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Characterization of a Probiotic Carbonated Beverage (*Lactobacillus casei* ssp. *rhamnosus*) Made from Sweet Whey Permeate and Fortified with Andean Blueberry (*Vaccinium meridionale* Swartz)

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Abstract

Sweet whey permeate derived from the cheese industry is a product resulting from a membrane separation process (ultrafiltration). It is rich in minerals, vitamins and lactose, which makes it a valuable substrate for functional products within a circular-economy framework. This study aimed to characterize a probiotic (*Lactobacillus casei* ssp. *rhamnosus*) carbonated beverage based on sweet whey permeate, enriched with Andean blueberry (*Vaccinium meridionale* Swartz) and sweetened with concentrated sugarcane juice (*Saccharum officinarum* L.). The beverages were prepared in two batches and standardized to 8.0°Bx soluble solids, using sweet whey permeate obtained by ultrafiltration (85%), whole Andean blueberry (3%), concentrated panelera cane juice (1.76%), citrate (0.05%), stabilizer (0.2%) and water (9.99%) for three of the treatments (F1, F2, and F3), while the remaining treatment (F4) contained no stabilizer and 10.04% water. The beverages were evaluated by semi-trained and trained sensory panels and characterized chemically, physicochemically, microbiologically and in terms of antioxidant capacity. The formulations differed significantly in soluble solids, pH, total acidity, zeta potential, total phenols and antioxidant capacity, but not in CO_2 volume or preference. F1 (ζ = −34.022 ± 1.6483 mV) and F2 (ζ = −34.844 ± 3.2859 mV) showed greater colloidal stability than F3 (ζ = −23.866 ± 1.5011 mV) and F4 (ζ = −15.166 ± 0.4725 mV), as more negative zeta potential values indicate stronger electrostatic repulsion between suspended particles. Total polyphenols ranged from 64.56 ± 1.06 to 87.48 ± 2.12 mg GAE/100 g of sample, and ABTS antioxidant capacity ranged from 35.38 ± 0.59 to 60.14 ± 12.05 mg Trolox/100 g of sample. Forced convection drying of the whole fruit resulted in a 13% loss of vitamin C. All beverages complied with Colombian regulations and Codex Alimentarius specifications and were well accepted by the sensory panels. These findings confirm the value of whey permeate as a raw material for the healthy food and beverage industry.

Keywords: agraz; Andean blueberry; whey; permeate; probiotic; sensory analysis; antioxidant capacity

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1. Introduction

Carbonated beverages are non-alcoholic drinks in which carbon dioxide (CO_2) is dissolved to produce effervescence. According to The Business Research Company (2025), the market for these beverages has grown steadily in recent years, reaching USD 122.34 billion in 2024 and is projected to reach USD 126.15 billion in 2025 [1]. This growth has been driven by consumer demand for convenience, the globalization of beverage brands, marketing and brand-development strategies, lifestyle trends and a dynamic consumer culture. As manufacturers seek to diversify the ingredients used in carbonated beverages and respond to the growing demand for healthier products, circular-economy principles and innovation trends in the dairy sector, which promote the valorization of dairy by-products, have positioned sweet whey as a valuable raw material and functional liquid with wide-ranging applications, primarily in the food, pharmaceutical and biotechnology industries [2–4].

Annual whey production is estimated to exceed 10 billion tons, of which only 50% is processed [5]. Whey can be valorized into environmentally safe foods and ingredients for various industries, including a functional beverage based on whey and carrots [6], functional probiotic carbonated whey beverages [7], whey and fruit beverages [8], fruit-flavored probiotic beverages based on goat whey fermented with *Bifidobacterium bifidum* or *Lactobacillus acidophilus* [9], yogurt flavored with Andean blueberry (*Vaccinium meridionale* Sw.) [10], an isotonic beverage based on sweet whey permeate with added cape gooseberry (*Physalis peruviana* L.) [11] and functional ice creams [12]. At the industrial level, membrane technology (ultrafiltration) has become a key processing step for concentrating whey proteins and obtaining whey permeate. Whey permeate has gained importance in recent years, with a market size of USD 877.00 million in 2025, driven by the growing demand for functional foods and beverages [13].

Probiotics are important for gut health and immune function because they help maintain the natural balance of the intestinal microbiota and contribute to the treatment of various diseases [14–17]; consequently, they play a key role in the development of functional foods and beverages [18–23]. One such probiotic is *Lactobacillus casei* ssp. *rhamnosus*, a facultatively heterofermentative species [24]. It is a Gram-positive, non-spore-forming, non-motile, catalase-negative bacterium that is facultatively anaerobic or microaerophilic. Although it is mesophilic, some strains can grow at temperatures below 15 °C or above 40 °C. Its growth requires vitamins such as folic acid, riboflavin, niacin and pantothenic acid, as well as calcium [25], and an optimal initial pH of 4.5 to 6.4 [26].

Whey permeate has been used to produce carbonated and isotonic beverages [11,27–31], often formulated with ingredients valued for their polyphenol content, antioxidant activity and associated health benefits. Among these ingredients, fruits of the genus *Vaccinium* have been widely studied [32–49]. In particular, the agraz, mortiño or Andean blueberry (*Vaccinium meridionale* Swartz), which has been characterized in several studies [50–57], is currently being investigated for use in the beverage industry because of the proven health benefits of its bioactive compounds [50,58–65].

Vaccinium meridionale Swartz, also known as Andean blueberry or Colombian bilberry and locally as “agraz” or “mortiño”, belongs to the genus *Vaccinium* of the family Ericaceae. It is distinguished by its high functional value and its high concentrations of anthocyanins, phenolic compounds, antioxidants and vitamins; it is therefore considered a “superfruit” with the capacity to counteract oxidative stress and improve health, and it offers considerable potential for the food industry. Similarly, sugarcane honey has great potential as an ingredient in various industries, particularly as a natural sweetener for foods and beverages. On a dry matter (DM) basis, it contains approximately 75% simple sugars, mainly sucrose (62.50%), glucose (14.38%) and fructose (14.39%), as well as protein and nitrogen (5.45% and 0.87% of DM, respectively), ash (16.61% of DM), potassium (K^+:

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45.209 ± 34.94 mg/kg), magnesium (Mg^{2+}: 2.109 mg/kg), calcium (Ca^{2+}: 2.050 mg/kg), sodium (Na^{+}: 551 mg/kg), phosphorus (PO_4^{3-}: 260 mg/kg), iron (Fe^{2+}: 166 mg/kg), manganese (Mn^{2+}: 23 mg/kg), zinc (Zn^{2+}: 6 mg/kg) and copper (Cu^{2+}: 4 mg/kg) [66].

Taken together, these considerations indicate that developing natural carbonated beverages from sweet whey with functional and probiotic properties is a technical and scientific challenge that aligns with circular-economy principles and helps mitigate the negative environmental impacts of cheese production in the dairy agro-industry. This research is relevant in the academic, scientific and technological spheres for several reasons: it uses promising plant species such as mortiño or agraz, whose high polyphenol content and antioxidant capacity provide health benefits to consumers; it reduces negative environmental impacts by valorizing whey; and it incorporates natural ingredients such as concentrated panelera cane juice as a sweetener and *Lactobacillus casei* ssp. *rhamnosus* as a probiotic culture. Moreover, information on the development of natural carbonated beverages in Colombia remains scarce. This led to the following research question: can forced carbonation be used to develop a functional beverage based on sweet whey permeate and whole Andean blueberry that incorporates probiotic properties while ensuring physicochemical stability, microbiological viability and adequate sensory and techno-functional attributes, in compliance with Colombian regulations and legislation?

To upgrade the sweet whey generated by fresh-cheese manufacturers to food-grade quality while minimizing its environmental impact, this study evaluated the physicochemical, sensory and microbiological quality of a probiotic (*Lactobacillus casei* ssp. *rhamnosus*) carbonated beverage based on sweet whey permeate, enriched with Andean blueberry (*Vaccinium meridionale* Swartz) and sweetened with concentrated panelera cane juice (*Saccharum officinarum* L.).

2. Materials and Methods

The raw materials used to develop the probiotic carbonated beverage based on sweet whey permeate and enriched with Andean blueberry were obtained and processed as described below.

2.1. Raw Materials and Their Processing

2.1.1. Sweet Whey Permeate

Sweet whey from fresh-cheese production, supplied by AURALAC S.A.S. (ANT, COL), was used to obtain the sweet whey permeate. The whey was ultrafiltered in a membrane separation system at the Dairy Products Laboratory of the National University of Colombia, Medellín Campus.

Preliminary treatment of the whey:

The sweet whey was first filtered through a mesh with a pore diameter of 0.1 mm to retain suspended particles and fat and was then centrifuged at 40 °C in a Westfalia skimmer (8050 rpm). After the fat and solids had been separated, the whey was pasteurized at 62 °C for 30 min in a batch pasteurizer (The Creamery Package Pasteurizer, Serial 8015, MFG Company, CHI, USA) and cooled to 45 °C for ultrafiltration.

Permeate Recovery by Membrane Filtration (Ultrafiltration—UF).

