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Block Freeze Concentration Processes for Goat Milk Aiming at the Elaboration of an Innovative Functional Fermented Milk

Maria Helena Machado Canella ¹, Amanda Alves Prestes ¹ , Erick Almeida Esmerino ², Eduard Hernández ³ , Adriano Gomes da Cruz ⁴, Tatiana Colombo Pimentel ^{5,*}  and Elane Schwinden Prudencio ^{1,6,*}

- ¹ Postgraduate Program in Food Engineering, Federal University of Santa Catarina, Technology Center, Trindade, Florianópolis 88040-900, SC, Brazil; helenacanella@gmail.com (M.H.M.C.); aprestes04@gmail.com (A.A.P.)
- ² Department of Food Technology, Federal Fluminense University, Niterói 24220-900, RJ, Brazil; erick.almeida@hotmail.com
- ³ Department of Agri-Food Engineering and Biotechnology, Universitat Politècnica de Catalunya (UPC) BarcelonaTech, 08860 Castelldefels, Barcelona, Spain; eduard.hernandez@upc.edu
- ⁴ Federal Institute of Education, Science and Technology of Rio de Janeiro (IFRJ), Department of Food, Rio de Janeiro 20270-021, RJ, Brazil; adriano.cruz@ifrj.edu.br
- ⁵ Federal Institute of Paraná (IFPR), 1400, Paranavaí 87703-536, PR, Brazil
- ⁶ Department of Food Science and Technology, Federal University of Santa Catarina, Itacorubi, Florianópolis 88034-001, SC, Brazil
- * Correspondence: tatiana.pimentel@ifpr.edu.br (T.C.P.); elane.prudencio@ufsc.br (E.S.P.)

Abstract: The development of functional dairy products has increasingly become a focus of the dairy industry, with goat milk gaining prominence due to its nutritional properties and digestibility. This study aimed to evaluate the effects of freeze concentration processes on skimmed goat milk, observing its potential prebiotic effects and impacts on the physical, chemical, microbiological, rheological, and sensory profiles of fermented milk, using the Preferred Attributes Elicitation (PAE) methodology. Skimmed goat milk was initially concentrated using the gravitational block freeze concentration technique. A fermented milk containing probiotics (FM1) was produced from this concentrate. In addition, two other samples were developed: one with skimmed goat's milk, 6% inulin, and probiotics (FM2) and another using whole goat's milk with probiotics as a control (FM3). The results indicated that the freezing concentration process resulted in a concentrate with 14.70 ± 0.06 g 100 g⁻¹ of total solids. Among the three types of fermented milk, FM1 presented the highest values of total solids and titratable acidity. Regarding color, both FM1 and FM2 tended towards yellowish and greenish tones, while FM3 presented a greater luminosity. During storage, all fermented milks maintained their probiotic properties. The freeze concentration process increased the viscosity of FM1, a characteristic also evidenced in the sensory evaluations using PAE. In contrast, FM2 presented a rheological behavior similar to that of the control (FM3). Regarding sensory acceptance, FM1 had lower acceptance regarding aroma, being described as having notes of "goat flavor" and "acid" and being "salty". The PAE methodology proved effective in characterizing the sensory qualities of the products, providing valuable information for developing new dairy products. These results offer an important theoretical basis for the industrial production of functional dairy products based on goat's milk, helping to evaluate quality characteristics and optimize manufacturing processes.

Keywords: goat milk; concentration; prebiotic; probiotic; PAE



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1. Introduction

Goat milk production has seen significant growth on a global scale, driven by its high nutritional value and the growing demand for goat-derived products. Goat milk stands out for its chemical composition, being rich in proteins of high biological value, essential fatty acids, minerals with high bioavailability, vitamins, and other bioactive compounds [1,2]. These factors make it a food with excellent nutritional qualities, serving as a promising

basis for developing various innovative products. Compared to cow's milk, goat milk has a lower content of α s1-casein and β -lactoglobulin, proteins associated with allergic reactions. In addition, its protein composition is more similar to human breast milk, making it less likely to trigger allergies. Another relevant aspect is that the fat globules in goat milk are smaller and more uniform, facilitating digestion and absorption by the body [3].

The market for dairy products based on goat milk is expanding mainly due to the growing demand for functional and differentiated foods that meet specific consumer niches. Goat milk products, such as cheeses, yogurts, and fermented milks, are increasingly valued by consumers seeking value-added options, whether for health, flavor, digestibility, or the provision of functional ingredients [4]. For example, the interaction between prebiotics and probiotics has been widely explored in the development of functional foods. Probiotics, which are live microorganisms beneficial to intestinal health, and prebiotics, which are non-digestible ingredients that promote the growth of beneficial bacteria in the gastrointestinal tract, form a synergy that favors the intestinal microbiota [5]. This combination has attracted the industry's and consumers' attention, as the benefits associated with digestive health, immunity, and metabolism are widely recognized. However, processing technologies must be improved to meet market demands for high-quality dairy products with functional characteristics. Conventional milk concentration methods, which use heat, can degrade the sensitive proteins, vitamins, and volatile compounds responsible for the aroma and flavor of products. These thermal processes often result in a significant reduction in the nutritional and sensory quality of the products. Freeze concentration, in turn, is an innovative technique that stands out for preserving the original characteristics of the food. By separating the solid-liquid phases at controlled low temperatures, this technology concentrates proteins, enzymes, and vitamins without heating, avoiding the adverse effects of heat. In addition, freeze concentration maintains volatile compounds and pigments, ensuring that the final product preserves its desirable sensory characteristics, such as flavor and texture [6–11].

