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Characterization of Goat Milk Fermented With *Streptococcus thermophilus*, *Limosilactobacillus mucosae* CNPC007 and Inulin With Addition of *Cereus jamacaru*

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ABSTRACT

The physicochemical characteristics, microbial viability, antioxidant parameters and sensory features of a goat milk supplemented with inulin, fermented with the commercial starter *Streptococcus thermophilus* QGE and the potentially probiotic indigenous culture *Limosilactobacillus mucosae* CNPC007 as adjunct, which was partially drained, and added with a jam-like dessert of mandacaru (*Cereus jamacaru*) were investigated. The survival of the indigenous culture in the final product under *in vitro* simulated conditions of the gastrointestinal tract was also evaluated. The final product presented physicochemical characteristics compatible with that required by the regulatory standards for fermented milks added of fruits. At the end of storage, *L. mucosae* CNPC007 achieved population higher than 7.8 log CFU g^{-1}. After passage through the *in vitro* enteric phase II in the gastrointestinal simulation, it was possible to recover the potentially probiotic microorganism above 6.00 log CFU g^{-1} for 11% of the samples evaluated. Regarding the sensory acceptability, average scores above 7 were obtained for all the attributes evaluated. The partial whey draining reduced the antioxidant capacity of the fermented milk base when compared with the unprocessed milk. The addition of mandacaru jam to the products resulted in a phenolic content of around 40 mg GAE mg^{-1}, twice the content found in the unprocessed milk, recovering the antioxidant capacity of the removed whey. These results show that the combination of *L. mucosae* CNPC007 culture with inulin and the mandacaru jam is promising for the improvement of the functionality of the fermented goat milk with high sensory acceptability.

1 | Introduction

In the last decade, there has been a growing interest in the use of goat milk and its derivatives as functional foods, given its nutritional and therapeutic properties. When goat milk and bovine milk are compared, it is observed that the first one has alkalinity, high digestibility, and hypoallergenic property (due to low content of α_{s1}-casein), besides undergoing more efficient lipid metabolism in the intestinal tract and having higher mineral content (zinc, calcium, phosphorus, potassium, and magnesium) [1, 2].

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It is also important to highlight the fat content and high whey protein of goat milk and its biological and functional value; however, there are technical difficulties for caprine products associated with the formation of a firm clot and sensory acceptance due to their peculiar taste and odour [3, 4].

In this sense, there is high resistance to the consumption of goat milk and its derivatives. Improvements in handling and processing techniques do not completely eliminate the odour but change or reduce its non-acceptance by the final consumer [5, 6].

On the other hand, the combination of goat milk and functional ingredients is one of the most promising areas of research for the current goat dairy industry [2]. In this context, prebiotic ingredients, probiotic cultures, and fruits can be added to the caprine products to overcome the difficulties related to the goat milk previously described.

The addition of inulin to fermented goat milk favours an increase in product viscosity due to the high degree of polymerization (DP). Inulin is an important prebiotic widely explored by the industry for the production of functional foods, both stimulating the proliferation and activity of desirable bacteria and influencing the stability and sensory aspects of these products. Also, this prebiotic can affect beneficially the biochemical processes that occur during storage and the activity of microorganisms present in the products [7, 8].

This fibre also positively interferes in the lipid metabolism, absorption of mineral ions from the intestine, control of blood sugar levels, and helps to prevent obesity [9]. The addition of inulin in dairy products induces the formation of microcrystals, promoting the sensation of the presence of fat in the mouth, therefore increasing viscosity, intensifying the perception of sweet taste, and balancing palatability [10].

Functional foods that contain prebiotics and probiotics are part of interest to the dairy industry for economic reasons and also because of scientific evidence related to their positive health benefits [11]. Probiotics are defined as live microorganisms that, when administered in adequate amounts, confers benefits to the host [12, 13]. These microorganisms must resist through the gastrointestinal tract passage in sufficient amounts so they can have the desired health effect [13, 14].

Nonetheless, commercial probiotic strains are expensive and increase the price of the product for the final consumer. The search for new probiotic strains among lactic bacteria isolated from raw food products and native fermented reduces the cost of probiotic cultures, benefiting small producers in the dairy sector. In addition, studies also revealed strains with promising technological properties to be applied in food processing [15, 16].

Limosilactobacillus mucosae (previously *Lactobacillus mucosae*) CNPC007 was isolated by researchers from Embrapa Goats and Sheep, Sobral, CE, Brazil, and brings a set of probiotic and technological properties verified by *in vitro* tests, being considered promising for use in functional dairy products. This strain has genes related to intestinal adhesion properties and bile salt tolerance and demonstrates the ability to survive under simulated gastrointestinal conditions [15, 17].

Regarding fruits, the mandacaru (*Cereus jamacaru*), a native species from the Caatinga vegetation, a biome exclusive of Brazil, also present in Paraíba state, Brazilan northeast, has nutritional characteristics that make it attractive for the food industry [18]. Studies carried out by Soares et al. [19] report that *C. jamacaru* fruits have high levels of calcium and potassium, low acidity, high sugar content, and high antioxidant potential due to the presence of phenolic compounds.

Aiming at this field, a fermented goat milk added of inulin, containing *L. mucosae* CNPC007 as adjunct culture and added of a jam of *C. jamacaru*, also containing inulin, was studied regarding their physicochemical characteristics, stability of the adjunct during storage and its survival under simulated gastrointestinal conditions *in vitro*, antioxidant properties and also sensory features. In order to improve the consistency of the final product, in this research the fermented milk base was partially drained, with the objective of increasing the sensory characteristics of the product, being the whey used in other studies by the research group.

