



# Whey block freeze concentration aiming a functional fermented lactic beverage with the addition of probiotic and guabiroba pulp (*Campomanesia xanthocarpa* O. Berg), a native Brazilian fruit

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## Abstract

The scientific importance involved in this study was the use of whey, a co-product of the cheese industry, and its performance during the freeze concentration process. Moreover, the best-concentrated whey from the freeze concentration process, about the total solids, proteins, and mineral contents, was used to prepare two functional fermented lactic beverages. Therefore, whey was subjected to the freeze concentration in blocks with gravitational thawing. Process performance indicated better yields and efficiency for the second stage of freeze concentration. Concentrated whey 2 was used to prepare two fermented lactic beverages added with probiotics: one without adding guabiroba pulp (control) and a beverage incorporated with 10% guabiroba pulp. Containing guabiroba pulp was not enough to modify the total solids, proteins, and mineral contents. However, it decreased pH values, changed the color to an orange hue, and decreased luminosity. The fermented lactic beverage added with probiotic and 10% guabiroba pulp showed 1.61× more phenolic compounds and an increase of 164% for each evaluated carotenoid content compared with the control beverage.

**Keywords:** Guabiroba; functional beverage; cheese whey; concentration; bioactive compounds.

**Practical Application:** Production of a fermented dairy beverage with the reuse of dairy waste, aiming to increase its functionality by adding native fruit pulp, with a rich composition of bioactive compounds.

## 1 INTRODUCTION

Whey is an important co-product of the cheese industry, and approximately 197.44 million tons of it are generated worldwide from cheeses made with cow's milk (FAOSTAT, 2023). However, the whey retains about 55% of the solids and 20% of the proteins present in milk, being about 0.6–0.8 g/100 g of protein, 0.4–0.5 g/100 g of fat, 4.5–5 g/100 g of lactose, and 8–10 g/100 g of mineral salts. Alternatives for using this co-product, aiming at its exploitation, have generated interest in both the small and large industrial sectors and the scientific area. In terms of improving whey's nutritional properties, it can be applied to concentration methods. Among these, Habib and Farid (2008) and Raventós et al. (2007) affirmed that the freeze concentration technology stands out, which employs low temperatures, bringing popularity as an alternative industrial concentration technique of the whey processing, such as vacuum evaporation and membrane technologies. Therefore, freeze concentration improves quality as it minimizes the effect of heat on sensitive components such

as proteins, water-soluble vitamins, and aromatic compounds (Moreno et al., 2015; Robles et al., 2016; Sánchez et al., 2010). Prestes et al. (2022) highlighted that freeze concentration is an important technology applied to focus liquid foods, maintaining their quality and preserving thermolabile compounds, flavor, and color. In dairy industries, this technological approach can significantly enhance milk's and whey's efficiency, concentrating its total dry matter. Also, such a technique provides additional advantages for the product's packaging, shipping, and storage. Barros et al. (2022) evaluated the freeze concentration of whey and observed that proteins mostly represent the higher total solids content of concentrated whey. Therefore, this study first focuses on using an emerging non-thermal technology in the concentration of a co-product from the dairy industry.

The growing consumer attention for a diet that goes beyond nutritional value, aiming to improve their well-being, has determined a great interest in the food industries in developing products with claims of functional properties. Among these products

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are probiotics. According to Hill et al. (2014), probiotics are live microorganisms that confer a health benefit on the host when administered in adequate amounts. These authors further considered two common benefits often associated with probiotics: supporting a healthy digestive tract and immune system. Hill et al. (2014) also acknowledged that evidence supports the beneficial relationship between some foods containing live beneficial microorganisms, especially fermented dairy products, and reduced risk of certain diseases. It accepts the following bacterial species when delivered in food at a level of  $1 \times 10^9$  colony-forming units (CFU) per serving as probiotics for which nonstrain-specific claims might be made: *Bifidobacterium (adolescentis, animalis, bifidum, breve, and longum)* and *Lactobacillus acidophilus, Lacticaseibacillus casei, Limosilactobacillus fermentum, Lactobacillus gasseri, Lactobacillus johnsonii, Lacticaseibacillus paracasei subsp. paracasei, Lactiplantibacillus plantarum subsp. plantarum, Lacticaseibacillus rhamnosus, and Ligilactobacillus salivarius*. This list represents a core group of well-studied species likely to impart some general benefits based on their contribution to healthy gut microbiota (Hill et al., 2014). In addition to using a concentrated product, and therefore with greater nutritional value, as well as a probiotic, this study envisaged using bioactive compounds from a native Brazilian fruit.