Fermented milk, one of the most consumed dairy products worldwide, can benefit from the application of prebiotics and probiotics and the freeze concentration process [5,10]. The addition of freeze-concentrated goat's milk to the formulation of fermented milks offers several advantages, such as an increased solids content, improved texture and creaminess, higher yield, improved fermentation potential, and reduced time (as the higher content facilitates the action of probiotic bacteria), better product stability, and flavor intensification [11].

With a high nutritional profile, especially regarding proteins and essential minerals, fermented goat milk emerges as an excellent base for creating innovative functional products. To ensure that these products meet consumer expectations, it is essential to carry out detailed sensory analyses, by using techniques such as the Preferred Attribute Elicitation (PAE) methodology. This method allows for the identification of the most relevant sensory attributes for product acceptance, such as flavor, texture, and aroma, using untrained consumers as evaluators. By involving consumers in the evaluation process and reaching a consensus on the most important attributes, PAE offers an effective approach to fine-tuning the development of new products, ensuring that they correspond to market preferences [12–14]. Therefore, the present study aims to evaluate the effects of applying cryoconcentration to skimmed goat's milk, observing its impact on the physical, chemical, microbiological, rheological, and sensory characteristics of the fermented milk, using the Preferred Attribute Elicitation (PAE) methodology. Addressing the interaction between concentration methods and adding functional ingredients such as prebiotics and probiotics aims to optimize the development of innovative and high-value dairy products.

2. Materials and Methods

2.1. Materials

The block freeze concentration procedure was realized with UHT (ultra-high temperature) skimmed goat's milk (Caprilat[®], CCA Laticínios, Rio de Janeiro, Brazil) (8.46 g

100 g⁻¹ of total solids, 3.00 g 100 g⁻¹ of total protein, and 4.30 g 100 g⁻¹ of total carbohydrates). The fermented milk was obtained using UHT skimmed goat's milk, UHT whole goat's milk (Caprilat[®], CCA Laticínios, Rio de Janeiro, Brazil) (11.8 g 100 g⁻¹ of total solids, 3.00 g 100 g⁻¹ of total protein, 3.50 g 100 g⁻¹ of lipids and 4.30 g 100 g⁻¹ of total carbohydrates), and thermophilic freeze-dried cultures (ABT4[®], Chr. Hansen, Hónsholm, Denmark) composed of *Streptococcus thermophilus*, *Bifidobacterium animalis* subsp. *Lactis*, and *Lactobacillus acidophilus*. Prebiotic inulin (Orafti[®] HPX, Orafti, Tienen, Belgium) with a degree of polymerization (DP) ≥ 23 and sucrose were also used. MRS agar (Merck[®], Darmstadt, Germany), M17 agar (Fluka, NeuUlm, Germany), lithium chloride (Vetec[®], Rio de Janeiro, Brazil), sodium propionate (Fluka[®], Neu-Ulm, Germany), bile (Sigma-Aldrich, St. Louis, MO, USA), and AnaeroGen[®] (Oxoid, Hampshire, UK) were used for the microbiological analysis.

2.2. Block Freeze Concentration

The block freeze concentration of skim goat milk followed the methodology described by Canella et al. [11]. The method separates two fractions obtained at the end of the freeze concentration stage, comprising one freeze-concentrated milk and one ice fraction. An initial volume of 20 L of skim goat milk was divided into 1 L batches and then frozen (-20 ± 2 °C) in polypropylene pots using a plate freezer (Frigostrella, Cotia, São Paulo, Brazil). Sequentially, the frozen samples were removed from the pots at each stage and placed on top of the stainless-steel screen in contact with the funnel (Figure 1). Partial defrosting was allowed using only gravitational force (by passive thawing). The room temperature was fixed at 20 ± 2 °C. The concentrated liquid passed through the funnel upon the thawing of frozen milk, which was collected and weighed. The freeze concentration stage was finished when the weight of the concentrate reached 50% of the initial frozen sample. Based on previous research [11], the concentrate from the first stage was collected and used to produce fermented milk, leading to energy savings.

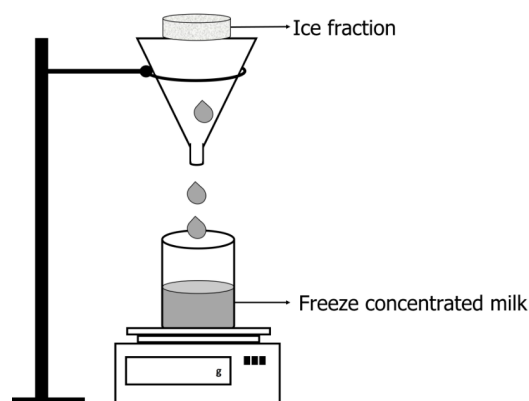


Figure 1. A block freeze concentration process was used to concentrate the skimmed goat milk.

2.3. Production of Fermented Milk

Three formulations of fermented milk (FM) were created. The first fermented milk (FM1) was prepared with freeze-concentrated skimmed goat's milk. The second fermented milk (FM2) was prepared using skimmed goat's milk and 6% prebiotic inulin. According to Hill et al. [5], using 6% inulin in fermented milk enhances the growth and survival of probiotics, improves acidification kinetics, and boosts the physicochemical properties of the product. These improvements contribute to better consumer acceptability and functional health benefits, making inulin a valuable addition to fermented milk products [5]. The third formulation (FM3) was prepared using whole goat's milk as a control. The culture added into the concentrated milk (inoculation 0.20 g L⁻¹) was a commercial freeze-dried culture for direct vatting with the ABT-4[®] culture (Chr. Hansen, Hónsholm, Denmark), composed of *Lactobacillus acidophilus* La-5, *Bifidobacterium animalis* ssp. *lactis* BB-12, and *Streptococcus*

thermophilus. The raw materials for each fermented milk were heated to 42 ± 1 °C and fermented for around 4 h. The fermented milk was cooled to 5 ± 1 °C and then stirred slowly. Fermented milk FM1, FM2, and FM3 were packaged in thermosealed plastic packages (Sulplack SPO-150, Caxias do Sul, Rio Grande do Sul, Brazil) with an aluminum lid and multilayer polyethylene and stored at a refrigerated temperature (5 ± 1 °C). Fermented milks were produced in triplicate. Aliquots of fermented milk were used for physical–chemical, rheological, and microbiological analyses on days 1 and 15 of storage at 5 ± 1 °C. Sensory analyses were performed on day 1 of storage of fermented milk.