2 | Material and Methods

2.1 | Obtaining the Mandacaru Fruits

Fully mature mandacaru fruits were harvested at a local farm in the municipality of Olivedos, Paraíba, Brazil (6° 59′ 27″ S, 36° 14′ 38″ W) in July 2018 and March 2019, and also from the municipality of Queimadas, Paraíba, Brazil (7° 21′ 28″ S 35° 53′ 52″ O) in May 2019. During the preparation of the samples, the fruits, homogeneous in colour and appearance, were selected, cleaned with cold water, sanitized with sodium hypochlorite and depulped for separation into pulp (including seeds) and skin. The mandacaru pulp was frozen under −18°C until be used in the dessert processing.

2.2 | Processing of the Jam From the Mandacaru Fruit Pulp

A jam-like dessert of the mandacaru fruit supplemented with inulin was prepared to be used as flavouring agent of the fermented goat milk. The ingredients used in this dessert and their proportions is shown in Table 1.

The sucrose syrup, consisting of commercial sugar (Estrela, Biosev, Brazil) dissolved in water under heating was added of the 0.2% citric acid solution. The mandacaru pulp, without

TABLE 1 | Proportion of ingredients used in the jam of mandacaru (*C. jamacaru*).

Ingredients	(%)
Mandacaru (*C. jamacaru*) fruit pulp	50%
Sucrose syrup[a]	43%
Inulin[b]	5%
0.2% citric acid solution[c]	0.7%
Cochineal red dye[d]	1.3%

[a]Previously prepared using 61.3% commercial sugar Estrela (Biosev, Ares, Brazil) and 38.7% distilled water.
[b]Orafit GR (distributed by Sweetmix, São Paulo, Brazil).
[c]Arcólor, São Paulo, Brazil.
[d]Obtained from Indústria de Laticínios de Campina Grande S/A, Campina Grande, Brazil.

addition of water, was processed separately in a blender (Walita, São Paulo, Brazil), weighed, and transferred for a container. In this container, the acidified sucrose solution, the prebiotic inulin, supplied as a powder ingredient (Orafti GR, 92% purity, degree of polymerization ≥ 10, Beneo, distributed by Sweetmix, São Paulo, SP, Brazil), and the cochineal red dye were also added. The container was maintained in a water bath at 85°C the total soluble solids content reached 61–65 °Brix. After this, the jam-like dessert was it was stored under 4°C until be used in the fermented milk formulation.

2.3 | Production of the Fermented Milk Base

The fermented milk base used in this study was composed by whole goat milk, the prebiotic inulin, also supplied as a powder ingredient (Orafti GR, 92% purity, degree of polymerization ≥ 10, Beneo, distributed by Sweetmix, São Paulo, SP, Brazil), the commercial starter culture *Streptococcus thermophilus* QGE (Biotech Fermentos e Coagulantes. Ltda., Alto Piquiri, PR, Brazil), and the autochthonous culture *L. mucosae* CNPC007. This fermented milk base was chosen from Galdino et al. [17] for formulation T35 and Pereira et al. [20], that confirmed its safety *in vivo*. For the production of the milk base, 75 g of the prebiotic inulin were solubilized in 1500 g of goat milk, thermally treated at 90°C for 15 min. Then, it was cooled down to 43°C for the addition of the *S. thermophilus* starter culture (0.004 g 100 mL^{-1}) and a pellet (0.005 g 100 mL^{-1}) of the activated autochthonous culture *L. mucosae* CNPC007. Then the milk base was fermented at 42°C until obtaining an acidity higher than 0.6 g of lactic acid 100 g^{-1}.

2.4 | Elaboration of the Fermented Milk Final Product With Mandacaru Jam

At the end of fermentation, the fermented milk base was partially drained with a sterilized cotton cloth, thus obtaining a curd with a firmer consistency (solids content of 19.43 g 100 g^{-1}). This curd was flavoured with the mandacaru jam in the proportion of 15 g 100 g^{-1}. The flavoured curd (95 g) was placed in plastic containers (ca. 100 mL), previously filled with mandacaru jam at the bottom, corresponding to 5 g 100 g^{-1} of the fermented milk final product, forming two phases. Three batches of the fermented goat milk with mandacaru jam were processed (independent replicates).

2.5 | Physicochemical Analysis

The analyses of pH and titratable acidity were carried out in triplicate before (T0) and after fermentation (TF), on the day after manufacture (D1), and after 7, 14, and 21 days of storage (D7, D14, and D21, respectively). The pH, density and titratable acidity analyses were also carried to the unprocessed (raw) milk.

The pH, in a potentiometer (model TEC-5, Tecnal Equipamentos para Laboratório Ltda, Piracicaba, Brazil), density at 15°C, using a thermolactodensimeter (Termômetros HG®, São Paulo, Brazil), and titratable acidity, expressed as g 100 g^{-1} lactic acid, were determined according to the standard methods 017/IV, 423/IV, and 426/IV of the Instituto Adolfo Lutz [21], respectively.

2.6 | Mean Composition Analysis

Total solids, ash, fat and protein content of the final products were determined on the 1st day of storage, in triplicate, for the three batches produced. The unprocessed milk was also analysed. Total solids were determined by drying 2 g samples at 70°C under vacuum using a vacuum (Lucadema Científica, São Paulo, Brazil, model 104/30). Ash was determined gravimetrically by heating the dried samples at 550°C in a muffle furnace (Fornitec – Indústria e Comércio Ltda, São Paulo, Brazil, model 10X12X15). The fat content of the unprocessed milk was determined by the Gerber method [21]. The fat content of the fermented goat milk with the mandacaru fruit jam (final product) was determined according to the method of Folch, Lees, and Sloane Santley [22]. Protein was estimated by measuring the nitrogen content of 0.2 g samples using the micro Kjeldahl method and multiplying by the conversion factor of 6.38 [23]. The total carbohydrate content was obtained by the difference to reach 100 g 100 g^{-1} of the total composition [24].

