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The Addition of Concentrated Cold-Pressed Guabiroba Juice to Yogurts: Effects on the Physicochemical Analyses, Antioxidant Activity, Carotenoid Content, Total Phenolic Compounds, and Mineral Profile

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Abstract: Cold-pressed guabiroba juice was subjected to block freeze concentration. The best process efficiency (PE) was obtained for the first stage of freeze concentration compared to the second stage (C2; 57.47%). Yogurt formulations were carried out with the concentrated juice from stage 1 (C1) (0, 10% (I10), and 15% (I15)). For all concentrated juices (C1 and C2), ice fractions from the first and second stages (I1 and I2), and yogurts (control, I10, and I15), physicochemical analyses were performed and antioxidant activity, carotenoid content, total phenolic content (TPC), and mineral profile were determined. Total soluble solids contents for the concentrated juices increased by 1.56 to 2 times compared to the cold-pressed guabiroba juice. Furthermore, an increase in TPC and carotenoids was observed. For the I15 sample, TPC increased by 4,556%, with the control and increased carotenoids, vitamin C, and mineral profiles (Ca, K, mg, and Na) contributing to increased antioxidant activity. The addition of concentrated guabiroba juice to yogurt formulations enhances the functional property of this dairy product by maintaining most of the bioactive compounds during cold-pressing associated with the freeze concentration.

Keywords: fermented milk; *Myrtaceae* family; bioactive compounds; phenolic compounds; antioxidant activity

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1. Introduction

Yogurt is the main fermented milk product consumed around the globe, since this dairy product, which different manufacturers produce, is widely accepted by consumers due to its rich nutritional and functional value, taste, and practicality [1]. To expand the

consumer market, the development of fermented milk products with new ingredients is on the rise, with the fusion of new flavors and relevant functional appeal. Adding juices, pulp, and pieces of fruit are among the ingredients that consumers most accept, and they increase the levels of bioactive compounds in dairy products [2–7].

Bioactive compounds, also called "phytochemicals", are produced by plant defense systems through antimicrobial, insecticidal, and antioxidant properties [8–10]. It was found that when these compounds are introduced into a healthy dietary routine, they can benefit the human body by reducing the incidence of chronic diseases such as diabetes, obesity, cancer, and cardiovascular and neurological problems [8,11,12]. Phenolic compounds, such as carotenoids and phytosterols, are bioactive in vegetables and fruits [13–15] and contain excellent functional value. For example, in previous studies, it was observed that there was an improvement in the antioxidant activity of yogurt when concentrated strawberry fruit was added [16]. Prestes et al. [17] stated that the addition of fruit bioactive compounds to fermented milk products improved their nutritional value and health properties. Lal et al. [18] verified the potential antioxidants and antimicrobials from *Myrica esculenta* fruit extract. Lima et al. [19] also confirmed evidence of fruit phenolic compounds as potential antimicrobials against pathogenic bacteria.

On the other hand, the natural pigment of the fruits, coming from bioactive compounds, can also reduce the addition of synthetic colorants to the formulation, which is extremely important due to recurrent allergenic conditions in people consuming artificial colorings [5,20–22]. Recent studies with unexplored native fruits show their bioactive composition as potential substitutes for artificial food colorants [23–25].

The guabiroba (*Campomanesia xanthocarpa* O. Berg) is a native Brazilian fruit rich in bioactive compounds, especially phenolic compounds, vitamin C, and carotenoids. The latter characterize an epicarp with an evident yellow-orange color due to the high concentrations of β-carotene and cryptoxanthin [23]. This native fruit has bioactive compounds also related to a potential prebiotic activity in yogurts, according to our previous study by Prestes et al. [7], who studied the addition of different concentrations of guabiroba pulp in fermented milk products to observe their functional properties in in vitro gastrointestinal simulation and the protective effect in *Bifidobacterium* BB-12 cells through all the gastric steps.

Fruit juices can be extracted from the original matrix and added to fermented milk formulations [3]. With an extraction without high temperatures, the nutritional and sensory compounds of the fruit are preserved. Cold-pressing processes produce cold-pressed juices, which first crush and press the fruit to extract its juice slowly. This process guarantees an efficient extraction and preserves the nutritional quality of the juice [26–29]. The contents of functional compounds, natural pigments, and flavorings in fruit juices can be increased when subjected to a concentration process. However, traditional industrial processes, such as evaporation, require high temperatures for water to boil, which reduces the concentration of most native bioactive compounds [30].

An alternative is the emerging freeze concentration technology in which liquid foods are concentrated over a pre-freezing step followed by separating pure ice crystals [31,32]. Gravitational-assisted block freeze concentration has been proven to be an efficient and economical technique for concentrating juices from different fruits with high amounts of bioactive compounds [16,30,33]. In this process, the raw material is completely frozen, followed by partial gravitational thawing. Therefore, this process is based on the fractional crystallization of water into ice and the sequential separation of the concentrated liquid by gravitational thawing. Thus, the ice block acts as a solid carcass through which the concentrated food passes. This process occurs until 50% of the initial frozen mass is thawed, producing a concentrated product. The concentrate obtained can be frozen and thawed again, always considering a thawing of 50% of the initial mass. Freezing the initial raw material and thawing results in a thawed and frozen fraction denoted as concentrate and ice, respectively. Both fractions result from the first step/stage of the gravitational-assisted block freeze concentration process. Then, the second step/stage of the gravitational-assisted block freeze concentration process consists in freezing the concentrated liquid obtained

from the first step and, successively, carrying out gravitational thawing (50% of the initial mass) a second time. The objective of each step/stage is to obtain an ice fraction composed of pure water and, after its removal, a concentrated liquid. However, the efficiency of this process depends on the initial composition of the raw material and must be studied in a specific way for each type of food. The main advantage of the block freezing concentration process would be removing water from food using low temperatures [30].

In order to encourage future industrial processes with native Brazilian fruit, this study first aimed to obtain cold-pressed guabiroba juice that was concentrated by the gravitational block freeze concentration process. In the sequence, the concentration of the freeze concentration stage, considered the most efficient, was chosen to be added to the yogurt formulation.

2. Materials and Methods

2.1. Chemicals

Standards of gallic acid (3,4,5-trihydroxybenzoic acid) (purity $\geq$ 90%), ABTS (2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), and Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) were obtained from Sigma-Aldrich (St. Louis, MO, USA). For elemental analysis, standard solutions (1000 mg.L^{-1}) of each element (K, Na, mg, Ca, Fe, Mn, Zn, Se, P, Ni, Cu, Ba, As, and Pb) were obtained from Spex Certiprep Chemical (Metuchen, NJ, USA); argon gas was obtained from Linde (Blumenau, Santa Catarina, Brazil). All analytical grades were obtained from Vertec (Rio de Janeiro, Rio de Janeiro, Brazil).

2.2. Cold-Pressing of Guabiroba Pulp

Guabiroba pulp was supplied by Embrapa Florestas (Colombo, PR, Brazil) (with 84.3% moisture, 0.18% protein, 7.75% carbohydrates, 0.88% fat, 6.26% dietary fiber, and 0.63% ash). A cold-pressed juice was slowly obtained with a hydraulic press (1 ton) (TE-098, TECNAL, Piracicaba, São Paulo, Brazil). The guabiroba pulp at room temperature (25 $\pm$ 2 °C), with a thick and viscous characteristic, was wrapped in fine-porosity cloths to filter all the juice with the application of pressure by the hydraulic press. The process was carried out three times for each batch of pulp to drain the juice completely. The cold-pressed guabiroba juice was stored at a temperature of 4 $\pm$ 2 °C until the next step.

