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Pilosocereus gounellei (xique-xique) flour: Improving the nutritional, bioactive, and technological properties of probiotic goat-milk yogurt

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ABSTRACT

In this study, we evaluate the technological, nutritional, and bioactivity effects on goat-milk yogurt of adding different concentrations of xique-xique flour: 1.0% (XY1%) and 2.0% (XY2%). The goat-milk yogurts (stored under refrigeration) also contained *Limosilactobacillus mucosae* CNPC007 (an autochthonous strain). The XY1% and XY2% treatments presented greater intensity in terms of color (yellow), and greater luminosity (L*) during storage than the control yogurt (CY). Up to the 14th day of storage, the XY1% and XY2% treatments presented greater viscosity values as well. During storage, as in any fermentation process, a reduction in lactose (hydrolysis) was observed, with a greater release of simple sugars glucose and galactose and a concomitant increase in the lactic acid content. The PCA confirmed that these behaviors were more evident from the 14th day of the XY1% treatment, and on the 28th day in XY2% treatment. After 28 days of storage, XY2% presented higher counts of *L. mucosae* CNPC007, with higher mineral, total phenolic compounds, and flavonoid contents, as well as greater antioxidant activity (by FRAP). Xique-xique flour can be used to produce goat-milk yogurt without negatively affecting its technological characteristics, adding both nutritional and functional value to the product.

1. Introduction

Increasingly, goat dairy milk products, such as cheese, yogurt, and fermented milk, have been gaining ground among consumers (Jia et al., 2016; Santis et al., 2019). Although there are still major technological and marketing challenges (Gomes et al., 2013; Ranadheera et al., 2012; Ribeiro et al., 2014; Yamazi et al., 2013), goat milk has some nutritional, functional, and technological advantages when compared to cow's milk, including higher concentration of conjugated linoleic acid (CLA), medium chain fatty acids, B complex vitamins (riboflavin, thiamine, and B12), greater digestibility and the ability to improve absorption of iron and copper (Clark & Mora García, 2017; Mituniewicz-Małek et al.,

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2014Mituniewicz-Małek, Ziarno, & Dmytrów, 2014; Nguyen, Afsarb, & Day, 2018; Silanikove et al., 2010; Verruck et al., 2019); these characteristics give this food matrix a high potential for the elaboration of dairy products, such as yogurt. However, due to its flavor, goat-milk yogurt is much less acceptable than cow's milk yogurt, attributed to its constitution of capric, caprylic, and caproic acids (Costa et al., 2017). There are technological strategies that can improve these sensory aspects.

One of the main techniques would be reformulation of this dairy milk product by adding new ingredients (Munekata et al., 2021), such as probiotic microorganisms. The addition of probiotics, live microorganisms that, when ingested in adequate amounts, provide benefits to the host, can add greater functional value to goat dairy derivatives (Pal, Dudhrejiya, & Pinto, 2017; Paula et al., 2020). In addition, the fermentation process promoted by probiotic microorganisms, together with starter cultures, improves the acceptance of sensory attributes such as flavor, taste and viscosity (Morais et al., 2022).

Insertion of microbial strains with probiotic potential into dairy products (*e.g.*, in fermented milk, yogurt and cheese), especially those of the Lactobacillus genus, brings benefits such as improved gastrointestinal tract function (Diez-Gutiérrez, Vicente, Barrón, Villarán, & Chávarri, 2020). In particular, the strain *Limosilactobacillus mucosae* CNPC007 (isolated from goat-milk) has been studied for its in vitro functional properties and its potential for use in fermented dairy derivatives. This strain has been reported to bring improvements to microbiological, physical-chemical, and sensory aspects of goat rennet cheese (Moraes, Santos, Barcelos, Lopes, & Egito, 2018) and goat milk Greek-style yogurt (Morais et al., 2022).

There are benefits to consumer health and consumer acceptance associated with the addition of probiotic microorganisms and bioactive compounds in yogurts, such as phenolics and dietary fibers, through incorporation of fruit and plant extracts. These, as well as benefiting consumer health, improve the technological (viscosity), and sensory aspects that affect both quality and consumer choice (Yadav et al., 2018). As examples, Moringa extract improved yogurt properties related to texture and bioactivity (Zhang et al., 2018), while Jujube pulp and fruit (with aronia, strawberries, raspberries and peach) helped to make the taste of goat's milk more palatable and improve its antioxidant characteristics (Cușmenco & Bulgaru, 2020; Feng et al., 2019). In yogurt, Jujube pulp and apple pomace also stimulated probiotic bacteria growth and improved the rheological properties (Feng et al., 2019; Wang, Kristo, & LaPoite, 2020).