2.4. Physical and Chemical Analyses

2.4.1. Determination of pH, Acidity, and Total Solids

The pH value of the fermented milk was measured at 20 °C using a previously calibrated digital pH meter (PHS-3 BW, BEL, Piracicaba, São Paulo, Brazil). The electrode was placed directly into a 20.0 g fermented milk sample and measurements were taken in triplicate. The titratable acidity of fermented milk was determined using a 0.1 M NaOH standard solution titration method. A fermented milk sample (10 g) was added to 1 mL of phenolphthalein solution (1% wt/vol in ethanol), and the mixture was titrated with standardized NaOH (0.1 M) until the color changed to pink. The volume of NaOH standard solution consumed was recorded [15]. Total titratable acidity (TTA) was calculated according to Equation (1):

$$TTA = \frac{V \times N \times f \times M}{W} \quad (1)$$

where the following symbols are used:

TTA: Total titratable acidity (grams of acid per 100 g of sample);

V: Volume of titrant (NaOH) used in the titration (mL);

N: Normality of the titrant solution (in equivalents L^{-1});

f: Conversion factor;

M: Molar mass of the predominant acid (lactic acid = 90 g mol^{-1});

W: Weight of the sample analyzed (g).

The total solids content was evaluated on the skimmed goat milk, freeze-concentrated skimmed goat milk, whole goat milk, and the fermented milk samples (FM1, FM2, and FM3). The mass loss measurement determined the total solid content after drying the samples at 105 °C up to constant weight and expressed as the dry matter/total mass content ($\text{g } 100 \text{ g}^{-1}$). The method was performed as described by the Association of Official Agricultural Chemists [16].

2.4.2. Color Parameters

The color of the fermented goat milk FM1, FM2, and FM3 was determined using a colorimeter Minolta Chroma Meter CR-400 (Konica Minolta, Osaka, Japan). The colorimeter was calibrated with a white standard plate and adjusted to operate with D65 lightning and a 10° observation angle. The CIELab color scale was used to measure the L^* , b^* , and a^* parameters, which indicate the luminosity (variation from black to white), variation from yellow ($+b^*$) to blue ($-b^*$), and variation from red ($+a^*$) to green ($-a^*$), respectively.

2.4.3. Rheological Properties

The rheological properties of the fermented skimmed goat milk samples FM1, FM2, and FM3 were evaluated using a rotational rheometer with a concentric cylinder (Brookfield Engineering Laboratories model DVIII Ultra, Stoughton, MA, EUA). The spindle used was the ULA. The measurements were collected using the Rheocalc[®] 32 software (version 3.2) (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA). The rheometer was thermostatically controlled by a water circulator (TE-184, TECNAL, São Paulo, Brazil) at a 4.0 ± 0.1 °C temperature. The flow curves were generated by a linearly increased shear rate

of 20 s^{-1} to 205 s^{-1} in the first 15 min (upward curve), returning to 20 s^{-1} in the following 15 min (downward curve).

2.5. Microbiological Analysis

The enumeration of *L. acidophilus* La-5 in the fermented goat milk FM1, FM2, and FM3 was performed at $37 \pm 1 \text{ }^\circ\text{C}$ for 72h on MRS agar modified with the addition of 0.15 g per 100 mL of bile (MRS-Bile). For *Bifidobacterium animalis* subsp. *lactis* counts, MRS agar with the addition of 0.20 g per 100 mL of lithium chloride and 0.30 g per 100 mL of sodium propionate (LPMRS) was used, and the plates were incubated at $37 \pm 1 \text{ }^\circ\text{C}$ for 72 h under anaerobic conditions in anaerobic jars containing AnaeroGen[®] (Vinderola and Reinheimer, 2000). An aerobic incubation was conducted at $37 \pm 1 \text{ }^\circ\text{C}$ for 48 h on M17 agar to count viable *S. thermophilus* cells (IDF, 1997). The volume of culture spread on the plates to enumerate viable colonies was equal to 0.1 mL. Bacterial enumerations were carried out in triplicate using the spread plate technique, and the results were given as log CFU g^{-1} .

2.6. Preferred Attribute Elicitation (PAE)

PAE was performed according to the methodology proposed by Grygorczyk et al. [14]. Consumers were selected based on the frequency of their consumption of fermented milk (at least weekly) (Ethics Committee, under number CAAE 54928016.4.0000.5688). Two PAE sessions were held with female and male consumers ($n = 15$ and 15) on the same day. Both consumer groups received 30 mL of each sample simultaneously, presented in 50 mL clear plastic cups labeled with random three-digit numbers. Consumers first rated the fermented goat's milk samples ($n = 3$). They were asked to rate their overall acceptance of the samples regarding appearance, aroma, flavor, texture, and overall impression on a 9-point hedonic scale (1 = dislike very much up to 9 = like very much). Then, they were asked to write down which attributes they liked or disliked in the evaluated samples. Thus, consumers were encouraged to think about the attributes of the fermented goat milk samples.