Bioactive compounds are synthesized by plants in the composition of fruits, flowers, leaves, seeds, or roots. These compounds can be metabolized by some microorganisms and animals (Patra et al., 2018). Among these compounds are polyphenols and carotenoids, which can act on human metabolism, reducing the incidence of degenerative diseases (Cutrim & Cortez, 2018). Sources of bioactive compounds, such as fruit extracts, pulps, and juices, are often studied as a functional additive in dairy products, becoming an important source for research and a trend for industries (Balthazar et al., 2019; Casarotti et al., 2018). Brazilian native fruits have been analyzed in studies related to their composition and consumption benefits (Azevedo et al., 2019), such as fruits of the *Myrtaceae* family that are known for their high content of bioactive compounds and antioxidant activity, including *Campomanesia xanthocarpa* O. Berg, which is popularly known as “guabiroba” (Silveira et al., 2019). Guabiroba is considered a native Brazilian functional fruit that has an acid-sweet taste and antioxidant compounds, such as polyphenols (Capeletto et al., 2016). These properties of guabiroba make the pulp suitable for consumption in nature or beverage compositions. However, guabiroba pulp is still not a widely used ingredient in commercial products (Barbieri et al., 2018). Therefore, this study aimed to use the concentrated whey from freeze concentration in blocks with gravitational thawing technology to elaborate a fermented lactic beverage added with probiotic and guabiroba pulp (*C. xanthocarpa* O. Berg), aiming to obtain a lactic beverage with probiotics rich in bioactive compounds.

## 2 MATERIAL AND METHODS

### 2.1. Material

The whey was obtained through the preparation of a fresh Minas Frescal type cheese (whole pasteurized milk; 10.98 g/100 g total solids, 2.98 g/100 g protein, 4.07 g/100 g carbohydrates, and

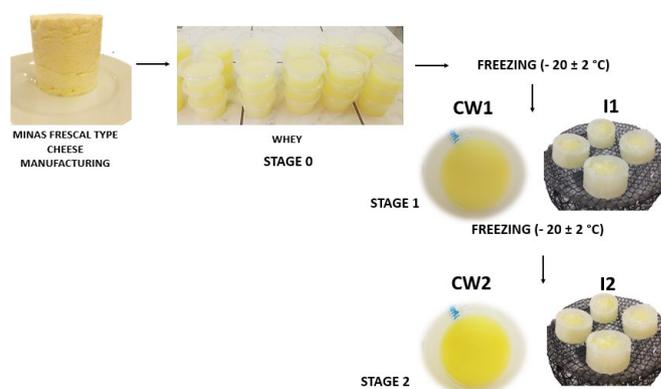
3.20 g/100 g fat, Tirol<sup>®</sup>, Treze Tílias, Brazil). Clotting enzyme (HA-LA<sup>®</sup>) with a coagulant power of 1:3,000 was purchased from Chr. Hansen (Valinhos, São Paulo, Brazil). The fermented beverages were prepared using a thermophilic culture of *Streptococcus salivarius* subsp. *thermophilus*, *Bifidobacterium* BB-12, and *L. acidophilus* LA-5 (BioRich<sup>®</sup>, Chr. Hansen, Valinhos, São Paulo, Brazil), sucrose (União<sup>®</sup>, Barra Bonita, São Paulo, Brazil), and glucose (Yoki<sup>®</sup>, Paranavaí, Paraná, Brazil). The fermented beverage was elaborated with guabiroba pulp addition. The fruits were collected in the “Pinho de Baixo” community, which is located in the interior of Irati, Paraná state, Brazil (S25o27c56”; W50o37’51”). Guabiroba was pulped and kindly provided for use in this study by EMBRAPA FLORESTAS (Colombo, PR, Brazil), with the following composition: 15.79 g/100 g of total solids, 0.18 g/100 g of protein, 7.75 g/100 g of carbohydrates, and 0.88 g/100 g of fat. Peptone water (Oxoid, Hampshire, UK), AnaeroGen<sup>®</sup> (Oxoid, Hampshire, UK), MRS Agar (Merck, Darmstadt, Germany), and M17 agar (Sigma-Aldrich, São Paulo, Brazil) were used for the microbiological assays. All reagents were analytical grade. DPPH (1,1-diphenyl-2-picrylhydrazyl), Folin-Ciocalteu reagent, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic) gallic acid, and catechin standards were purchased from Sigma-Aldrich (St. Louis, MO, USA).

### 2.2 Whey obtaining

Through the previous production of Minas Frescal type cheese described by Souza and Saad (2009), whey, classified as a residue of the process, was obtained. For cheese production, 48 L of whole pasteurized milk was heated to  $37 \pm 1^\circ\text{C}$  for the addition of the coagulating enzyme, followed by incubation at  $37 \pm 1^\circ\text{C}$  for 40 min. After this period, the clot was gently cut into cubes, stirred, drained, and placed in perforated cylindrical containers, each with a capacity of 500 g, to separate the whey. A filtration process was carried out with the clot, obtaining the filtered whey which was frozen ( $-20 \pm 2^\circ\text{C}$ ) until the freeze concentration process and further analysis.

