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The Functional Carbonated Beverage Properties of Guabiroba Juice Using the Ice Fraction from Gravitational Block Freeze Concentration

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Abstract: The freeze concentration of liquid foods generates a by-product that has few academic studies and no industrial application: the ice fraction of each concentration stage. Sugar-free carbonated beverages were produced from the addition of 20% residual ice fraction (stage 1—I120 and stage 2—I220) of the gravitational block freeze concentration process, and the result was compared with a control beverage produced with 20% guabiroba juice (J20). The physicochemical properties, carotenoid content, total phenolic content (TPC), vitamin C, and antioxidant activity were analyzed for all samples. There was no significant difference between J20 and I220 for the total solid content and total soluble solids. For the total phenolic compounds (TPC), the I220 content was 151.3% higher than that of the original juice J20 and, for antioxidant activity, 295.8% higher for ABTS and 130.2% higher for DPPH. The I220 beverage presented 159% more vitamin C content than the beverage containing juice (J20). The same behavior was observed for each carotenoid content, with 168% more for the I220 sample. The total color difference revealed no difference visible to the naked eye for the three formulated beverages ($\Delta E < 3.0$; $p < 0.05$). The promising results of the bioactive compounds from guabiroba juice retained in the ice fraction can add value to this process waste in the formulation of new products due to the remaining functional appeal of the original fruit matrix.

Keywords: *Myrtaceae* family; guabiroba juice; non-alcoholic beverage; process waste; carotenoids; phenolic compounds; antioxidant activity

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1. Introduction

The *Campomanesia xanthocarpa* O. Berg, popularly known as "guabiroba" or "gabiroba" is a fruit native to the Atlantic Forest and Cerrado savannah of South America, which covers a region running from the central west (Cerrado regions) and south of Brazil to south Argentina. There are around 15 varieties of guabiroba with botanical synonyms (*C. crenata*, *C. dusenii*, *C. littoralis*, *C. malifolia*, and *C. rhombea*) [1–3]. However, due to the small and regional production aimed at rural producers, there is no commercialization

of guabiroba on an industrial scale since knowledge about the characteristics of the fruit is not widespread. Due to its rich nutritional and functional composition, recent studies have aimed to explore the guabiroba fruits and apply them in various products to spread knowledge about this native fruit [4–9]. Table A1 (Appendix A) shows several products in studies with guabiroba, using different parts of the fruit, including the whole fruit, pulp, pomace, dehydrated residue, and juice.

Emerging non-thermal technologies must be applied to maintain the fruit juice's nutritional aspects because it usually maintains the high contents of bioactive compounds in guabiroba products in industrial processes. The cold-pressing process allows the juice from the whole fruit to be extracted through slow crushing and simultaneous pressure at room temperature [10,11]. For the concentration of liquid products rich in bioactive compounds, technologies that operate at low temperatures are also fundamental for preserving the nutritional value of the fruit.

The gravitational block freeze concentration process is an efficient and economical technology that can concentrate fruit juices through a previous freezing step followed by the separation of pure ice crystals [12,13]. This technique, which can be carried out in several stages, concentrates approximately 50% of the solid fraction contained in the juice. Depending on the process efficiency, there may be significant losses of the solid content in the ice fraction, which is considered a process waste and does not have, to date, technological applications for its reuse (Table A1 in Appendix A). The applicability of the residual ice fraction in the development of new products can contribute to circular economy, in which economic development is associated with better use of natural resources, optimization of manufacturing processes with less dependence on raw materials, prioritizing recyclable inputs and renewable sources, and becoming a key to the development of sustainability [14]. Previous studies with the freeze concentration of cold-pressed guabiroba juice [11] showed significant retention of bioactive compounds in the ice fraction, which can be reused in the formulation of other food products, such as beverages.

Carbonated beverages are among the non-alcoholic beverages most consumed by children and adults, because they are socially accepted. Typically, this drink is formulated to offer the consumer a pleasant fruity flavor, and reconstituted fruit juices from 2 to 20% of the volume of the product can be added. Carbohydrates are the traditional source of sweetness, mainly high-fructose corn syrup (HFCS) or sucrose [15,16]. However, diet and healthier versions are formulated without added sugars, replacing them with synthetic sweeteners (saccharin, aspartame, and sucralose, among others). In addition to the replacement of sugars by their substitutes of natural origin (stevia and polyols), there is a tendency to add fruits or functional plants as raw material in carbonated beverages, with emphasis on sources rich in bioactive compounds and antioxidants, which is the fastest-growing segment of the functional food market [17–19].