2.3. Block Freeze Concentration Process

Gravitational-assisted block freeze concentration was performed according to Canella et al. [34]. Firstly, the cold-pressed guabiroba juice was divided into plastic containers of 200 mL and then frozen at -20 ± 2 °C. After the guabiroba juice was completely frozen, 50% of the initial volume was defrosted at room temperature (20 $\pm$ 2 °C), obtaining two fractions, the concentrate guabiroba juice (C1) and the ice fraction (I1) (Figure 1A).

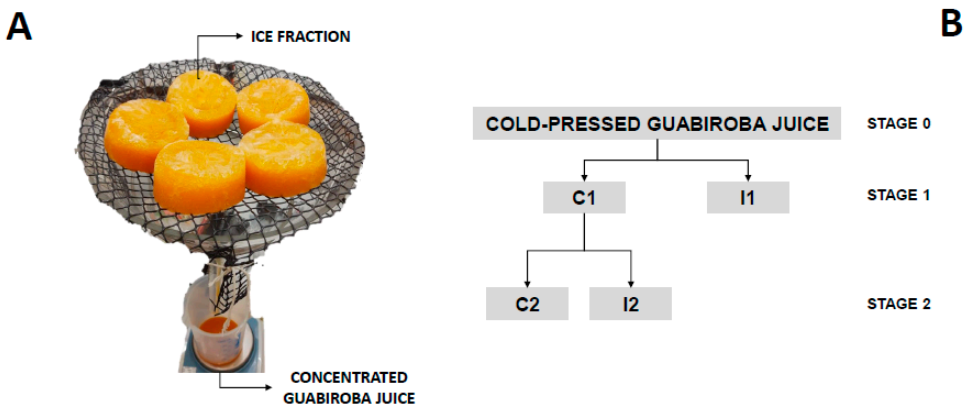


Figure 1. (**A**) Block freeze concentration of cold-pressed guabiroba juice; (**B**) diagram of guabiroba juice freeze concentration. Note: C1 and C2 refer to concentrates of stages 1 and 2, respectively; I1 and I2 refer to ice fractions of stages 1 and 2, respectively.

The defrosted liquid (C1) was used as a feed solution for the second stage, obtaining a second concentrate juice (C2) and ice (I2) (Figure 1B). A portion of each stage (C1 and C2) and ice fraction (I1 and I2) was collected and stored at −20 ± 2 °C until physicochemical, phenolic, and elemental analysis.

2.4. Freeze Concentration Parameters

The freeze concentration parameters were evaluated by the concentration factor (CF) according to the calculus of the methodology proposed by Aider and Ounis [35] using Equation (1):

$$Concentration\ Factor\ (CF) = \frac{TS_n}{TS_0} \quad (1)$$

TS_n is the total soluble solids content (g.100 mL^{-1}) of the concentrated guabiroba juice, and TS_0 is the total soluble solids content (g.100 mL^{-1}) of the initial guabiroba juice.

The process efficiency (PE) was calculated based on the increase in the total soluble solids (TS) in the concentrated juice (g.100 mL^{-1}) relative to the TS remaining in the ice (g.100 mL^{-1}) from each freeze concentration stage, according to Equation (2):

$$Process\ Efficiency(\%) = \frac{TS_C - TS_I}{TS_C} \cdot 100 \quad (2)$$

TSc is the total soluble solids content (g.100 mL^{-1}) in the concentrate, and TS_I is the total soluble solids content (g.100 mL^{-1}) in the ice at the end of each freeze concentration stage.

The experimental mass balance of each stage was calculated to validate the experimental results and compared to the theoretical value [36,37] using Equation (3):

$$Wpred = \frac{C_i - C_c}{C_g - C_c} \quad (3)$$

where W_{pred} is the predicted ice mass ratio (kg ice/kg concentrated guabiroba juice), Ci is the total soluble solids content of the initial guabiroba juice (g.100 mL^{-1}), Cc is the total soluble solids content of the concentrate fraction (g.100 mL^{-1}), and C_g is the total soluble solids content of the ice fraction (g.100 mL^{-1}). The root mean square (RMS) deviation was calculated using Equation (4) to determine the deviation between experimental and theoretical results.

$$RMS\ (\%) = 100\sqrt{\frac{\sum(Wexp - Wpred\ /\ Wexp)2}{N}} \quad (4)$$

W_{exp} and W_{pred} are the experimental and predicted ice mass ratios, respectively, and *N* is the number of test repetitions.

2.5. Yogurt Production

For yogurt production, commercial UHT (ultra-high-temperature) skimmed milk (4.5% carbohydrate, 3.75% protein, 0% lipid, and 8.05% total solids content) was employed in Florianópolis, Santa Catarina State, Brazil, with an incorporation of thermophilic started culture (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, Yoflex®, Chr. Hansen, Hønsholm, Denmark) according to the manufacturer's instructions. The incubation for the fermentation step was performed at 42 ± 2 °C until reaching pH 4.6 and cooled at 4 ± 2 °C for 24 h. The yogurt was stirred and divided into three samples (control, I10, and I15). The control sample was prepared with no addition of concentrated guabiroba juice, and I10 and I115 were prepared with 10% and 15% concentrate (m/m) (due to the proportion starting from 10% having a greater emphasis on the supply of bioactive compounds according to our previous study [7]), respectively, of the most efficient stage of freeze concentration. The samples were stored at −20 ± 2 °C until further analysis.

2.6. Physicochemical Analysis

For all the samples (C1, C2, I1, I2, and the yogurt formulations: control, I10, and I15), the total solids content (g.100 g^1) was obtained by the oven-drying method until constant weight at 105 ± 2 °C (315 SE-Fanem, Campinas, São Paulo, Brazil) [38]. Crude protein was

determined by the Kjeldahl method [38], fat content by the Soxhlet method [38], and fixed mineral residue (ash) by subjecting the samples to 550 °C (J 200—Jung, Brazil) [38]. The titratable acidity was also determined, according to the Association of Official Analytical Chemist (AOAC) [38].

The pH analysis was performed with a digital pH meter DM 20 (Digimed, São Paulo, Brazil), and total soluble solids (°Brix) were measured at 20 °C (±1 °C) on a Tropen model I refractometer. Color intensity was measured with a U-1800 UV–Vis spectrophotometer (Hitachi, Kyoto, Japan) at 420 nm, and the total color difference (ΔE*) between the samples was performed according to Equation (5) [39]:

$$\Delta E^* = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2} \quad (5)$$

where ΔL* is the luminosity difference, Δa* represents the intensity of the red color, and Δb* is the intensity of the yellow color.

A glass pycnometer was used for density analysis (g/cm^3) and calculated according to Equation (6):

$$\rho s = \frac{(m_3 - m_1)}{(m_2 - m_1).pH_20} \quad (6)$$

where ρs is the density of the solutions, m_1 is the mass of the empty pycnometer (g), m_2 is the mass of the pycnometer with water (g), m_3 is the mass of the pycnometer with solutions (g), and pH_2O is the density of water.

The concentration of vitamin C was determined by the Tillmans method [38], which consists of reducing 2,6-dichlorophenolindophenol with ascorbic acid, and the results were expressed as mg ascorbic acid.100 mL^{-1}.

2.7. Phenolic and Antioxidant Analysis

The samples (C1, C2, I1, I2, and control, I15, and I10 yogurt formulations) were analyzed for total phenolic content (TPC) according to the Folin–Ciocalteu method [40] at 720 nm in a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). The results were expressed in milligrams of gallic acid equivalent per liter of the sample (mgGAE.mL^{-1}) (calibration curve linearity range: R^2 = 0.99).

For antioxidant activity, the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed by Brand-Williams et al. [41]. The analysis read was performed in a spectrophotometer at 515 nm, and the results were expressed in micromoles of Trolox equivalent per liter of the sample (μmolTE.L^{-1}). For the ABTS [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate)] assay, according to Re et al. [42], the analysis read was at 734 nm in a spectrophotometer, and the results were expressed in micromoles of Trolox equivalent per liter of sample (μmolTE.L^{-1}). All these analyses were performed in triplicate.