The discussion was carried out through a round table mediated by the researcher. Consumers were asked to name which attributes were different between the samples. The elicited attributes were noted in a table. When the consumers had no more attributes to add, they were asked to group them as they saw fit. Attributes considered unimportant by the consumer group have been removed. Consumers grouped attributes into appearance, aroma, flavor, and texture groups. Then, ten-point scales were generated for the attribute groups, and consumers defined anchor descriptor terms based on the intensity of the attributes.

Furthermore, consumers knew they could add attributes if those selected did not encompass the attributes important to the samples within a group. The number of attributes was reduced considering, in consensus, the attributes that had the same meaning and those that would not be easily evaluated. Then, the consumers were asked to classify the attributes presented in the scales, considering their contribution to the taste of the products. Consumer groups knew attributes could be ranked similarly if they were considered equally important. There was a 30 min break, and then the participants received the 3 samples of fermented goat milk and the evaluation form. They were asked to rate the sensory descriptors of each fermented milk sample using ten-point scales with the anchor terms they chose. Consumers were provided with water and biscuits to clean their palate between samples. Consumers used paper ballots to rate the strength of attributes for each sample individually. The PAE session was carried out over 70 min.

2.7. Statistics

All results are expressed as the mean \pm standard deviation. One-way analysis of variance (ANOVA) and Tukey's test were used to determine significant differences ($p < 0.05$) between results. All statistical analyses were performed using STATISTICA 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA). The PAE data were evaluated by Generalized Procrustes Analysis (GPA), using a matrix dataset composed of 3 rows (fermented milk

formulations) and a total of 300 columns (number of attributes \times consumers, 10 in tributes and 30 consumers). Although the GPA can deal with different amounts of data elucidated by each consumer, there is a need for the consumers to group the sensory attributes into appearance, aroma, flavor, and texture during the PAE methodology [14] to ensure that a similar amount of matrix data is used for each consumer during the GPA. For sensory analyses, GPA and ANOVA were performed using XLSTAT 2019.2 software (Adinsoft, Paris, France).

3. Results and Discussion

3.1. Physical and Chemical Analyses and Microbiological Analyses

After carrying out the block freeze concentration process on the skimmed goat milk, it was possible to obtain a concentrate with $14.70 \pm 0.06 \text{ g } 100 \text{ g}^{-1}$ of total solids. The initial skimmed goat milk showed $8.46 \pm 0.01 \text{ g } 100 \text{ g}^{-1}$ of total solids. The concentrate had a higher total solids content than whole goat milk, which, on average, has $11.8 \text{ g } 100 \text{ g}^{-1}$ of total solids. The fermented milk samples were prepared using the concentrate obtained from the block freeze concentration process and probiotics (FM1), skimmed goat milk and 6% of inulin (FM2), and whole goat milk (FM3), aiming to produce functional dairy products using goat milk.

Table 1 presents the results obtained for total solids, pH, titratable acidity, color parameters, and microbiological analyses of fermented milk samples FM1, FM2, and FM3 on days 1 and 15 of storage. By using concentrated milk to prepare the fermented milk, a product with a high content of total solids (FM1) can be obtained when compared to the other fermented milks (FM2 and FM3) ($p < 0.05$). The goat milk concentration also influenced the titratable acidity and pH values for the FM1 fermented milk ($p < 0.05$) on all days of storage. The higher acidity can be explained by the organic acids, such as lactic acid, citric acid, and formic acid, commonly found in fermented milk made with goat milk [17,18], since the substrates used by the bacteria to produce these organic acids have been concentrated. Furthermore, the highest pH for the FM1 sample could be associated with this sample having the highest titratable acidity value. According to Li et al. [19], during storage, the pH of fermented milk could be affected by the initial acidity, i.e., a higher initial acidity value increases the fermented milk's pH value. Ozcan et al. [20] reported that a higher colloidal calcium phosphate content is related to an increase in pH. Therefore, the freeze concentration process could also be responsible for increasing the colloidal calcium phosphate content in the raw material (concentrate) used in the creation of the fermented milk (FM1) sample. In this case, the freeze concentration process also further influences the pH behavior of the FM1 sample. The buffering capacity of dairy products is closely related to their protein concentration and composition. Higher levels of proteins, especially casein, enhance the buffering capacity, making the products more resistant to pH changes.

On the other hand, the FM2 and FM3 fermented milk samples showed increased acidity and decreased pH values during the storage period ($p < 0.05$). The increase in acidity is attributed to the post-acidification (lactic acid production) of fermented dairy products. Lactic acid may result from the metabolism of starter cultures or probiotics during fermentation [17]. Furthermore, during refrigerated storage (between 0 and 5 °C), β -galactosidase enzymes, which are produced by the bacteria added to fermented milks, are responsible for the catabolism of lactose during the fermentation process, resulting in a decrease in pH.

The FM3 fermented milk showed the greatest luminosity among the samples (FM1, FM2, and FM3) during the storage period ($p < 0.05$). It was verified that the greater luminosity resulting from the presence of colloidal particles, such as milk fat globules and casein micelles, is capable of dispersing light in the visible spectrum [21,22]. This fact may explain the lower values ($p < 0.05$) recorded for the luminosity (L^*) of the FM1 and FM2 fermented milk samples, which were both made with skimmed goat milk. The higher concentration of total solids in the FM1 fermented milk influenced the high tendency

($p < 0.05$) for the other samples to show a yellow color (b^*). According to Balde and Aider [23], the increase in a yellow color in milk is associated with an increase in protein content. A greater tendency towards green coloration was observed in samples FM1 and FM2 on the first day of storage. According to Nozière et al. [24], the parameter a^* of milk color can be influenced by the concentration of natural pigments present in milk, such as riboflavin. This pigment is a green compound in the aqueous phase which is found in significant amounts in goat milk [25]. The use of concentrated and skimmed milk as ingredients in the FM1 and FM2 samples corroborate this explanation.