The present study aimed to elaborate on a functional carbonated beverage using the ice fraction of cold-pressed guabiroba juice resulting from the freeze concentration processes. From the beverages elaborated, the total solid content, crude protein content, fat content, fixed mineral reside, titratable acidity, color analysis, total phenolic content, antioxidant activity, and carotenoid content were determined.

2. Materials and Methods

2.1. Chemicals

Standards of gallic 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). Xylitol was obtained from Growth supplements (Tijucas, Santa Catarina, Brazil), citric acid from ACS Científica (São Paulo, São Paulo, Brazil), sodium benzoate from Vogler (Cabo de Santo Agostinho, Pernambuco, Brazil), potassium sorbate from ACS Científica (São Paulo, São Paulo, Brazil), and EDTA (ethylenediaminetetraacetic acid) from ACS Cientifica (São Paulo, São Paulo, Brazil).

2.2. Cold Pressing and Freeze Concentration Processes of Guabiroba Juice

In partnership with Embrapa Florestas (Colombo, Paraná, Brazil), guabiroba pulp was obtained and, with a hydraulic press (1 ton) (TE-098, TECNAL, Piracicaba, São Paulo, Brazil), the cold-pressed juice was obtained and frozen at −20 ± 2 °C until the next steps.

According to Canella et al. [20], the gravitational assisted block freeze concentration was performed. Figure 1 contains a schematic view of the process: the cold-pressed guabiroba juice (J) was fractionated in 200 mL plastic containers and then frozen at −20 ± 2 °C. After completely freezing the juice, 50% of the initial mass was defrosted at room temperature (20 ± 2 °C), obtaining two fractions at the first stage: the concentrated guabiroba juice (C1) and the ice fraction (I1). The defrosted liquid (C1) was used as a feed solution for the second stage, obtaining a second concentrated juice (C2) and ice (I2). For stage 1 of the freeze concentration process, the concentration factor (CF) and process efficiency (PE) were 1.6 and 76%, respectively. For stage 2, CF = 2.1 and PE = 58%, according to Aider and Ounis [21].

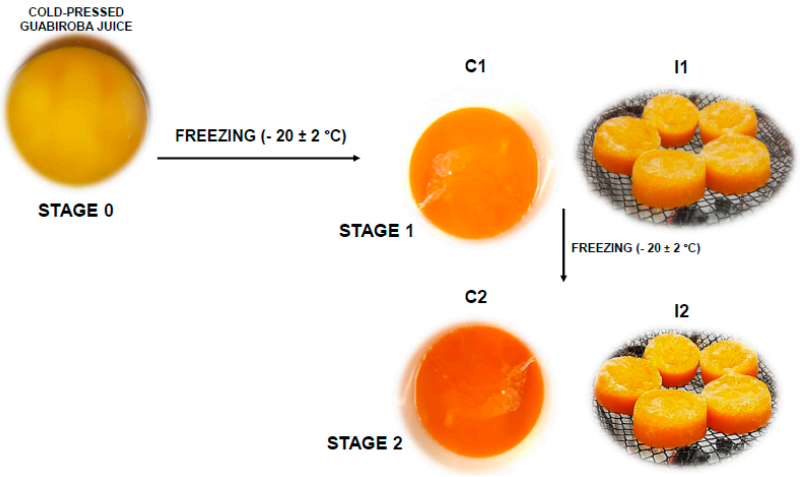

Figure 1. Visual scheme of block freeze concentration carried out with the cold-pressed guabiroba juice in two stages. Note: C1 and C2 refer to concentrates of stages 1 and 2, respectively; I1 and I2 refer to ice fractions of stages 1 and 2, respectively.

The concentrated guabiroba juice (C1 and C2) was used for other food applications previously (composition shown in Table 1 [11]. In contrast, the ice fraction, which is the residue of the process and is not yet applicable, was used in this work and stored for future analysis.

Table 1. Mean nutritional composition of guabiroba fruit and guabiroba fractions.