2.8. Carotenoid Content

The carotenoid content was determined according to the methodology proposed by Rodriguez-Amaya [43] with modifications. In a spectrophotometer, total alpha-carotene, beta-carotene, gamma-carotene, and cryptoxanthin contents were obtained at the following wavelengths: 450, 444, 452, and 462 nm, respectively. The results were expressed in micrograms of carotenoids per 100 g of sample (μg.100 mL^{-1}).

2.9. Elemental Profile by Optical Emission Spectrometer (ICP OES)

The multi-mineral composition of concentrate guabiroba juice, ice fractions, and yogurt formulations was determined using an inductively coupled plasma optical emission spectrometer (ICP OES). The spectrometer (iCAP 6300 DUO, Waltham, MA, USA) had a concentric nebulizer, a cyclone spray chamber, and an automatic sampler (CETAC ASX-520 Thermo Scientific, Waltham, MA, USA). The samples were digested with nitric acid and hydrogen peroxide in a microwave oven (DGT-100 Plus Provecto microwave oven, Provecto Analítica, Jundiaí, São Paulo, Brazil). Argon was used as the main, auxiliary, and

nebulizer gas. The monitored elements were K, Na, mg, Ca, Fe, Mn, Zn, Se, P, Ni, Cu, Ba, As, and Pb. The operating parameters were 1300 W radio frequency power, an auxiliary gas flow of 1.0 L.min^{-1}, and a nebulizer gas flow rate of 0.38 L.min^{-1}. All determinations were performed in triplicate, and recovery tests at three levels were performed to check the method's accuracy.

2.10. Statistical Analysis

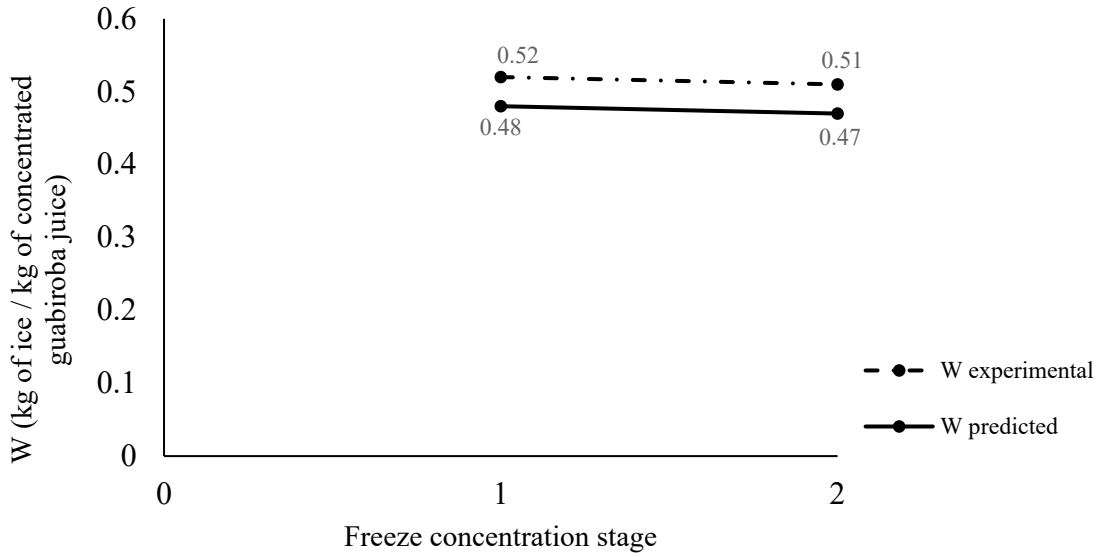
The results were expressed as means ± standard deviation. The data analysis was performed using STATISTICA 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA) (https://edelivery.tibco.com/storefront/index.ep). One-way analysis of variance (ANOVA) was implemented to determine the significant differences ($p < 0.05$). All samples were produced in triplicates, and three parallel measurements were made for each replication.

3. Results and Discussion

3.1. Mass Balance and the Performance of the Block Freeze Concentration

The mass balance of the freeze concentration process is shown in Figure 2A. Therefore, when comparing the mass balance of theoretical data (W predicted) in each freeze concentration stage with experimental data (W experimental), it is possible to observe that good agreement was reached. With the RMS, it is possible to determine the deviation between theoretical and experimental results with a good adjustment observed in the first stage (8.10%) and the second (7.92%). These concentration stages obtained deviations below 25%, which becomes an acceptable adjustment according to Lewicki [44]. The RSM results are also in agreement and close to the values obtained in studies by Canella et al. [45] (6.9%), Hernández et al. [46] (7.3%), and Petzold et al. [47] (9.5%).

These calculated parameters were based on total soluble solids content, which ranged from 15.36 to 33.0 g.100 mL^{-1} for the guabiroba juice and the concentrates, statistically differing in each stage of concentration (Figure 2B) ($p < 0.05$). With the increase in freeze concentration stages, there was a progressive increase in concentration factor (1.56 to 2.14), directly related to the total soluble solids in the concentrated fraction [45]. Similar results were obtained in studies with the freeze concentration of orange juice by Haas et al. [33], with concentration factors ranging from 1.61 to 3.53.



(**A**)

Figure 2. *Cont.*

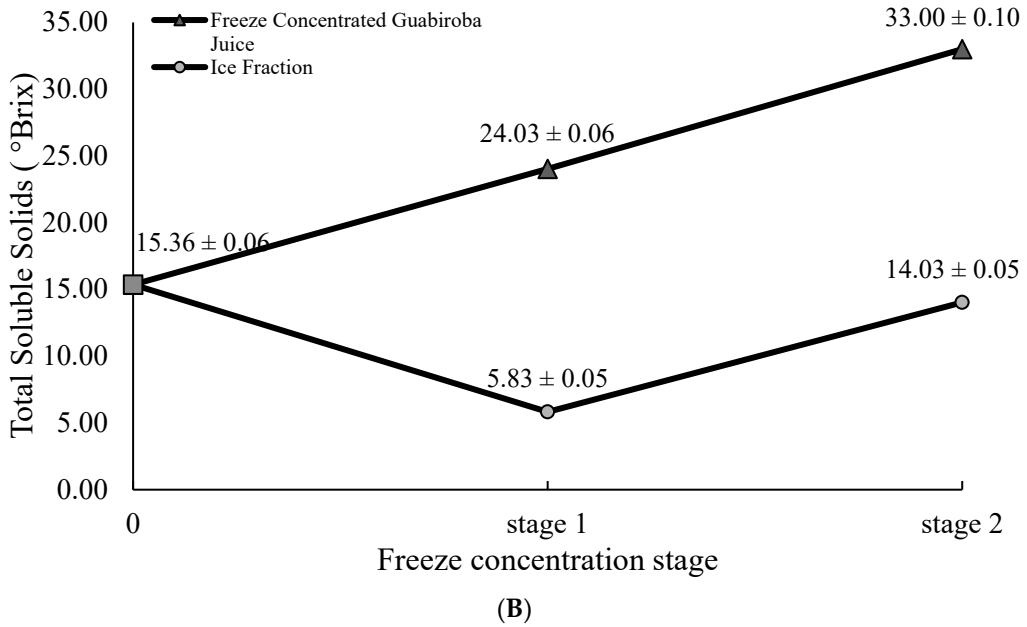


Figure 2. (**A**) Experimental and predicted ice mass ratios as a function of freeze concentration stages of guabiroba juice. (**B**) Changes in soluble solids of concentrated guabiroba juice and ice fractions obtained by two-stage block freeze concentration.