### 2.3 Block freeze concentration process

The block freeze concentration process with gravitational thawing was used in whey concentration, following the methodology described by Canella et al. (2018). At each stage of the freeze concentration process, two fractions were obtained



**Figure 1.** Scheme of the cryoconcentration process with gravitational thawing.

and named concentrated whey (CW) and ice (I) (Figure 1). An initial volume of 7.2 L of whey was separated into pots containing approximately 200 g. Plastic containers containing whey were frozen at  $-20\pm 2^\circ\text{C}$  in a freezer unit (Consul®, Biplax CRD41D, São Bernardo do Campo, Brazil). After complete freezing of whey, 50% of the initial volume was thawed at room temperature ( $20\pm 2^\circ\text{C}$ ), obtaining two fractions, concentrated whey (CW1) and ice (I1). The concentrated fraction (CW1) was again frozen in plastic pots containing approximately 200 g at  $-20\pm 2^\circ\text{C}$  and used as a feed solution in the second freeze concentration step, resulting in concentrated whey (CW2) and on ice (I2). After each step, a portion of concentrated (CW1 and CW2) and ice fractions (I1 and I2) were collected and stored at  $-20\pm 2^\circ\text{C}$  for further analysis and use in preparing the fermented beverages.

### 2.3.1 Concentration factor

The concentration factor of the freeze concentration process, that is, its yield, was calculated using Equation 1, according to Aider and Ounis (2012),

$$\text{CF}\% = \frac{\text{TS}_n}{\text{TS}_o} \times 100 \quad (1)$$

Where:

$\text{TS}_n$ : the total solids content (g/100 g), mineral salts (g/100 g), or proteins (g/100 g) in the concentrated whey at each concentration stage;

$\text{TS}_o$ : the total solids content (g/100 g), mineral salts (g/100 g), or proteins (g/100 g) in the initial whey.

The CF value was determined at each concentration stage as a function of the increase in total solids (g/100 g), mineral salts (g/100 g), and proteins (g/100 g) in the cryoconcentrate ( $\text{TS}_n$ ) and about whey initial ( $\text{TS}_o$ ).

### 2.3.2 Process efficiency

The efficiency of the freeze concentration (PE) process was determined based on the increase in total solids in the concentrate (g/100 g) concerning total solids, mineral salts (g/100 g), or proteins (g/100 g) remaining in the ice fraction of each stage of freeze concentration. PE was calculated by Equation 2, according to Aider and Ounis (2012),

$$\text{PE}\% = \frac{\text{TS}_{n-\text{TS}_i}}{\text{TS}_n} \times 100 \quad (2)$$

Where:

$\text{TS}_n$ : the content of total solids (g/100g), mineral salts (g/100g), or proteins (g/100g) in the concentrated whey fractions;

$\text{TS}_i$ : the content of total solids (g/100 g), mineral salts (g/100 g), or proteins (g/100 g) on ice.

The concentrated whey used in the preparation of fermented beverages was chosen based on the evaluation of the FC and EP results.

## 2.4 Fermented beverage elaboration

Two fermented beverages were prepared according to the methodology of Almeida et al. (2001), with modifications and previous studies (data not shown) (Table 1).

The concentrated whey of the stage that presents the best performance of the freeze concentration process was used to prepare the beverages. Two fermented beverages (control beverage and guabiroba beverage) were prepared by heating concentrated whey to  $42\pm 2^\circ\text{C}$ , followed by adding sucrose (8 g/100 g) and glucose (4 g/100g) to increase the speed of the fermentation process by starter cultures and probiotic cells and inoculating with 0.05 g/100 g of thermophilic culture (*L. acidophilus* LA-5, *Bifidobacterium* sp. BB-12, and *Streptococcus thermophilus*), as recommended by the manufacturer. The incubation for the fermentation step was carried out at  $42\pm 2^\circ\text{C}$ , measuring the pH, and cooled at  $4\pm 2^\circ\text{C}$  for 24 h. The control beverage was prepared only with concentrated whey, without adding guabiroba pulp, and the beverage with guabiroba was ready with 10 g/100 g of pulp, as proposed by Prestes et al. (2022). The samples were kept refrigerated ( $4\pm 1^\circ\text{C}$ ) until analysis.

## 2.5 Physicochemical analysis

### 2.5.1 Proximate composition

For all samples of concentrated whey, ice fraction, and fermented beverages (control and with guabiroba pulp), the total solids content (g/100 g) was obtained by gravimetry drying in an oven at  $105^\circ\text{C}$  about 2 g of the sample for at least 16 h or until reaching a constant weight (AOAC, 2019). Protein content was performed using the Kjeldahl method. Approximately 0.2–0.5 g of the sample was added to digester tubes containing the protein catalyst, concentrated sulfuric acid with the process occurring at  $100^\circ\text{C}$  (temperature increased every hour until reaching  $350^\circ\text{C}$ ) until complete digestion. Distillation was carried out with sodium hydroxide and titration with hydrochloric acid. The results were expressed in g/100 g ( $N \times 6.38$ ). The hydrogen ion potential of the samples was determined in a digital pH meter (Kasvi®, São Paulo, São Paulo, Brazil) with a previous calibration with buffer solutions (pH=4.0 and pH=7.0) at room temperature.

**Table 1.** Formulation of the fermented beverage control and the fermented beverage with guabiroba pulp (10%) made with the concentrated whey from the best freeze concentration performance.