The sweet whey permeate was obtained using a pilot-scale membrane separation system (PERINOX, SP). A spiral-wound polyether sulfone membrane (3838 K131-HYV) with a molecular weight cut-off of 10,000 Da was used to produce the ultrafiltration permeate (UFP). The system was fed with 300 L of sweet whey (batch mode) and operated at a transmembrane pressure of 2 bar and a constant temperature of 45 °C. This temperature was selected because it is below both the maximum permissible temperature for the membrane (50 °C) and the denaturation temperature of whey proteins, and above the bacterial

growth temperature (37 °C). The volumetric concentration factor (VCF) was set at 18; these operating conditions were obtained through the optimization reported by Agudelo et al. [20].

Lactose hydrolysis of the permeate

Lactose in the ultrafiltration permeate (UFP) was hydrolyzed with β-galactosidase (lactase) from *Bacillus licheniformis* (Saphera® 2600 L, Novozymes (CPH, DK)) at 4 °C for 16 h, at the dosage specified in the technical data sheet provided by the manufacturer (Interenzimas S.A.S., BOG, COL).

The degree of hydrolysis (%H) was calculated using the following expression [67]:

$$\%H = \frac{Ci - Cf}{Ci} x100$$

where *Ci* is the initial lactose concentration (mg/mL) in the unhydrolyzed sample and *Cf* is the final lactose concentration (mg/mL) in the hydrolyzed sample.

2.1.2. Andean Blueberry (*Vaccinium meridionale* Swartz)

Commercially ripe Andean blueberry (*Vaccinium meridionale* Swartz) fruits were purchased at the local market (Medellín, Colombia) and selected by ripeness in accordance with Colombian standard NTC6391 [68] and the classification of Buitrago et al. [69], which is based on skin color: 3 (red), 4 (reddish purple) and 5 (dark purple). The whole fruit (pulp, skin and seeds) was used. Leaves and stems were removed manually, and the fruits were washed with potable water and disinfected with 100 ppm sodium hypochlorite [56,70]. The fruits were then ground whole (Talsa Ll30 industrial blender, MED, COL) and dried by forced convection at 55 °C with an air velocity of 0.5 m/s (Memmert, UNE 600, SC, DE) in a 3 mm layer until the moisture content was below 13% w.b. The dried material was milled (IKA MF 10.2 impact mill, STF, DE) and sieved for 15 min through a 2 mm sieve in a Ro-Tap shaker (model RX29), W.S. Tyler, OH, USA; mesh sizes 30 to 60). The product was stored frozen (−18 °C) until analysis and use in the formulations.

2.1.3. Concentrated Panelera Cane Juice (*Saccharum officinarum* L.)

Concentrated panelera cane juice (75°Bx) was supplied by a certified sugarcane mill in the municipality of Girardota (ANT, COL).

2.1.4. Probiotic Microorganism *Lactobacillus casei* ssp. *rhamnosus*

The freeze-dried probiotic culture *(Lactobacillus casei* subsp. *rhamnosus*) BIO-RHM was supplied by Igea Colombia S.A.S., BOG, COL, with a cell count concentration of ≥3000 × 10^9 CFU/10 UD (100–200 L).

2.2. Characterization of Raw Materials

The raw materials used to develop the beverage were subjected to the physicochemical and microbiological analyses listed in Table 1.

Table 1. Analytical methods for the physicochemical and microbiological characterization of the raw materials (whole and dehydrated powdered Andean blueberry, hydrolyzed ultrafiltration permeate and concentrated panelera cane juice).

Parameter	Whole Andean Blueberry (Pulp, Skin, Seeds)—Fresh Macerated Fruit	Dehydrated Powdered Andean Blueberry	Hydrolyzed Ultrafiltration Permeate (HUFP)	Concentrated Panelera Cane Juice **
Moisture Content (% w.b.)	AOAC 925.45A/90 Gravimetric oven drying method [71]	AOAC 925.45A/90 Gravimetric oven drying method [71]		AOAC 925.45A/90 Gravimetric oven drying method [71]
Total Acidity (% citric acid)	AOAC 947.05/90 [71]	AOAC 947.05/90 [71]	AOAC 947.05/90 [71]	AOAC 947.05/90 [71]
pH	AOAC 981.12/90 [71]	AOAC 981.12/90 [71]	AOAC 981.12/90 [71]	AOAC 981.12/90 [71]
Water activity—aw (25 °C)	AOAC/98 [72]	AOAC/98 [72]		AOAC/98 [72]
Soluble Solids (°Bx)	Refractometry AOAC method 932.12/90 [71]	Refractometry AOAC method 932.12/90 [71]	Refractometry AOAC method 932.12/90 [71]	Refractometry AOAC method 932.12/90 [71]
Refractive Index		Refractometry AOAC method 932.12/90 [71]		
Freezing Point (°C)			Cryoscopy method NTC5135 [73]	
Density (Kg/m^3)				AOAC 33.2.03 (925.22) [74]
Total Polyphenols (mg GAE/100 g sample)	Folin–Ciocalteu spectrophotometric method [75]	Folin–Ciocalteu spectrophotometric method [75]		Folin–Ciocalteu spectrophotometric method [75]
ABTS Antioxidant Capacity (mg Trolox/100 g sample) *		ABTS 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (method described in [76], with some modifications made by [77])		ABTS 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (method described in [76], with some modifications made by [77])
Antioxidant Capacity by DPPH (mg Trolox/100 g sample)		DPPH method (1,1-diphenyl-2-picrylhydrazyl) [78], with some modifications by [77]		DPPH method (1,1-diphenyl-2-picrylhydrazyl [78], with some modifications by [77]
Antioxidant Capacity by ORAC (µmol TE/g sample)		Oxygen radical scavenging, using fluorescence as a probe (AOAC method 2012.23) [79]		
CIEL*a*b* Color System (L*, a*, b*)	Colorimetric method (Konica Minolta CR-400 series colorimeter), NTC5622 [80]	Colorimetric method (Konica Minolta CR-400 series colorimeter), NTC5622 [80]	Colorimetric method (Konica Minolta CR-400 series colorimeter), NTC5622 [80]	Colorimetric method (Konica Minolta CR-400 series colorimeter), NTC5622 [80]

Total Sugars (g/100 g)		UV–vis spectrophotometric method (MRE-001 V09:2018, Section 5:18) [81]	UV–vis spectrophotometric method (MRE-001 V09:2018, Section 5:18) [81]	UV–vis spectrophotometric method (MRE-001 V09:2018, Section 5:18) [81]
Lactose (g/L)			High-performance liquid chromatography (HPLC) [82,83]	
Glucose (g/L)	High-performance liquid chromatography (HPLC) [82,83]		High-performance liquid chromatography (HPLC) [82,83]	High-performance liquid chromatography (HPLC) [82,83]
Galactose (g/L)			High-performance liquid chromatography (HPLC) [82,83]	
Fructose (g/100)	High-performance liquid chromatography (HPLC) method [82,83]			High-performance liquid chromatography (HPLC) method [82,83]
Sucrose (g/100)	High-performance liquid chromatography (HPLC) method [82,83]			High-performance liquid chromatography (HPLC) method [82,83]
Crude Protein (g/100 g)		Kjeldahl volumetric method, with conversion factor 6,25—AOAC 920.05/90 [71]	Kjeldahl volumetric method, with conversion factor 6,25—AOAC 920.05/90 [71]	Kjeldahl volumetric method, with conversion factor 6,25—AOAC 920.05/90 [71]
Ash (g/100 g)		AOAC method 945.46/90 [71]—ISO5984:2002 [84]	AOAC method 945.46/90 [71]—ISO5984:2002 [84]	AOAC method 945.46/90 [71]—ISO5984:2002 [84]
Total fat (g/100 g)		Soxhlet gravimetric method	AOAC Gerber method 200.18/2001 [85]	
Total non-enzymatic dietary fiber (g/100 g)		AOAC method 993.21:1996 [86]		
Gross calorific value (cal/g)		Calorimetric method (PRE-012 V00:2018) [87]	Calorimetric method (PRE-012 V00:2018) [87]	Calorimetric method (PRE-012 V00:2018) [87]
Citric acid (g/100 g)		High-performance liquid chromatography (HPLC) method [82,83]	High-performance liquid chromatography (HPLC) method [82,83]	
Malonic acid (g/100 g)		High-performance liquid chromatography (HPLC) method [82,83]		