Guabiroba Fractions	Total Solids (g.100 g^{-1})	Protein (g.100 g^{-1})	Lipid (g.100 g^{-1})	Carbohydrate (g.100 g^{-1})	Ash (g.100 g^{-1})	Fiber (g.100 g^{-1})
Guabiroba fruit	19.6 ± 0.1 ^{c}	1.3 ± 0.1 ^{b}	1.3 ± 0.1 ^{c}	8.3 ± 0.1 ^{c}	0.6 ± 0.1 ^{d}	8.1 ± 0.1 ^{b}
Guabiroba pulp	15.7 ± 0.1 ^{e}	0.2 ± 0.1 ^{e}	0.9 ± 0.1 ^{d}	7.8 ± 0.1 ^{d}	0.6 ± 0.1 ^{d}	6.2 ± 0.1 ^{c}
Guabiroba pulp residue	3.9 ± 0.1 ^{f}	1.1 ± 0.1 ^{b}	0.4 ± 0.1 ^{d}	0.5 ± 0.1 ^{g}	<0.1	1.9 ± 0.1 ^{d}
Pulp residue dehydrated	27.8 ± 0.1 ^{a}	2.2 ± 0.1 ^{a}	5.8 ± 0.1 ^{b}	4.5 ± 0.1 ^{e}	0.7 ± 0.1 ^{e}	14.6 ± 0.1 ^{a}
Cold-pressed juice	15.6 ± 0.1 ^{e}	0.4 ± 0.1 ^{d}	10.0 ± 0.1 ^{a}	0.8 ± 0.1 ^{f}	2.5 ± 0.1 ^{c}	1.9 ± 0.1 ^{d}
* Concentrate 1	18.0 ± 0.1 ^{d}	0.4 ± 0.1 ^{d}	<0.1	14.1 ± 0.1 ^{b}	3.4 ± 0.1 ^{b}	<0.1
* Concentrate 2	24.5 ± 0.1 ^{b}	0.6 ± 0.1 ^{c}	<0.1	35.5 ± 0.1 ^{a}	4.9 ± 0.1 ^{a}	<0.1

Table 1. *Cont.*

Guabiroba Fractions	Total Solids (g.100 g^{-1})	Protein (g.100 g^{-1})	Lipid (g.100 g^{-1})	Carbohydrate (g.100 g^{-1})	Ash (g.100 g^{-1})	Fiber (g.100 g^{-1})
* Ice fraction 1	4.4 ± 0.1^{f}	0.2 ± 0.1^{e}	<0.1	4.0 ± 0.1^{e}	<0.1	<0.1
* Ice fraction 2	9.7 ± 0.1^{e}	0.4 ± 0.1^{d}	<0.1	8.9 ± 0.1^{c}	0.3 ± 0.1^{f}	<0.1

Note: Results are expressed as mean ± standard deviation. Superscript lowercase letters Within a column, different superscript lowercase letters denote significant differences ($p < 0.05$); * Concentrates 1 and 2 come from the first and second stages of the cold-pressed guabiroba juice freeze concentration process, respectively. Ice fractions 1 and 2 come from the first and second stages of the cold-pressed guabiroba juice freeze concentration process, respectively.

2.3. Carbonated Beverage Manufacturing

Firstly, concentrated syrup formulations were obtained separately, containing 20% of the original cold-pressed guabiroba juice (J), ice fraction from the first stage (I1), and the second stage (I2) from the freeze concentration process (Table 2). There was an addition of 7.5 g.CO_2.L^{-1} at 2.1 to 4.5 °C in the beverages with a carbonator (Omve, CF 121, Utrecht, The Netherlands). In total, 100 L of three different beverages (containing 40 kg of concentrated syrup) were produced, obtaining a carbonated beverage with the original cold-pressed guabiroba juice (J20) and two others containing the ice fraction from the first stage (I120) and from the second stage of the freeze concentration process (I220) (Figure 2).

Table 2. Formulation of 40 kg of concentrated syrup with cold-pressed guabiroba juice (J) (20%) and ice fractions (I1 and I2—20%) from the block freeze concentration process.

Formulation (kg)	
Water	11.61
Xylitol	8.25
Citric acid	0.10
Sodium benzoate	0.02
Potassium sorbate	0.01
EDTA	0.002
J, I1, or I2	20.00
Total (kg)	40.00

Figure 2. Carbonated beverages with 20% of cold-pressed guabiroba juice (J20), ice fraction from the first stage of freeze concentration (I120), and ice fraction from the second stage of freeze concentration (I220).

2.4. Physicochemical Analysis

For the guabiroba pulp, the physicochemical analyses were carried out according to the Association of Official Analytical Chemists (AOAC) [22] (84.3% moisture, 0.18% protein, 7.75% carbohydrates, 0.88% fat, 6.26% dietary fiber, 0.63% ash).

For all the beverages (I120 and I220), the total solid content (g.100 g^{-1}) was obtained by the oven drying method until constant weight at 105 ± 2 °C [22]. Crude protein was determined by the Kjeldahl method [22], fat content by the Soxhlet method, and fixed mineral reside (ash) by subjecting the samples to 550 °C [22]. The titratable acidity was also determined, according to the Association of Official Analytical Chemists [22].