Formulation (g)	Control beverage	Guabiroba beverage
Concentrated whey chosen	879.5	779.5
Sucrose	80	80
Glucose	40	40
Thermophilic culture	0.5	0.5
Guabiroba Pulp	0	100
<b>Total (g)</b>	<b>1,000</b>	<b>1,000</b>

### 2.5.2 Instrumental analysis

The color parameters of the fermented beverages (control and with guabiroba pulp) were determined using the Minolta Chroma Meter CR-400 colorimeter (Konica Minolta, Osaka, Japan), which was adjusted to operate with D65 illuminant and an observation angle of 10°. The colorimeter was calibrated with a standard white plate, and the CIELab color scale was used to measure the parameters L\*, a\*, and b\*. The L\* parameter varied from 0 to 100 and indicated the brightness (variation from black to white); the b\* axis was the change from yellow (+b\*) to blue (-b\*); and the a\* axis showed the transition from red (+a\*) to green (-a\*). The total color difference ( $\Delta E^*$ ) between the two beverages was calculated according to Okpala et al. (2010), as described in Equation 3:

$$\Delta E^* = (\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2 \quad (3)$$

where:

$\Delta L^*$ : the difference in luminosity;

$\Delta a^*$ : the intensity of the red color;

$\Delta b^*$ : the intensity of the yellow color.

### 2.6 Microbiological analysis

For the probiotic count (*Bifidobacterium* BB-12 and *Lactobacillus* LA-5), 25 g of beverage was diluted in 225 mL of phosphate buffer (pH 7, 0.1 mol/L) followed by homogenization using a magnetic stirrer for 10 min. The mixtures were serially diluted in peptone water and inoculated in depth on MRS agar. The inverted plates were incubated at 37°C for 72 h in anaerobic jars using an AnaeroGen® sachet (Vinderola & Reinheimer, 1999). *S. thermophilus* count was carried out by the pour plate technique using M17 agar with lactose solution (10 g/100 mL) incubated aerobically at 37°C for 48 h (IDF 1997). The total viable count was expressed as colony-forming units per gram of beverages (CFU/g).

### 2.7 Extraction for phenolic analysis and antioxidant activity

Preparation of fermented lactic beverage extracts was conducted according to the method used by Shori and Baba (2013). Therefore, 10 g of fermented lactic beverages were mixed with 2.5 mL of distilled water, adjusted to pH 4 using HCl (1 M), and incubated at 45°C for 10 min. In the sequence, the mixture was centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was collected, adjusted to pH 7 using 0.1 M NaOH, and centrifuged again at 10,000 rpm for 20 min at 4°C.

#### 2.7.1 Diphenyl-1-picrylhydrazyl radical scavenging activity

The DPPH method was performed according to Brand-Williams et al. (1995). Standard Trolox solution (3000  $\mu\text{mol/L}$ ) was used to obtain the calibration curve (linearity range:  $R^2=0.99$ ). A volume of 100  $\mu\text{L}$  of the sample was pipetted into tubes with

the addition of DPPH solution (0.00336 g in 100 mL) with a reaction time of 30 min without light and at room temperature. The analysis read was performed in a spectrophotometer (UV-1800, Shimadzu, Brazil) at 515 nm, and the results were expressed in micromoles of Trolox equivalents per liter of the sample ( $\mu\text{mol TE/L}$ ).

#### 2.7.2 Total phenolic content

The total phenolic content was evaluated using the Folin-Ciocalteu method (Singleton & Rossi, 1965) with a calibration curve obtained with a standard solution of gallic acid (1–9 mg/L) (calibration curve linearity range:  $R^2=0.99$ ). In tubes, the sample extract was added (between 0.1 and 1 mL), with the addition of 1.25 mL of Folin-Ciocalteu's reagent and 5 mL of 15% sodium carbonate solution. The analysis read was performed in a spectrophotometer at 720 nm. The results were expressed in milligrams of gallic acid equivalent per liter of the sample (mg GAE/mL).

#### 2.7.3 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical cation decolorization activity

For the 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) method, according to Re et al. (1999), the standard Trolox solution (3,000  $\mu\text{mol/L}$ ) was used to obtain the calibration curve (linearity range:  $R^2=0.99$ ). A volume of 30  $\mu\text{L}$  of the sample was pipetted into tubes with the addition of 3 mL of analysis solution (ABTS solution 7 mmol/L and potassium persulfate 140 mmol/L) with a reaction time of 2 h without light and at room temperature. The analysis read was at 734 nm in a spectrophotometer, and the results were expressed in micromole of Trolox equivalent per liter of the sample ( $\mu\text{mol TE/L}$ ).

