Autochthonous adjunct culture of *Limosilactobacillus mucosae* CNPC007 improved the techno-functional, physicochemical, and sensory properties of goat milk Greek-style yogurt

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ABSTRACT

We evaluated the performance of *Limosilactobacillus mucosae* CNPC007 as an autochthonous adjunct culture in the production of goat milk Greek-style yogurt. The techno-functional, physicochemical, and sensory characteristics of the control yogurt (containing only starter culture, CY) and the probiotic yogurt (with the probiotic strain added, PY) were assessed during 28 d of refrigerated storage. Furthermore, we determined the survival of the strain throughout the gastrointestinal tract under simulated conditions. The PY yogurt had a lower extent of proteolysis index and a higher depth of proteolysis index. These results indicate that the proteolytic enzymes of *L. mucosae* may have a possible action in PY. The PY formulation exhibited viscosity almost 1.5 times as high as CY over the refrigeration period, probably due to higher production of exopolysaccharides by the probiotic strain, which directly interferes with the microstructure, texture, and viscosity of the product. The PY formulation received higher scores for color, flavor, and global acceptance at 1 d of storage and higher texture scores at 28 d. The counts of *L. mucosae* remained high (>7 log cfu/g and >8.5 log cfu/g) throughout mouth-ileum digestion and storage, respectively, in PY. The autochthonous adjunct culture of *L. mucosae* CNPC007 can be used for production of a novel potentially probiotic goat yogurt without

negatively affecting the general characteristics of the product quality, adding value associated with maintaining its functional potential.

Key words: fermented goat milk, proteolysis, viscosity, gastrointestinal conditions

INTRODUCTION

Goat dairy milk products, such as cheese, yogurt, and fermented milk, have been gaining ground among consumers (Jia et al., 2016; de Santis et al., 2019). Despite its technological and market challenges (Ranadheera et al., 2012; Gomes et al., 2013; Yamazi et al., 2013; Ribeiro et al., 2014), goat milk has some nutritional, functional, and technological advantages over cow milk, including greater digestibility and the ability to improve absorption of iron and copper (Silanikove et al., 2010; Mituniewicz-Malek et al., 2014; Clark and Mora García, 2017; Verruck et al., 2019); these characteristics give this food matrix great potential for the elaboration of dairy products, such as yogurt.

Protein-rich yogurts such as Greek-style yogurts are consumed worldwide and exhibit varying compositions and designations, depending on the place of origin (Aryana and Olson, 2017). These yogurts are characterized by 9 to 10% protein content, creamy texture, and low fat content (Wouters, 2012). Further health benefits result from the addition of probiotic bacteria to dairy products (de Oliveira et al., 2014; Ribeiro et al., 2014; Martins et al., 2018) such as yogurt (Aryana and Olson, 2017), ice cream (Balthazar et al., 2018), and cheese (Silva et al., 2018). The addition of probiotics, live microorganisms that, when ingested in adequate amounts,

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provide benefits to the host, can add greater functional value to goat dairy derivatives (Pal, Dudhrejiya, and Pinto, 2017; de Paula et al., 2020). Probiotics act through several mechanisms of action that culminate in important functions for the consumer's health. Among the properties of these microorganisms, the following stand out: prevention and treatment of gastrointestinal diseases, modulation of the immune system and intestinal microbiota, anticholesterolemic, anti-dyslipidemic and antihypertensive effects, and anticancer, antimicrobial, and antioxidant activities (Fijan, 2014; Terpou et al., 2019).

The genus *Lactobacillus* contains several potentially probiotic species, including *Limosilactobacillus mucosae*, a name first proposed in 2000 (Roos et al., 2000). The adhesion of this lactic acid bacteria to gastrointestinal mucus enables efficient intestine colonization. The ability of *L. mucosae* to modulate the intestinal immune system and inhibit pathogenic bacteria (by various mechanisms of action, including the production of organic acids and bacteriocins) renders it a valuable strain in probiotic food development (Bilková et al., 2011). Additionally, the strain's ability to produce exopolysaccharides can be explored in the formulation of dairy products with a richer texture and lower degree of syneresis (London et al., 2015). In particular, the *Limosilactobacillus mucosae* strain CNPC007 was isolated from goat milk by a group of researchers from the Brazilian Agricultural Research Corporation (Embrapa). This strain brings together a set of probiotic and technological properties previously verified from in vitro tests (de Moraes et al., 2017) and is considered promising for application in functional dairy products (de Moraes et al., 2018).