Lactic acid (g/L)		High-performance liquid chromatography (HPLC) method [82,83]	
Propionic acid (g/L)		High-performance liquid chromatography (HPLC) method [82,83]	
Formic acid (g/L)		High-performance liquid chromatography (HPLC) method [82,83]	
Tartaric acid (g/100 g)	High-performance liquid chromatography (HPLC) method [82,83]		
Vitamin C (µg/100 g)	Liquid chromatography (HPLC) method AOAC2012.11:2013 (modified) [88]		
Calcium (g/100 g)	Atomic absorption spectrophotometric method, Colombian Technical Standards, NTC 5151 [89] and NTC 4981 [90]	Atomic absorption spectrophotometric method, Colombian Technical Standards, NTC 5151 [89] and NTC 4981 [90] (mg/kg)	Atomic absorption spectrophotometric method, Colombian Technical Standards, NTC 5151 [89] and NTC 4981 [90] (mg/kg)
Magnesium (mg/kg)	Atomic absorption spectrophotometric method, Colombian Technical Standards, NTC 5151 [89] and NTC 4981 [90]	Atomic absorption spectrophotometric method, Colombian Technical Standards, NTC 5151 [89] and NTC 4981 [90]	Atomic absorption spectrophotometric method, Colombian Technical Standards, NTC 5151 [89] and NTC 4981 [90] (mg/kg)
Potassium (g/100 g)	Atomic absorption spectrophotometric method, Colombian Technical Standards, NTC 5151 [89] and NTC 4981 [90]	Atomic absorption spectrophotometric method, Colombian Technical Standards, NTC 5151 [89] and NTC 4981 [90]	Atomic absorption spectrophotometric method, Colombian Technical Standards, NTC 5151 [89] and NTC 4981 [90]
Sodium (g/100 g)	Atomic absorption spectrophotometric method, Colombian Technical Standards, NTC 5151 [89] and NTC 4981 [90] (mg/kg)	Atomic absorption spectrophotometric method, Colombian Technical Standards, NTC 5151 [89]	Atomic absorption spectrophotometric method, Colombian Technical Standards, NTC 5151 [89] and NTC 4981 [90] (mg/Kg)

		and NTC 4981 [90] (mg/kg)	
Iron (mg/100 g)	Atomic absorption spectrophotometric method, Colombian Technical Standards, NTC 5151 [89] and NTC 4981 [90]		
Mesophilic aerobic microorganism count (CFU/g or mL)	ISO 4833-1:2013 method [91]	ISO 4833-1:2013 method [91]	ISO 4833-1:2013 method [91]
Mold and yeast (CFU/g or mL)	ISO 21527-1:2008 method [92]		
Total coliform count/g	NTC 4458:2018 method [93]	NTC4458:2018 method [93]	NTC 4458:2018 method [93]
Escherichia coli count (CFU/g or mL)	NTC 4458:2018 method [93]	NTC4458:2018 method [93]	NTC 4458:2018 method [93]

* A 0.5 g sample was diluted to 10 mL with methanol, and the solution was stored in an amber bottle under refrigeration (4 °C) until analysis. ** Concentrated panelera cane juice with a soluble solids content of 65–75°Bx (concentrated at atmospheric pressure). w.b.: wet basis; °Bx: degrees Brix; GAEs: gallic acid equivalents; CFU: colony-forming unit; ABTS: (2′2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic)acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl; ORAC: oxygen radical absorbance capacity; Note: all measurements were performed in triplicate, and results are expressed as mean ± standard deviation.

Probiotic culture (*Lactobacillus casei* ssp. *rhamnosus*)

The viable count of the probiotic culture (*Lactobacillus casei* ssp. *rhamnosus*) used in the beverages was determined as colony-forming units per mL by plate counting on de Man, Rogosa and Sharpe agar (*Lactobacilli* MRS Agar), and the cells were examined in Gram-stained smears (adapted from Marín et al. [94] and Vera et al. [95]).

2.3. Preparation of the Beverage

2.3.1. Formulation of the Beverages

Preliminary tests of the beverage without stabilizers showed phase separation by sedimentation, which indicated that stabilizers were required. Ten beverages were therefore formulated and divided into two batches: Batch 1 (five beverages), without probiotic culture and with different stabilizers (CMC, xanthan gum, a mixture of CMC and xanthan gum, Goma PRO 181 AG, or no stabilizer), and Batch 2 (five beverages), with probiotic culture and stabilized with Goma PRO 181 AG (gellan gum and acacia or Arabic gum) at different concentrations. Four beverages (two from each batch) were selected through sensory analysis by a semi-trained panel. All ten beverages were formulated to 8.0°Bx soluble solids with HUFP (85%), whole Andean blueberry (3%), sodium citrate (except N28; 0.2%) and concentrated panelera cane juice at a fixed level for each batch (Batch 1: 2.645%; Batch 2: 1.603%; these values varied according to the soluble solids content of the concentrated juice). The remaining components varied: Batch 1 was formulated with CMC, xanthan gum and PRO 181 AG gum (A27, CMC 0.05%; B31, xanthan gum 0.05%; C79, CMC 0.025% and xanthan gum 0.025%; M25, PRO 181 AG gum 0.1%), whereas in Batch 2 Goma PRO 181 AG ranged from 0.05% to 0.3% (Z73, 0.05%; M25, 0.1%; T12, 0.2%; S96, 0.3%). In each batch, water was added to make up 100% of the formulation, and beverage N28 was formulated without stabilizers or sodium citrate.

The ingredients were mixed (Turbomix FC Carpigiani, CA2618 Series, BO, IT) at 45 °C, double-filtered and homogenized (St Regis homogenizer, serial 5DD13-2941, CHI, IL, USA) at 103.4–138.0 bar. The mixture was then pasteurized at 62 °C for 30 min in a batch pasteurizer (Pastomaster 120 XPL P, Carpigiani, BO, IT) and rapidly cooled to 37–39 °C. At this temperature, the freeze-dried probiotic culture (*Lactobacillus casei* ssp. *rhamnosus*), suspended in deionized, demineralized water, was added and homogenized, and the mixture was held for 1 h. The mixture was then stored at 4 °C for approximately 24 h before carbonation. The product was bottled in 330 mL amber glass bottles, and samples were taken for sensory, physicochemical and microbiological analyses.

2.3.2. Forced Carbonation of the Beverage

The beverages were carbonated in a hermetically sealed 9.5 L stainless-steel Cornelius© tank. Food-grade CO_2 was used, and its inlet pressure was regulated with a pressure-reducing valve; the carbonated product was dispensed through a food-grade hose fitted with a pressure nozzle. Forced carbonation was carried out to a low (grade 3) to medium (level 2) carbonation level [96,97] for 2 h, in several cycles of storage and agitation, at 4 °C and 3.0 bar, with 9 L of beverage.

The bottles were filled by counter-pressure filling. Once each bottle reached the fill volume (330 mL), it was capped, sealed, checked for gas leaks and immediately stored under refrigeration (4 °C) until the physicochemical, microbiological and sensory analyses were performed.

2.3.3. Sensory Characterization of the Beverages

Evaluation of sensory attributes using a semi-trained panel

A semi-trained panel specializing in dairy products—comprising seven technical experts from the Dairy Products Laboratory at the National University of Colombia, Medellín Campus—selected the four beverages for further analysis. Participation was strictly voluntary and followed the standard quality-control and tasting protocols of the food and beverage industry, posing no health risk. The ten beverages initially produced were evaluated on a five-point hedonic scale. To select the two best beverages from each batch, the scores were analyzed with the nonparametric Friedman test, with each panelist as a blocking factor (5% significance level), followed by all pairwise comparisons with Holm's correction of *p*-values for multiplicity.

After the four beverages with the highest scores and Friedman rank sums (two per batch) had been selected, their sensory characteristics were re-evaluated by a semi-trained panel of 14 members, who rated odor, color, flavor, texture and overall appearance on a scale from 1 (dislike extremely) to 5 (like extremely). Preference was rated on an 8 cm horizontal line (ISO 22935-3 IDF 99-3:2023 [98], ISO 6658 [99], ISO 8586:2023 [100], ISO 8587:2010 [101], ISO8589 [102] and NTC3930:2015 [103]). Each attribute was analyzed using the Friedman test (5% significance level), with each panelist as a blocking factor, and all pairwise comparisons were performed with Holm's correction of *p*-values for multiplicity.

In addition, a quantitative descriptive test was conducted on a rating scale from 1 (absent) to 6 (very strong intensity), together with a preference test on an 8 cm horizontal line. The following attributes and descriptors (adapted from Beucler et al. [28] and consistent with the sensory lexicon for the descriptive analysis of beverages), as well as preference, were evaluated: appearance (presence of foreign matter, froth, bubbles and phase separation); color (brightness, uniformity, characteristic agraz color and atypical colors); odor and flavor (fruity odor, milky color, characteristic agraz odor, atypical odors, and sweet, salty, sour, bitter, milky, characteristic Andean blueberry and atypical flavors); trigeminal sensations, aftertaste and persistence (astringent, refreshing, effervescent and

spicy sensations, aftertaste, persistence 1 and persistence 2); texture (viscosity, fluidity and density); microstructure (smooth or soft, grainy or sandy); and other attributes defined by the evaluator.

Each descriptor was analyzed using the nonparametric Friedman test, with each panelist as a blocking factor (5% significance level), followed by all pairwise comparisons with Holm's correction of *p*-values for multiplicity.

Sensory evaluation with a trained panel

A ranking test was then performed to evaluate differences among the samples in the intensity of a single attribute, several attributes or an overall impression according to a specified criterion (ranking), following NTC 3930:2015 [103]. The test was conducted by a panel of seven trained judges, in accordance with the Colombian Technical Guide GTC 280:2017 [104], to rank the intensity of Andean blueberry flavor in the samples.