### 2.8 Carotenoid content

Both fermented beverages (control beverage and guabiroba beverage) were evaluated according to Rodriguez-Amaya (2001), with modifications. To extract carotenoids, 1 g of the sample and 20 mL of acetone were weighed in a 50-mL Falcon® tube. After mixing in the vortex (Biomixer®, Jacareí, São Paulo, Brazil), the tube containing the mixture was placed in ultrasound for 30 min. The extract was separated using a filter paper and a funnel. In a burette, 4 mL of petroleum ether was added, followed by the extracted liquid and 3 mL of type 2 ultrapure water. The burette was left to rest, waiting for phase separation. When there was no separation, a few drops of NaOH solution were dripped, and separation was awaited. After separation, the lower fraction (colorless) was removed for disposal, keeping only the colored phase in the burette. The colored phase was released to a volumetric flask, passing through a filter paper with sodium sulfate, retaining any aqueous residue. The burette was cleaned with petroleum ether, avoiding loss of extract. Carotenoid content was obtained in a spectrophotometer, using a wavelength of 450 nm for  $\beta$ -carotene, a wavelength of 444 nm for  $\alpha$ -carotene, a wavelength of 452 nm for  $\beta$ -cryptoxanthin, and a wavelength of 462 nm for  $\lambda$ -carotene. The carotenoid content was calculated using Equation 4:

$$\text{Carotenoid content } \mu\text{g}/100 \text{ g} = \frac{\text{Abs} \times \text{Vol mL (dilution)} \times \text{A1 cm}^{-1} \times \text{weight of sample} \times 106}{\text{Vol mL}} \quad (4)$$

where:

A: the absorbance;

V: the total extract volume (mL);

A1%: the molar absorptivity=2,592 ( $\beta$ -carotene);

A1%: the molar absorptivity=2,800 ( $\alpha$ -carotene);

A1%: the molar absorptivity=3,100 ( $\lambda$ -carotene);

A1%: the molar absorptivity=2,386 ( $\beta$ -cryptoxanthin).

### 2.9 Statistical analysis

The results were expressed as mean  $\pm$  standard deviation. Data were analyzed using the STATISTICA 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA). To determine significant differences ( $p < 0.05$ ), an analysis of variance was carried out, followed by *post hoc* analysis with the Tukey's test. All samples were produced in triplicates, and three parallel measurements were made for each replication.

## 3 RESULTS AND DISCUSSION

The results obtained for the freeze concentration of whey about the total solids, proteins, and mineral salt contents indicate that these values differed ( $p < 0.05$ ) in the following order: CW2 > CW1 > whey > I2 = I1 (Table 2). It is noteworthy that this study used two stages of freeze concentration because, according to Aider et al. (2007) and Aider and Ounis (2012), from the third and fourth stages of the whey freeze and of the skimmed milk freeze concentration, respectively, relatively high amounts of total solids are trapped in the ice fraction. Following these authors, this happens because, with increasing viscosity of the dairy raw material to be concentrated, the ability to obtain pure ice crystals decreases, and therefore, the general efficiency of the freeze concentration process also decreases. These authors also concluded that the increase in the viscosity of the solution resulted in a decrease in the phenomena of mass and heat transfer in the system. On the contrary, Samsuri et al. (2015) also stated that large ice crystals contain fewer impurities and lower solids content than smaller crystals, and such behavior is noted when slow freezing is used, as used in this study. The behavior obtained for total solids, proteins, and mineral salt contents of whey in our study was expected compared with these studies cited above.

Canella et al. (2020) carried out the freeze concentration process in blocks with vacuum thawing of goat milk, obtaining values for the concentrate yield of ~85% for the total solids content in two stages of freeze concentration. In the second stage of the freeze concentration, the yields of the concentrates obtained in this study were 348.35, 321.50, and 300%, with the total solids, proteins, and mineral salts, respectively. In the study by Canella et al. (2020), it was also observed that the efficiency of the freeze concentration process for the total solids content was around 90%, while our method was verified in the second stage of freeze concentration with an efficiency of >95%. For proteins and mineral contents, the efficiency of our freeze concentration process was also approximately 90%. However, in the whey freeze concentration process carried out by Aider et al. (2007), the concentration factor for the total solids content in stage 2 was equal to 351%, and therefore, this value was similar to the second stage of this study. However, in the study realized by these authors, at this same freeze concentration stage, the protein content concentration factor was 213 and 24.81% for the somatory of the following mineral salts: potassium, sodium, calcium, and magnesium, being, therefore, lower than values obtained in this study.

Regarding the pH, the data obtained showed a difference ( $p < 0.05$ ) between all values (Table 2). Igartúa et al. (2022) stated that the whey pH values are approximately 7.0. According to Ho et al. (2021), pH around 6 and 6.5 cannot affect the whey's functional properties, such as solubility, foaming, and emulsifying properties. Through the results obtained in this first stage of the study, that is, due to the higher levels of total solids, proteins, and mineral salts, as well as due to the excellent values obtained for CF and PE, CW2 (concentrated whey 2) was chosen for the elaboration of fermented beverages. The results for the physicochemical composition and color parameters of both beverages are shown in Table 3.

Between the two fermented beverages elaborated with whey concentrate (CW2), no differences were found ( $p > 0.05$ ) between the contents of total solids, proteins, and minerals; that is, the 10% guabiroba pulp did not show differences about these contents. It is relevant to point out that guabiroba pulp was added after the fermentation process because, according to Ning et al. (2021), some concentrations of organic acids contained in fruit pulps and juices can induce the separation of proteins; in this case, the proteins contained in concentrated whey (CW2). The values for the pH of the fermented beverages after the fermentation process, and therefore, even without the addition of guabiroba pulp, showed differences between them

**Table 2.** Results of the chemical composition and pH (mean  $\pm$  standard deviation) of whey and samples and the results of the concentration factor and the efficiency for each freeze concentration stage.