In this study, we evaluated the effects promoted by the addition of an autochthonous adjunct culture of *Limosilactobacillus mucosae* CNPC007 on the techno-functional, physicochemical, and sensory characteristics of Greek-style goat yogurt, including the survival of the strain along the simulated gastrointestinal tract.

MATERIALS AND METHODS

Raw Material and Ingredients

Milk from Toggenburg goats was provided by a cooperative in Nova Floresta, Paraíba, Brazil. Commercial goat milk powder (Caprilat) and sugar (União, Brazil) were used in the yogurt formulations. The indigenous culture *Limosilactobacillus mucosae* CNPC007 was obtained from the Collection of Microorganisms of Interest to the Food and Agroenergy Industry of Embrapa Agroindustry Tropical (Fortaleza, Ceará, Brazil), and

was cultivated according to de Moraes et al. (2017). The starter culture (Y 472, Clerici-Sacco, Brazil), composed of *Streptococcus salivarius* ssp. *thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, was acquired commercially.

Milk Quality Control

The titratable acidity, pH, total dry extract, defatted dry extract, protein, fat, and lactose content of goat milk were determined according to the methods of AOAC International (2016). The raw material was assessed for the most probable number (MPN) of total coliforms and thermotolerants (MPN/g), molds and yeast counts in cfu/g, and total count of aerobic mesophilic bacteria (cfu/g) and detection of absence of *Salmonella* spp. per 25 mL, considering the criteria established in the current Brazilian legislation (Ministry of Agriculture, Livestock, and Supply, 2000) and standard procedures described elsewhere by the American Public Health Association (APHA, 2015).

Inoculum and Yogurt Preparation

The inoculum of the probiotic bacteria in milk was prepared in 2 stages. Inoculum 1 was prepared by diluting 0.1 g of lyophilized *L. mucosae* CNPC007 in 10 mL of reconstituted powdered goat milk (Caprilat) in sterile water and incubated for 22 h (stationary phase) at 37°C. Final inoculum was prepared in a proportion of 1/2 of inoculum 1 + 10 mL of powdered milk reconstituted in sterile water, and incubated for 22 h at 37°C, with final counts ranging from 10 to 11 log cfu/g. The counts were confirmed by using serial dilutions of the inoculum with sterile peptone water at a concentration of 0.1 g/100 mL (Sigma-Aldrich). Then 10 µL of the proper dilutions (10^{-1} to 10^{-6}) were poured onto de Man, Rogosa, and Sharpe agar (Oxoid) and acidified to pH 5 (IDF, 1995) by the microdrop technique. The plates were incubated aerobically at 37°C for 48 h. The results were expressed in log cfu/g.

Yogurts were made from pasteurized goat milk (65°C/30 min), combined with 10% sucrose and 10% powdered goat milk, and subjected to heat treatment ($90 \pm 0.5^\circ\text{C}/10$ min). After cooling to 40°C, the milk had a starter culture added (*Strep. salivarius* ssp. *thermophilus* and *L. delbrueckii* ssp. *bulgaricus*; control yogurt, **CY**), or a starter culture and 100 g of final inoculum containing *L. mucosae* CNPC007 were added (probiotic yogurt, **PY**). The 2 yogurt formulations (Table 1) were incubated in biochemical oxygen demand at $45 \pm 0.5^\circ\text{C}$ for 6 h. Fermentation was terminated at pH 4.5. After overnight refrigeration, the yogurt was drained in a cloth bag for about 18 to 20 h,

Table 1. Yogurt formulations

Code ¹	Proportions of microorganism
CY	0.4 g starter culture/L of milk
PY	0.4 g/L starter culture + 100 g <i>L. mucosae</i> final inoculum/L of milk

¹CY = control yogurt; PY = probiotic yogurt with added *Limosilactobacillus mucosae* CNPC007.

as suggested by Şanlıdere Aloğlu and Öner (2013). The yogurt was stored at $4 \pm 0.5^\circ\text{C}$ until analysis.