Because no order among the products was assumed, the samples were compared using the Friedman test (rank-based analysis of variance), with the following null (H0) and alternative (H1) hypotheses, where X denotes the sample:

H0. $X1 = X2 = X3$

H1. $X1 \leq X2 \leq X3$

2.4. Physicochemical and Microbiological Characterization of the Beverages

The four beverages selected through sensory analysis were evaluated for the physicochemical and microbiological parameters listed in Table 2.

Table 2. Analytical methods used for physicochemical and microbiological characterization of the beverages.

Parameter	Beverages
Total acidity (% citric acid)	AOAC method 947.05/90 [71].
pH	AOAC method 981.12/90 [71].
Soluble solids (°Bx)	Refractometry, AOAC method 932.12/90 [71].
Zeta Potential (ζ) (mV)	Zetasizer light scattering [105], at 25 °C [106,107].
Phase separation	Measurement of the change in height observed in 50 mL graduated cylinders, as described in [108], with some modifications, at refrigeration temperature (4 °C) and over 32 days.
CO_2 volume (g/L)	The amount of CO_2 dissolved in the beverage was determined based on the method proposed by NTE INEN 1082 (1984) [109] and compared with the requirements of NTC 2740 (2020) [97], with the pressure and temperature of the carbonated beverage recorded for this purpose.
Total polyphenols (mg GAE/100 g sample)	Folin–Ciocalteu spectrophotometric method, described in [75].
Antioxidant capacity by ABTS (mg Trolox/100 g sample)	ABTS method (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid). The ABTS method described in [76] was used, with some modifications made by [77].
Antioxidant capacity by DPPH (mg Trolox/100 g sample)	DPPH method (1,1-diphenyl-2-picrylhydrazyl). (77, 78)
Mesophilic aerobic microbial count (CFU/g or mL)	ISO 4833-1:2013 method [91].
Mold and yeast count (CFU/g or mL)	ISO 21527-1:2008 method [92].
Total coliform count/g	NTC 4458:2018 method [93].
Escherichia coli count (CFU/g or mL)	NTC 4458:2018 method [93].

°Bx: degrees Brix; GAEs: gallic acid equivalents; CFU: colony-forming unit; ABTS: (2′2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic)acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl.

2.5. Microbiological Analyses

The following microbiological analyses were performed: mesophilic aerobic microorganisms (CFU/g or mL; ISO 4833-1:2013 [91]), *Escherichia coli* (CFU/g or mL; NTC 4458:2018 [93]), molds and yeasts (CFU/g or mL; ISO 21527-1:2008 [92]), total coliforms (count/g; NTC 4458:2018 [93]) and *Lactobacillus* (count/g; ISO 15214:1998 [110]).

CFU count of the probiotic culture

Colony-forming units (CFUs) of the probiotic culture in the beverages were counted following procedures adapted, with slight modifications, from Marín et al. [94] and Vera et al. [95]. One milliliter of beverage was added to 9 mL of peptone water and mixed until homogeneous (1:10 dilution), and serial dilutions were prepared up to $1:10^{10}$. De Man, Rogosa and Sharpe agar (*Lactobacilli* MRS Agar) was used as the culture medium. One milliliter of each dilution was placed in a sterile Petri dish, MRS agar was added and mixed, and the plates were left to stand for 10 min until the agar solidified. The plates were incubated at 37 ± 2 °C for 48 h under anaerobic conditions; plates containing 25–250 colonies were counted, and the counts were multiplied by the corresponding dilution factor. In parallel, smears of the colonies were prepared using Gram staining, a basic technique for visualizing bacteria that stains the cell wall violet or red depending on the wall's structural characteristics [111]. The smears were examined under an electron microscope with 40× and 100× objectives to identify the microorganisms.

3. Results and Discussion

3.1. Characterization of Fresh Andean Blueberry (Vaccinium meridionale Swartz)

The macerated fresh fruit (pulp, seeds and peel) contained 9.52 g/100 g glucose, 3.83 g/100 g sucrose and 9.41 g/100 g fructose. The glucose and fructose contents differ by 4.8% and 5.9%, respectively, from those reported by Murcia et al. [112] for *Vaccinium meridionale* Swartz grown in a cold climate. These authors found values of 10% to 14% depending on the growth stage. Vivas et al. [12] reported, for Andean blueberry pulp, a moisture content of 77.64% w.b., a water activity (aw) of 0.98, soluble solids of 13.59°Bx, a pH of 3.0 and a titratable acidity of 1.35% citric acid, with CIELab color parameters typical of dark purple: L* (10.2), a* (3.5) and b* (7.4). These values are similar to those obtained in this study, with differences of less than 0.5% in moisture content, 0.0% in water activity, 2.06% in soluble solids and 1.11% in total acidity. Murcia et al. [112] obtained lower mean values for total soluble solids and pH and a higher total acidity (13.27°Bx, 2.57 and 2.39%, respectively), indicating that the compositional differences may be attributed to the degree of ripeness of the fruit at each growth stage. Changes in pH and total acidity may result from an increase in organic acid content, whereas total soluble solids (TSS) reflect sugar accumulation and, consequently, greater sweetness during ripening. At these stages, polysaccharides synthesized during growth are degraded into simple sugars, while the characteristic tart flavor of Andean blueberry is attributed mainly to malic and citric acids or, in some species, to the additional synthesis of tricarboxylic acids. This uncommon situation would explain the increase in total acidity during Andean blueberry ripening; at more advanced stages, however, acidity decreases as a result of respiration and gluconeogenesis. In their evaluation of freeze-dried lingonberries (*Vaccinium vitis-idaea* L.), Vilkickyte et al. [113] found that sugar content can vary with genotype, with the highest levels observed in berries harvested at full ripeness at the end of the growing season from sunny, well-drained sites with higher solar radiation and ambient temperature.

The whole Andean blueberry (pulp, skin and seeds) had a total polyphenol content of 389.03 ± 97.83 mg GAE/100 g of fresh fruit, ranging from 206.36 ± 5.40 to 528.77 ± 28.34 mg GAE/100 g of fresh fruit. These values differ from those reported for the same fruit by Garzón et al. [51] and Gaviria et al. [53], namely 758.6 ± 62.3 mg GAE/100 g of fresh fruit (48.71% higher than the value obtained here) and 609 ± 39 mg GAE/100 g of fresh fruit (36.12% higher), respectively. For other *Vaccinium* species, such as *V. corymbosum*, *V. ashei* and *V. angustifolium*, Prior et al. [39] reported values between 190 and 473 mg GAE/100 g of fresh fruit (71.98% lower and 21% higher than the value obtained here, respectively) and a value of 525 mg GAE/100 g of fresh fruit (34.95% higher) for *V. myrtillus* from North America. Vasco et al. [43] reported a value of 882 mg GAE/100 g of fresh fruit for *V. floribundum* (126.72% higher than the value obtained here). This high variability in total polyphenol content depends on numerous factors, including genotype, cultivar, growing location, environmental and climatic conditions, soil composition, pre- and post-harvest management, degree of ripeness, extraction method, technological processing and differences among wild clones [32,40,113–120].

Accordingly, reported total polyphenol values (per 100 g of fresh fruit) vary widely, with wild species generally showing higher values than cultivated ones: 424.84–819.12 mg GAE/100 g [34], 251–614 mg GAE/100 g [35], 367–1286 mg GAE/100 g [37], 181 and 390 mg GAE/100 g [39], 414.48 ± 31.90–726.94 ± 66.03 mg GAE/100 g [44], 198 ± 101–525 ± 0.0 mg GAE/100 g [121], 358.7 ± 2.4 mg GAE/100 g [33], 175–351 mg GAE/100 g [122], max. 357 mg GAE/100 g [123], max. 399.3 mg GAE/100 g [41], max. 125.1 mg GAE/100 g [42], 289.5–670.9 mg/100 g [35], 175.8 mg GAE/100 g [124], 190 mg GAE/100 g [45], 208–595.5 mg GAE/100 g [38], 298 mg GAE/100 g [125], 443.6 ± 17 mg GAE/100 g [126] and 151 ± 19 mg GAE/100 g [127]. These differences arise from factors that influence the biosynthesis and accumulation of secondary metabolites (total polyphenols, anthocyanins and antioxidant capacity), such as altitude, cultivation techniques and agronomic management, precipitation, growing location, ambient temperature, wind speed, soil, radiation intensity and genotype [128].

3.2. Characterization of the Dehydrated Whole Fruit Powder

Drying is an important process for plant products, not only for preservation but also for adapting raw materials to further processing, and it has a significant effect on product quality [129]. The food industry therefore faces the challenge of preserving the compositional quality of the fresh product, which has prompted research on various drying methods, including forced-convection drying, in which control of parameters such as product moisture content and drying temperature is essential to obtain stable and technologically viable products [129].

Table 3 presents the composition of the dehydrated whole Andean blueberry powder.

Table 3. Composition of the dehydrated whole Andean blueberry powder.