Table 1. Physical–chemical and microbiological properties of fermented milk FM1, FM2, and FM3 on days 1 and 15 of storage at 4.0 ± 1.0 °C.

Analyzes	FM1		FM2		FM3	
	Day 1	Day 15	Day 1	Day 15	Day 1	Day 15
Total solids ($\text{g } 100 \text{ g}^{-1}$)	25.27 ± 0.01 ^{aA}	25.10 ± 0.08 ^{aA}	11.01 ± 0.02 ^{aC}	11.08 ± 0.13 ^{aC}	12.82 ± 0.38 ^{aB}	12.63 ± 0.23 ^{aB}
Titrate acidity ($\text{g } 100 \text{ g}^{-1}$)	1.49 ± 0.01 ^{aA}	1.57 ± 0.04 ^{aA}	0.68 ± 0.02 ^{bB}	0.76 ± 0.01 ^{aB}	0.66 ± 0.01 ^{bB}	0.80 ± 0.02 ^{aB}
pH	5.02 ± 0.01 ^{bA}	5.34 ± 0.01 ^{aA}	4.62 ± 0.01 ^{aB}	4.46 ± 0.01 ^{bB}	4.56 ± 0.01 ^{aC}	4.36 ± 0.01 ^{bC}
L^*	80.39 ± 0.54 ^{aB}	74.58 ± 0.32 ^{bB}	79.07 ± 0.40 ^{aC}	73.00 ± 0.13 ^{bC}	83.01 ± 0.39 ^{aA}	77.98 ± 0.13 ^{bA}
b^*	9.14 ± 0.09 ^{aA}	9.30 ± 0.12 ^{aA}	4.58 ± 0.02 ^{aC}	4.26 ± 0.01 ^{bC}	5.14 ± 0.02 ^{aB}	4.79 ± 0.02 ^{bB}
a^*	-2.23 ± 0.02 ^{bB}	-1.82 ± 0.05 ^{aA}	-2.64 ± 0.03 ^{bC}	-2.37 ± 0.01 ^{aB}	-1.98 ± 0.01 ^{bA}	-1.82 ± 0.01 ^{aA}
<i>Lactobacillus acidophilus</i> LA-5 ($\log \text{CFU g}^{-1}$)	8.13 ± 0.01 ^{aC}	7.10 ± 0.29 ^{bB}	8.66 ± 0.01 ^{aB}	8.68 ± 0.01 ^{aA}	8.99 ± 0.09 ^{aA}	9.00 ± 0.02 ^{aA}
<i>Bifidobacterium</i> BB-12 ($\log \text{CFU g}^{-1}$)	8.12 ± 0.01 ^{aC}	6.98 ± 0.01 ^{bB}	8.73 ± 0.04 ^{aB}	8.73 ± 0.01 ^{aA}	8.98 ± 0.01 ^{aA}	9.00 ± 0.02 ^{aA}
<i>Streptococcus salivarius</i> subsp. <i>thermophilus</i> ($\log \text{CFU g}^{-1}$)	8.20 ± 0.02 ^{aC}	7.15 ± 0.21 ^{bB}	8.72 ± 0.01 ^{aB}	9.05 ± 0.08 ^{aA}	8.99 ± 0.01 ^{aA}	9.01 ± 0.02 ^{aA}

^{a,b,c} Means \pm standard deviation in the same row with different lowercase letters are significantly different between storage times for each fermented milk ($p < 0.05$); ^{A,B,C} Means \pm standard deviation on the same line with different capital letters are significantly different between fermented milk for the same storage time ($p < 0.05$). Formulations: FM1 (fermented milk combined with freeze-concentrated skimmed goat milk); FM2 (fermented milk combined with skimmed goat milk with the addition of 6% inulin); and FM3 (fermented milk combined with whole goat milk).

3.2. Viscosity Analysis

Concerning probiotic count, Bedani et al. [26], Muñoz et al. [27], and Verruck et al. [28] observed that the inclusion of prebiotics like inulin in the food matrix further improves the survival rates of *Bifidobacterium* BB-12 by providing a bifidogenic effect. For this reason, in the FM2 sample, there was no reduction in the bifidobacteria count. On the other hand, FM3 was composed of whole milk. Concerning this sample (FM3), the presence of fat resulted in improved bifidobacteria (probiotic) survival. A study carried out by Verruck et al. [29] verified that a higher milk fat content supports better bifidobacteria survival. These authors observed that dairy products with a higher milk fat content show better stability and lower reductions in *Bifidobacterium* BB-12 populations over time. The lower reductions in the *Bifidobacterium* BB-12 count occurred because organic fermented milk has higher levels of unsaturated fatty acids like trans-vaccenic and α -linolenic acids than conventional milk [30,31].

Furthermore, to be classified as a probiotic, a product must contain a minimum of 6 log CFU/mL of viable microorganisms. Note that the *Bifidobacterium* BB-12 count in the FM2 sample on day 15 was equal to 6.98 ± 0.01 . This result guarantees that the product could be classified as a probiotic. Also, in the present study, two probiotic strains were used, so even if there were a reduction in the bifidobacteria count, we would not have any problems regarding the classification of this product as a probiotic because we still had the addition of *Lactobacillus acidophilus* LA-5 with a count that was also ≥ 6 log CFU/mL. Therefore, until day 15, all three fermented milks could be considered as probiotics.