Yogurts were evaluated at times 1, 7, 14, 21, and 28 d of cold storage ($4 \pm 0.5^\circ\text{C}$), regarding their technological, physicochemical, microbiological, and sensory characteristics. The protective effect of the food matrix on the survival of lactic acid bacteria in simulated conditions of the gastrointestinal tract (GIT) was assessed at 7 d of cold storage.

Yogurt Characterization

Technological Analyses. Apparent viscosity, water retention capacity (WRC), and syneresis of yogurts were assessed in triplicate throughout storage. The susceptibility of yogurt to the separation of water from the clot (syneresis) was determined by draining of 30 g samples for 5 h at 4°C (Riener et al., 2010). Syneresis (%) was computed by Equation 1:

$$\text{Syneresis} = \frac{\text{Whey mass after filtration}}{\text{Fermented milk mass}} \times 100. \quad (1)$$

The WRC was determined by centrifuging yogurt samples under $2,397 \times g$, 15 min, 10°C , with a refrigerated centrifuge (model CT-5000R, Cientec; Harte et al., 2003). Apparent viscosity (mPa·s) of 7.5-mL samples was measured with a Brookfield viscometer (model DV II+Pro) with spindle SC4-21, coupled to a thermostatic bath, at 4°C and a speed of 40 rpm.

Physicochemical Analyses. Yogurts were submitted, in triplicate, to acidity in lactic acid, pH, total sugars, and proteolysis analyses according to the methods of the Association of Official Analytical Chemists (AOAC, 2016). The following tests were performed: pH with a digital pH meter (Q400, Quimis); acidity in lactic acid by titration; and total sugars by Fehling reduction. The extent of proteolysis index and the depth of proteolysis index were determined by the micro-Kjeldahl method (AOAC, 2016) and Equations [2] and [3] (Andreatta et al., 2007):

$$\text{EPI} = \frac{\text{SN at pH 4.6}}{\text{TN}} \times 100; \quad (2)$$

$$\text{DPI} = \frac{\text{SN in TCA}}{\text{TN}} \times 100, \quad (3)$$

where EPI is the extent of proteolysis index (%); DPI is the depth of proteolysis index (%); SN represents the soluble nitrogen; TN, the total nitrogen; and TCA represents trichloroacetic acid.

Sanitary Quality and Lactic Acid Bacteria Viability

Quality control tests comprised the *Escherichia coli* count, as well as the total mold and yeast counts in colony-forming units per gram (cfu/g), and detection of absence of *Salmonella* spp. per 25 g (APHA, 2015; Brazil, National Health Surveillance Agency, 2019). The lactic acid bacteria viability included the counts of *Strep. salivarius* ssp. *thermophilus* (APHA, 2015), *Limosilactobacillus mucosae* CNPC007 (London et al., 2015), and *Lactobacillus* ssp. *bulgaricus* (Lima et al., 2009).

Survival of Probiotic Bacteria Under Simulated Gastrointestinal Conditions

The survival of lactic acid bacteria in yogurts stored at $4 \pm 0.5^\circ\text{C}$ for 7 d was assessed during GIT simulated digestion, according to de Oliveira et al. (2014) and Madureira et al. (2011).

Inoculation of Fermented Milk Matrices. For PY yogurt formulation, 5 samples labeled C1, C2, C3, S1, and S2 were produced: C1 and C2 are duplicate control yogurts, which were inoculated with the tested probiotic strain but not exposed to simulated gastrointestinal conditions; C3 refers to the control yogurt inoculated with probiotic strain and exposed to simulated gastrointestinal conditions (used in pH adjustments over the stages of simulated digestion); S1 and S2 are yogurts inoculated and exposed to simulated gastrointestinal conditions. All samples were prepared in sterile 50-mL vials, containing 25 g of yogurt.

Simulation of Gastrointestinal Conditions. The gastrointestinal tract was simulated as per the conditions described in Table 2 and as follows:

- Stage 1 (Before simulation): Samples were evaluated before simulation of ingestion.
- Stage 2 (Mouth): Chewing was simulated using a saliva solution prepared with 100 U/mL of α -amylase (Sigma-Aldrich) diluted in 1 mM CaCl_2 solution. Saliva solution was added to 25-g samples at a rate of 0.6 mL/min for 2 min. The pH was adjusted to 6.9 using 0.1 M NaHCO_3 solution.
- Stages 3 to 8 (Esophagus–stomach): Pepsin solution was added at a rate of 0.05 mL/mL for 90 min.