Parameter	Average Value
Moisture Content (% w.b.)	2.660 ± 0.523
Total acidity (% citric acid)	1.032 ± 0.034
pH	2.66 ± 0.270
Water activity—aw (25 °C)	0.26 ± 0.050
Soluble solids (°Bx)	48 ± 0.000
Refractive index	1.34275
Total polyphenols (mg GAE/100 g sample)	2207.1475 ± 6.87
Antioxidant capacity by ABTS (mg Trolox/100 g sample)	2333.99 ± 5.497
Antioxidant capacity by DPPH (mg Trolox/100 g sample)	2084.21 ± 7.11

Antioxidant capacity by ORAC (μmol TE/g sample)	312.89
L	16.6183 ± 1.129
a*	12.095 ± 1.023
b*	7.908 ± 0.567
Total sugars (g/100 g)	39.2–40.98
Crude protein (g/100 g)	6.05–6.1
Ash (g/100 g)	3.31–3.42
Total fat (g/100 g)	1.2–1.22
Total non-enzymatic dietary fiber (g/100 g)	24.5–24.55
Gross calorific value (cal/g)	3759–3777
Citric acid (g/100 g)	9.77–10.06
Malonic acid (g/100 g)	0.31–1.02
Tartaric acid (g/100 g)	1.54
Vitamin C (mg/100 g)	28.923
Calcium (g/100 g)	0.21–0.24
Magnesium (mg/kg)	688–711
Potassium (g/100 g)	0.865–0.89
Sodium (g/100 g)	0.1–0.13
Iron (mg/100 g)	2.847
Mesophilic aerobic microbial count (CFU/g or mL)	<10
Mold and yeast count (CFU/g or mL)	5
Total coliform count/g	<10
Escherichia coli count (CFU/g or mL)	<10

w.b.: wet basis; °Bx: degrees Brix; GAEs: gallic acid equivalents; CFU: colony-forming unit; ABTS: (2′2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic)acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl; ORAC: oxygen radical absorbance capacity.

As shown in Table 3, the dried Andean blueberry is a highly acidic product, as reflected in its acidity and pH values, a characteristic partly attributable to its high citric acid content, followed by tartaric and malonic acids. It also provides vitamins and minerals of nutritional relevance, such as vitamin C, calcium, iron, potassium, magnesium and sodium. Its moisture content was lower than that reported by Vivas et al. [12] (5.2%), whereas the color parameters L*, a* and b* were similar. For freeze-dried Andean blueberry, Quintero et al. [50] reported higher moisture, fat and carbohydrate contents (14.65 ± 0.13%, 3.48 ± 0.24% and 78.85 ± 0.19%, respectively) than those obtained here for Andean blueberry dehydrated by forced convection, whereas their total protein and ash contents (1.39 ± 0.25% and 1.62 ± 0.03%, respectively) were lower than those found in this study (6.05–6.1 g/100 g and 3.31–3.42 g/100 g). As indicated by Ismail & Göğüş [130], these physical and chemical changes during drying result mainly from water removal, which causes shrinkage and changes in porosity and, more generally, in the macro- and microstructure owing to physical and chemical transformations of the cellular structure. Enzymatic and non-enzymatic browning markedly alter the color. High temperatures and long drying times, particularly in the presence of oxygen, may reduce the vitamin and mineral content of dried fruits. These authors also explain that drying reduces the nutritional value of dried fruits by degrading components such as phenolic compounds and flavonoids and by reducing antioxidant activity. All of these changes depend on the type of fruit, the drying method and the processing conditions.

Vitamin C has low thermal stability and is readily oxidized; therefore, any high-temperature process leads to losses relative to the fresh material, ranging from 20% to 90% depending on temperature, processing time and oxygen exposure [131]. However, Muñoz et al. [132], studying the bioaccessibility of phenolic compounds in fresh and dried

blueberries (*Vaccinium corymbosum* L.), evaluated the effects of preservation methods (freeze-drying, osmotic dehydration and convection drying) on antioxidant capacity (DPPH and ORAC), total polyphenols and total anthocyanins, and found that convection drying best preserved total polyphenol content and antioxidant capacity, whereas freeze-drying and convection drying did not differ significantly in total anthocyanins. Ávila et al. [55] reported 8 mg/100 g of fresh fruit in Andean blueberry packed in plastic crates and stored for 9 days at 2 °C and 90% relative humidity, a difference of 72.34% in vitamin C (ascorbic acid) relative to the value obtained in this study after forced-convection drying (28.923 mg/100 g of fresh fruit). Compared with the fresh fruit (34.2 mg/100 g of product), a 12.5% loss of vitamin C was observed, which may be attributed to variations in temperature, air velocity and relative humidity, as well as to the drying method; reductions of up to 70% have been reported. With respect to freeze-drying, the vitamin C values obtained in this study are less than 30% compared with those reported by Onwude et al. [133], because freeze-drying operates at lower process temperatures. Samples prepared for quantification must also be protected from light, and other factors that affect analytical performance must be considered, such as analyte stability (sensitivity to heat, pH, light, oxygen, metals and enzymes), low analyte concentrations, the diversity of food types, the complexity of the food matrix, interactions between analytes and other components (e.g., proteins, lipids and sugars) and the presence of multiple active forms of the analyte [134].

This loss of vitamin C is a multifactorial phenomenon in which heat- and mass-transfer thermodynamics, degradation kinetics and the biochemistry of plant tissues converge. Ascorbic acid acts as a potent natural water-soluble antioxidant in fresh fruit; however, its unsaturated enediol structure makes it highly susceptible to environmental factors such as oxygen, light, metal ions and, critically, elevated temperature. During forced-convection drying, the fruit is exposed to a continuous flow of mechanically driven hot air, and vitamin C losses result not from a single cause but from the simultaneous action of two main degradation pathways: oxidative (aerobic) degradation and direct thermal degradation. In the oxidative pathway, the circulating hot air provides a constant supply of oxygen in the drying chamber. Ascorbic acid is first reversibly oxidized to dehydroascorbic acid (DHAA), which retains vitamin activity but is thermodynamically unstable and readily undergoes irreversible hydrolysis to 2,3-diketogulonic acid, thereby losing its nutritional value and becoming a precursor of non-enzymatic browning reactions (e.g., polymerization with amino acids). In thermal degradation, the molecules break down directly at high temperatures. Furthermore, transition metals naturally present in plant tissues (e.g., iron and copper) catalyze the oxidation of ascorbic acid through the formation of oxygen free radicals. The internal resistance of the fruit and the retention time also play a role: because of the structural firmness and waxy cuticle of the fruit, internal moisture transfer is slow. Consequently, the process is dominated by the falling-rate drying period, which requires prolonged exposure times when air temperatures are not sufficiently high. At the temperatures used, the instantaneous degradation rate decreases but drying is prolonged, and this extended exposure to oxygen in the convection air leads to cumulative oxidative losses that can be as severe as those caused by intense heat [55,118,133,135,136].

The total polyphenol content obtained in this study (2207.1475 ± 6.87 mg GAE/100 g of fresh fruit; range 897.56 ± 6.22 to 3103.90 ± 52.12 mg GAE/100 g of fresh fruit) was higher than that reported by Antícona et al. [33] for commercial dried blueberries, which ranged from 391.7 ± 2.4 mg GAE/100 g of fresh fruit (red cranberries) to 2336.9 ± 8.7 mg GAE/100 g of dried fruit (black cranberries), and higher than that reported by Quintero et al. [50] (139.29 ± 5.69 mg GAE/g of freeze-dried cranberries). Antícona et al. [33] found that, among fresh blueberries, juices, pulp and dried products, the dried products contained the highest amount of these compounds (1011.5 mg GAE/100 g of dried fruit) compared with blueberry pulp, fresh blueberries and juices (379, 358.7 and 234 mg GAE/100 g,

respectively), and concluded that blueberry variety determines phenolic content, with black blueberries (*V. myrtillus* L.) containing more phenolic compounds than red ones.

Antícona et al. [33] attribute the high total polyphenol concentration in dried blueberries to the considerable water loss during processing, which increases the proportion of solid matter. Howard et al. [135 reported that processing (depulping) of *Vaccinium meridionale* Swartz for gelatin production typically degrades both anthocyanin and non-anthocyanin phenolic compounds and reduces antioxidant activity, a finding corroborated by López et al. [136], who observed that the antioxidant capacity and the anthocyanin and phenolic contents of sour cherry decrease with increasing drying temperature and time. Howard et al. [128] also found that destemming had the greatest impact on anthocyanins, whose total content was significantly higher in the pomace (747.6 ± 59.2 mg cyanidin 3-glucoside (cyn 3-glu)/100 g of fresh pomace) than in the pulp (102.7 ± 8.3 mg cyn 3-glu/100 g of fresh pulp). Abd et al. [137], evaluating changes in total phenolic content (TPC) and antioxidant activity in grapefruit (*Citrus grandis* (L.) Osbeck) peels after oven drying (50 °C and 60 °C) and freeze-drying, observed only a slight difference (4–6%) in TPC between oven-dried and fresh peels, indicating that post-drying methods had little effect on the retention of the antioxidant potential of these by-products. These authors reported that the highest retention of bioactive compounds occurred in fresh peels (437–1876 mg GAE/100 g DM), followed by peels oven-dried at 50 °C and 60 °C (892–1336 mg GAE/100 g DM) and freeze-dried peels (555–1267 mg GAE/100 g DM), and concluded that oven drying was the most suitable post-drying method for retaining bioactive compounds in grapefruit by-products.