2.7 | Microbiological Analysis and Resistance of *L. mucosae* to *In Vitro* Gastrointestinal Conditions

The analyses of viability of *S. thermophilus* and *L. mucosae* were carried out in triplicate before (T0) and after fermentation (TF), on the day after manufacture (D1), and after 7, 14, and 21 days of storage (D7, D14, and D21, respectively). The *L. mucosae* survival throughout the *in vitro* simulated gastrointestinal conditions was evaluated in the final product added of mandacaru jam after 21 days of storage.

To evaluate microbial populations in the different sampling periods, 1 mL of each dilution was pour plated in De Man, Rosa & Sharpe agar (Reagen, Quimiobrás Indústrias Químicas SA, Rio de Janeiro, Brazil) acidified to a pH of 5.4 to determine *L. mucosae*, and in M17 medium (Difco, USA) with lactose addition (Vetec – Química Fina Ltda., Rio de Janeiro, Brazil, 5 g L^{-1}) for *S. thermophilus* determination. Both culture media were incubated at 37°C for 48 h according to the methodology described by Buriti et al. [25].

The evaluation the resistance of *L. mucosae* present in the final product added of mandacaru jam at 21 days of storage to simulated gastrointestinal conditions was conducted in triplicate samples using the *in vitro* methodology described by Galdino et al. [17]. Portions of 25 g samples were diluted in 225 mL 0.5% NaCl solution. A 10 mL aliquot dilution of fermented milk was added of 0.3 mL HCl 1 N and solutions of pepsin from porcine mucosa (Sigma, USA, 0.1 mL) and lipase (Amano lipase F-AP15, from *Rhizopus oryzae*, Sigma, 0.01 mL) also dissolved in NaCl 0.5%, achieving 10.41 mL (3.07 g/L pepsin; 0.9 mg/L lipase) with pH between 1.5 and 2.5, which was incubated at 37 ± 1°C for 2 h, under agitation (150 rpm). Next, 0.17 ml NaCl 0.5% and 2 mL sodium phosphate solution pH 12 ($NaH_2PO_42H_2O$, 14 g; NaOH 1 N 150 mL; H_2O distilled *q.s.p* 1 L) containing bile (Bovine bile, Sigma, 0.1017 g) and pancreatin (Pancreatin from porcine pancreas, Sigma, 0.0101 g) were added, achieving a content of 12.58 mL (9.53 g/L bile and 0.953 g/L pancreatin) with pH between 4.5 and

6.0, which was incubated again at 37°C and agitated at 150 rpm for 2 h. After 4 h from the beginning of the assay, a 1 mL aliquot of sodium phosphate solution pH 12 containing bile (0.010 g) and pancreatin (0.0010 g) was added, achieving a final volume of 13.58 mL (9.57 g/L bile and 0.9570 g/L pancreatin), with pH between 6.0 and 7.0, which was again incubated at 37°C and agitated at 150 rpm for 2 h, completing 6 h of assay. Aliquots containing the simulated gastric and enteric juices with final product added of mandacaru jam were collected after 30 min, 2 h, 4 h and 6 h of the in *vitro assay*, which were serially diluted and pour plated into MRS agar pH 5.4 and incubated for 48 h.

2.8 | Phenolic Extraction for Analysis of Phenolic Content and Antioxidant Activity

The extracts prepared from samples of unprocessed goat milk, partially drained fermented goat milk, drained whey and fermented goat milk added with mandacaru pulp (final product, D1, and after 7, 14, and 21 days of storage, D7, D14, and D21, respectively) were obtained according to the methodology proposed by dos Santos et al. [26] with some adaptations.

2.8.1 | Total Phenolic Analysis

The total phenolic content was determined according to the methodology of dos Santos et al. [26] with some modifications. All procedures were performed in the dark. An aliquot of 60 μL prepared extract was transferred to 15-mL plastic test tubes and sequentially added of distilled water (2340 μL), and Folin Ciocalteu reagent (Sigma-Aldrich Chemie GmbH, Steinheim, Germany; 150 μL). All samples were allowed to rest at room temperature for 8 min. After this period, an aliquot of 450 μL of Na_2CO_3 solution (Neon Commercial, São Paulo, Brazil, 30 g/100 mL^{-1}) was added and mixed with the samples, which were left to stand at room temperature for 30 min. The absorbances of the reactions were measured at 750 nm in a spectrophotometer UV SP-2000 (Spectrum, Xangai, China). A standard curve was previously constructed using gallic acid (Vetec, Sigma-Aldrich, Duque de Caxias, Brazil) for the comparison of the absorbance results. The total phenolic content was expressed in terms of mg gallic acid equivalent (mg GAE)100 g^{-1} de sample.

2.8.2 | DPPH Assay and Antioxidant Capacity Calculation

The antioxidant capacity of the same samples was tested using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity, according to the protocol proposed by Karaaslan et al. [27], with adaptations.

The results obtained were expressed as a percent of DPPH radicals scavenging, following equation (1):

$$\text{DPPH radicals scavenging (\%)} = \frac{\text{ABSC} - \text{ABSA}}{\text{ABSC}} \times 100 \quad (1)$$

ABSC = is the control absorbance (absorbance of the DPPH solution without sample extract).

ABSA = is the absorbance with the sample extract.