With the decrease in the availability of free water and the advancement of the freeze concentration stages, there is a greater retention of solid content in the concentrated juices of the original juice, increasing the concentration factor [47]. On the other hand, process efficiency decreases throughout the freeze concentration stages due to the increase in total solids not only in the concentrated juice but also in its ice fraction as the stages of the process pass (5.83 g.100 mL^{-1} for I1 and 14.03 g.100 mL^{-1} for I2) [33]. According to Raventós et al. [48] and Sánchez et al. [49], the viscosity of the concentrate solution increases as the total solids content increases (15.61 to 24.46), and these particles tend to be retained at the ice–liquid interface, decreasing the speed of the diffusion process and also the process efficiency.

In the freeze concentration process of fruit juices, the total soluble solids content (°Brix) is a good indicator of the process efficiency and the amount of solids that is retained both in the concentrated juice and the ice fractions, being an important parameter of the number of stages of this process to be carried out. In our preliminary studies with whey and milk, process efficiency is high up to the second or third stage of block freeze concentration [34,50–52]. In this study, the highest process efficiency of the freeze concentration of cold-pressed guabiroba juice was obtained in stage 1 (75.73%), similar to the same stage obtained for freeze-concentrated orange juice (81.80%) [33]. In addition, It was observed a significant difference ($p < 0.05$) between the total solids retained in the ice fractions (4.28 g.100 mL^{-1} for I1 and 9.72 g.100 mL^{-1} for I2), which emphasizes the decrease in the process efficiency for stage 2. Therefore, C1 was chosen to be added to the formulation of yogurts at different concentrations (0, 10, and 15%).

Table 1 shows the performance of the freeze concentration process with values of concentration factor and process efficiency.

Table 1. Process efficiency and concentration factor of block freeze concentration of cold-pressed guabiroba juice.

Stages	Samples	Total Soluble Solids (g.100 mL^{-1})	CF	PE (%)	RSM (%)
Stage 0	Cold-pressed guabiroba juice	15.36 ± 0.06 ^{cA}	-	-	-
Stage 1	C1	24.03 ± 0.06 ^{b}	1.56 ± 0.01	75.73 ± 0.18	8.10
	I1	5.83 ± 0.05 ^{C}	-	-	
Stage 2	C2	33.00 ± 0.10 ^{a}	2.14 ± 0.01	57.47 ± 0.01	7.92
	I2	14.03 ± 0.05 ^{B}	-	-	

Note: Results are expressed as the mean ± standard deviation among two batches performed in triplicate for each freeze concentration stage, with three repetitions for total soluble solids, CF, and PE. ^{a,b,c} Within a column, different superscript lowercase letters denote significant differences ($p < 0.05$) between the guabiroba juice and C1 and C2. ^{A,B,C} Within a column, different superscript uppercase letters denote significant differences ($p < 0.05$) between the guabiroba juice and the I1 and I2 fractions.

3.2. Physicochemical Properties and Mineral Profile of Concentrated Guabiroba Juice

Table 2 shows the physicochemical parameters for cold-pressed guabiroba juice, the freeze concentrates, and the ice fractions.

Table 2. Physicochemical parameters for cold-pressed guabiroba juice and its freeze concentrates.

Analysis	Cold-Pressed Juice (J)	C1	I1	C2	I2
Moisture (g.100 mL^{-1})	84.39 ± 7.26 ^{Ca}	81.93 ± 0.87 ^{b}	95.62 ± 0.04 ^{A}	75.54 ± 0.36 ^{c}	90.28 ± 0.04 ^{B}
Total solids (g.100 mL^{-1})	15.61 ± 7.26 ^{Ab}	18.07 ± 0.87 ^{c}	4.38 ± 0.12 ^{C}	24.46 ± 0.37 ^{a}	9.72 ± 0.82 ^{B}
Protein (g.100 mL^{-1})	0.40 ± 0.01 ^{Ac}	0.43 ± 0.01 ^{b}	0.20 ± 0.01 ^{C}	0.61 ± 0.02 ^{a}	0.36 ± 0.02 ^{B}
Lipid (g.100 mL^{-1})	<0.01	<0.01	<0.01	<0.01	<0.01
Ash (g.100 mL^{-1})	1.50 ± 0.35 ^{Ac}	3.41 ± 2.55 ^{b}	0.01 ± 0.01 ^{C}	4.94 ± 0.95 ^{a}	0.29 ± 0.01 ^{B}
Titratable acidity (g.100 mL^{-1})	0.96 ± 0.04 ^{Ac}	1.28 ± 0.28 ^{b}	0.76 ± 0.63 ^{C}	2.10 ± 0.05 ^{a}	0.88 ± 0.01 ^{B}
pH	3.87 ± 0.03 ^{Ba}	3.75 ± 0.01 ^{b}	3.87 ± 0.02 ^{B}	3.72 ± 0.03 ^{c}	3.90 ± 0.02 ^{A}
Density (g.cm^{3})	1.07 ± 0.01 ^{Ab}	1.09 ± 0.05 ^{b}	1.01 ± 0.01 ^{B}	1.13 ± 0.10 ^{a}	1.02 ± 0.01 ^{B}
L*	33.33 ± 0.18 ^{a}	33.06 ± 0.13 ^{a}	33.00 ± 0.16	32.69 ± 0.17 ^{b}	33.41 ± 0.33
a*	3.82 ± 0.07 ^{Ab}	3.90 ± 0.02 ^{b}	3.44 ± 0.03 ^{B}	4.62 ± 0.03 ^{a}	3.46 ± 0.10 ^{B}
b*	16.69 ± 0.18 ^{A}	16.62 ± 0.15	16.13 ± 0.03 ^{B}	16.90 ± 0.03	15.64 ± 0.15 ^{C}
ΔE (JxC1)			0.29		
ΔE (JxC2)			1.04		
ΔE (C1xC2)			0.86		
ΔE (JxI1)			0.73		
ΔE (JxI2)			1.11		
ΔE (C1xI1)			0.73		
ΔE (C2xI2)			1.85		
ΔE (JxC1)			0.29		

Note: Results expressed as the mean ± standard deviation. ^{a,b,c} Within a column, different superscript lowercase letters denote significant differences ($p < 0.05$) between the guabiroba juice and C1 and C2. ^{A,B,C} Within a column, different superscript uppercase letters denote significant differences ($p < 0.05$) between the guabiroba juice and the I1 and I2 fractions. ΔE = total color difference between two different samples.

For moisture results (Table 2), the freeze-concentrated juices had significantly lower levels (75.54 to 81.9 g.100 mL^{-1}) compared to the original juice (84.39 g.100 mL^{-1}) ($p < 0.05$). These data were expected since, at each stage of the block freeze concentration, about 40 to 50% of the free water content is directed to the ice fraction [53]. From this, there is a significant increase ($p < 0.05$) in the total solids content (18.07 g.100 mL^{-1} for C1 and 24.46 g.100 mL^{-1} for C2) and ash (3.41 g.100 mL^{-1} for C1 and 4.49 g.100 mL^{-1} for C2) as the freeze concentration stages advance.

The protein content within the measured total solids also increased with the evolution of the block freeze concentration stages (0.43 g.100 mL^{-1} for C1 and 0.61 g.100 mL^{-1} for C2). The protein content of the whole guabiroba fruit is around 0.4 to 5.5 g.100 g^{-1} [26,54] and, considering that the cold-pressed juice does not contain other parts of the fruit such as pulp, peel, and seeds, the protein content is lower. Based on this principle, in the cold-pressing process, the juice is extracted from the guabiroba pulp without peel and seeds, which contain the lipid content of the fruit (1.5 to 1.9 g.100 g^{-1}) [26,55]. Due to the absence of extracting the lipid fraction of the peels and seeds for the juice, the total lipid content was below the quantification limit of the method (<0.01 g.100 mL^{-1}; Table 2).