		Whey	CW1	I1	CW2	I2	CF1 (%)	PE1 (%)	CF2 (%)	PE2 (%)
Chemical composition (g/100 g)	Total solids	6.06 <sup>a</sup> $\pm$ 0.24	11.62 <sup>b</sup> $\pm$ 0.01	0.93 <sup>d</sup> $\pm$ 0.05	21.11 <sup>a</sup> $\pm$ 0.06	0.97 <sup>d</sup> $\pm$ 0.02	190.10	91.93	348.35	95.40
	Proteins	0.93 <sup>b</sup> $\pm$ 0.06	1.73 <sup>b</sup> $\pm$ 0.08	0.27 <sup>d</sup> $\pm$ 0.01	2.99 <sup>a</sup> $\pm$ 0.03	0.31 <sup>d</sup> $\pm$ 0.04	186.02	84.39	321.50	89.63
	Mineral salts	0.56 <sup>a</sup> $\pm$ 0.12	1.01 <sup>b</sup> $\pm$ 0.03	0.14 <sup>d</sup> $\pm$ 0.03	1.68 <sup>a</sup> $\pm$ 0.02	0.16 <sup>d</sup> $\pm$ 0.01	180.36	86.14	300.00	90.47
pH		6.17 <sup>a</sup> $\pm$ 0.01	6.25 <sup>a</sup> $\pm$ 0.01	6.20 <sup>d</sup> $\pm$ 0.01	6.52 <sup>a</sup> $\pm$ 0.05	6.41 <sup>b</sup> $\pm$ 0.00	-	-	-	-

CW1 and CW2: concentrated whey from the first and second stages of the freeze concentration process, respectively; I1 and I2: ice from the first and second stages of the freeze concentration process; CF1 and CF2: the concentration factor from the first and second stages of the freeze concentration process; PE1 and PE2: process efficiency from the first and second stages of the freeze concentration process, respectively; <sup>a-c</sup>different and superscript lowercase letters, expressed in the same line, indicate significant differences between samples ( $p < 0.05$ ).

( $p < 0.05$ ). Likewise, these differences ( $p < 0.05$ ) were observed between the control and fermented beverages incorporated with 10% guabiroba pulp. According to Meena et al. (2022), the difference ( $p < 0.05$ ) in the pH values in the two cases may be due to variations in the different amounts of CW2 used and, subsequently, concerning other factors related to the constitution and enzymatic action of guabiroba, as well as changes in the chemical state of the fruit, transformed into pulp.

Due to the orange color of the guabiroba pulp, the color of the fermented beverage incorporated with the guabiroba pulp presented a color between yellow and red, and the luminosity ( $L^*$ ) decreased ( $p < 0.05$ ). The value for  $\Delta E^*$  was  $> 3$ , and according to Dantas et al. (2021), this confirms that the two fermented beverages produced have color differences that can be detected by the human eye, which can also be established in Figures 2A and 2B.

Regarding the microbiological count, a slight reduction ( $p < 0.05$ ) can be observed for the fermented beverage incorporated with 10% guabiroba pulp. Similar results were obtained by Ning et al. (2021) in yogurts added with passion fruit pulp. These authors credited this behavior to the reduction in pH values and to the higher contents of phenolic compounds presented by the juice, which could negatively affect the viability of the bacteria. However, both fermented beverages could be considered potential probiotic products because their count was  $\geq 10^6$  CFU/g. According to Castro et al. (2013), this should be considered a possible advantage because it demonstrates the possibility of developing products with high whey concentrations and high microorganism counts that are beneficial to human health, such as probiotic bacteria. These results confirm the ability of the whey beverage to serve as a food matrix or as a food system that could be supplemented with counts of probiotic

bacteria that are capable of delivering human health benefits, mainly toward *Bifidobacterium* BB-12 and *L. acidophilus* LA-5 counts (Castro et al., 2013). This finding corroborated with a highlighted by Farias da Cruz et al. (2022) that dairy products were the most used vehicles for probiotic administration.

Notably, phenolic contents in several dairy products are restricted, and according to Pereira et al. (2012), guabiroba pulp is rich in phenolic compounds. Due to the greater content of these compounds, the fermented beverage with 10% guabiroba pulp can benefit consumers' health (Table 4). It was possible to verify that the beverage with 10% guabiroba pulp presented 1.61 times more total phenolic compounds and 2.55 times more DPPH and ABTS results than the control beverage

**Table 3.** Results of the chemical composition, pH (mean  $\pm$  standard deviation), and microbiological of the two fermented beverages (control beverage and guabiroba beverage with 10% pulp), both elaborated from whey concentrate 2 (WC2)\*.

