution for the third stage. Samples of concentrate (C1, C2, C3) and ice were (I1, I2, I3) taken from each step for further analysis and

stored at -18 ± 2 °C for analysis. All cryoconcentrates and their ice were evaluated to determine concentration performance, physicochemical and phenolic composition and antioxidant capacity.

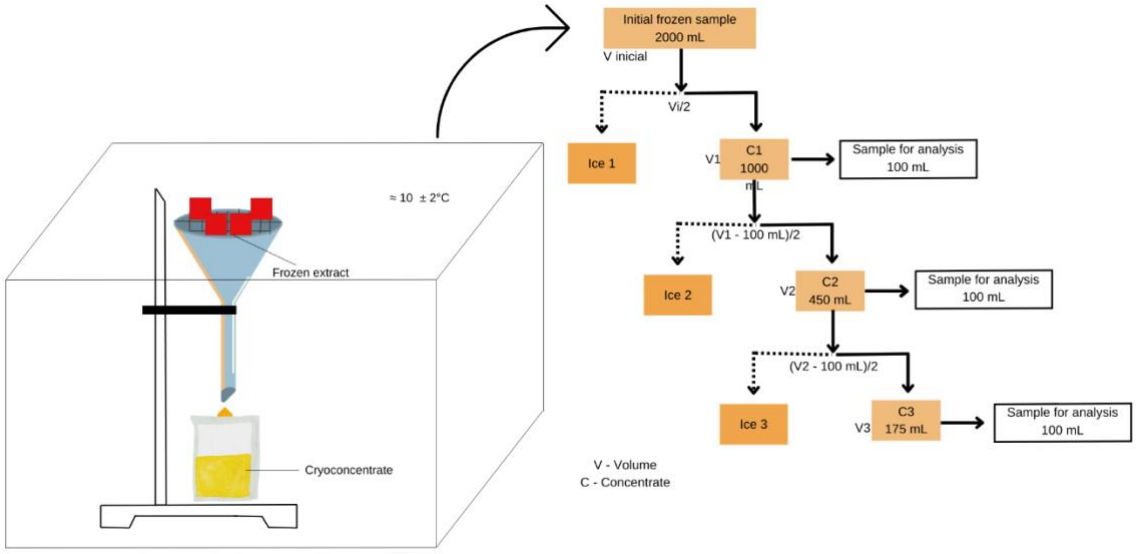

Figure 1. General diagram of BFC process by gravitational method of the acerola juice.

2.3. Physicochemical analysis

The physicochemical analysis was carried with orange juice, concentrated orange juice, and ice fraction samples. The pH was determined with digital pH meter DM 22 (Digimed, São Paulo, Brasil), and total soluble solids (Brix) were measured at 20 ◦C (±1 ◦C) on a refractomer model PAL-1 ATAGO). The titratable acidity and total dry matter content were also determined, according to AOAC (2005). All analyzes were performed in triplicate.

2.4. Block freeze concentration performance

The concentration factor (CF) at each freeze concentration stage was determined as a function of the increase in the concentration of the solution in relation to the quantity of total dry matter content in the initial acerola juice, as proposed by Aider and Ounis (2012). The total dry matter content was determined by measuring the mass loss after drying at 105 °C for 24 hours, and expressed as dry matter content/total mass (g.100 g^{-1}) (AOAC, 2005). The CF was calculated as the ratio between DMn is the total dry matter content (g) of the concentrated fluid in each freeze concentration stage and DM0 is the total dry matter content (g) of the initial acerola juice. The results were expressed in %.

As described by Belén *et al.* (2012), the efficiency of the freeze concentration process (PE) (%) was determined based on the increase in the content of total phenolics compounds (TP) of the concentrated fluid in relation to the IC remaining in the ice, as calculated through the following equation:

$$PE(\%)=\frac{TPC_n-TPI_n}{TPC_n} \quad (1)$$

TPC_n and TPI_n are the concentration of total phenolic compounds in concentrated juice and ice fractions.