The amount of sample needed to reduce the initial concentration of DPPH by 50% (EC_{50}) is calculated (initially in g of sample per L of 100 μM of DPPH solution) after constructing the percent inhibition by the extract concentration curve. The final result of the total antioxidant capacity is expressed in g of DPPH sample g^{-1}, according to Rufino et al. [28] following Equation (2):

$$\text{Total antioxidant capacity (g sample g}^{-1}\text{ DPPH)} = \frac{(EC_{50}\ (\text{g L}^{-1}))}{\mu\text{M DPPH} \times 394.3} \times 10^6 \quad (2)$$

μM DPPH is the DPPH (in μM) consumed by the sample to decrease absorbance by 50% during the assay and 394.3 is the molar mass of DPPH in g mol^{-1}.

2.9 | Sensory Analysis

The sensory evaluation of the present study was approved (CAAE: n° 09918118.9.0000.5182) by the Hospital Universitário Alcides Carneiro, Federal University of Campina Grande, UFCG, Paraíba, Brazil, and was carried out at the Laboratory of Sensory Analysis of Federal University of Campina Grande, Campina Grande Campus. The samples were evaluated after 7, 14, and 21 days of storage, and the investigation of indicator microorganisms in the products (coliforms at 35°C, coliforms at 45°C and *Salmonella* sp.) was carried out in the first week of storage, in order to guarantee the safety of the judges. Sensory analysis was performed through acceptability tests, using a 10-point hybrid hedonic scale (0=didn't like it very much; 5 = neither like it nor dislike it; 10 = like it very much) [29, 30], focusing on attributes appearance, colour, texture, odour, flavour and also of overall acceptability. Fifty-four untrained judges participated in the research, mainly students, professors, staff, researcher fellows from the State University of Paraíba (UEPB) and UFCG, and other healthy volunteers who were willing to participate in the study.

The samples were kept refrigerated before the tests and served, in a monadic and random way, in plastic containers coded with three random digits. During one session each consumer analysed three trials. The judges were also instructed to report the sensory attributes related to flavour, sweetness, consistency, appearance and colour, as well as whether they liked or disliked the samples, being free to mention none or more than one attribute.

2.10 | Statistical Analysis

The results were presented as a mean ± standard deviation. For statistical analysis, data on pH, acidity, *L. mucosae* and *S. thermophilus* before and after fermentation (T0 and TF) and during storage (1, 7, 14, and 21 days) were submitted to Friedman's non-parametric analysis of variance and, when necessary, to the Wilcoxon test for the identification of contrasts. For total phenolics, DPPH radical scavenging, EC_{50} and total antioxidant capacity (at 1, 7, 14, and 21 days), and sensory evaluation (at 7, 14, and 21 days) the same tests were used to compare the final product during storage. The analyses were carried out using the Statistica 7.0 software (Statsoft Inc., Tulsa, OK, USA), with $p < 0.05$ as statistically significant.

For the survival of *L. mucosae* in the final product submitted to the *in vitro* simulation of gastrointestinal conditions, the exact binomial test was carried out, considering the null hypothesis (H_0) that the viability proportion above 6 log CFU g^{-1} during the simulation should be at least 25% [31].

3 | Results and Discussion

3.1 | Characterization of Unprocessed Goat Milk

The milk used in the production of the products of the present study was obtained from regional producers and, for this reason, the analyzes for its characterization are important to guarantee the quality of the fermented milks. The results of of density, acidity, pH, total solids, moisture, ash, proteins, lipids, and carbohydrates by difference for the unprocessed milk are shown in Table 2. All the parameters of the unprocessed milk met the requirements of the Normative Instruction n°. 27 for goat milk [33].

3.2 | Mean Composition of Fermented Goat Milk Added With Mandacaru Jam

Table 3 presents the results of the average composition of goat milk after fermentation (TF) and final product, after curd draining and the addition of mandacaru jam. These samples differed significantly between them for almost all compositional parameters studied ($p < 0.05$), except for the ash content in fresh weight base, showing the significant effect of curd draining and the addition of mandacaru jam in the proximate composition of the final product.

According to Brazilian legislation [32], the protein content must be at least 2.9 g 100 g^{-1} for fermented milk and may have lower protein content in fermented milk with added, sugary, and/or flavoured milk, and should not be reduced to a proportion greater than the percentage of non-dairy food substances. The fermented milk flavoured with mandacaru jam of the present study obtained a value higher than the minimum established. Also, according to the legislation, fermented milk is considered partially skimmed when it has a fat content between 0.6 and

TABLE 2 | Mean values (mean ± standard deviation) of the physical-chemical analysis of goat milk.

Parameters		Mean ± standard deviation	Standards*
Density (g L^{-1})		1.030 ± 0,000	1.028–1.034
Acidity (g 100 g^{-1})		0.150 ± 0,003	0.130–0.180
pH		5.99 ± 0,02	—
Total solids at 70°C (g 100 g^{-1})		14.19 ± 0.30	—
Moisture		85.81 ± 0.30	—
Ash (g 100 g^{-1})	Fresh weight sample	0.906 ± 0.020	Min. 0.70
	Dried weight sample	6.40 ± 0.10	—
Fat content (%)	Fresh weight sample	4.2 ± 0.3	Min. 2.9
	Dried weight sample	29.35 ± 1.43	—
Protein content (g 100 g^{-1})	Fresh weight sample	3.71 ± 0,20	Min. 2.8
	Dried weight sample	26.18 ± 1.60	—
Carbohydrates by difference (g 100 g^{-1})	Fresh weight sample	5.40 ± 0.16	Min 4.3
	Dried weight sample	23.90 ± 1.45	—

Note: * According to the Brazilian regulatory standards for goat milk [32]. — = parameter not required. Min. = minimum.

TABLE 3 | Mean composition (± standard deviation) of goat's milk plus inulin after fermentation (fermented milk, TF) and fermented goat curd (after draining) with the addition of mandacaru jam (final product).