Freeze concentration caused significant effects ($p < 0.05$) both for pH and total titratable acidity in concentrated juices and ice fractions (Table 2). The increase in acidity is related to the increase in the concentration of organic acids as the stages of this process advance [30]. Due to the concentration of solids content, the density of C2 increased ($p < 0.05$) in the cold-pressed juice. This behavior emphasizes the efficiency of solids retention by the process described in Table 1.

For color parameters, C2 differed significantly concerning C1 and cold-pressed juice. For the a* coordinate, the progress of the process intensifies the orange color of the concentrated juices (C2 = 4.62; C1 = 3.90; juice = 3.82) (Figure 3C; Table 2) but decreases the luminosity, characterized by L* (C2 = 32.69; C1 = 33.06; juice = 33.33; Table 2). The b* coordinate did not differ statistically ($p < 0.05$) from C1 and cold-pressed juice, indicating a yellow hue for all samples. The yellow/orange color of the cold-pressed guabiroba juice and the concentrated juice is related to the high concentration of carotenoids in the fruit [23,26] (Table 3). Since part of the solid content is also retained in the ice fraction, a yellow-orange color is also observed for I1 and I2 (I1: a* = 3.44; b* = 16.13; I2: a* = 3.46; b* = 15.64; Figure 3B). The intensity of the color is so noticeable that for the total color difference, expressed by ΔE*, in all comparison pairs between samples, the values were less than 3.0. Martínez-Cervera et al. [56] reported that when ΔE* is lower than 3.0, it cannot be visually perceived by the human eye, which is notable in Figure 3A–C.

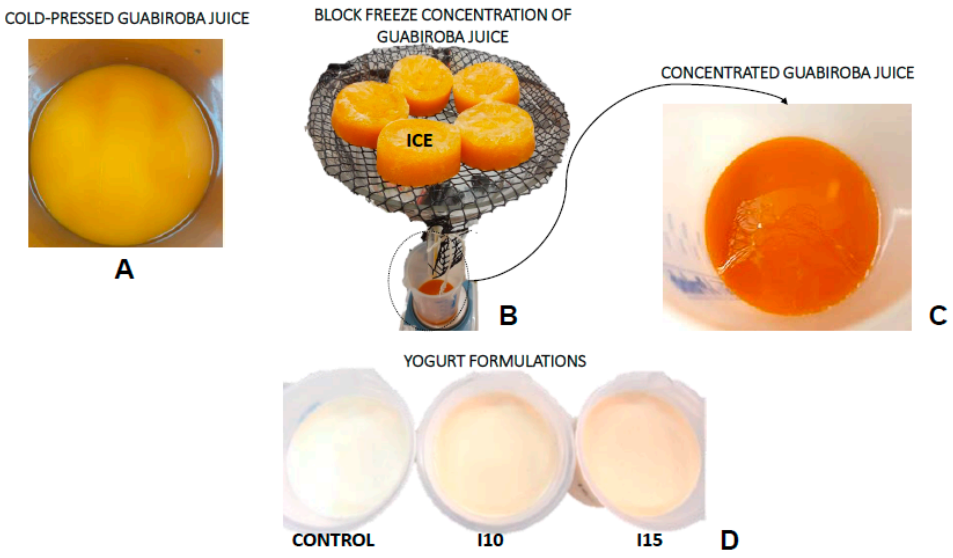


Figure 3. Cold-pressed guabiroba juice (**A**), ice fraction from freeze concentration process (**B**), concentrated guabiroba juice (**C**), and yogurt formulations (**D**).

Table 3. Phenolic and carotenoid content, antioxidant activity, and vitamin C of cold-pressed guabiroba juice, freeze-concentrated juices, and ice fractions.

Analysis	Cold-Pressed Juice	C1	I1	C2	I2
TPC ($mg.L^{-1}$)	3434.01 ± 0.26^{Ac}	5944.98 ± 0.23^{b}	2392.16 ± 0.11^{C}	9480.25 ± 2.37^{a}	2954.05 ± 0.79^{B}
α-carotene ($\mu g.100\ mL^{-1}$)	357.10 ± 0.80^{Ac}	651.96 ± 0.18^{b}	155.59 ± 3.21^{C}	2003.88 ± 2.91^{a}	212.61 ± 1.17^{B}
β-carotene ($\mu g.100\ mL^{-1}$)	385.76 ± 2.49^{Ac}	704.28 ± 7.94^{b}	168.80 ± 4.75^{C}	2164.68 ± 3.22^{a}	229.68 ± 6.34^{B}
γ-carotene ($\mu g.100\ mL^{-1}$)	322.54 ± 4.01^{Ac}	588.87 ± 2.01^{b}	140.53 ± 5.10^{B}	1809.96 ± 9.31^{a}	192.04 ± 2.08^{C}
Cryptoxanthin ($\mu g.100\ mL^{-1}$)	419.06 ± 7.19^{Ac}	765.08 ± 5.41^{b}	249.51 ± 2.23^{B}	2351.58 ± 6.34^{a}	182.59 ± 8.13^{C}
DPPH ($\mu molTE.L^{-1}$)	2304.00 ± 138.59^{Ac}	5240.00 ± 93.40^{b}	93.40 ± 40.24^{C}	$16,640.00 \pm 131.37^{a}$	1015.07 ± 40.46^{B}
ABTS ($\mu molTE.L^{-1}$)	$41,477.78 \pm 688.61^{Ac}$	$58,700.00 \pm 648.31^{b}$	1067.00 ± 470.14^{C}	$101,477.78 \pm 554.09^{a}$	2177.00 ± 330.00^{B}
Vit C ($mgAA.100\ mL^{-1}$)	466.66 ± 46.19^{Ac}	533.33 ± 23.09^{b}	293.33 ± 23.10^{C}	666.66 ± 20.3^{a}	373.33 ± 24.00^{B}

Note: Results expressed as the mean ± standard deviation. ^{a,b,c} Within a column, different superscript lowercase letters denote significant differences ($p < 0.05$) between the guabiroba juice and C1 and C2. ^{A,B,C} Within a column, different superscript uppercase letters denote significant differences ($p < 0.05$) between the guabiroba juice and the I1 and I2 fractions. TPC = total phenolic content; TE = Trolox equivalent; AA = ascorbic acid.

The levels of total phenolic content (TPC) ranged from 3434.01 to 9480.25 $mg.L^{-1}$ (Table 3), increasing as the block freeze concentration stages progressed, with an approximate increase of 276% in the last stage of freeze concentration (C2). Similar behaviors were observed in orange juice [33], apple juice [32], and blueberry juice [30]. As the freeze concentration steps progress, there is a decrease in the water available in the concentrates and, consequently, higher levels of total solids (Table 2). Among these are the concentrated bioactive compounds, which increase the levels compared to the original cold-pressed juice. However, the process efficiency tends to decrease as the freeze concentration steps advance (PE = 75.73% for stage 1 and 57.47% for stage 2), and there is a saturation in the solids content, with an increase in its viscosity. This phenomenon was studied by Sánchez et al. [36] by the decrease in the speed of the diffusion process, and, with this, there is an increase in retention at the ice/concentrate interface. This behavior explains the high total solids content in the ice fraction of stage 2 (I2) and the bioactive compounds of this same fraction (Table 3).