2.5. Determination of total phenolic compounds

Total phenolic compounds polyphenols were determined spectrophotometrically on a UV-1600 (Pró-Análise, São Paulo, Brazil) spectrophotometer using the Folin-Ciocalteu assay, according to Singleton and Rossi (1965). The absorbance of samples was measured at 765 nm and results expressed as gallic acid equivalents (mg GAE.100 mL^{-1} juice). All analyzes were performed in triplicate.

2.6. Determination of antioxidant activity

The determination of antioxidant activity was performed by the DPPH method (1,1-diphenyl-2-picryl-hydrazyl) according to Rufino *et al*. (2007) with some modifications. The absorbance reading was performed at 515 nm in a spectrophotometer (model UV-1600, Pró-Análise, São Paulo, Brazil). Results were expressed in TEAC (antioxidant activity equivalent in Trolox) as µ MolTrolox. g^{-1} sample. All analyzes were performed in triplicate.

2.7. Statistical analysis

In this study, all data were presented as mean ± standard deviation (SD). Tukey's least significant difference test (LSD), with a significance level of 5%, was used to compare the means. All analyzes were performed using the software Statistica (v. 7.0, TIBCO Software Inc., Palo Alto, CA, USA).

3.Results and discussions

3.1. Physicochemical analysis

The increase in the soluble solids content in the natura juice concentrate and in the ice, in each stage of the cryoconcentration process, is shown in Figure 2.

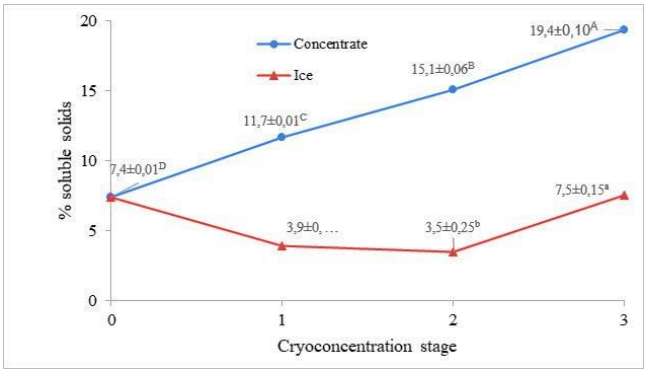

Figure 2. Soluble solids content of concentrated fractions and residual ice as a function of cryoconcentration steps for fresh juice.

Data are expressed as mean ± SD (n = 3) of the soluble solids content in the concentrated samples and ice in each. Different superscript lowercase letters indicate a significant difference ($p < 0.05$) between feed and concentrated fluid at each stage. Different superscript capital letters indicate a significant difference ($p < 0.05$) between food and ice at each stage.

The concentration of soluble solids in the concentrates increased significantly ($p < 0.05$) in all steps, when compared to the initial juice. The total soluble solids content of acerola juice increased from 7.4 ºBrix to 19.4 ºBrix in the concentrate of the third stage, representing about 2.6 times of the initial value. In the concentration of orange juice, Haas et al. (2022) obtained significantly from 17.37 to 38.07 ◦Brix in juice concentrates. Meneses et al. (2021) achieved an increse from 4% (w/w) to 14.1% (w/w) in the three stages of green tea concentrantion. Petzold *et al.* al. (2015) achieved cryoconcentration in blueberry and pineapple juices, which resulted, after 3 repetitions, in an increase from 13ºBrix to 33ºBrix in both juices, that is, a concentration of 2.5 times.

Figure 3 shows the total solids content in each stage of cryoconcentration for acerola juice. There was an increase in solids content ($p < 0.05$) in all stages of concentrate.

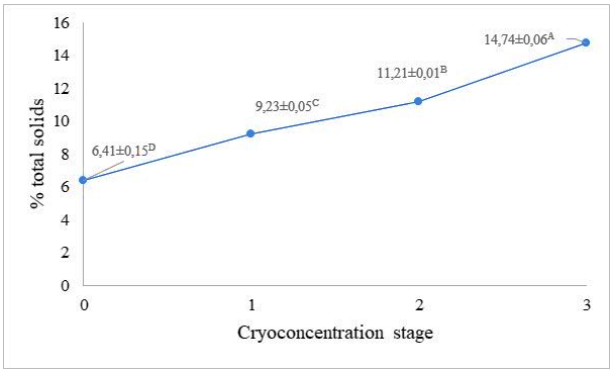

Figure 3. Total solid content in concentrated fractions of cryoconcentration.
Different superscript capital letters indicate a significant difference ($p < 0.05$) between juice and concentrate at each stage.