Parameters		Fermented milk (TF)	Final product
Total solids at 70°C (g 100 g^{-1})		19.4 ± 0.10^{a}	27.81 ± 2.14^{b}
Moisture		80.6 ± 0.10^{b}	72.19 ± 2.14^{a}
Ash (g 100 g^{-1})	Fresh weight sample	0.794 ± 0.050^{a}	0.772 ± 0.024^{a}
	Dried weight sample	4.10 ± 0.27^{b}	2.80 ± 0.18^{a}
Fat content (%)	Fresh weight sample	4.03 ± 0.06^{b}	2.54 ± 0.61^{a}
	Dried weight sample	20.76 ± 0.23^{b}	9.06 ± 1.75^{a}
Protein content (g 100 g^{-1})	Fresh weight sample	5.8 ± 0.30^{b}	3.25 ± 0.17^{a}
	Dried weight sample	27.21 ± 1.50^{b}	11.73 ± 0.80^{a}
Carbohydrates by difference (g 100 g^{-1})	Fresh weight sample	9.31 ± 0.30^{a}	21.25 ± 1.64^{b}
	Dried weight sample	28.51 ± 1.57^{a}	48.60 ± 3.00^{b}

Note: ^{a,b} different superscript lowercase letters overwritten in the same row differ significantly between milk after fermentation (TF) and the final product for the same parameters ($p < 0.05$).

2.9 g 100 g^{-1} [32]. Fermented goat milk added to mandacaru jam had a fat content of 2.55 g 100 g^{-1}. The fat content of fermented milk was lower than that of unprocessed goat milk, a fact justified by the process of removing the whey from the product after fermentation, thus causing loss of fat which is partially adhered to the materials used during the process.

The carbohydrate content of milk (5.4 g 100 g^{-1}) increased after fermentation (9.31 g 100 g^{-1}). This fact can be explained because, even after draining the fermented milk with the addition of inulin, part of the inulin remained in the clotted mass, improving its consistency as will be seen later in the results of the sensory analysis, as well as the fraction corresponding to mandacaru jam was equally added inulin. In addition, mandacaru jam was also sweetened, being also a source of sugars, contributing to the total carbohydrate content.

3.3 | Physical-Chemical and Microbiological Evaluation during the Storage Period

The changes in the mean values of pH, titratable acidity, in the populations of *L. mucosae*. and *S. thermophilus* during 21 days of storage are shown in Table 4.

There were significant changes in the pH values of the final product between the 1st and 7th day of storage ($p < 0.05$). On the 21st day, there was a significant increase in titratable acidity about the first day of storage ($p < 0.05$). The increase in titratable acidity occurs due to the active metabolism of the added

TABLE 4 | Values of pH, titratable acidity and *L. mucosae*. and *S. thermophilus* populations in goat's milk plus inulin before (T0) and after fermentation (TF) and in the final product added of mandacaru jam after 1, 7, 14 and 21 days of storage at 4 ± 1°C (D1, D7, D14, and D21, respectively).

	Parameters			
Sampling periods	pH	Acidity (g lactic acid 100 g^{-1})	*L. mucosae* Log CFU g^{-1}	*S. thermophilus* Log CFU g^{-1}
T0	6.04 ± 0.23^{a}	0.180 ± 0.025^{d}	7.93 ± 0.31^{a}	7.92 ± 0.33^{c}
TF	4.50 ± 0.30^{bc}	0.670 ± 0.066^{c}	8.02 ± 0.40^{a}	8.56 ± 0.11^{ab}
D1	4.48 ± 0.25^{b}	0.680 ± 0.077^{bc}	8.13 ± 0.40^{a}	8.35 ± 0.43^{b}
D7	4.40 ± 0.15^{c}	0.694 ± 0.060^{bc}	8.02 ± 0.18^{a}	8.70 ± 0.17^{a}
D14	4.37 ± 0.24^{bc}	0.734 ± 0.050^{ab}	7.75 ± 0.28^{a}	8.53 ± 0.26^{b}
D21	4.47 ± 0.28^{bc}	0.751 ± 0.080^{a}	7.80 ± 0.40^{a}	8.52 ± 0.42^{ab}

Note: ^{a,b} different superscript lowercase letters overwritten in the same column differ significantly over sampling periods for the same parameters ($p < 0.05$).

TABLE 5 | Survival of probiotic cultures (minimum and maximum values, log CFU g^{-1}) under *in vitro* gastrointestinal conditions GP (gastric phase), EPI (enteric phase I), and EPII (enteric phase II) in the final product added of mandacaru jam after 21 days of storage.

Simulation phases	Item	Final product
Initial	Value (log CFU g^{-1})	7.34–8.00
	% of samples above 6 log CFU g^{-1}	100 (9/9)^{a}
GP 30 min	Value (log CFU g^{-1})	4.02–5.02
	% of samples above 6 log CFU g^{-1}	0 (0/18)^{b}
GP 2 h	Value (log CFU g^{-1})	4.02–5.70
	% of samples above 6 log CFU g^{-1}	0 (0/18)^{a}
EPI	Value (log CFU g^{-1})	4.60–6.04
	% of samples above 6 log CFU g^{-1}	5.5 (1/18)^{b}
EPII	Value (log CFU g^{-1})	4.41–6.10
	% of samples above 6 log CFU g^{-1}	11 (2/18)
Overall	% of samples above 6 log CFU g^{-1}	4 (3/72)^{b}

Note: ^{a,b} Superscript lowercase letters differ significantly in the binomial exact test ($p < 0.05$) for the viability proportion above 6 log UFC g^{-1} considering the null hypothesis (H_0) that such proportion during the *in vitro* gastrointestinal simulation assay should be, at least, 25% below the value obtained before the beginning of the *in vitro* assay.

cultures, a phenomenon known as post-acidification [34]. During fermentation and storage, there were significant differences ($p < 0.05$) in the parameters results of pH, acidity, and *S. thermophilus* population. During the fermentation process, the population of *S. thermophilus* increased by 0.6 log cycle, achieving viability higher than 8.5 log CFU g^{-1} at the end of this period (TF). This behaviour was not verified for *L. mucosae*, which remained around 8.0 log CFU g^{-1} during the fermentation process, without significant differences ($p > 0.05$). For acidity values, a significant difference ($p < 0.05$) was observed between the beginning of fermentation and after 6 h of fermentation, which was already expected due to the acidification caused by the metabolism of lactic acid cultures in the fermentation process. During storage, there was a significant difference on the 21st day.