Table 2. Processing conditions used in simulated digestion

Stage	Compartment	Condition	Stirring speed (rpm)	Final pH	Exposure time (min)
1	Before mouth	—	—	—	—
2	Mouth	Saliva solution	200	6.9	2
3	Esophagus–stomach	Stomach solution (pepsin)	130	5.5	10
4				4.6	10
5				3.8	10
6				2.8	20
7				2.3	20
8				2.0	20
9	Duodenum	Intestinal solution (pancreatin + bile salts)	45	5.0	30
10	Ileum		45	6.5	60

Pepsin solution (Sigma-Aldrich) was prepared in HCl at 0.1 *N* in a ratio of 25 mg/mL (Aura, 2005). The pH of each stage was adjusted according to Table 2, using 1 *M* HCl solution.

- Stage 9 (Duodenum): The intestinal solution was added to the samples at a rate of 0.25 mL/mL (Laurent et al., 2007). Such solution was prepared using 2 g/L pancreatin and 12 g/L bile salts (Sigma-Aldrich), diluted in 0.1 *M* NaHCO₃ solution. The pH adjustment was made with a 0.1 *M* NaHCO₃ solution.
- Stage 10 (Ileum): A 0.1 *M* NaHCO₃ solution was used to adjust pH to 6.5.

All enzymatic solutions were prepared in sterile filtered vials using a 0.22- μ m filter membrane (Milipore). After sterilization, all solutions were kept in an ice bath throughout the simulation period. A 37°C incubation chamber with mechanical stirring (TE-424 TECNAL Orbital Shaker, Incubadora) was used to simulate both body temperature and intestinal peristaltic movements similar to those of each digestive compartment. For each stage of simulation of the GIT, the viable cell counts of lactic acid bacteria added to yogurts were determined by preparation and sowing of decimal serial dilutions with sterilized peptone water (0.1 g/100 mL, Sigma-Aldrich; London et al., 2015).

Sensory Analysis

Microbiological tests preceded the sensory analysis to ensure the yogurts met legislated sanitary standards (Brazil, National Health Surveillance Agency, 2019). The authors declare that the present study was carried out in accordance with the Research Ethics Committee of the Health Sciences Center of the Federal University of Paraíba–CEP/CSS (João Pessoa, Brazil). The approval number is CAAE 02226912.0.0000.5188, and written consent was obtained. Informed written consent was obtained from willing participants before the start of the study.

The participants filled out the informed consent form before the sensory tests. Consumers were recruited through personal contact and invitations via social media, and individuals who were healthy, consumed fermented dairy products, had already consumed goat milk or goat milk dairy products, and had available time for the sensory analyses were selected. One hundred regular consumers of fermented dairy products (45% men, 55% women; aged 18–45 yr; mean age 22 yr) participated in the sensory acceptance test. All of the participants consumed yogurts, fermented milks, or fermented whey beverages at least 3 times a week.

The sensory attributes evaluated included appearance, color, aroma, flavor, texture, and overall acceptance of the yogurt formulations. Ten-gram samples at $4 \pm 0.5^\circ\text{C}$ were presented to the panelists in 50-mL white plastic cups labeled with 3-digit numbers following a monadic sequential order. Consumers evaluated the sensory acceptance on a 9-point hedonic scale (1 = disliked very much; 5 = neither liked nor disliked; 9 = liked very much; García-Gómez et al., 2019). A glass of water and biscuits were provided for cleansing the palate during evaluation of the different yogurt formulations. Yogurts were considered accepted when they received an average score of 5.0 or higher.

Statistical Analysis

Data were submitted to Student's *t*-test or ANOVA followed by Tukey's test with significance declared at $P \leq 0.05$, using Statistica software, version 13 (StatSoft). Graphs were plotted using Matlab, version R2019b.