The concentrated juice presented increase of the total solids content, in the concentrate of each stage of the cryoconcentration process. The concentration factor increased ($p < 0.05$) in all stages when compared to the initial juice. The concentration factor increased ($p < 0.05$) in all stages when compared to the initial acerola juice. Values of 143.97 ± 0.04% were obtained in the first stage, 121.44 ± 0.01% in the second stage, 131.47 ± 0.04% in the third stage. This same behavior of the concentration factors found for fresh juice and concentrates corroborates Adorno *et al.* (2016), for strawberry juice, who reported a significant increase in the total solids content in the concentrates obtained in the four stages of cryoconcentration in blocks.

The same was observed by Haas *et al.* (2022), for orange juice and by Almeida *et al.* (2023), by concentrating *Morinda citrifolia L.* tea.

Table 1 presents the results of the analysis of pH and titratable acidity of the concentrate and of the ice obtained during the three stages of cryoconcentration of acerola juice.

3.2. Total phenolic content

The results of the content of total phenolic compounds in acerola juice and the concentrates from cryoconcentration are shown in Table 2. Analyzing the effect of cryoconcentration in each stage, an increase in all three is observed when compared to the initial juice. There was also an increase ($p < 0.05$) in the content of phenolic compounds present in the juice ice fractions. In the second stage, the CFT content was reduced, but in the third stage there was a significant increase ($p<0.05$) when compared to the previous stage. This behavior of the CFT content in the ice fractions can be explained, according to Aider *et al.* (2007), by the fact that the content of phenolic compounds behaves this way due to the formation of hydrogen bonds, since it has the ability to bind to a large number of water molecules. With the increase of phenolic compounds in the solution, the interstitial water becomes less available for freezing, as a result, during the process of separating the concentrated fluid from the ice, the frozen phase retains greater amounts of phenolic compounds.

The highest efficiency was observed in the second stage of cryoconcentration for fresh juice, with a value of 70.49±0.20%.

Table 1. Physicochemical composition of initial juice, cryoconcentrated acerola juice and ice fractions obtained by block freeze concentration

Samples	Physicochemical parameters	
	pH	Titratable acidity
IJ	$3,66^{b}$±0,02	$0,90^{d}$±0,01
C1	$4,15^{a}$±0,15	$1,54^{c}$±0,03
C2	$3,90^{ab}$±0,15	$1,68^{b}$±0,01
C3	$3,65^{b}$±0,15	$2,19^{a}$±0,01
I1	$3,87^{A}$±0,16	$0,05^{C}$±0,01
I2	$3,53^{A}$±0,25	$0,03^{B}$±0,01
I3	$3,53^{A}$±0,15	$0,09^{A}$±0,01

Results are expressed as mean ± standard deviation (n = 3). Different lowercase letters indicate statistical difference ($p \leq 0.05$) between initial orange juice and cryoconcentrated juices (C1, C2 and C3). Different uppercase letters indicate statistical difference between ice fractions (IF1, IF2 and IF3) at every stage. OJ, initial juice; I1, ice fraction of stage 1; I2, ice fraction of stage 2; I3, ice fraction of stage 3; C1, cryoconcentrated orange juice of stage 1; C2, cryoconcentrated orange juice of stage 2; C3, cryoconcentrated orange juice of stage 3.
Titratable acidity expressed in citric acid (g/100 mL).

Table 2. Total phenolic content for the cryoconcentrated and ice fractions and process efficiency of the three-stage cryoconcentration of orange juice

Stage		TPC (mg GAE.100 mL^{-1})	Eficiency (E%)
Acerola juice		$0,524^{Ab}$±0,01	
Stage 1	C1	$0,578^{ab}$±0,02	49,21±0,10
	I1	$0,293^{BC}$±0,07	
Stage 2	C2	$0,594^{ab}$±0,10	70,49±0,20
	I2	$0,175^{C}$±0,01	
Stage 3	C3	$0,662^{a}$±0,03	39,21±0,70
	I3	$0,403^{BC}$±0,10	

Data are expressed as mean ± SD (n = 3) of the total phenolic content in the concentrated samples and ice in each cryoconcentration stage. Different superscript lowercase letters indicate a significant difference ($p < 0.05$) between the initial juice and concentrates of each cryoconcentration stage. Different uppercase letters indicate a significant difference between the initial juice and the ice of each cryoconcentration stage.