The addition of certain fruit juices and pulps can be harmful to the survival capacity of some species and strains of probiotic microorganisms in food products, mainly due to acidity and the presence of antimicrobial compounds [35–37]. However, no significant differences in the *L. mucosae* population were verified ($p > 0.05$) throughout the storage period for the product of the present study, remaining above 7 log CFU g^{-1}. These results corroborated with those verified by Buriti et al. [25], since in that study the probiotic strains analysed (*Lacticaseibacillus rhamnosus* LR-32 and *Bifidobacterium animalis* subsp. *lactis* BB-12) maintained good viability, above 7 log CFU g^{-1} in the presence of fruit pulps (guava and soursop). According to Martinez, Bedani and Saad [38], the concentration of probiotic bacteria in a portion of food required to perform activities beneficial to health is at least between 6 and 8 log CFU g^{-1} of the food or 8 to 10 log CFU. g^{-1} per day, based on human clinical trials reported in those authors' reviews. On the other hand, *S. thermophilus* populations differed significantly during storage ($p < 0.05$). Although biologically with changes of only 0.35 log cycle or lower, on the 7th day of storage, there was a significant increase in the starter culture population and after 14 days there was a significant decrease in this population when compared to the 7th day. However, this reduction was not significant when compared to the 1st day of storage ($p > 0.05$). At the end of storage, the population of *S. thermophilus* remained above 8 log CFU g^{-1}. Considering the sum of *Lactobacillaceae* and *S. thermophilus*, the viability of these microorganisms remained within the requirements of the legislation throughout storage, above 6 log CFU g^{-1} of viable lactic acid bacteria in fermented milk [32].

Feng et al. [39] in their study observed that the addition of fruit pulp decreases the population of *Lactobacillus bulgaricus* and *S. thermophilus*. However, Barbosa and Gallina [40] did not observe significant differences ($p > 0.05$) in the starter culture