Color analysis was determined with a spectrophotometer at 420 nm on a U-1800 UV–Vis—Hitachi (Kyoto, Japan), previously calibrated with distilled water at room temperature (25 ± 2 °C), and the total color difference (ΔE*) between the samples was determined according to Equation (1) [23]:

$$\Delta \mathbf{E}^* = \sqrt{(\Delta \mathbf{L}^*)^2 + (\Delta \mathbf{a}^*)^2 + (\Delta \mathbf{b}^*)^2} \quad (1)$$

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

For each sample, the concentration of vitamin C was determined according to the Association of Official Analytical Chemists [22] through the reduction of 2,6-dichlorophenolindo phenol by ascorbic acid. The results were expressed as mg ascorbic acid.100 mL^{-1}.

2.5. Total Phenolic Content and Antioxidant Activity

Total phenolic content (TPC) was measured for all the carbonated beverages according to the Folin–Ciocalteu method [24], with a calibration curve generated from a standard gallic acid solution (1–9 mg.L^{-1}), showing a linearity of R^2 = 0.9927 (y = 0.559x + 0.0967). The sample extract (0.1 to 1 mL) was combined in tubes with 1.25 mL of Folin–Ciocalteu reagent and 5 mL of a 15% sodium carbonate solution. The absorbance was measured at 720 nm using a spectrophotometer, and the results were reported in milligrams of gallic acid equivalents per liter of sample (mg.GAE.mL^{-1}).

For antioxidant activity analysis, the DPPH method followed the procedure described by Brand-Williams et al. [25]. A standard Trolox solution (3000 µmol.L^{-1}) created the calibration curve, showing a linearity of R^2 = 0.9901 (y = −0.003x + 0.6788). A 100 µL sample was pipetted into tubes, followed by adding the DPPH solution (0.00336 g in 100 mL). The reaction occurred for 30 min in the dark and at room temperature. The absorbance was measured using a spectrophotometer (UV-1800, Shimadzu, Barueri, Brazil) at 515 nm. The results were reported in micromoles of Trolox equivalents per liter of sample (µmol TE.L^{-1}).

For the ABTS [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate)] assay, as outlined by Re et al. [26], a standard Trolox solution (3000 µmol.L^{-1}) was utilized to generate the calibration curve, demonstrating a linearity of R^2 = 0.9907 (y = −0.002x + 0.6). A 30 µL sample was added to tubes, followed by 3 mL of the analysis solution (comprising 7 mmol.L^{-1} ABTS and 140 mmol.L^{-1} potassium persulfate). The reaction was allowed to proceed for 2 h in the dark at room temperature. Absorbance readings were taken at 734 nm using a spectrophotometer, and the results were expressed as micromoles of Trolox equivalents per liter of sample (µmol TE.L^{-1}). All these analyses were performed in triplicate.

2.6. Carotenoid Content

The carotenoid content was measured according to Rodriguez-Amaya [27] with modifications. For carotenoid extraction, 1 g of the sample was mixed with 20 mL of acetone in a 50 mL Falcon® tube. The mixture was vortexed (Biomixer®, Jacareí, São Paulo, Brazil) and placed in an ultrasound bath for 30 min. Afterward, the extract was filtered using filter paper and a funnel. In a burette, 4 mL of petroleum ether was added, followed by the

filtered extract and 3 mL of ultrapure water (type 2). The burette was left undisturbed for phase separation. If separation did not occur, a few drops of NaOH solution were added to facilitate the process. Once separated, the colorless lower phase was discarded, keeping the colored phase in the burette. The colored fraction was transferred to a volumetric flask through filter paper containing sodium sulfate to remove any aqueous residue. The burette was rinsed with petroleum ether to avoid extract loss. The carotenoid content was measured using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at 450 nm for β-carotene (molar absorptivity = 2592), 444 nm for α-carotene (molar absorptivity = 2800), 452 nm for β-cryptoxanthin (molar absorptivity = 2386), and 462 nm for λ-carotene (molar absorptivity = 3100). The results were expressed in micrograms of carotenoids per 100 milliliters of sample (μg.100 mL^{-1}).

2.7. Statistical Analysis

All the results were expressed as means ± standard deviation. One-way analysis of variance (ANOVA) was used to determine the significant differences ($p < 0.05$), followed by post hoc analysis with Tukey's test. All samples were produced in triplicates, and three parallel measurements were made for each replication. The data analysis was performed using STATISTICA 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results and Discussion