Guabiroba is one of the fruits of the *Myrtaceae* family, with one of the highest levels of phenolic compounds in its composition (9033.20 $mg.100\ g^{-1}$) [26,56–58]. The cold-pressed juice, even if it does not contain fractions of the peel, seeds, and pomace, where the phenolic compounds are also concentrated, contains high levels of phenolic compounds (3434.01 $mg.L^{-1}$) when compared to orange juice (715.70 $mg.L^{-1}$) and apple juice (740 to 873 $mg.L^{-1}$) [32,33]. In the same way, the content of individual carotenoids increased with the progress of the concentration stages, with an average increase of 561% for each carotenoid content for C2 (α, β, γ-carotene, and cryptoxanthin; Table 3). According to Schmidt et al. [59], *Campomanesia xanthocarpa* O.Berg is a potential source of carotenoids, with the β-carotene contents of the whole fruit (12.30 to 3400.00 $mg.100\ g^{-1}$) being related to a great potential of vitamin A when compared to other fruits (papaya = 0.04 $mg.100\ g^{-1}$; orange = 0.09 $mg.100\ g^{-1}$). The intensity of the orange color, on the other hand, is related to the significant levels of cryptoxanthin (419.06 $\mu g.100\ mL^{-1}$ for cold-pressed juice, 765.08 $\mu g.100\ mL^{-1}$ for C1, and 2351.58 $\mu g.100\ mL^{-1}$ for C2; Table 3), which

are also prominent when compared to other fruits (papaya = 0.50 mg.100 g^{-1} and apricot = 0.60 mg.100 g^{-1}) [26].

Fruits from the *Myrtaceae* family have been demonstrated to contain high levels of vitamin C. The whole guabiroba fruit has average levels of 17.80 to 233 mg.100 g^{-1} [26]. For the cold-pressed juice and concentrates, the contents ranged from 466.66 to 666.66 mg.100 mL^{-1} (Table 3). In orange juice's block freeze concentration process, Haas et al. [33] obtained levels in the second stage of 40 mg.100 mL^{-1}. On the other hand, the concentrated juice of the second stage of this work (C2) reached 666.66 mg.100 mL^{-1}, corresponding to more than 16 times the content of orange juice. This content offers potential benefits to human health due to its antioxidant capacity, which acts on the mechanism of scavenging free radicals related to aging processes and degenerative diseases [26,59]. Thus, high levels of vitamin C, total phenolics, and carotenoids present in both juices and concentrates are related to the great antioxidant activity of all samples. For the DPPH assay, the C2 content reached 16,640.00 µmolTE.L^{-1}, and for the ABTS method, 101,477.78 µmolTE.L^{-1} (Table 3), indicating that the antioxidant activity also increased with the progress of the freeze concentration. A similar behavior was also obtained in apple juices, according to Zielinski et al. [32], and in orange juices by Haas et al. [33]. Since it is a non-thermal process, the freeze concentration preserves bioactive compounds and increases antioxidant activity levels. In addition, the cold-pressing process prevents heat and oxidative damage to bioactive compounds by pressing the whole fruit at low/room temperature [29].

The mineral profile of cold-pressed juice, concentrates, and ice fractions is shown in Table 4.

Table 4. Elemental profile of cold-pressed guabiroba juice, freeze-concentrated juices, and ice fractions obtained using block freeze concentration.

Elements (µg.mL^{-1})	Cold-Pressed Guabiroba Juice	C1	I1	C2	I2
Ca	35.00 ± 2.00 ^{Ab}	18.00 ± 1.00 ^{c}	21.00 ± 1.00 ^{B}	52.00 ± 4.00 ^{a}	26.00 ± 2.00 ^{B}
K	1409.00 ± 26.00 ^{Ac}	1483.00 ± 21.00 ^{b}	573.00 ± 14.00 ^{C}	2609.00 ± 45.00 ^{a}	1028.00 ± 30.00 ^{B}
Mg	198.00 ± 3.00 ^{bA}	173.00 ± 3.00 ^{c}	58.00 ± 1.00 ^{C}	290.00 ± 3.00 ^{a}	118.00 ± 4.00 ^{B}
Na	16.00 ± 1.00 ^{Ac}	30.00 ± 1.00 ^{b}	5.00 ± 1.00 ^{C}	79.00 ± 2.00 ^{a}	14.00 ± 1.00 ^{B}
Fe	0.22 ± 0.02 ^{c}	0.80 ± 0.10 ^{b}	<LOQ	1.90 ± 0.10 ^{a}	<LOQ
Mn	2.20 ± 0.10 ^{Ab}	1.42 ± 0.10 ^{c}	0.74 ± 0.10 ^{C}	3.30 ± 0.10 ^{a}	1.96 ± 0.10 ^{B}
Se	0.28 ± 0.04 ^{b}	0.26 ± 0.08 ^{b}	<LOD	1.19 ± 0.03 ^{a}	<LOD
Zn	3.41 ± 0.10 ^{Ab}	2.21 ± 0.10 ^{c}	1.26 ± 0.10 ^{C}	5.30 ± 0.10 ^{a}	3.14 ± 0.10 ^{B}
P	143.00 ± 1.00 ^{Ab}	126.00 ± 3.00 ^{c}	40.00 ± 2.00 ^{C}	223.00 ± 5.00 ^{a}	86.00 ± 2.00 ^{B}
Ni	0.14 ± 0.06	<LOD	<LOD	0.11 ± 0.01	< LOD
Cu	0.84 ± 0.03 ^{Ab}	0.53 ± 0.02 ^{c}	0.34 ± 0.01 ^{C}	1.43 ± 0.05 ^{a}	0.73 ± 0.01 ^{B}
Ba	0.69 ± 0.02 ^{Ab}	0.55 ± 0.01 ^{c}	0.33 ± 0.01 ^{B}	0.97 ± 0.03 ^{a}	0.39 ± 0.01 ^{B}
As	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Pb	<LOQ	<LOQ	<LOD	<LOD	<LOQ

Note: Results expressed as the mean ± standard deviation. ^{a,b,c} Within a column, different superscript lowercase letters denote significant differences ($p < 0.05$) between the guabiroba juice and C1 and C2. ^{A,B,C} Within a column, different superscript uppercase letters denote significant differences ($p < 0.05$) between the guabiroba juice and the I1 and I2 fractions. LOD = limit of detection; LOQ = limit of quantification.

The levels of four macroelements (Ca, K, mg, and Na) and seven microelements (Fe, Se, Zn, Cu, Mn, P, and Ba) varied significantly ($p < 0.05$) between the juice and concentrates. With the advancement of the freeze concentration stages and with the high concentration of solids and removal of water, there was a greater concentration of

the main macroelements for C2 (K: 2609 μg.mL^{-1}; mg: 290 μg.mL^{-1}; Na: 79 μg.mL^{-1}; Ca: 52 μg.mL^{-1}; with increases of 185%, 146%, 493%, and 148%, respectively). These results are in agreement with the literature since the guabiroba fruit is a good source of potassium (208.40 mg.100 g^{-1}), which is naturally present in fruit in the form of potassium tartrate, and calcium (28.40 mg.100 g^{-1}) [23,55]. For micronutrients, which are also essential to maintain proper functioning of the body's biological functions, guabiroba is rich in iron (0.60 mg.100 g^{-1}), phosphorus (14.90 mg.100 g^{-1}), manganese (13.50 mg.100 g^{-1}), and zinc (0.40 mg.100 g^{-1}) [26,55]. In the present study, interesting values of these micronutrients were also obtained for stage 2 (P: 223 μg.mL^{-1}; Fe: 1.9 μg.mL^{-1}; Mn: 3.3 μg.mL^{-1}; Zn: 5.3 μg.mL^{-1}; with approximate increases of 155%). Arsenic and Pb were below the detection and quantification limits. These results are important because metallic elements are potentially toxic at a low level of exposure to the human body, affecting the respiratory and vascular systems, the kidneys, the heart, and the liver [60].

These results show that, as in any process, the block freeze concentration can generate total solid losses that tend to be retained in the ice fraction. Considerable contents of phenolic compounds, carotenoids, and some minerals such as Na, mg, Fe, and P make this by-product interesting to be applied in other food formulations as a result of the retention of functional and nutritional compounds, and also by the remarkable orange color, related to the concentration of carotenoids (Tables 3 and 4, Figure 3B). Therefore, ice fractions can be reused and destined for processing beverages, jams, or candies, adding a new functional and sensorial value.