Figure 2 shows the viscosity of the fermented milk samples on days 1 (a) and 15 (b) of storage. As expected, the higher total solids concentration of the FM1 fermented milk obtained by the freeze concentration process resulted in a product with a higher viscosity during storage. The proportional interaction between the solids content and the viscosity agrees with previous publications [32–35]. The viscosity of a system is dependent on the

volume fraction occupied by the contributing particles in combination with the inherent viscosity of the continuous phase. In skimmed milk systems, proteins determine the volume fraction of suspended material. This increase in viscosity occurs because the removal of water causes an increase in the volumetric fraction of dispersed particles and increases micelle–micelle interactions, making the distance between micelles smaller [32]. It was also possible to observe that adding inulin to fermented milk sample FM2 allowed for the production of a product with the same viscosity characteristics as fermented milk made with whole milk (FM3). This behavior likely occurs due to an increase in the total solids in FM2 with the addition of inulin and this prebiotic's ability to retain water, reducing the mobility of the protein matrix and increasing the viscosity of the products [36]. As can be seen, the viscosity of all protein samples decreased as the shear rate increased. This fact indicated that the samples were pseudoplastic fluids with shear thinning and exhibited non-Newtonian behavior. The increase in the shear rate destroyed the protein network structure, reducing the apparent viscosity.

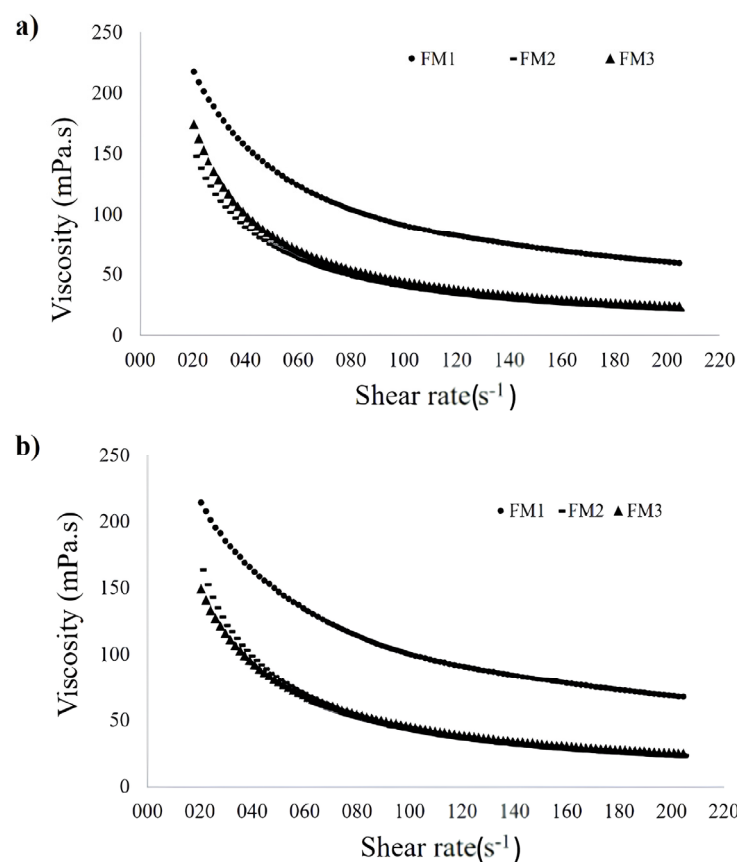


Figure 2. Viscosity versus the shear rate of fermented milk FM1 (fermented milk made with freeze-concentrated skim goat milk), FM2 (fermented milk made with skim goat milk with the addition of 6% inulin), and FM3 (fermented made milk with whole goat milk) on days 1 (a) and 15 (b) of storage at 4.0 ± 1.0 °C. (n = 3).

Figure 3 shows the flow curves obtained from the three fermented goat milk samples. A similar rheological behavior was observed for the FM2 and FM3 samples. The FM1 sample had higher shear stress values than the other samples. The results indicate that the concentration of milks solids increased the viscosity of FM1, contributing to the formation of a stronger protein network, resulting in higher viscosity and gel strength. The formation of a hysteresis curve, the thixotropy, can explain changes in the rheological behavior of a product. Oliveira et al. [37] reported that thixotropy, a phenomenon commonly detected in fragile agglomerated particles, such as fermented milk, occurs when these particles are subjected to a shear force. In this case, the three-dimensional structure initially formed in

the fermentation process is lost and can practically be recovered after rest. It is possible to notice in Figure 3 that all of the fermented milk samples presented hysteresis during storage. The formation of a thixotropic ring shows that, under the action of shear force, the alteration of the internal organization structure is greater, resulting in a lower recovery speed. However, after 15 days of storage, it is possible to observe that all samples required a lower shear force to obtain the product flow curves. This fact indicates that during storage, the gel structures formed during the samples' fermentation became weaker.