3.1. Effects of Freeze Concentration on Solid Retention of Carbonated Beverages

During the freeze concentration process, as the stages advance, there is a progressive increase in the concentration factor (1.60 for C1 and 2.10 for C2), directly related to the increase in the concentration of total solids in the concentrated fraction (Figure 1). This behavior for the two stages was also similar in studies with gravitational block freeze concentration for orange juice (1.61 and 3.53 for C1 and C2, respectively) [12], for blueberry juice (1.70 and 2.6, in unidirectional conditions) [28], and for *Morinda citrifolia* L. tea (1.51 and 2.95) [29]. Petzold et al. [30] described that the free water availability in the concentrated fraction decreases with the advancement of the freeze concentration stages, leading to greater solid retention of the original juice and an increase in the concentration factor. However, the process efficiency tends to decrease as the freeze concentration steps advance (PE = 76% for stage 1 and 58% for stage 2). Sánchez et al. [31] describe this phenomenon as the increase in viscosity in the concentrated fraction as the solid content also increases, which declines the particle diffusion speed, retaining them at the ice–liquid interface. For the freeze concentration of orange juice, Haas et al. [12] obtained similar behavior for process efficiency (81.80% for stage 1 and 76.47% for stage 2) as well as in studies with pineapple juice (59.0% and 54.9%) and blueberry juice (66.08% and 58.01%) [32].

In works that use the concentrated fraction, applying the concentrate from the first stage of freeze concentration is interesting due to its significant efficiency and solid retention [33]. However, as this study focuses on reusing the residual ice fraction in developing new products, the higher the solids retained in this waste the most suitable its applicability. Thus, the technological aptitude of this process allows the use of all its fractions.

According to the physicochemical results of the formulated beverages (Table 3), the soluble solid levels ranged from 10.58 to 12.82°Brix. In agreement with the standards established by Brazilian legislation [34], non-alcoholic carbonated beverages must have a minimum content of 10.5 °Brix. Similarly, the total acidity must have a minimum content of 0.1 g of citric acid.100 mL^{-1} and a pH between 2.7 and 3.5. According to Table 3, the acidity between the samples ranged from 0.35 to 0.51 g.100 mL^{-1} and the pH from 3.88 to 4.03, which follows established legislation. Casas-Forero et al. [13] described that the freeze concentration is a method that can cause significant effects both in total acidity and pH due to the increase in organic acids as the process stages advance.

Table 3. Physicochemical properties for carbonated beverages.

Analysis	J20	I120	I220
Moisture (g.100 mL^{-1})	89.81 ± 0.10 ^{b}	91.06 ± 0.10 ^{a}	88.55 ± 0.22 ^{c}
Total solids (g.100 mL^{-1})	11.45 ± 0.10 ^{a}	8.94 ± 0.10 ^{b}	10.98 ± 0.22 ^{a}
Total soluble solids (g.100 mL^{-1})	12.82 ± 0.30 ^{a}	10.18 ± 0.15 ^{b}	11.91 ± 0.29 ^{a}
Protein (g.100 mL^{-1})	0.27 ± 0.10	0.24 ± 0.10	0.27 ± 0.10
Lipid (g.100 mL^{-1})	<0.01	<0.01	<0.01
Ash (g.100 mL^{-1})	0.30 ± 0.10 ^{a}	0.18 ± 0.10 ^{b}	0.25 ± 0.10 ^{a}
Titratable acidity (g.100 mL^{-1})	0.43 ± 0.10 ^{b}	0.35 ± 0.10 ^{c}	0.51 ± 0.10 ^{a}
pH	3.88 ± 0.10 ^{b}	4.03 ± 0.10 ^{a}	3.95 ± 0.10 ^{b}
L*	39.40 ± 0.72 ^{b}	41.82 ± 0.48 ^{a}	36.80 ± 0.25 ^{c}
a*	2.14 ± 0.10 ^{b}	1.19 ± 0.10 ^{c}	2.57 ± 0.10
b*	20.83 ± 0.14	20.12 ± 0.77	20.35 ± 0.86
ΔE (J20 × I120)		2.91	
ΔE (J20 × I220)		2.65	
ΔE (I120 × I220)		2.69	

Notes: Results are expressed as 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. J20 is a carbonated beverage with 20% of the original cold-pressed juice. I120 corresponds to the carbonated beverage with a 20% ice fraction from the first stage of block freeze concentration; I220 corresponds to the carbonated beverage with 20% from the second block freeze concentration.

There was no difference ($p < 0.05$) between the samples for total protein content. During the cold-pressing process to obtain guabiroba juice, there is no migration of pulp, peel, or seeds into the juice, which could cause a decrease in its protein content. Based on the same fact, cold-pressed juice does not contain seed fractions, which are the portions of the guabiroba fruit that have a low fat content (1.5–1.9 g.100 g^{-1}) [35], which led to obtaining beverages with lipid levels below the quantification limit of the applied method (<0.01 g.100 mL^{-1}; Table 3).