3.3. Physicochemical, Bioactive Properties and Mineral Profile for Yogurt Formulations

The choice of yogurt as the raw material to be added to guabiroba pulp is because this is a widely consumed fermented dairy product known for its health benefits. Therefore, incorporating fruit-derived bioactive compounds into yogurt can be justified by their synergistic health benefits and improved nutritional profile, ensuring the product remains nutritionally balanced. The exclusive use of fermented milk as a sample with fruit bioactive compounds is justified by its ability to increase the bioactivity of the final product. Yogurt can be an excellent medium to deliver the health benefits of fruit-derived bioactive compounds to consumers.

The best process efficiency obtained in stage 1 was the determining factor for adding C1 in the yogurt formulation, containing 0%, 10%, and 15% (control, I10, and I15) of the concentrated juice, with the physicochemical results shown in Table 5.

With a gradual increase in the concentration of C1 between the samples, there was an increase in the content of total solids (10.99 to 11.98 g.100 mL^{-1}), total soluble solids (6.60 to 10.50 g.100 mL^{-1}), ash (0.74 to 0.79 g.100 mL^{-1}), and density (0.97 to 1.01 g.cm^3), with the highest levels found for yogurt with 15% concentrated guabiroba juice ($p < 0.05$).

Dairy products are sources of protein with average levels of 3.4 g.100 mL^{-1} for milk and 5 g.100 mL^{-1} for yogurt [61]. However, when concentrated guabiroba juice was added to the formulation, there was a dilution of protein contents in samples I10 (3.51 g.100 mL^{-1}) and I15 (3.43 g.100 mL^{-1}), when compared with the control sample (3.75 g.100 mL^{-1}), statistically differing ($p < 0.05$, Table 5). Similar results were also obtained by Bianchini et al. [62] in yogurts with uvaia pulp (3.12 to 3.35 g.100 mL^{-1} of protein) from the *Myrtaceae* fruit family, with a control sample (4.02 g.100 mL^{-1} of protein). For the total lipid content, skimmed milk was used to manufacture yogurts, and, in the concentrated guabiroba juice, they were not detected by the method; thus, the results of the lipid content were not significant for all samples. The organic acids in the concentrated form in C1 increased the acidity between samples (0.9 to 1.04 g.100 mL^{-1}), with a slight decrease in pH value (4.31 to 4.19).

Color parameters are important factors in improving the quality of yogurts with added pulp or fruit juices due to influences on the sensory acceptance of the product. In addition, the predominant color of fruits can decrease or end by adding artificial pigments that are potential allergens in a wide range of people. The addition of C1 decreased the lightness (L*; 78.58 to 73.78) of the yogurts ($p < 0.05$) and increased the tendency to red (a*; −3.23 to −0.09)

and yellow (b*; 8.69 to 16.40) (Table 5). These parameters were expected due to the intense orange pigmentation of guabiroba juice (Figure 3). For the total color difference (ΔE), the comparison between the control sample with the yogurts with the addition of C1 showed a value higher than 3 (Table 5), and, according to Dantas et al. [63], this confirms that the three yogurts produced have color differences that the human eye can detect. However, for I10 and I15 samples, the value of ΔE was 2.33. Therefore, the two samples have no visible perception (Figure 3D).

Table 5. Physicochemical properties for yogurt formulations with freeze-concentrated guabiroba juice.

Analysis	Control (C)	I10	I15
Moisture (g.100 mL^{-1})	88.41 ± 0.32 ^{a}	88.09 ± 0.09 ^{b}	88.01 ± 0.03 ^{c}
Total solids (g.100 mL^{-1})	10.99 ± 0.32 ^{c}	11.59 ± 0.08 ^{b}	11.98 ± 0.18 ^{a}
Total soluble solids (g.100 mL^{-1})	6.60 ± 0.10 ^{c}	8.83 ± 0.04 ^{b}	10.50 ± 0.01 ^{a}
Protein (g.100 mL^{-1})	3.75 ± 0.39 ^{a}	3.51 ± 0.01 ^{b}	3.43 ± 0.16 ^{c}
Lipid (g.100 mL^{-1})	<0.01	<0.01	<0.01
Ash (g.100 mL^{-1})	0.74 ± 0.08 ^{c}	0.77 ± 0.03 ^{b}	0.79 ± 0.03 ^{a}
Titratable acidity (g.100 mL^{-1})	0.90 ± 0.04 ^{c}	0.99 ± 0.02 ^{b}	1.04 ± 0.02 ^{a}
pH	4.31 ± 0.01 ^{a}	4.23 ± 0.02 ^{b}	4.19 ± 0.01 ^{c}
Density (g.cm^{3})	0.97 ± 0.01 ^{b}	1.01 ± 0.03 ^{a}	1.01 ± 0.10 ^{a}
L*	78.58 ± 2.91 ^{a}	74.13 ± 0.01 ^{b}	73.78 ± 0.04 ^{b}
a*	−3.23 ± 0.12 ^{ac}	−0.59 ± 0.24 ^{b}	−0.09 ± 0.02 ^{a}
b*	8.69 ± 0.19 ^{c}	14.16 ± 1.75 ^{b}	16.40 ± 0.03 ^{a}
ΔE (CxI10)		7.52	
ΔE (CxI15)		9.65	
ΔE (I15xI10)		2.33	

Note: Results expressed as the mean ± standard deviation. ^{a,b,c} Within a column, different superscript lowercase letters denote significant differences ($p < 0.05$) between the samples. ΔE = total color difference between two different samples.

Regarding the bioactive compounds shown in Table 6, there was an increase with the addition of guabiroba juice concentrate, with the highest values obtained for the I15 sample ($p < 0.05$). TPC ranged from 208.96 mg.L^{-1} to 9506.58 mg.L^{-1}, with a representative increase of 45.5 times (approximately 4,556%) for I15, compared with the control sample. The presence of phenolic compounds in fermented milk products is related to several benefits to the human body. According to our previous study [7], with the addition of 5% and 10% guabiroba pulp in yogurts, it was found that the maximum addition of 10% stood out in terms of prebiotic action and increased levels of antioxidant compounds when compared to the minimum proportion of 5% in the dairy product. Therefore, in this study, the greater the addition of concentrated guabiroba juice to the yogurt sample, the greater the contribution to the levels of bioactive compounds and antioxidant activity.

These results also potentiate the functional activity of the guabiroba composition since studies with fermented milk products with the addition of fruits of the same scientific family did not obtain the same high concentration of total polyphenols [64,65]. Bianchini et al. [66] found the TPC content equal to 118.0 mg.100 g^{-1}, while Fidelis et al. [4] obtained a TPC content equal to 43.6 mg.100 g^{-1}, for yogurts with uvaia and camu-camu extracts, respectively. According to our previous studies, from the concentration of 10% of guabiroba pulp in probiotic yogurts, there was an increase in the concentration of total phenolic compounds and antioxidant activity after gastric simulation, which characterized

the fermented milk as a potential symbiotic product, attributing prebiotic characteristics to the bioactive compounds present in the guabiroba composition [7].

Table 6. Phenolic and carotenoid content, antioxidant activity, and vitamin C of yogurt formulations.