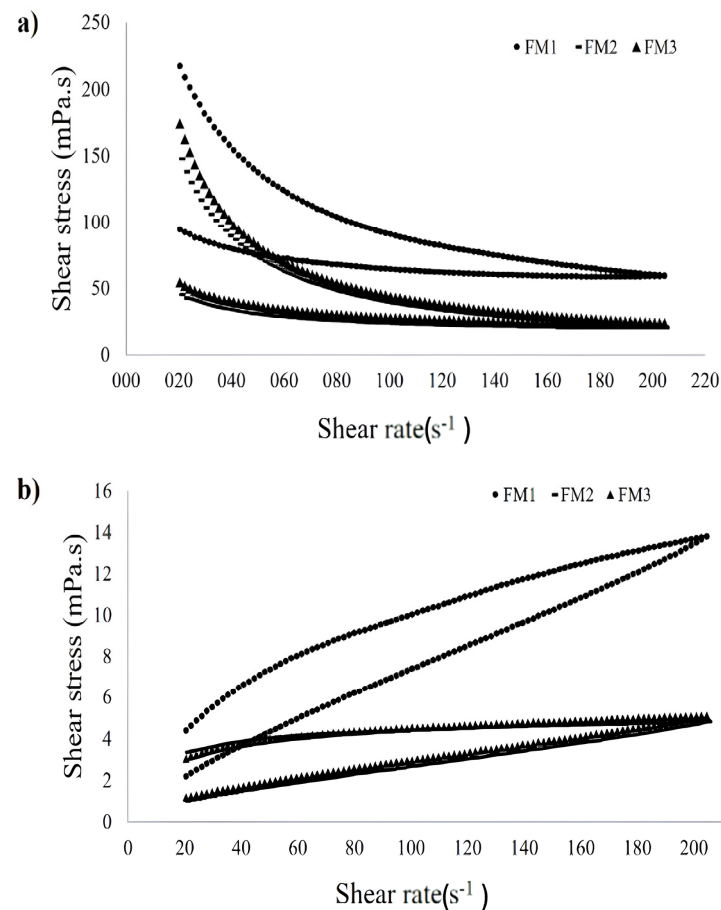


Figure 3. Flow curve of fermented milk FM1 (fermented milk made with freeze-concentrated skim goat milk), FM2 (fermented milk made with skimmed goat milk with the addition of 6% inulin), and FM3 (fermented milk made with whole goat milk) on days 1 (a) and 15 (b) of storage at 4.0 ± 1.0 °C. (n = 3).

3.3. Microbiological Analysis

The counts of the probiotic cultures *Lactobacillus acidophilus* LA-5, *Bifidobacterium* BB-12, and *Streptococcus thermophilus* remained above $6 \log \text{CFU g}^{-1}$ on the first and fifteenth day of storage (Table 1). This indicates that all fermented milks can be classified as probiotics, according to Hill et al. (2014) [5], who define probiotic products as those containing at least 10^6 to 10^7 CFU g⁻¹ or mL⁻¹ of viable cells at the time of consumption.

However, a reduction in the number of viable cells of all microorganisms was observed during storage in the fermented milk FM1, possibly due to the high concentration of organic acids in the product, which may have inhibited bacterial growth. In contrast, prebiotic inulin in FM2 and fat in FM3 contributed to better preservation of the probiotic microorganisms, helping to maintain their viability over time.

3.4. Preferred Attribute Elicitation (PAE)

The results of the sensory acceptance test for the fermented milks are shown in Table 2. Consumers in the PAE assigned scores of 5.73–7.37 on a 9-point scale to samples FM2 and FM3, suggesting that they liked the products slightly or moderately. However, milk fermented with freeze-concentrated skimmed goat milk (FM1) was evaluated with a score of 4.03–7.27, showing a lower score in the flavor attribute and consequently influencing the lower acceptance of this product according to overall impressions. The use of goat milk concentration technology in the preparation of fermented milk had a significant effect on product acceptance ($p > 0.05$) due to the flavor and overall impression attributes, as only the FM2 and FM3 fermented milks were equally accepted ($p > 0.05$).

Table 2. Sensory acceptance of FM1, FM2, and FM3 fermented milk.

Attributes	Fermented Milk		
	FM1	FM2	FM3
Appearance	7.27 ± 1.26 ^a	7.13 ± 1.18 ^a	7.37 ± 1.22 ^a
Aroma	6.30 ± 1.57 ^a	6.20 ± 1.64 ^a	6.83 ± 1.34 ^a
Flavor	4.03 ± 1.99 ^b	5.73 ± 1.79 ^a	6.03 ± 1.58 ^a
Texture	6.30 ± 1.90 ^a	6.73 ± 1.18 ^a	6.50 ± 1.31 ^a
Overall Impression	5.00 ± 2.03 ^b	6.43 ± 1.12 ^a	6.23 ± 1.36 ^a

^{a,b,c} Means ± standard deviation on the same line with different letters significantly differs between formulations for the same sensory attribute ($p < 0.05$). Sensory acceptance (appearance, aroma, taste, texture, and overall impression) using a 9-point hedonic scale (1 = dislike extremely; 2 = dislike very much; 3 = dislike moderately; 4 = dislike slightly; 5 = neither like nor dislike; 6 = like slightly; 7 = like moderately; 8 = like a lot; 9 = like very much, $n = 30$). Formulations: FM1 is fermented milk made with freeze-concentrated skimmed goat milk; FM2 is fermented milk made with skimmed goat milk with 6% inulin; and FM3 is fermented milk made with whole goat milk.

Figure 4a shows results of the two-dimensional GPA of group 1 for the fermented milk formulations made using freeze-concentrated skimmed milk, prebiotic-added skimmed milk, and whole goat milk (FM1, FM2, and FM3) on day 1 of storage. The variation is mainly explained by the F1 axis (77.66%) followed by the F2 axis (22.34%), explaining 100.00% of the data variability. This result is key, as untrained consumers were used as evaluators in the PAE methodology [18]. The first component (F1) was mainly related to the attributes associated with the appearance (yellow and white color) and flavor (goat, salty, and sweet). At the same time, the second component (F2) was associated with the attributes of flavor (sour and sweet), texture (viscous), and aroma (butter aroma).

Figure 4b shows the two-dimensional GPA of group 2, where the variation is also mainly explained by the F1 axis (80.32%) followed by the F2 axis (19.68%), totaling 100.00%. It was observed that, in this group, the first component (F1) was related to attributes associated with all groups: appearance (yellow and white color), flavor (goat taste, salty and sweet), texture (viscous), and aroma (smell goat). In both groups, the first component (F1) separated the formulations based on the type of raw material used to prepare the product; since the formulation that used concentrated skimmed milk is located on the left (FM1), the formulation that used skimmed milk with a prebiotic (FM2) is located in the center. The formulation made with whole milk (FM3) is on the right. In both groups, the FM1 fermented milk generally had a greater goat flavor, acid taste, yellowish color, viscosity, and saltiness. Meanwhile, the FM2 fermented milk was characterized by a sweet taste, and FM3 fermented milk had a whiter color and greater intensity of butter and fermentation aromas.