3.2. Carbonated Beverage Functionality

The formulation of carbonated beverages was carried out per Brazilian legislation covering the standards for producing soft and non-alcoholic carbonated beverages (Table 2) [34,36]. Since guabiroba is a native Brazilian fruit, all products obtained from this fruit must be within the standards established by local legislation, which regulates non-alcoholic carbonated beverages with the addition of a minimum amount of 5% fruit juice or pulp, carbonation with pure industrial carbon dioxide equal to or greater than 2.5v [34], the desired amount of xylitol and citric acid, a maximum of 500 mg.L^{-1} and 800 mg.L^{-1} of sodium benzoate and potassium sorbate, respectively, and a maximum of 35 mg.L^{-1} of EDTA (ethylenediaminetetraacetic acid), for preservative and stabilizing properties [37]. The addition of a natural sweetener (xylitol) was chosen to completely replace sucrose, fructose, or glucose to guarantee a healthy appeal to the product. According to Zhang et al. [38], sugar-containing carbonated beverages are extremely common consumed worldwide and contain large amounts of fructose. This consumption is positively associated with hyperuricemia, the precursor of gout, and is related to cardiovascular disease, hypertension, and renal disease. Chun et al. [39] reported that high consumption of sugary carbonated beverages is associated with a predisposition to heart disease, even in healthy individuals without a genetic predisposition or history of coronary heart disease, cancer, or diabetes.

Furthermore, the consumption of sugary carbonated beverages is directly associated with the increase in obesity. These beverages are high in calories and low in nutrients, resulting in excessive calorie consumption without satisfying hunger. In addition, the sugar in carbonated beverages rapidly raises blood glucose levels, leading to increased insulin resistance and accumulation of body fat. Thus, in relation only to the added natural sweetener, the formulation of the carbonated beverages in this study can be targeted

for consumption by adults and children without restrictions. Furthermore, beverages containing bioactive compounds such as carotenoids, phenolic compounds, and vitamins can provide numerous health benefits [19]. Nowadays, this implementation becomes important due to the daily intake of fruit by the world population (at least 400 g of fruit and vegetables per day) being lower than that recommended by the WHO (World Health Organization) and the FAO (Food and Agriculture Organization), which leads to functional appeal strategies for industrial formulations containing fruit and being well accepted by the consumer market [40].

Usually, the ice fraction from the freeze concentration process is not reused. However, the data in Tables 3 and 4 prove that using ice remaining from the guabiroba juice concentration can be an intelligent strategy on an industrial scale due to the significant levels of retained solids and bioactive compounds. For total phenolic compounds (Table 4), the beverage containing 20% of the ice fraction from the second stage (I220) stood out when compared to the other formulations ($p < 0.05$). Concerning the carbonated beverage containing guabiroba juice (J20) (543.11 mgGA.L^{-1}), the TPC for I220 was higher by 151.3%. Originally, the guabiroba fruit stood out for containing high levels of phenolic compounds (9033.20 mg.100 g^{-1}), classified as one of the fruits from the *Myrtaceae* family with the highest levels of these compounds, which, consequently, are transferred to the fruit fractions [35,41]. Furthermore, obtaining the guabiroba juice by cold pressing in this work maintains most of the nutritional compounds sensitive to high temperatures, being also a beneficial technology for improving the product's functionality.

Table 4. Carbonated beverages' total phenolic, carotenoid, vitamin C, and antioxidant activity.

Analysis	J20	I120	I220
TPC (mgGA.L^{-1})	543.11 ± 96.82 ^{c}	548.28 ± 33.97 ^{b}	821.59 ± 181.46 ^{a}
α-Carotene (μg.100 mL^{-1})	31.18 ± 3.21 ^{c}	42.52 ± 1.17 ^{b}	71.42 ± 0.80 ^{a}
β-Carotene (μg.100 mL^{-1})	33.76 ± 4.75 ^{c}	45.93 ± 6.34 ^{b}	77.15 ± 2.49 ^{a}
γ-Carotene (μg.100 mL^{-1})	28.11 ± 5.10 ^{c}	38.41 ± 2.08 ^{b}	64.51 ± 4.01 ^{a}
Cryptoxanthin (μg.100 mL^{-1})	36.52 ± 2.23 ^{c}	49.91 ± 8.13 ^{b}	83.81 ± 7.19 ^{a}
DPPH (μmolTE.L^{-1})	159.15 ± 0.33 ^{c}	205.27 ± 0.96 ^{b}	207.16 ± 1.57 ^{a}
ABTS (μmolTE.L^{-1})	83,998.33 ± 5831.24 ^{c}	136,350 ± 8267.21 ^{b}	248,460.00 ± 7839.71 ^{a}
Vitamin C (mgAA.100 mL^{-1})	58.66 ± 2.74 ^{c}	74.66 ± 3.25 ^{b}	93.33 ± 4.85 ^{a}

Note: Results are expressed as 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. DPPH is 2,2-diphenyl-1-picrylhydrazyl assay. ABTS is 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) assay. GA = gallic acid; TE = Trolox equivalent; AA = ascorbic acid. J20 is a carbonated beverage with 20% of the original cold-pressed juice. I120 corresponds to the carbonated beverage with a 20% ice fraction from the first stage of block freeze concentration; I220 corresponds to the carbonated beverage with 20% from the second block freeze concentration.