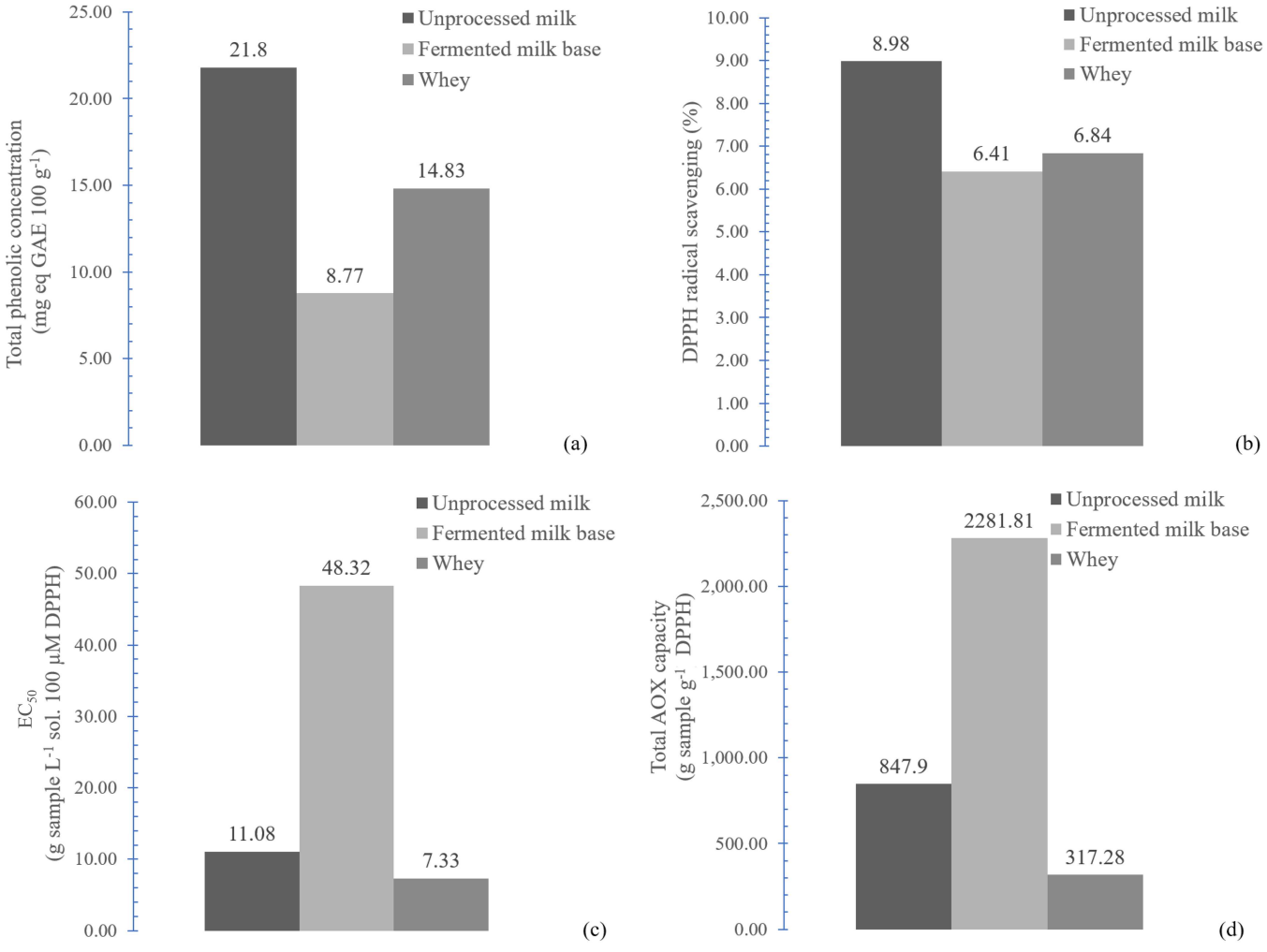


FIGURE 1 | Total phenolic content (a), percent of DPPH radicals scavenging (b), EC_{50} (c), and total antioxidant capacity (d) of unprocessed (raw) milk, fermented milk base (curd after whey draining), and the resulting whey.

population in yogurt made with mango pulp, corroborating the results obtained in this study.

3.4 | Survival of Probiotics under Simulated Gastrointestinal Conditions

Table 5 shows the results of lactobacilli survival after simulating the gastrointestinal conditions of fermented goat milk flavoured with mandacaru jam (final product) after 21 days of storage.

Before the simulation, the gastrointestinal conditions, the population of lactobacilli obtained was from 7.34 to 8.00 log of CFU g^{-1} after 21 days of storage, in accordance with what is proposed by the literature, in which the population of these microorganisms must remain viable in the product during the storage in an amount above 6–7 log CFU g^{-1} to provide the consumer with amounts of at least 8–9 log CFU in the daily serving. In the present study, the greatest reduction in viable cell count was observed after the gastric phase, and the recovery of lactobacilli count was recorded after the last enteric phase.

During the gastric phase (pH–1.8) a decrease of 3.32 to 2.30 log cycles was observed about the initial population of the native culture, similar results were found by Galdino et al. [17], the authors described that after the gastric phase, the populations of *L. mucosae* CNPC007 showed a decrease of up to 2.14 log cycles in fermented milk and from 2.14 to 3.11 log cycles in MRS broth.

At the end of the enteric phase (pH–6.64), there was a small recovery of these microorganisms about the previous phase and a decrease from 2.03 to 1.90 about the initial population of lactobacilli, also in the study by Galdino et al. [17]. After the end of enteric phase II (pH 6.2–6.7), the populations were, in general, higher than those observed in the previous phases from the second hour of the trial. This decrease between the gastric and enteric phases is due to the temporary stress caused by the pH variation in these phases. The pH of the gastric phase causes damage to the cells of microorganisms, which can be repaired in the enteric phase [41].

Although there was a significant decrease in the population of the native culture during the simulation of gastrointestinal conditions, it can be stated that these microorganisms can survive this condition. Complementary studies should be carried out to verify if the amount reached is capable of developing benefits *in vivo*.

3.5 | Total Phenolics and Antioxidant Activity of Fruits

Figure 1 shows the results of total phenolic content (a), DPPH radical scavenging (b), EC_{50} (c) and total antioxidant capacity (d) of unprocessed goat milk and goat milk after fermentation.

It is possible to observe in Figure 1a that there was a decrease of almost 50% in the amount of total phenolic compounds after fermentation of goat milk, a fact that could be justified by the consumption of these compounds by lactic acid bacteria during fermentation [42] and also due to the loss of phenolic compounds during whey draining since they are water-soluble molecules [43]. Although in the present study, desiccation was carried out to improve the consistency of the fermented milk, the whey of this product, a source of bioactive and containing part of the inulin used in the fermentation, was destined for the elaboration of dairy drinks and other products studied by this research group, thus bringing benefits to these other products.

The potential of goat milk in DPPH radical scavenging was close to 9% while for fermented milk it was 6.41% (Figure 1b). According to Li et al. [42], lactic acid bacteria consume antioxidant compounds and simultaneously produce oxygenated compounds (free radicals) during fermentation. This fact can be confirmed, since, in Figure 1c. it is noted that the amount of fermented milk required to consume half the concentration of the DPPH radical was almost five times greater than that of unprocessed milk. It is observed, therefore, that the milk lost its antioxidant capacity after fermentation (Figure 1d) since for fermented milk it takes five times the amount of sample to exert an antioxidant effect (consumption of 1 g of DPPH) compared to unfermented milk.

Table 6 have presented the mean values of phenolic compounds content and antioxidant potential of fermented milk added to mandacaru jam on days 1, 7, 14 and 21 of storage (D1, D7, D14, D21, respectively).

Based on the phenolic content of the fermented milk, it is possible to state that the addition of goat's milk jam collaborates with the phenolic compounds content of the final product since it resulted in a concentration twice as high as that found in the fermented milk with 30% higher than that found in raw milk. These results can be justified by the importance of the nutritional value of the fruit of *C. jamacaru* fruits, having high antioxidant potential due to the presence of phenolic compounds

TABLE 6 | Mean values (mean ± standard deviation) of phenolic compounds content and antioxidant potential of the final product added of mandacaru jam on days 1, 7, 14 and 21 of storage (D1, D7, D14, and D21, respectively).