The high attribution of a goat flavor to FM1 fermented milk may be related to the possible hexanoic acid concentration responsible for the goat flavor/aroma [38]. According to Fazilah et al. [38], there is a consumer preference for products with a reduced goat aroma, which explains the lower score of the FM1 product in the acceptance test concerning taste. This fermented milk's greater acidity and yellowness can also be related to the acidity and

color parameters in Table 1. At the same time, the attribute of a greater viscosity can also be seen in Figure 2.

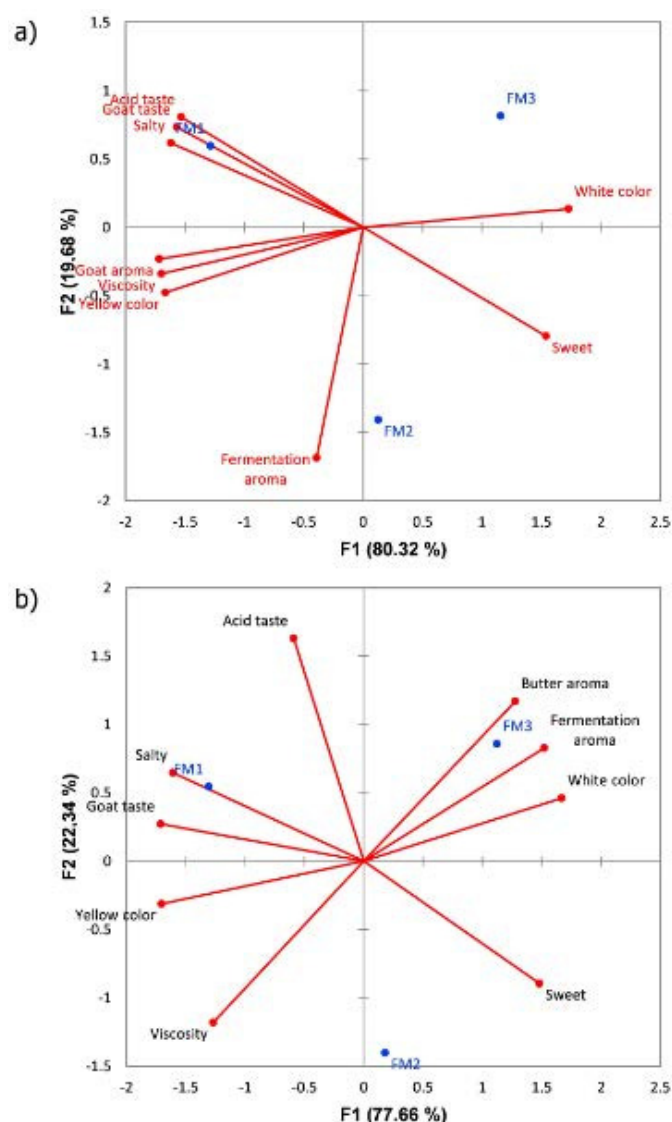


Figure 4. Sensory attributes generated using the PAE method for group 1 (a) and group 2 (b) for samples FM1 (fermented milk elaborated with freeze-concentrated skim goat milk), FM2 (fermented milk elaborated with skimmed goat milk with the addition of 6% inulin), and FM3 (fermented milk elaborated with whole goat milk). The descriptive data from the PAE sessions were combined and normalized using Generalized Procrustes Analysis.

This study proved that fermented milk prepared using concentrated skimmed goat milk does not have adequate sensory acceptance, mainly due to the product's taste. It also proved that replacing goat milk fat with the prebiotic inulin had a significant effect on the acceptance of the fermented milk ($p > 0.05$) for all evaluated attributes (appearance, aroma, flavor, texture, and general impression) since samples FM2 and FM3, which were fermented products, were equally accepted ($p > 0.05$). It was possible to demonstrate that the PAE methodology can provide important information for the characterization of fermented goat milk, with the results correlating with physicochemical and rheological analyses.

Therefore, based on this study's results, it can be seen that PAE consumers should have accept the sensory properties of low-complexity products at the beginning of the product development process. For the food industry, the results are important because they show that significant results can be obtained using PAE without the need for panel training,

resulting in substantial savings of time and resources. Furthermore, PAE can identify the most important attributes for consumer acceptance, guiding the industry and allowing it to focus on these attributes for product reformulation.

4. Conclusions

It was possible to obtain a concentrate with a high value of total solids, which allowed for the production of the fermented milk sample FM1 which presented a higher concentration of total solids and titratable acidity than the other formulations. The freeze concentration process used for FM1 and the addition of inulin to FM2 influenced them to show a greater tendency towards yellow and green coloration. At the same time, FM3 showed greater luminosity when compared with FM1 and FM2. All of the fermented milks were considered as probiotics following storage. The freeze concentration process also influenced a higher FM1 viscosity described during the PAE. With the addition of inulin to FM2, it was possible to produce a fermented milk with a rheological profile similar to that made with whole milk (FM3). However, the freeze concentration of the FM1 sample made with skimmed goat milk interfered with the acceptance of the product since terms such as goat flavor, acidic, and salty flavor were attributed to it. Thus, the PAE methodology is advised to assess consumer acceptance of food products, especially in the initial product development process, which allows for the use of fewer individuals. Finally, it was possible to associate high nutritional value, lower allergenic potential, and better digestibility with the goat milk, making it a promising matrix for developing innovative functional foods with therapeutic potential to meet current market demand. Goat milk products with increased bioactive ingredients and functional properties can lead to innovative and increased consumer demand, making them a valuable resource for functional food development.

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