The results found corroborate those reported by Adorno *et al.* (2016) for strawberry juice, Nunes *et al.* (2015) for aqueous extract of yerba mate and Benedetti *et al.* (2015) for tofu whey concentration. However, they differ from those reported by Boaventura *et al.* (2013) for aqueous extract of yerba mate and Belén *et al.* (2012) for wastewater from tofu production. According to these authors, the greater efficiency of cryoconcentration is usually presented in the first stage, with a decline occurring with the evolution of the stages due to the increase in the retention of solids in the ice. The results found suggest that a lower retention of CFT in ice promotes greater efficiency in the process.

3.3. Antioxidant activity assays

Table 3 presents the DPPH results for the antioxidant capacity of fresh juice and concentrates, expressed in µmol TEAC. mL-1. A significant increase ($p<0.05$) in the antioxidant activity of all concentrates (C1, C2, C3) can be observed when compared to fresh juice. Concentrate C3 had an increase of approximately 1.71 times in antioxidant potential. Haas *et al.* (2022) and Almeida *et al.* (2023) observed an increase in antioxidant activity in the concentrated fractions of cryoconcentration, by the DPPH method, for orange juice and *Morinda citrifolia* L. tea., respectively. Higuera (2013) and Moreno *et al.* (2014) also reported an increase in antioxidant activity (determined by the DPPH method) of coffee aqueous extract concentrates and coffee extract, respectively, corroborating the present study. The main bioactive compounds in acerola are vitamin C and carotenoids. Among the carotenoids, anthocyanins stand out, which have a suitable chemical structure to act as an antioxidant, as they can donate hydrogens or electrons to free radicals. The greater antioxidant activity is related to the presence of hydroxyl groups in the 3' and 4' positions of ring B, which confer high stability to the formed radicals (Cao *et al.*, 1997). The free hydroxyl groups in positions 3 and 5, together with the carbonyl group in position 4' are electron donors (Rice-Evans *et al.*, 1996).

Therefore, anthocyanins are important free radical scavengers. Also due to their chemical structure, anthocyanins can act as singlet oxygen deactivators.

Table 3. Antioxidant activity via DPPH in initial juice and cryoconcentrated fractions obtained by block freeze concentration

Samples	DPPH method (µmol TROLOX. g^{-1})
Acerola juice	$16,42^{b}$±2,12
C1	$23,72^{c}$±1,04
C2	$22,68^{c}$±0,61
C3	$28,12^{a}$±1,69

Data are expressed as mean ± SD (n = 3) of the total phenolic content in the concentrated samples and ice in each cryoconcentration stage. Different superscript lowercase letters indicate a significant difference ($p < 0.05$) between the initial juice and concentrates of each cryoconcentration stage.

4. Conclusions

This study showed that it is possible to increase the content of bioactive compounds, represented by the content of phenolic compounds and antioxidant activity, by the block cryoconcentration method, for in natura acerola juice. Cryoconcentration proved to be an alternative to preserve the nutritional quality of acerola juice and promoted an increase in the concentration factor in relation to the total solids content, with an average increase of approximately 144% in the first stage, 121% in the second stage and 131 % in the third step.Concentrated fluids showed an increase in the content of compounds in all stages of cryoconcentration, especially in the final stage (C3). In addition, there was 260% in the soluble solids content and 240% in the acidity of the concentrated acerola juice (C3).The antioxidant capacity measured by the DPPH method increased significantly about 1.7 times when compared to the initial juice, with the highest concentration found in C3.The results of this study indicate that the method of cryoconcentration in blocks, applied to acerola juice, increased the content of total and soluble solids, the antioxidant activity and the content of total phenolic compounds, proving to be a viable method for the concentration of compounds biological agents and an important technology for the concentration of acerola juice.

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