RESULTS AND DISCUSSION

Goat Milk Quality Control

Microbiological analyses revealed total and thermo-tolerant coliform counts <3 MPN/mL and mold and yeast counts <1 cfu/mL, in addition to absence of *Sal-*

Table 3. Goat milk physicochemical properties

Property ¹	Mean $\pm$ SD	Literature reference
pH	6.75 $\pm$ 0.00	6.49 $\pm$ 0.05 ² and 6.75 $\pm$ 0.00 ³
Titrateable acidity (g of lactic acid/100 g)	0.11 $\pm$ 0.01	0.12 $\pm$ 0.02 ² and 0.13 $\pm$ 0.01 ³
TDE (g/100 g)	13.55 $\pm$ 0.20	13.56 $\pm$ 0.03 ² and 13.18 $\pm$ 1.28 ⁴
DDE (g/100 g)	10.40 $\pm$ 0.10	11.24 $\pm$ 0.02 ²
Protein (g/100 g)	3.99 $\pm$ 0.02	4.67 $\pm$ 0.15, ⁴ 3.59 $\pm$ 0.09, ⁵ and 3.57 $\pm$ 0.07 ³
Fat (g/100 g)	3.15 $\pm$ 0.02	6.82 $\pm$ 0.04, ² 5.20 $\pm$ 0.05, ⁴ 3.51 $\pm$ 0.14, ⁵ and 3.01 $\pm$ 0.01 ³
Lactose (g/100 g)	3.82 $\pm$ 0.00	3.17 $\pm$ 0.42 ⁵ and 4.30 $\pm$ 0.04 ³

¹TDE = total dry extract; DDE = defatted dry extract.

²Rafiq et al. (2016).

³Machado et al. (2017).

⁴Vyhmeister et al. (2019).

⁵Fabersani et al. (2018).

monella spp. and *Listeria monocytogenes*, confirming the goat milk as being suitable for human consumption and for use as a raw material for preparing yogurts. The milk presented acidity in lactic acid ($\pm$ SD) of 0.11 $\pm$ 0.01 g/100 g; pH of 6.75 $\pm$ 0.00; total solids 13.55 $\pm$ 0.20 g/100 g; protein 3.99 $\pm$ 0.02 g/100 g; fat 3.15 $\pm$ 0.02 g/100 g; lactose 3.82 $\pm$ 0.00 g/100 g; and fixed mineral residue 0.70 $\pm$ 0.01 (Table 3). Most of these results were in agreement with studies by other authors (Rafiq et al., 2016; Machado et al., 2017; Fabersani et al., 2018; Vyhmeister et al., 2019).

Technological and Physicochemical Characteristics of Yogurt

We observed an increase in acidity and consequent reduction in the pH of the 2 samples throughout storage (Figure 1), which is explained by the continued production of acids by lactic acid bacteria (from the starter culture or probiotic strain, or both). The formulation containing *L. mucosae* (PY) exhibited lower total sugar content than the CY sample after 7 d of storage onward. This behavior was possibly due to the action of starter bacteria (*Strep. salivarius* ssp. *thermophilus* and *L. delbrueckii* ssp. *bulgaricus*) in co-culture with the autochthonous bacterium *L. mucosae* CNPC007 present in the PY formulation, which may have contributed to a higher consumption of sugar after 7 d of storage. Zhang et al. (2020), in a study on yogurt making by co-cultivation of *Lactobacillus plantarum* WCFS1 with yogurt starter cultures, also observed this same behavior for the formulation of yogurt in coculture with *L. plantarum*, indicating higher consumption of sugars when other groups of microorganisms are added in addition to the starter culture.

The depth of proteolysis index (Figure 2) shows that the action of microbial enzymes on the yogurt proteins increased throughout storage in both formulations (P

≤ 0.05). The extent of proteolysis index refers to the natural proteinases of milk and the action of coagulating agents added in the processing of dairy products, which degrade proteins in peptides of high molecular weight (Narimatsu et al., 2003). In this study, we did not use coagulating agents in the processing of yogurts. As the extent of proteolysis index increased in CY and decreased in PY, we concluded that the proteolytic action of microbial enzymes exceeded the activity of milk proteinases in the formulation with *L. mucosae* CNPC007.

Syneresis increased by about 1% over storage time in both samples ($P \leq 0.05$), reaching a final value of around 25% (Figure 2). According to Ramirez-Santiago et al. (2010), syneresis in yogurts occurs due to rearrangements in the casein network, which promote the expulsion of serum. Syneresis values up to 39% are considered satisfactory (Aportela-Palacios et al., 2005). However, WRC increased up to 21 d for PY ($P \leq 0.05$) and dropped dramatically at 28 d in both samples, especially in PY (Figure 2). Exopolysaccharides commonly produced by *L. mucosae* may explain the highest WRC value in 21 d of storage (Figure 2; London et al., 2015). However, we conclude that, after a certain period, the breakdown of the yogurts' protein network promoted by lactic acid bacteria from the starter culture and by *L. mucosae* surpassed the production of exopolysaccharides, culminating in a reduction in WRC and increased syneresis (Machado et al., 2017).