		Control beverage	Guabiroba beverage
Composition (g/100 g)	Total solids	28.48 $\pm$ 0.82	28.53 $\pm$ 0.28
	Proteins	2.99 $\pm$ 0.08	2.70 $\pm$ 0.15
	Mineral salts	1.66 $\pm$ 0.11	1.50 $\pm$ 0.08
pH	After the fermentation process	4.79 <sup>a,A</sup> $\pm$ 0.01	4.48 <sup>b,D</sup> $\pm$ 0.01
	Beverages	4.67 <sup>a,B</sup> $\pm$ 0.01	4.56 <sup>b,C</sup> $\pm$ 0.01
Color parameters	$L^*$	39.04 <sup>a</sup> $\pm$ 0.03	35.13 <sup>b</sup> $\pm$ 2.21
	$a^*$	-3.86 <sup>b</sup> $\pm$ 0.02	4.52 <sup>a</sup> $\pm$ 0.01
	$b^*$	7.12 <sup>b</sup> $\pm$ 0.04	21.31 <sup>a</sup> $\pm$ 0.01
	$\Delta E^*$		16.53
Total count of cultures (CFU/g)	Probiotic culture	5.85 $\times 10^{8a}$	3.95 $\times 10^{8b}$
	<i>Streptococcus thermophilus</i>	5.71 $\times 10^{8a}$	4.01 $\times 10^{8b}$

\*The probiotic culture count comprises the count of *Bifidobacterium* BB-12 and *Lactobacillus acidophilus* LA-5, where the total count of cultures was expressed as log colony-forming units per gram of beverage ( $\log_{10}$  CFU/g); <sup>a,b</sup>Within a row, different superscript lowercase letters denote significant differences ( $p < 0.05$ ) between samples; <sup>A-D</sup>Within a row or column, different superscript uppercase letters indicate significant differences ( $p < 0.05$ ) between samples.



**Figure 2.** (A) Control fermented beverage and (B) guabiroba fermented beverage (10% pulp) made from whey concentrate 2 (WC2), (C) "Guabiroba" fruit (*Campomanesia xanthocarpa* O. Berg), and (D) "Guabiroba" pulp.

**Table 4.** Results (mean  $\pm$  standard deviation) of and carotenoid content of two fermented beverages (control beverage and guabiroba beverage with 10% pulp), both elaborated from whey concentrate 2 (CW2).

		Control beverage	Guabiroba beverage
Antioxidant activity	Total phenolic (mg GAE/mL)	1.68 $\pm$ 0.25	2.70 $\pm$ 0.36
	DPPH ( $\mu\text{mol TE/L}$ )	601.45 <sup>b,B</sup> $\pm$ 70.41	1533.67 <sup>a,A</sup> $\pm$ 117.95
	ABTS ( $\mu\text{mol TE/L}$ )	681.40 <sup>b,B</sup> $\pm$ 89.10	1741.44 <sup>a,A</sup> $\pm$ 108.23
Carotenoids ( $\mu\text{g}/100\text{mL}$ )	$\beta$ -Carotene	75.90 $\pm$ 0.04	200.82 $\pm$ 0.05
	$\alpha$ -Carotene	70.27 $\pm$ 0.32	185.89 $\pm$ 0.06
	$\gamma$ -Carotene	63.47 <sup>b</sup> $\pm$ 7.28	167.91 <sup>a</sup> $\pm$ 14.01
	$\beta$ -Cryptoxanthin	82.46 <sup>b</sup> $\pm$ 9.47	218.16 <sup>a</sup> $\pm$ 17.03

mgGAE/mL: milligrams of gallic acid per milliliter;  $\mu\text{mol TE/L}$ : micromole of Trolox equivalents per liter; <sup>a,b</sup>Within a row, different superscript lowercase letters denote significant differences ( $p < 0.05$ ) between samples; <sup>A,B</sup>Within a row or column, different superscript uppercase letters indicate significant differences ( $p < 0.05$ ) between samples.

(without the addition of pulp). Thus, the antioxidant potential was also found in the beverage made only with CW2. According to Arranz et al. (2019), whey proteins can exhibit antioxidant activity. Bovine whey proteins are rich in branched chains and sulfur-containing amino acids. Several studies have evaluated whey proteins as antioxidants. They could potentially be used in beverage formulations to deliver much-needed protein and serve to boost the antioxidant intake levels of the elderly consumer (Arranz et al., 2019). Bielecka et al. (2022) stated that histidine and other hydrophobic amino acids represent the antioxidant activity of whey. Peptides with high antioxidant capacity are released during whey protein hydrolysis. It is noteworthy that selected bacterial strains and enzymes can be used to stimulate protein hydrolysis and the synthesis of biologically active peptides to design novel products with proven health benefits. Therefore, fermented products are classified as a good source of bioactive peptides (Bielecka et al., 2022). Rosa et al. (2023) affirmed that probiotic strains might show different metabolic activities during fermentation, further concentrating on bioactive peptides. These authors observed that probiotic addition, regardless of the probiotic strain, increased the antioxidant activities of whey beverages. More specifically, Rosa et al. (2023) verified that *Lactobacillus* LA-5 and *Bifidobacterium* BB-12 showed higher  $\alpha$ -glucosidase inhibition, improvements in the high saturated hypercholesterolemic index, and peptides with angiotensin-converting-enzyme-inhibitory, antimicrobial, immunomodulatory, and antioxidant activities. Their findings suggest that probiotic fermented whey beverages may exert antioxidant properties. Rosa et al. (2023) affirmed that the products should be processed with LA-5 or BB-12 for better biological activity. However, the increase in antioxidant activity was notable for the guabiroba beverage with 10% pulp. Raphaelli et al. (2021) described that guabiroba has good functional and nutritional properties due to its high antioxidant potential. Prestes et al. (2022), evaluating the influence of guabiroba pulp added to fermented milk, observed that the addition of compounds with a potential prebiotic function, such as the phenolic compounds of guabiroba, enhances the higher selective fermentation, resulting in a higher concentration of antioxidant metabolites. Therefore, bioactive substances present in plants have become popular as complementary or alternative therapeutic agents to manage or treat chronic diseases (Ning et al., 2021). Farias et al. (2020) reported that the genus *Campomanesia*, which comprises guabiroba, includes species that are used against fever, dysentery, and urinary tract diseases and is considered a fruit that contains bioactive compounds.