Parameters	Time (day)	Mean ± standard deviation
Total phenolic content (mg eq GAE 100 g^{-1})	D1	40.33 ± 11.17^{a}
	D7	34.41 ± 6.05^{a}
	D14	38.94 ± 11.92^{a}
	D21	34.54 ± 11.26^{a}
DPPH radical scavenging (%)	D1	16.53 ± 9.54^{a}
	D7	11.83 ± 7.40^{ab}
	D14	14.67 ± 9.90^{b}
	D21	12.46 ± 7.60^{b}
EC_{50} (g sample L^{-1} sol. 100 μM DPPH)	D1	7.27 ± 1.39^{a}
	D7	8.22 ± 1.76^{a}
	D14	7.03 ± 1.90^{a}
	D21	8.15 ± 2.04^{a}
Total AOX capacity (g sample g^{-1} DPPH)	D1	279.37 ± 55.00^{b}
	D7	331.20 ± 41.00^{a}
	D14	279.66 ± 31.57^{b}
	D21	332.69 ± 85.57^{ab}

Note: [a,b] the same lowercase letters do not differ significantly over time for the same parameters ($p < 0.05$).

[19]. During storage, the total phenolic content did not differ significantly in 21 days ($p > 0.05$), evidencing the stability of these compounds in the product, even containing viable lactic acid bacteria.

However, the DPPH radical scavenging capacity of the fermented milk was significantly ($p < 0.05$) higher on the first day of storage compared to the 14th and 21st days. However, between the second and the last week of storage, there were no significant changes ($p > 0.05$). On the other hand, the minimum amount necessary to reduce the initial concentration of DPPH by half did not differ significantly ($p > 0.05$) during storage, similarly to what was verified for the total phenolic content.

The total antioxidant capacity only differed significantly ($p < 0.05$) on the 7th day of storage. In general, an average of 300 g of product is needed to capture 1 g of DPPH. Considering both the 200 g portion recommended for fermented milk and the 120 g portion for jam on each occasion of consumption of these products [44], on average, one and a half portions of the product studied might be able to exert the total antioxidant capacity.

3.6 | Sensory Analysis

Fifty-four tasters participated in this analysis, 13 men and 41 women, aged between 18 and 58 years. The results of the sensory analysis are presented in Table 7.

The scores for the appearance, colour, and consistency parameters started high, close to or higher than 8, but reduced significantly between 7 and 14 days of storage. This characteristic is probably related to the acidity of the product, which already shows a tendency to increase in 14 days. Post-acidification has been described as a phenomenon harmful to the texture and appearance of the product [34], which would justify the lower scores from day 14 onwards.

The addition of some types of fruit, such as those grown in the semiarid region, and of fibres such as inulin-type fructans, is an alternative to improve the viscosity and acceptability of goat dairy products, in addition to contributing with nutrients that are not contained in milk, mainly food fibres [25].

TABLE 7 | Overall acceptability values (mean ± standard deviation) obtained in the sensory analysis with 54 tasters for the final product added of mandacaru jam after 7, 14 and 21 days of storage (D1, D7, D14, and D21, respectively).

	Time (day)		
Parameters	**D7**	**D14**	**D21**
Appearance	7.91 ± 2.24^{a}	7.44 ± 2.25^{b}	$7.50 \pm 1{,}95^{ab}$
Colour	8.22 ± 1.84^{a}	7.54 ± 2.34^{b}	$7.44 \pm 2{,}06^{b}$
Texture	8.04 ± 2.11^{a}	7.35 ± 2.40^{b}	$7.22 \pm 2{,}60^{b}$
Odour	7.70 ± 2.02^{s}	7.57 ± 2.26^{a}	$7.30 \pm 2{,}17^{a}$
Flavour	7.54 ± 2.38^{a}	7.15 ± 2.44^{a}	$6.74 \pm 2{,}53^{a}$
Overall acceptability	7.83 ± 1.83^{a}	7.11 ± 2.24^{a}	$6.90 \pm 2{,}23^{a}$

Note: ^{a,b} the same lowercase letters do not differ significantly over time for the same parameters.

However, the storage time factor did not affect the general acceptability of the panellists. Despite the downward trend in grades, there was no significant difference ($p > 0.05$) due to the high standard deviation. The same occurred with the parameters of odour and flavour that are related to each other.

According to Slačanac et al. [4], during fermentation of goat milk, the nutritional value increases and the characteristic flavour becomes less noticeable, which could justify the higher scores for all parameters evaluated on day 7.

4 | Conclusion

Fermented goat milk flavoured with madacaru jam presented average composition within the parameters established by Brazilian legislation, remained stable for 21 days in terms of total phenolic content, EC_{50}, as well as having good global sensory acceptance (grades above 7 on a scale of 0 to 10).

The strain *L. mucosae* CNPC007 showed good viability in the presence of jam from mandacaru fruit with values above 7 log CFU g^{-1} and in the simulation of gastrointestinal conditions in vitro, after enteric phase II, there was a recovery of the population of autochthonous culture that reached 6 log CFU g^{-1} in the final product after 21 days of storage.

The addition of mandacaru fruit jam favoured an increase in the content of phenolic compounds in the final product since it resulted in a concentration 30% higher than that found in raw milk.

The combination of the potentially probiotic native culture *L. mucosae* CNPC007 with inulin and mandacaru jam showed favourable results for the fermented goat milk, in addition to being a source of bioactive compounds that contributes to the use of this fruit during its harvest.

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Ethics Statement

The sensory evaluation of the present study was approved by the Hospital Universitário Alcides Carneiro, Federal University of Campina Grande, UFCG, Paraíba, Brazil (CAAE: n° 09918118.9.0000.5182). All participants gave their informed consent prior to the assays.

Conflicts of Interest

The authors declare no conflicts of interest. Karina Maria Olbrich dos Santos and Antonio Silvio do Egito are employed by Brazilian Agricultural Research Corporation (EMBRAPA).

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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