The apparent viscosity of yogurt decreased after 28 d of storage ($P \leq 0.05$) only in the PY formulation (Table 4). However, PY exhibited viscosity (mPa·s) almost 1.5 times as high as CY over 28 d ($P \leq 0.05$), which can be explained by the effects of exopolysaccharides produced by the probiotic strain on the microstructure, texture, and viscosity of yogurt. Yang et al. (2014) and London et al. (2015) observed the same effect in yogurt with added lactic acid bacteria, including *L. mucosae*. Ac-

According to those authors, exopolysaccharides contribute to the formation of a microstructure with denser pores and, therefore, confer higher viscosity to yogurts. For this reason, London et al. (2015) recommend the use of exopolysaccharide-producing strains of *L. mucosae* to confer a richer texture in low-fat yogurts, such as Greek-style yogurt.

Food-grade polysaccharides from lactic acid bacteria are well known as nontoxic, biodegradable, and ecological ingredients that act as natural thickeners, emulsifiers, stabilizers, binders, gelifiers, coagulants, and suspension agents in food and cosmetic products (Jindal and Kattar, 2018). However, the texture of food products generally depends on both the fermentation bacteria and the process parameters. Some lactic acid bacteria can improve texture by producing metabolites in the medium or hydrolyzing fibers added to the food during processing (Escobar et al., 2012). In addition, bacterial exopolysaccharides may offer several human health benefits, such as immunomodulatory, antitumor, and antioxidant activities, blood cholesterol reducing ability, and prebiotic properties (Yildiz and Karatas, 2018).

Microbiological Characterization of Yogurt and Survival of *L. mucosae* CNPC007 Under Simulated Gastrointestinal Conditions

The results of hygienic sanitary microbiological analysis revealed that all prepared goat yogurt formulations were suitable for human consumption throughout the assessed refrigerated storage period, because the counts for *E. coli*, molds, and yeasts and absence of *Salmonella* spp. were in accordance with the criteria recommended by current Brazilian legislation (Brazil, National Health Surveillance Agency, 2019), indicating good manufacturing practices.

Probiotic properties are attributed to some *L. mucosae* strains due to their adhesion to the intestinal mucosa, resistance to passage through the gastrointestinal tract, immunomodulatory capacity, and production of exopolysaccharides and bacteriocins (London et al., 2015; de Moraes et al., 2018). Therefore, tests of viability during storage (Figure 3) and *L. mucosae* survival of digestion (Figure 4) were performed to evaluate the ability of Greek-style goat yogurt to carry this probiotic strain.

Figure 1. Acidity in lactic acid (g/100 g), pH, and total sugars (g/100 g) of control yogurt (CY, red) and probiotic yogurt with added *Limosilactobacillus mucosae* CNPC007 (PY, blue) over 28 d of storage. Error bars indicate SD.

Table 4. Apparent viscosity (mPa·s) of yogurt over storage

Storage time (d)	Formulation ¹	
	CY	PY
1	1,390.33 ± 54.65 ^{A,b}	2,399.00 ± 55.15 ^{A,a}
7	1,019.16 ± 105.01 ^{AB,b}	1,574.00 ± 26.96 ^{B,a}
14	958.17 ± 115.96 ^{AB,b}	1,426.00 ± 121.75 ^{B,a}
21	853.46 ± 66.35 ^{B,b}	1,626.00 ± 76.21 ^{B,a}
28	1,265.00 ± 349.68 ^{AB,b}	1,718.25 ± 150.86 ^{B,a}

^{a,b}Means ± SD with different lowercase letters in the same row represent differences between formulations according to Student's *t*-test ($P \leq 0.05$).

^{A,B}Means ± SD with different uppercase letters in the same column represent differences between storage times according to Tukey test ($P \leq 0.05$).

¹CY = control yogurt; PY = probiotic yogurt with added *Limosilactobacillus mucosae* CNPC007.