Analysis	Control	I10	I15
TPC ($mg.L^{-1}$)	208.96 ± 0.07 ^{c}	879.13 ± 0.18 ^{b}	9506.58 ± 1.30 ^{a}
α-carotene ($\mu g.100\ mL^{-1}$)	120.46 ± 27.94 ^{c}	270.57 ± 31.56 ^{b}	273.07 ± 18.41 ^{a}
β-carotene ($\mu g.100\ mL^{-1}$)	130.13 ± 16.21 ^{c}	292.28 ± 14.50 ^{b}	294.13 ± 28.14 ^{a}
γ-carotene ($\mu g.100\ mL^{-1}$)	108.80 ± 14.21 ^{c}	244.39 ± 10.98 ^{b}	246.64 ± 12.76 ^{a}
Cryptoxanthin ($\mu g.100\ mL^{-1}$)	141.36 ± 9.31 ^{c}	317.52 ± 15.77 ^{b}	320.45 ± 10.49 ^{a}
DPPH ($\mu molTE.L^{-1}$)	200.91 ± 40.69 ^{c}	685.40 ± 49.50	884.00 ± 60.93
ABTS ($\mu molTE.L^{-1}$)	885.33 ± 30.36 ^{c}	1750.33 ± 69.60 ^{b}	7558.89 ± 90.29 ^{a}
Vit C ($mgAA.100\ mL^{-1}$)	0.83 ± 0.58 ^{c}	17.16 ± 2.02 ^{b}	24.83 ± 0.58 ^{a}

Note: Results expressed as the mean ± standard deviation. ^{a,b,c} Within a column, different superscript lowercase letters denote significant differences between the samples ($p < 0.05$). TPC = total phenolic content; TE = Trolox equivalent; AA = ascorbic acid.

Individual carotenoids in yogurts increased significantly ($p < 0.05$) with the addition of C1, mainly for the sample I15, increasing ~226% for α, β, γ-carotene, and cryptoxanthin, when compared to the control sample (Table 6). In addition to the visible change in the color of yogurts, due to the high concentration of carotenoids in concentrated guabiroba juice (Figure 3D), the supplementation of these bioactivities is extremely beneficial to health. The ingestion of β-carotene, the precursor activity of vitamin A, and other specific carotenoids promotes antioxidant effects. It reduces the incidence of non-communicable degenerative diseases, such as cancer, cardiovascular, and ocular diseases [66].

The concentration of vitamin C with the addition of C1 was very positive for the composition of yogurts (with an increase of 2,991% for I15 compared to the control; Table 6); since dairy products are not sources of vitamin C, the incorporation of natural additives rich in this bioactive compound enriches the nutritional composition and functional properties of the product. According to the Brazilian Pediatric Society [67], the recommended daily vitamin C intake is 65 mg for ages 14 to 18. Therefore, consuming 100 g of yogurt with 15% concentrated juice provides approximately 38.2% of the daily intake for this vitamin. Antioxidant activities significantly differed with the addition of concentrated guabiroba juice, with an increase of 440% for DPPH and 854% for ABTS for I5 compared to the control sample (Table 6). The high contents of phenolic compounds, carotenoids, and vitamin C of the guabiroba composition are related to high antioxidant properties. Furthermore, during fermentation in fermented milk products, *L. bulgaricus* and *St. thermophilus* can release bioactive peptides through endogenous enzymatic pathways. These bioactive peptides have specific structures according to their composition, size, and amino acid sequence and also have antioxidant properties [68,69], which explains the presence of antioxidant activities for both DPPH (200.91 $\mu molTE.L^{-1}$) and ABTS (885.33 $\mu molTE.L^{-1}$) assays in the control yogurt.

Regarding the multielement profile of yogurts, four macroelements (Ca, K, mg, and Na) and six microelements (Mn, Se, Zn, P, Cu, and Ba) differed ($p < 0.05$) among the samples. Fe, Ni, As, and Pb values were below the detection limit and the method's quantification (Table 7).

Table 7. Elemental profile of yogurts developed with concentrated guabiroba juice from stage 2 of block freeze concentration.

Elements (μg.mL^{-1})	Control	I10	I15
Ca	776.00 ± 11.00 ^{c}	1089.00 ± 63.00 ^{b}	1094.00 ± 34.00 ^{a}
K	780.00 ± 12.00 ^{c}	1521.00 ± 95.00 ^{b}	1758.00 ± 63.00 ^{a}
Mg	74.00 ± 4.00 ^{c}	141.00 ± 9.00 ^{b}	160.00 ± 4.00 ^{a}
Na	260.00 ± 3.00 ^{c}	407.00 ± 12.00 ^{b}	430.00 ± 24.00 ^{a}
Fe	0.05 ± 0.03	<LOQ	<LOQ
Mn	0.03 ± 0.01 ^{c}	0.43 ± 0.10 ^{b}	0.62 ± 0.10 ^{a}
Se	0.30 ± 0.02 ^{c}	0.78 ± 0.08 ^{b}	0.93 ± 0.04 ^{a}
Zn	2.85 ± 0.20 ^{c}	4.93 ± 0.30 ^{b}	5.23 ± 0.10 ^{a}
P	520.00 ± 11.00 ^{c}	800.00 ± 50.00 ^{b}	814.00 ± 26.00 ^{a}
Ni	<LOD	<LOQ	<LOD
Cu	0.04 ± 0.01 ^{c}	0.21 ± 0.01 ^{b}	0.28 ± 0.01 ^{a}
Ba	0.07 ± 0.01 ^{b}	0.26 ± 0.01 ^{a}	0.26 ± 0.01 ^{a}
As	<LOQ	<LOQ	<LOQ
Pb	<LOQ	<LOQ	<LOQ

Note: Results expressed as the mean ± standard deviation. ^{a,b,c} Within a column, different superscript lowercase letters denote significant differences ($p < 0.05$) between samples. LOD = limit of detection; LOQ = limit of quantification.

Due to its higher solids content, yogurt with 15% concentrated guabiroba juice had the highest concentration of these elements. The supplementation of 15% guabiroba juice promotes the main minerals of its composition: sodium (430 μg.mL^{-1}), calcium (1094 μg.mL^{-1}), potassium (1758 μg.mL^{-1}), manganese (160 μg.mL^{-1}), and phosphorus (814 μg.mL^{-1}), and can be delivered to the customers by the addition in the yogurt. Dairy products are a source of calcium, which explains the high contents among the samples (776 to 1094 μg.mL^{-1}). For children and adolescents between 14 and 18 years, the ideal dose of calcium consumption is 1300 mg per day [66]. A portion of 100 g of yogurt I15 can provide about 8.41% of the required daily calcium intake. The ingestion of this mineral is important because it composes the structure of bones and teeth, blood plasma, regulates blood pressure, and helps the development of the muscles [70]. Potassium is also highly concentrated in guabiroba composition; therefore, the I15 can provide about 5% of the recommended daily intake in a portion of 100 g. This mineral is the main intracellular cation that helps the normal functioning of the cells [66,70]. The same portion of I15 yogurt can provide approximately 4% and 6.5% of the daily intake for manganese and phosphorus, respectively. The ingestion of these micronutrients is also essential for the human body. Manganese is present in the formation of bones and reactions involving amino acids, cholesterol, and carbohydrates. Phosphorus composes all cell membranes and is part of the structure of bones and teeth [71].

The results of the present study show that concentrated guabiroba juice improved the components naturally present in yogurt, obtaining a dairy product with higher functional and nutritional properties.

4. Conclusions

The freeze concentration of cold-pressed guabiroba juice provided satisfactory process efficiency for the first stage and can be used to formulate yogurts. The evident concentration of bioactive compounds and minerals in the freeze-concentrated juice provided yogurts with a high concentration of phenolic compounds, carotenoids, vitamin C, and antioxidant activity, emphasizing the yogurt produced with 15% concentrated juice from

the first stage. The non-thermal processes of obtaining the juice by cold-pressing and freeze concentration allowed for maintaining most of the bioactive compounds that are sensitive to high temperatures, with a high concentration in the composition of the dairy products. The innumerable benefits of the bioactive compounds present in guabiroba can encourage future applications of this pioneering study on an industrial scale since the consumer market craves products with healthy and functional appeal. Furthermore, the ice fractions, considered by-products of the freeze concentration process, can be reused in the formulation of several food products since there were significant retentions of bioactive compounds, minerals, and the evident orange color of the guabiroba juice, which makes reuse interesting from an economic point of view.

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