The higher concentration of bioactive compounds for sample I220 is due to the successive concentration of solids, mostly retained at the solid–liquid interface since the process efficiency in concentrating solid particles in the concentrated fraction is lower for stage 2. It should be noted that the first-stage concentrate was used in the second stage of the freeze concentration process (Figure 1). In this way, feed with a higher solid content was used. As the freeze concentration stages consist of reducing the mass of the feed by 50%, when using a liquid with a higher solid content, a higher content of bioactive compounds may be retained in the ice fraction (stage 2, I220) (Table 4), as well as for the total solid content of I220 (Table 3). This behavior is related to the decrease in process efficiency observed in stage 2 of the freeze concentration process.

Furthermore, the TPC in the ice fractions of the second stage of guabiroba juice freeze concentration was so prominent (I220 = 821.59 mgGA.L^{-1}) that, when compared to orange juice freeze concentration by Haas et al. [12], only 20% of the addition of the ice fraction was superior to whole orange juice with 715.7 mgGA.L^{-1}. The I220 beverage also obtained higher TPC levels when compared to studies with Red Delicious apple juice, also subjected

to a freeze concentration process (740 mgCA.L^{-1}) [42]. The same behavior applies to the antioxidant activity, directly related to the TPC and other bioactive components such as carotenoid content and vitamin C [43]. The I220 sample presented the highest antioxidant activity ($p < 0.05$) among the three samples for both the DPPH (207.16 µmolTE.L^{-1}) and ABTS (248,460.00 µmolTE.L^{-1}) assays, and when compared to the J20 beverage, the activities were higher by 130.2% and 295.8% for DPPH and ABTS, respectively (Table 4). Compared to studies with whole apple juice (1255.0 µmolTE.L^{-1}) and concentrated juice by two stages of freeze concentration (8254.0 µmolTE.L^{-1}) [42], only 20% of the ice fraction of guabiroba juice in the beverages promoted greater antioxidant activity for the ABTS assay, highlighting the intense functional property of the original fruit fractions. The DPPH and ABTS analyses are important for assessing the antioxidant capacity of fruit juices. These methodologies measure the efficiency of compounds present in fruits in neutralizing free radicals, which are linked to oxidative stress and the development of chronic diseases. The DPPH test uses a stable radical to verify the electron-donating capacity of antioxidants, while ABTS measures the neutralizing capacity of specific radicals [25,26]. The natural antioxidants present in fruit juices are essential for combating lipid oxidation. Lipid oxidation, which occurs when fats are exposed to oxygen, results in the formation of free radicals, causing food spoilage. Natural antioxidants, such as vitamin C, flavonoids, and polyphenols, present in guabiroba fruit, help neutralize these free radicals, slowing or preventing the oxidative process.

Among the formulated beverages, there was a considerable vitamin C content (58.66–93.33 mgAA.100 mL^{-1}), also related to the significant antioxidant activity due to its ability to capture and neutralize free radicals. One of the most important nutritional properties of *Campomanesia xanthocarpa* O. Berg is the high content of vitamin C in its composition, ranging from 17.80 to 233 mg.100 g^{-1} according to our previous studies [35]. Orange juice is a common product in consumers' daily lives and is a routine source of vitamin C. According to Haas et al. [12], the vitamin C content of orange juice is 33.3 mgAA.100 mL^{-1} and, compared with carbonated beverages, the I120 sample presented 2.2× more and, for the I220 sample, 3x the vitamin C content than pure orange juice.

The functional prominence of the I220 sample can also be discussed about the carotenoid levels (α-, β-, γ- carotene, and cryptoxanthin). With the J20 beverage, the average content of all carotenoids was 168% higher. Santos [44] and Schmidt et al. [45] described the guabiroba fruit as a potential source of carotenoids, mainly for β-carotene concentration, a precursor of vitamin A (retinol), (12.30 to 3400.00 mg.100 g^{-1}) which stands out when compared to the levels of other conventional fruits, such as papaya with 0.04 mg.100 g^{-1} [35]. The beverage containing only 20% of the ice fraction (I220 = 0.07 mg.100 mL^{-1}) surpassed the β-carotene content found in whole papaya fruit. In addition to contributing to the increase in antioxidant activity, the intensity of the orange color of both guabiroba fruit and its fractions (which can also be noted in the carbonated beverages in this work in Figure 2) is also related to the high levels of carotenoids, mainly for the cryptoxanthin content, with 36.52–83.81 µg.100 mL^{-1} among the samples.