The counts of *L. bulgaricus* ssp. *delbrueckii* (Figure 3) increased by about 0.5 log cfu/g over the 28 d of storage. The PY and CY formulations showed an increase ($P \leq 0.05$) from 8.59 ± 0.02 log cfu/g to 8.99 ± 0.01 log cfu/g and 8.65 ± 0.08 log cfu/g to 9.00 ± 0.01 log cfu/g, respectively. Counts of *Strep. salivarius* ssp. *thermophilus* (Figure 3) decreased in both formulations

($P \leq 0.05$), with a particular drop in the CY sample. The reduction in *Strep. thermophilus* was about 0.5 log cfu/g in PY (9.38 ± 0.11 log cfu/g to 8.98 ± 0.02 log cfu/g) and almost 1.5 log cfu/g in CY (9.38 ± 0.05 log cfu/g to 8.06 ± 0.05 log cfu/g). This type of behavior is expected in the yogurt fermentation process, in which strains of *Strep. salivarius* ssp. *thermophilus* and *L. delbrueckii* ssp. *bulgaricus* show synergistic interactions (protocooperation). *Lactobacillus delbrueckii* ssp. *bulgaricus* initially grows more slowly than *Strep. thermophilus* but remains viable for a much longer time. *Streptococcus salivarius* ssp. *thermophilus*, in turn, grows more quickly at the beginning of the process, when the pH of the milk is close to neutral; but, toward the end, due to greater production of lactic acid and sensitivity to acidity, it is surpassed by *L. bulgaricus*. Thus, at the end of the process, a much larger number of *L. bulgaricus* than *Strep. thermophilus* is found (McKevith and Shortt, 2003; Narvhus and Abrahamsen, 2021).

The yogurt containing *L. mucosae* CNPC007 (Figure 3) showed a reduction in the count of this probiotic strain ($P \leq 0.05$), from 9.53 ± 0.04 log cfu/g to 8.96 ± 0.01 log cfu/g. Possibly, competition for substrate between *L. bulgaricus* and *L. mucosae* further contrib-

Figure 2. Syneresis (%), water retention capacity (WRC, %), depth of proteolysis index (DPI, %), and extent of proteolysis index (EPI, %) of control yogurt (CY, red) and probiotic yogurt with added *Limosilactobacillus mucosae* CNPC007 (PY, blue) over 28 d of storage. Error bars indicate SD.

uted to the reduction of the probiotic strain during storage. In parallel with the antagonistic effect between *L. bulgaricus* and the probiotic strain, synthesis of bacteriocins and organic acids may have occurred (London et al., 2015; de Moraes et al., 2018), in addition to release of microbial metabolites. Even so, over the entire storage period, the PY formulation showed probiotic bacteria counts much higher than the minimum recommended to produce beneficial health effects (6.0 to 7.0 log cfu in 100 g; Terpou et al., 2019).

Figure 4 illustrates the survival of *L. mucosae* CNPC007 along the simulated gastrointestinal tract. The counts of the probiotic strain in the sample that was not exposed to in vitro digestion are also shown. As expected, greater stability and higher counts ($P \leq 0.05$) were observed in the sample that was not exposed to simulated digestion. The greatest drop in *L. mucosae* CNPC007 counts occurred in stomach conditions ($P \leq 0.05$). Upon reaching the duodenum, at about 90 min of exposure, a considerable drop occurred in the number of viable cells compared with the counts found during mouth digestion (more than 2 log cfu/g), with counts remaining stable until reaching the ileum. At the end of

the 182 min of simulated digestion, the yogurt showed sufficient viability to be considered a probiotic product, with an average viable cell population of *L. mucosae* around 7 log cfu/g (Terpou et al., 2019). This behavior indicates a possible protective effect of the food matrix on the survival of *L. mucosae* CNPC007, contributing to the product's probiotic effects.