Quintero et al. [50] reported an ORAC antioxidant capacity of 637.17 ± 13.03 μmol TE/g DM for freeze-dried Andean blueberry, which is higher than the value obtained in this study (321.44 μmol TE/g DM). This difference is probably because forced convection affects the antioxidant capacity of the fruit more than other drying methods, such as freeze-drying [50,58,129]. Abd et al. [137] noted that most bioactive compounds are sensitive to oxidation, which may occur during drying depending on operating conditions, and that their stability can be affected by oxygen, temperature fluctuations, light, water activity, pH and pro-oxidants (e.g., enzymes, minerals and metals).

Regarding microbiological quality, the product complies with Colombian legislation for this type of product (Resolution 3929 of 2013) [138], with counts of mesophilic microorganisms, molds and yeasts, and total and fecal coliforms within the established limits.

Table 4 presents the characterization of the hydrolyzed ultrafiltration permeate.

Table 4. Characterization of hydrolyzed ultrafiltration permeate (HUFP).

Parameter	Average Value
Total acidity (% lactic acid)	0.066 ± 0.005
pH	6.24 ± 0.125
Soluble solids (°Bx)	5.956 ± 0.540
Total fat (%)	0.3
Lactose (g/L)	0.59
Freezing point (°C)	−0.71717 ± 0.03
L	44.917 ± 0.234
a*	−1.166 ± 0.250
b*	3.856 ± 0.267
Total sugars (g/100 g)	3.715–3.73
Crude protein (g/100 g)	0.15–0.2
Ash (g/100 g)	0.475–0.48
Fat content (g/100 g)	0.3

Gross calorific value (cal/g)	176–193
Citric acid (g/L)	1.52–1.68
Lactic acid (g/L)	0.01
Propionic acid (g/L)	0.95–0.97
Formic acid (g/L)	0.42
Calcium (mg/kg)	290–314
Magnesium (mg/kg)	57
Potassium (g/100 g)	0.105–0.11
Sodium (mg/kg)	419–485
Mesophilic aerobic microorganism count (CFU/g or mL)	4500
Total coliform count/g	195
Escherichia coli count (CFU/g or mL)	<10

°Bx: degrees Brix; CFU: colony-forming unit.

The values in Table 4 are similar to those reported by Pérez et al. [139] and Vargas et al. [30] for unhydrolyzed permeates with respect to total protein (≤2.5%), ash (0.481 ± 0.032), calcium (283.063 ± 27.472 mg/kg), magnesium (61.813 ± 5.256 mg/kg), potassium (0.14 ± 0.10% w/w), total acidity (0.081 ± 0.004% lactic acid), pH (6.47 ± 0.15) and soluble solids (5.50 ± 0.05°Bx), with percentage differences of 1.26% lower for ash, 2.39% higher for calcium, 8.44% lower for magnesium, 27.27% lower for potassium, 22.72% higher for acidity, 3.69% higher for pH and 7.66% lower for soluble solids. Beucler et al. [28] reported, for a whey permeate, a fat content below 0.1%, 260 ± 100.4 mg/L calcium, 1340 ± 509.5 mg/L potassium, 59.8 ± 23 mg/L magnesium, 450 ± 195.7 mg/L sodium, 335 ± 133.3 mg/L total phosphates, 0.175 ± 0.095 mg/L zinc, a mean total protein content of 2.48 ± 1.09 g/L, total solids of 4.2 ± 1.6%, a mean pH of 6.45 ± 0.08 and an initial glucose content of 0.8 ± 1.0 g/L. These data are largely consistent with those of the present study, except for potassium and glucose, which can be explained by differences in the type and initial composition of the whey used.

The color parameters (L*, a*, b*) indicate a yellowish-green hue (negative a* = green; positive b* = yellow) and an intermediate L* value, similar to those reported in [140]. Regarding caloric content, Jelen et al. [141] indicate that products containing large amounts of whey can have a high caloric content owing to the presence of lactose. When the product is hydrolyzed, its caloric content is reduced, as is the case with the addition of fructose or other non-caloric sweeteners. These authors reported values of 49–58 kcal/100 g for beverages formulated with 80–85.3% whey. As expected, the value obtained for the hydrolyzed ultrafiltration permeate is much lower than those reported for these whey-based beverages.

Table 5 presents the sugar contents of the unhydrolyzed (UFP) and hydrolyzed (HUFP) permeates.

Table 5. Sugar content in whey permeate.

	Glucose (g/L)	**Lactose (g/L)**	**Galactose (g/L)**
Unhydrolyzed permeate (UFP)	0	46.44	0
Hydrolyzed permeate (HUFP)	23.07	0.59	22.72

The degree of hydrolysis of the ultrafiltration permeate under refrigeration (4 °C) for 24 h exceeded 85%, as evidenced by the glucose and galactose contents relative to the lactose content (Table 5), which makes it an ideal raw material for products intended for individuals with lactose intolerance. When β-galactosidase is added and allowed to act for a given period, whether under refrigeration, as in this case, or at higher temperatures, it cleaves the glycosidic bond of the disaccharide lactose to form the monosaccharides

glucose and galactose, provided that conditions are suitable for its activity (pH, temperature, reaction time, initial substrate and enzyme concentrations and ionic strength) [142–144]. For the UFP, Vargas et al. [30] reported a lactose content 7.77% higher (50.052 ± 0.134 g/L). Beucler et al. [28], who produced a beverage from whey permeate, reported a glucose content (0.8 ± 1.0 g/L) higher than that found in this study (0.0 g/L) and, in the hydrolyzed permeate (35 °C, 3 h of hydrolysis, Maxilac® enzyme), a similar value (23.4 ± 5.1 g/L).

3.3. Characterization of Concentrated Panelera Cane Juice (Saccharum officinarum L.)

Table 6 presents the physicochemical and microbiological characteristics of the concentrated panelera cane juice used as a sweetener.

Table 6. Characterization of concentrated panelera cane juice.

Parameter	Average Value
Moisture content (% d.b.)	27.732 ± 0.327
Total acidity (% citric acid)	0.487 ± 0.0768
pH	5.593 ± 0.0152
Water activity-aw (25 °C)	0.832 ± 0.180
Soluble solids (°Bx)	69.866 ± 0.251
Density (kg/m^3)	1.347 ± 0.001
Total phenols (mg GAE/100 g sample)	536.64 ± 1.44
Antioxidant capacity by ABTS (mg Trolox/100 g sample at w.b.)	369.42 ± 6.40
Antioxidant capacity by DPPH (mg Trolox/100 g sample at w.b.)	308.80 ± 7.86
L	23.298 ± 0.479
a*	3.882 ± 0.827
b*	3.06 ± 0.343
Total sugars (g/100 g)	67.92
Crude protein (g/100 g)	0.6
Ash (g/100 g)	0.79
Gross calorific value (cal/g)	2714
Calcium (mg/kg)	131
Magnesium (mg/kg)	157
Potassium (g/100 g)	0.19
Sodium (mg/kg)	126
Mesophilic aerobic microbial count (CFU/g or mL)	165
Total coliform count/g	<10
Escherichia coli count (CFU/g or mL)	<10

d.b.: dry basis; °Bx: degrees Brix; GAEs: gallic acid equivalents; CFU: colony-forming unit; ABTS: (2′2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic)acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl.

As shown in Table 6, concentrated panelera cane juice provides substantial amounts of energy, minerals and sugars and is a significant source of polyphenols and antioxidant capacity. The values reported by Delgado et al. [145] differ from those obtained in this study, with differences of 13.074% (higher), >100% and 17.55% (lower) in °Bx, protein and total sugars, respectively. These authors describe concentrated cane juice as a viscous, very dark brown liquid obtained from sugarcane in sugar mills, with a mean soluble solids content of 78–82°Bx, 1063 mg K, 290 mg Ca, 200 mg Mg, 69 mg P, 6.0 mg Fe, 9.5 μg folic acid and 6–8 g ash, values that may vary. In addition, the following values were obtained in this study: soluble solids of 79°Bx, 2.3 g/100 g protein, 56 g/100 g total sugars, 1050 mg/100 g K, 280 mg/100 g Ca, 69 mg/100 g P, 180 mg/100 g Mg, 6.0 mg/100 g Fe, 1.0% insoluble solids and 6.1% ash, among other vitamins and minerals. These results support

the observation by Quiroga et al. [146] that the chemical composition of concentrated sugarcane juice varies widely in nutrient content, flavor, color, viscosity and total sugar content, depending on factors such as soil type, ambient temperature, humidity, production season, variety, production practices at each processing plant and storage conditions.

3.4. Sensory Characterization of the Beverages Using a Hedonic Test

Table 7 presents the results of the sensory evaluation of the ten initially formulated beverages, divided into two batches, by the semi-trained panel. Based on the highest scores and Friedman rank sums, beverages B31 and C79 from Batch 1 and Z73 and N28 from Batch 2 were selected.

The beverages in Batch 2 received the highest scores, all with mean scores above 4.0 except beverage S96. Notably, the probiotic culture, which was not added to the Batch 1 beverages, appeared to have a marked influence on the Batch 2 beverages. In both batches, beverage N28, which contained no stabilizers, performed satisfactorily and ranked among the highest-scoring beverages.