According to Table 3, the a* parameter, related to the intensity of the red color, was greater for the I220 sample (I220 = 2.57; J20 = 2.14; I120 = 1.19), correlating the higher carotenoid contents with the intense orange color of both the guabiroba juice and its respective ice fractions. For the b* parameter, which relates to the intensity of the yellow color, there was no significant difference for all three samples, emphasizing the color intensity of the guabiroba pulp remaining in the fruit fractions. Therefore, for the total color difference, expressed by ΔE* (Table 3), there was no noticeable difference to the naked eye because the values were less than 3.0. Martínez-Cervera et al. [46] described that, when the total color difference is lower than 3.0, the change in the color of the samples or their tone is not visually noticeable, as shown in Figure 2. The increase in the concentration of total solids in samples is directly related to the increase in compounds with antioxidant properties. Therefore, these compounds could intensify the final product's color [47]. This characteristic makes the beverage formulation even more functional due to the lack of

need to use dyes from synthetic sources, which can be allergenic. In addition to the health benefits of the carotenoid content, these carbonated beverages can be aimed at all groups of people, as food allergies among consumers are increasingly evident.

The production of sugar-free carbonated beverages with functional properties has become a promising trend for the beverage market. With the increasing demand for health-conscious products, companies seek alternatives combining flavor, low-calorie content, and health benefits. One of the main prospects is using waste from agro-industrial processes rich in bioactive compounds such as antioxidants and vitamins. In this case, the residual ice fraction from the freeze concentration process of the fruit juice can be incorporated into carbonated beverage formulations, adding nutritional value and functionality. In addition to reducing waste, this approach contributes to sustainability, promoting a circular economy. The technology for extracting bioactive compounds and for their use in functional beverages must improve, making it possible to develop beverages that, besides being sugar-free, offer health benefits, such as improved digestion, immune strengthening, and combating oxidative stress, aligning health and innovation. Based on the results obtained in this study, it is recommended that sensory tests and a cost analysis of the product be carried out before the future industrial carbonated beverage is launched.

4. Conclusions

The ice fraction from a freeze concentration process has no academic or industrial applicability, being considered a process waste. This unprecedented work combined the development of a new product with functional properties with the intelligent reuse of this residual fraction. The ice resulting from the block freeze concentration of cold-pressed guabiroba juice had a significant retention of polyphenols, vitamin C, and carotenoids, with the latter compounds responsible for the residue's striking orange color. The formulation of a carbonated beverage without added sugar with 20% of the ice fraction from the second freeze concentration stage (I220) showed higher levels of bioactive compounds due to the lower process efficiency of solid migration to the concentrated fraction, resulting in greater contents in the ice fraction. Thus, in addition to developing a carbonated beverage containing guabiroba juice, the reuse of ice fractions from a block freeze concentration becomes an outstanding strategy due to the high retention of bioactive compounds from the original fruit. This innovative product has an interesting economic appeal due to the reuse of process waste and its healthy properties due to the absence of sugars and synthetic dyes. Therefore, in the future, both the incorporation of the freeze concentration process and the development of products with guabiroba fractions have the potential to be applied on a large scale, disseminating knowledge of this native fruit with a rich functional composition and contributing to waste management and circular economy.

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Appendix A

Table A1. Products developed with guabiroba fruit and its fractions.

Guabiroba Fractions	Product	References
Guabiroba fruit	*In natural* fruit	[2]
Guabiroba pulp	Juice Cold-pressed juice Liqueur Jam Ice cream *Dulce de leche* Cheese Fermented milk Cake Frozen pulp	[1,5,6,8–11]
Guabiroba pulp residue	Pulp dehydrated residue Cake Cookies	[4]
Pulp residue dehydrated	Guabiroba flour Cookies Meat seasonings and sauces Cereal bar Cake Bread	[4]
Cold-pressed juice	Refrigerated cold-pressed juice Fermented lactic beverage Ice cream Concentrated from two stages of the freeze concentration process Ice fraction from two stages of the freeze concentration process	[1,9–11]
Concentrates 1 and 2	Fermented milk	[9–11]
Ice fractions 1 and 2	Not yet applied	-

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