Sensory Acceptance Test

Both yogurt formulation samples received the same sensory acceptance scores in terms of appearance and aroma ($P > 0.05$), both at the beginning and at the end of storage (Table 5). On the first day of storage, PY and CY received the same score for texture ($P > 0.05$). However, PY received the highest scores for color, flavor, and overall acceptability both at the beginning and at the end of storage ($P \leq 0.05$), demonstrating that the *L. mucosae* strain potentiated better sensory acceptance of these attributes evaluated in this formulation. The PY formulation also received higher texture scores at 28 d ($P \leq 0.05$), corroborating the viscosity results that this formulation had higher

Figure 3. Viability of lactic acid bacteria (*Lactobacillus delbrueckii* ssp. *bulgaricus*, *Streptococcus salivarius* ssp. *thermophilus*, and *Limosilactobacillus mucosae* CNPC007) in control yogurt (CY, red) and probiotic yogurt with added *Limosilactobacillus mucosae* CNPC007 (PY, blue) over 28 d of storage. Error bars indicate SD.

viscosity than CY, which may have led to higher texture scores.

According to the panelists, the 2 samples' appearance and color remained unchanged over time ($P > 0.05$). However, the aroma, flavor, and overall acceptability improved over storage in both samples ($P \leq 0.05$). The PY formulation received scores between 7 to 9 throughout storage ("liked it moderately" to "liked it extremely"). In other words, the addition of *L. mucosae* did not cause negative effects on the general evaluation of yogurt and provided an increase in the acceptance of the product.

CONCLUSIONS

The addition of the *L. mucosae* strain CNPC007 provided a more viscous and creamier Greek-style yogurt

than the control. In addition, the formulation with added *L. mucosae* showed higher sensory acceptance scores for color, flavor, texture, and global assessment. The viability and GIT tests indicated that the probiotic yogurt developed is an efficient vehicle of probiotic bacteria for human consumption, as lactic acid bacteria counts remained high ($>7 \log \text{ cfu/g}$) throughout storage and simulated gastrointestinal conditions, respectively. These results indicate a great technological potential of *L. mucosae* CNPC007 in the development of functional goat milk yogurts.

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Sadly, Rita Queiroga (Federal University of Paraíba) died as a consequence of COVID-19 shortly before publication of this article, after 30 years of dedicated

Figure 4. Viable cell counts of *Lactobacillus mucosae* CNPC007 in Greek-style goat yogurt when exposed (blue) and not exposed (red) to simulated digestion. The pH values in each digestion stage are shown at the top of the graph. PY = probiotic yogurt with added *L. mucosae* CNPC007. Error bars indicate SD.

Table 5. Sensory acceptance of yogurts at beginning and end of storage (n = 100)

Property	Day of storage	Formulation ¹	
		CY	PY
Appearance	1	7.71 ± 0.59 ^{a,A}	7.72 ± 0.61 ^{a,A}
	28	7.69 ± 0.60 ^{a,A}	7.81 ± 0.53 ^{a,A}
Color	1	7.88 ± 0.50 ^{a,B}	8.08 ± 0.60 ^{a,A}
	28	7.90 ± 0.48 ^{a,B}	8.16 ± 0.51 ^{a,A}
Aroma	1	7.20 ± 0.85 ^{b,A}	7.20 ± 0.70 ^{b,A}
	28	7.61 ± 0.57 ^{a,A}	7.50 ± 0.66 ^{a,A}
Flavor	1	6.56 ± 0.78 ^{b,B}	7.51 ± 0.54 ^{a,A}
	28	6.98 ± 0.91 ^{a,B}	7.43 ± 0.54 ^{a,A}
Texture	1	7.81 ± 0.53 ^{a,A}	7.93 ± 0.97 ^{a,A}
	28	7.43 ± 0.83 ^{b,B}	8.03 ± 0.56 ^{a,A}
Overall acceptance	1	6.84 ± 0.83 ^{b,B}	7.87 ± 0.65 ^{b,A}
	28	7.06 ± 0.34 ^{a,B}	8.04 ± 0.45 ^{a,A}

^{a,b}Comparisons between storage times for the same formulation. Values in the same column marked with different lowercase letters differed according to Student's *t*-test ($P \leq 0.05$) for a given property.

^{A,B}Comparisons between formulations at times 1 and 28 d of storage. Values in the same row marked with different uppercase letters differed according to Student's *t*-test ($P \leq 0.05$) for a given property.

¹CY = control yogurt; PY = probiotic yogurt with added *Limosilactobacillus mucosae* CNPC007.

research focused on dairy products from goats and, latterly, donkey milk and cactus. This study received no external funding. The authors have not stated any conflicts of interest.

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