Table 7. Sensory evaluation of the formulated beverages.

Score	Batch 1					Batch 2				
Beverage	A27	B31	C79	M25	N28	Z73	M25	T12	N28	S96
Total liking score	22	25	23	22	23	30	29	28	32	27
Friedman rank sums	19.5	24.5	21.5	19.5	20.0	22.5	19.0	18.0	28.0	17.5
Mean	3.14 ± 0.90	3.57 ± 0.98	3.29 ± 0.76	3.14 ± 1.07	3.29 ± 0.95	4.29 ± 0.49	4.14 ± 0.69	4.00 ± 0.82	4.57 ± 0.79	3.86 ± 0.69

Mean values are presented as mean ± standard deviation. Note: Batch 1: A27 = citrate and CMC; B31 = citrate and xanthan gum; C79 = citrate, xanthan and CMC; M25 = citrate and PRO gum; N28 = UFP, Andean blueberry, concentrated panelera cane juice and water. Batch2: Z73 = citrate and PRO gum (0.05%); M25 = citrate and PRO gum (0.1%); T12 = citrate and PRO gum (0.2%); N28 = UFP, Andean blueberry, concentrated panelera cane juice and water; S96 = citrate and PRO gum (0.3%).

As shown in Table 7, the highest-scoring beverages, which were selected for further analysis, were B31 (25 points) and C79 (23 points) in Batch 1 and Z73 (30 points) and N28 (32 points) in Batch 2. Notably, beverage N28 was included in both batches and received high scores (29 and 32, respectively), despite containing no added stabilizers or citrate. The highest scores were obtained by the Batch 2 beverages, which were formulated with a single type of stabilizer (Ingredion Goma Pro 181) at different concentrations; among these, the beverage with the lowest concentration (0.05%) received the highest score, followed by N28, which contained no added stabilizers. These four beverages (B31, C79, Z73 and N28) were selected for further analysis to identify the best formulation.

Table 8 presents the physicochemical characterization of the four beverages selected on the basis of the sensory analyses conducted by the semi-trained panel.

Table 8. Physicochemical and sensory characterization of the four beverages selected by the semi-trained panel.

Formulation	pH	Total Acidity (% Citric Acid)	Soluble Solids (°Bx)	CO_2 Volume (g/L)	Zeta Potential-ζ (mV)	Total Phenols (mg GAE/100 g Sample at w.b.)	Antioxidant Capacity by ABTS (mg Trolox/100 g Sample at w.b.)	Antioxidant Capacity by DPPH (mg Trolox/100 g Sample at w.b.)	Preference Test
F1	4.17 ± 0.005 ^{abc}	0.31 ± 0.01 ^{a}	8.2 ± 0.00 ^{a}	2.95 ± 0.03 ^{a}	−34.022 ± 1.6483 ^{a}	87.48 ± 2.12 ^{a}	58.56 ± 1.20 ^{a}	32.9 ± 1.50 ^{a}	4.085 ± 1.933 ^{a}
F2	4.19 ± 0.03 ^{ac}	0.40 ± 0.01 ^{b}	7.8 ± 0.20 ^{b}	2.92 ± 0.03 ^{a}	−34.844 ± 3.2859 ^{a}	82.51 ± 1.07 ^{b}	60.14 ± 12.05 ^{a}	29.27 ± 0.79 ^{b}	3.9885 ± 1.995 ^{a}
F3	4.14 ± 0.005 ^{ab}	0.31 ± 0.01 ^{a}	8.0 ± 0.00 ^{ab}	2.99 ± 0.01 ^{a}	−23.866 ± 1.5011 ^{b}	76.16 ± 1.07 ^{c}	50.72 ± 3.38 ^{ab}	35.72 ± 0.76 ^{c}	5.3000 ± 0.964 ^{b}
F4	4.19 ± 0.00 ^{ac}	0.33 ± 0.00 ^{a}	8.0 ± 0.00 ^{ab}	2.98 ± 0.02 ^{a}	−15.166 ± 0.4725 ^{c}	64.56 ± 1.06 ^{d}	35.38 ± 0.59 ^{b}	23.78 ± 0.52 ^{d}	5.507 ± 2.021 ^{b}

The results are presented as mean ± standard deviation. Values in the same column with different superscript letters differ significantly ($p < 0.05$). F1: with xanthan gum stabilizer (B31). F2: with hydrocolloid mixture stabilizer: xanthan gum—CMC (C79). F3: with Ticaloid® PRO 181 AG hydrocolloid system (gellan gum, acacia gum) (Z73). F4: without stabilizer (N28).

As shown in Table 8, the beverages differed significantly ($p < 0.05$) in pH, total acidity, soluble solids, zeta potential, total phenols and antioxidant capacity (ABTS and DPPH), with beverage F2 (C79) showing the greatest variability, mainly in acidity and antioxidant capacity. No significant differences ($p > 0.05$) were observed in CO_2 volume or in the preference test, in which beverages F3 (Z73) and F4 (N28) received the highest ratings. CO_2 losses during counter-pressure filling and handling were minimized by applying the principles of fluid thermodynamics, Henry's law and fluid mechanics.

Beverages containing fruit juices or fruit products with added hydrocolloids are more stable than those without such additives [31,147]. A similar trend was observed in this study, in which the more negative zeta potential (ζ) values indicate greater stability of beverages F1 (B31) and F2 (C79). A higher absolute zeta potential increases the electrostatic repulsion between suspended particles, which maintains turbidity and reduces phase separation during storage.

The pH, total acidity, soluble solids, total polyphenols and antioxidant capacity of the beverages differ from those reported by Zárate et al. [31] for a non-carbonated soft drink made from hydrolyzed sweet whey permeate and supplemented with cape gooseberry (*Physalis peruviana* L.) and Andean blueberry (*Vaccinium meridionale* Swartz) pulp, which was more acidic, had a higher soluble solids content and showed lower total polyphenol content (56.40% difference) and antioxidant capacity, both by ABTS (22.74% difference) and by DPPH (33.31% difference). Likewise, in a non-carbonated isotonic beverage based on hydrolyzed sweet whey permeate containing cape gooseberry [11], total polyphenols and antioxidant capacity were lower than those obtained here (differences of 88.98% for phenols, 87.19% for ABTS and 86.02% for DPPH).

Table 9 presents the microbiological characterization of the four selected beverages.

Table 9. *Microbiological characterization of the four beverages selected by the semi-trained panel.*

Formulation	Mesophilic Aerobic Microorganism Count (CFU/g or mL)	Mold and Yeast Count (CFU/g or mL)	Total Coliform Count (CFU/g)	*Escherichia coli* Count (CFU/g or mL)
F1	100	10	<10	<10
F2	105	5	<10	<10
F3	115	<10	<10	<10
F4	100	10	<10	<10

The results are presented as the mean ± standard deviation of the measurements. Values in the same column with different letters indicate statistically significant differences ($p < 0.05$). F1: with xanthan gum stabilizer (B31). F2: with hydrocolloid mixture stabilizer: xanthan gum—CMC (C79). F3: with Ticaloid® PRO 181 AG hydrocolloid system (gellan gum, acacia gum) (Z73). F4: without stabilizer (N28). CFU: colony-forming unit.

As shown in Table 9, all beverages complied with the microbiological specifications for pasteurized fruit beverages established in NTC 3549 of 2022 [148] and in Resolution 3929 of 2013 of the Colombian Ministry of Health and Social Protection [138].

The results of the sensory characterization performed by the semi-trained panel are shown in Figure 1.