Other bioactive compounds present in guabiroba are carotenoids. Therefore, it can also be observed in Table 4 the results obtained for the carotenoid contents, such as  $\beta$ -carotene,  $\alpha$ -carotene,  $\gamma$ -carotene, and  $\beta$ -cryptoxanthin of the fermented beverage control and the fermented beverage incorporated with 10% guabiroba pulp. Carotenoids are natural pigments present in plants that have chemical structures that differ in their functional groups, which allows them to be classified into two groups: xanthophylls that contain oxygen as an active group and carotenes that have only the hydrocarbon, without the presence of no functional group. Commonly encountered oxygen substituent groups are hydroxyls, for example,  $\beta$ -cryptoxanthin. Carotenoids also promote health benefits, even when present in pulps added to

products. Carotenoids are phytochemicals among the most important food constituents helping to prevent cancer, in addition to being absorbed and converted by the human body into vitamin A, such as  $\beta$ -carotene. Vitamin A plays an important role in the human body because it directly participates in the chemistry of vision, cell differentiation, the reproduction system, growth, and the formation of organs and bones (Farias et al., 2020).

The fermented beverage incorporated with 10% guabiroba pulp presented an increase of 164% for each evaluated carotenoid compared with those offered by the control beverage. Stinco et al. (2019) assessed the bioaccessibility of 22 commercial milk and fruit beverages made in Spain, and all of them had  $\beta$ -carotene (2.50–567.70  $\mu\text{g}/100\text{ mL}$ ) in their composition, with 12 containing  $\alpha$ -carotene (0.40–646.00  $\mu\text{g}/100\text{ mL}$ ) and 9 containing  $\beta$ -cryptoxanthin (2.90–475  $\mu\text{g}/100\text{ mL}$ ). These authors concluded that the great variability of carotenoids is related to the food matrix. Even so, when comparing the values obtained in this study with the commercial data and the control sample, verifying that the carotenoid contents were relevant was possible. Finally, this study provides a new and unprecedented approach to a functional product obtained from concentrated whey from the second stage of freeze concentration in preparing a fermented beverage with 10% guabiroba pulp. It is noteworthy that whey beverages are important for dairy industries due to their economic and environmental values because whey is a by-product of the low cost of the cheese industry. In addition, the reduced-cost alternative to conventional yogurt has increased fermented whey beverage consumption. On the contrary, the growing interest in products derived from native plants has gained much attention internationally, which is very important for countries like Brazil, with rich biodiversity. This pioneering study may make it possible in the future to use a by-product of the dairy industry in the preparation of this innovative fermented beverage with potential functional properties. Future studies still must be carried out focusing on the viability of probiotic cells by *in vitro* methods and sensory analysis for the overall evaluation of the product by future consumers.

#### 4 CONCLUSION

The best performance concerning the total solids, proteins, and mineral salt contents was found for the concentrated whey from the second stage of freeze concentration, which was used for elaborating both probiotic fermented beverages (without and with 10% guabiroba pulp). Both fermented beverages could be considered potential probiotic products because their count was  $\geq 10^8$  CFU/g. It was possible to verify that the incorporation of 10% guabiroba pulp in the probiotic fermented beverage was not enough to modify their contents of total solids, proteins, and minerals. However, a decrease in pH values was observed, besides changing their colors to an orange hue with a reduced luminosity. However, the beverage with the addition of 10% guabiroba pulp showed a great advantage, which could classify it as a functional product, because it presented 1.61 times more phenolic compounds and an increase of 164% for each of the evaluated carotenoids (namely,  $\beta$ -carotene,  $\alpha$ -carotene,  $\gamma$ -carotene, and  $\beta$ -cryptoxanthin) when compared with the beverage without the addition of guabiroba pulp. Finally, it could be

concluded that it was possible to elaborate a functional fermented beverage with 10% guabiroba pulp due to the probiotic count and improving its nutritional properties.

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