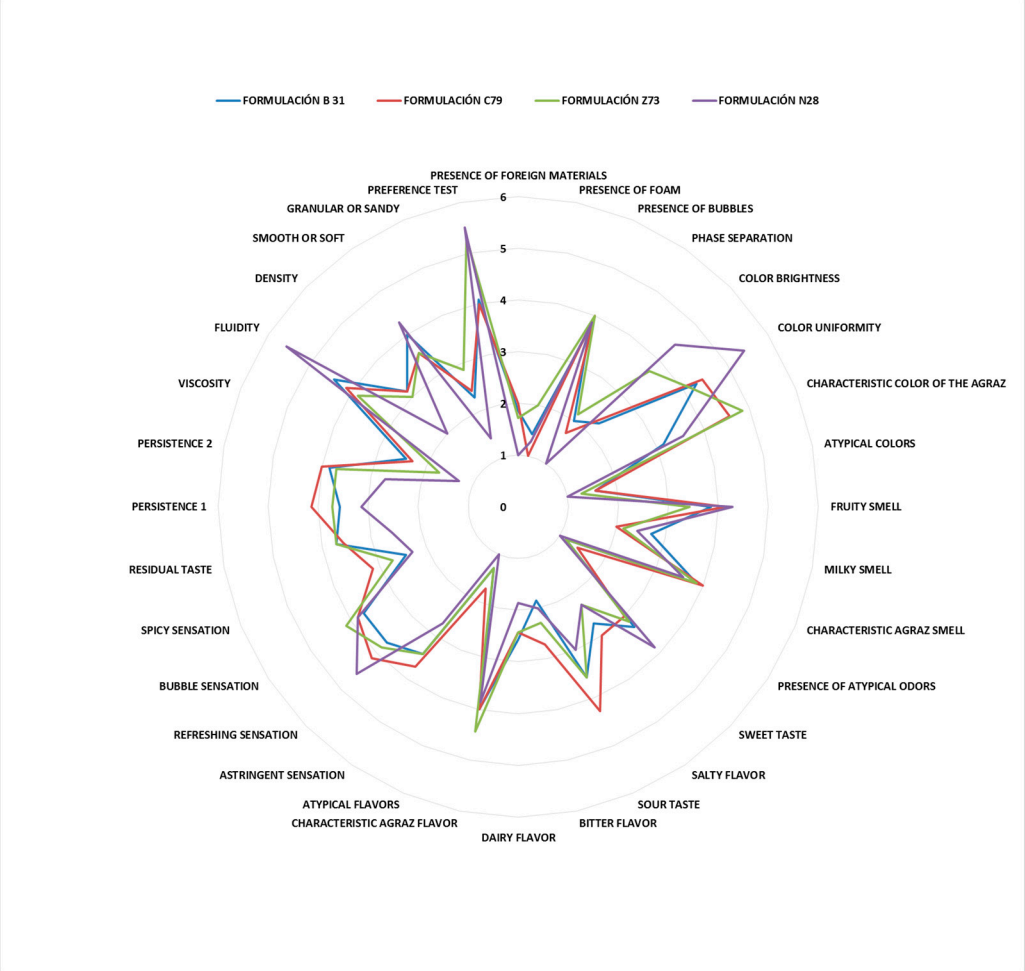


Figure 1. *Sensory characterization of the four carbonated beverages.* F1: with xanthan gum stabilizer (B31). F2: with hydrocolloid mixture stabilizer: xanthan gum—CMC (C79). F3: with Ticaloid® PRO 181 AG hydrocolloid system (gellan gum, acacia gum) (Z73). F4: without stabilizer (N28).

Of the four beverages analyzed (Figure 1), F4 (N28) received the highest ratings for the descriptors refreshing sensation, mouthfeel, presence of bubbles, color uniformity, fruity aroma and sweet taste, although it had the lowest total phenolic content (64.56 ± 1.06 mg GAE/100 g sample) and the lowest antioxidant capacity by ABTS and DPPH (35.38 ± 0.59 and 23.78 ± 0.52 mg Trolox/100 g sample, respectively). All beverages exhibited a very pronounced, highly acidic flavor characteristic of Andean blueberry.

As described in Section 2.3.3, these four beverages were also evaluated by a panel of seven trained judges using a ranking test (Table 10).

Table 10. Sum of ranks in the ranking test applied by the trained panel.

F1:B31	F2:C79	F3:Z73	F4:N28
235	441	64	169

F1: with xanthan gum stabilizer (B31). F2: with hydrocolloid mixture stabilizer: xanthan gum—CMC (C79). F3: with Ticaloid® PRO 181 AG hydrocolloid system (gellan gum, acacia gum) (Z73). F4: without stabilizer (N28).

The Friedman test revealed significant differences ($p < 0.05$) among the four samples evaluated by the trained panel, and the rank-sum analysis (Table 10) identified beverage F2 (C79) as the best, owing to its greater Andean blueberry flavor intensity compared with the other three samples.

3.5. Microbiological Characterization of the Selected Beverage

The probiotic count of the beverage met the requirements of current national legislation (>10^6 CFU/mL; Resolution 810 of 2021 of the Ministry of Health and Social Protection [149]) and of NTC 805 for fermented milks [150], as well as the values used by the food industry. Shori [18] and Asaithambi et al. [151] indicate that the success of new probiotic foods depends on their ability to deliver a sufficient number of viable cells to beneficially modify the intestinal microbiota of the host. Viable probiotic counts in the final product should therefore be at least 10^6–10^7 CFU/mL [18], a level at which interactions between phenolic compounds and the gut microbiota have beneficial effects in humans [152]. The probiotic count (Table 11) exceeded these values. Likewise, the counts of mesophilic aerobes, *E. coli* and molds and yeasts were within the limits established for pasteurized fruit beverages in NTC 3549 of 2022 [148] and for foods and beverages for human consumption in Resolution 1407 of 2022 of the Ministry of Health and Social Protection [153].

Table 11. Microbiological and probiotic characterization of the selected beverage.

Lactic Acid Bacteria Count (*Lactobacillus casei* subsp. *rhamnosus*) (CFU/mL)	**Mesophilic Aerobic Microorganism Count (CFU/mL)**	**Total Coliform Count/mL**	***Escherichia coli* Count (CFU/mL)**	**Mold and Yeast Count (CFU/mL)**
8.36×10^6	<10	<10	<10	<100

CFU: colony-forming unit.

Sireswar et al. [154] indicate that clinical, epidemiological and biochemical studies have established that optimal nutrition, including probiotics and fruit phenolics, can reduce the risk and morbidity of certain chronic diseases. This observation is based on potential synergies between probiotic strains and the bioactive components of food matrices, including carbonated beverages, in which carbonation is thought to extend shelf life [155]. However, no systematic studies have examined the effect of carbonation on the quality or shelf life of some of these products, such as fruit juices.

Wen et al. [156] state that *Lactobacillus casei* is intentionally introduced into fruit and vegetable juices as a probiotic because of its high activity and survival capacity. However, Pourjafar et al. [157] found that free (non-microencapsulated) *L. acidophilus* and *L. rhamnosus* in a commercial yogurt-type beverage (Doogh) increased acidity and metabolite production during refrigerated storage (42 days), especially after day 21, impairing its organoleptic properties compared with the microencapsulated microorganisms. Vinderola et al. [158] suggested that adding fruit juices or pulps may reduce the viability of some probiotic species and strains in food products. This was not the case for *L. casei* ssp. *rhamnosus* in this study, which showed adequate survival without significantly altering the sensory attributes or physicochemical composition of the beverage.

Carbonation prevents spore germination and inhibits the growth of contaminating microorganisms, provided that each production stage is properly controlled and measures are taken to ensure microbial stability with respect to ingredients, formulation and processing. During formulation, control of pH, carbonation and preservative concentration is essential to inhibit the growth of microorganisms that could cause spoilage. Complementary critical measures for producing stable plant-based beverages include hygiene control of equipment, the processing environment and packaging [159].

4. Conclusions

The carbonated beverage formulated with hydrolyzed permeate can be considered a functional product because it contains the probiotic *Lactobacillus casei* ssp. *rhamnosus* at levels exceeding those required by current national legislation (1×10^6 CFU/g; Resolution 810 of 2021 of the Colombian Ministry of Health and Social Protection on nutritional and front-of-package labeling), as well as compounds with antioxidant capacity, thereby meeting current consumer demand for nutritional benefits. Stabilizers are required to prevent destabilization mechanisms that would otherwise slightly affect fundamental carbonation attributes, such as effervescence and bubbly mouthfeel.

This probiotic beverage, developed from an innovative combination of matrices (sweet whey permeate, Andean blueberry, concentrated panelera cane juice and probiotics) and technologies (ultrafiltration, lactose hydrolysis, forced-convection drying and carbonation), broadens consumer options in the market for healthy and functional soft drinks. It also demonstrates the value of upcycling a complex residual matrix (permeate) combined with a natural sweetener (concentrated panelera cane juice) instead of refined sugar, in line with healthy-eating and clean-label trends.

Although this study has focused on the comprehensive physicochemical, antioxidant and sensory characterization of the prototypes in their initial stage (demonstrating the technological feasibility of using whey permeate and the impact of the stabilizer on colloidal stability), a current limitation is the lack of monitoring of probiotic viability and changes in bioactive compounds during shelf life. Nevertheless, the baseline characterization conducted in this study establishes the platform and essential selection criteria (identifying F1 and F2 as the optimal formulations due to their zeta potential values greater than $|-34|$ mV) for the design of future studies aimed at evaluating degradation kinetics, shelf life and the survival of the *Lactobacillus casei* ssp. *rhamnosus* inoculum under refrigerated conditions.

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Institutional Review Board Statement: Ethical review and approval were waived for this study because it met the criteria for minimal-risk research. The sensory evaluation was limited to the organoleptic analysis (taste, color, aroma, texture and appearance) of safe, harmless non-alcoholic beverages produced in accordance with food quality standards. Participation was strictly voluntary and followed the standard quality-control and tasting protocols of the food and beverage industry, posing no health risk.

Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

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Abbreviations

The following abbreviations are used in this manuscript:

w.b.	wet basis
D.M.	dry matter
°Bx	degrees Brix
aw	water activity
TSS	total soluble solids
GAEs	Gallic acid equivalents
ABTS	(2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid)
DPPH	2,2-diphenyl-1-picrylhydrazyl
ORAC	Oxygen radical absorbance capacity
TAC	Total antioxidant capacity
TEAC	Trolox equivalent antioxidant capacity
TE	Trolox equivalents
CFU	Colony-forming unit
UFP	unhydrolyzed permeate
HUFP	hydrolyzed permeate

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