Xique-xique (*Pilosocereus gounellei*) is a cactus that has considerable potential as an additive. It is commonly located in dry climatic regions, such as in Northeastern Brazil (Furtado et al., 2019). Studies point to the therapeutic effectiveness of xique-xique as a gastro-protective agent (Sousa et al., 2018) as evaluated in *in vivo* tests, for example, reducing colitis in rats (Assis et al., 2019). Applications using xique-xique in food products are scarce, yet emerging, and fully feasible (Toit, Wit, Osthoff, & Hugo, 2018).

Products such as juice of xique-xique (Assis et al., 2019; Ribeiro et al., 2020), yogurt with xique-xique jelly added (Bezerril et al., 2021a), cereal bars with xique-xique (Araújo, Reis, & Oliveira, 2019Araújo, dos Reis, & de Oliveira, 2019), and cookies with xique-xique (Machado et al., 2021), have all been satisfactory in terms of nutritional, physical-chemical, and sensorial parameters, signaling the birth of a new, economically viable product. However, up to this writing, there were no studies using xique-xique flour in dairy products, such as yogurt. In this study, we evaluated technological, nutritional, and bioactive properties of these, during refrigerated storage, upon adding different xique-xique flour concentrations to goat-milk yogurt already supplemented with *Limosilactobacillus mucosae* CNPC007 (an autochthonous Brazilian strain), and pineapple jelly.

2. Material and methods

2.1. Raw material and ingredients

The starter culture (YF-L903 - *Streptococcus salivarius* subsp. *thermophillus,* and *Lactobacillus delbruecki* subsp. *bulgaricus*) was commercially acquired from the Christian Hansen® company (Valinhos, Minas Gerais, Brazil). The indigenous culture *Limosilactobacillus mucosae* CNPC007 was obtained from the "Collection of Microorganisms of Interest to the Food and Agroenergy Industry", at Embrapa Agroindustry Tropical (Fortaleza, Ceará, Brazil). Xique-xique cladodes (two 30 kg lots, May 2017) were obtained from a privately owned cultivation located in the municipality of Boa Vista, in the state of Paraíba, Brazil (latitude 7.16762352, longitude −36.1432815). The plant was identified by the Agricultural Sciences Center at the Federal University of Paraíba (CCA/UFPB), and the species certification (No. 17562) was deposited at the Herbário Prof°. Jaime Coelho Morais (CCA/UFPB). The collection was authorized by the Brazilian Biodiversity Information System (No. 62681), and the National System for the Management of Genetic Heritage (SISGEN, No. AA17429). Pineapple and crystal sugar (União®, Limeira, São Paulo, Brazil) were obtained commercially.

2.2. Xique-xique flour processing

The xique-xique flour was prepared according to Machado et al. (2021), where xique-xique cladodes were carefully sanitized (soil removal and decontamination) with running water and sodium hypochlorite (100 ppm/15 min). The central stem was stripped, and the pulp and peel were removed. The central stems were then cut into 1 cm slices, which were autoclaved (121 ± 1 °C/20 min). Subsequently, the samples were cooled at room temperature, followed by drying in an air circulation oven (40 ± 1 °C) until reaching approximately 4% moisture. After drying, the xique-xique was ground in a knife mill (Willey, Solab®, Piracicaba, São Paulo) and screened with a 100-mesh sieve on a sieve shaker. The flour was vacuum sealed in sterile polyethylene bags at approximately 100 g per bag, rolled in aluminum foil, and frozen (−20 ± 1 °C) until use.

2.3. Jelly, inoculum, and yogurt preparation

To prepare a jelly, a pineapple pulp to sugar proportion of 70:30 (w/w) was used. The mixture was cooked for 45 min, with manual stirring until it reaching 62–65 °Brix, which was measured using a digital refractometer (Hanna® brand, model HI 96801). The pineapple jelly was then transferred, still hot, to a previously sterilized glass container and stored at room temperature 27 ± 2 °C until used in processing the yogurts.

The final inoculum (FIn) of probiotic bacteria into the goat-milk was prepared in two stages. Inoculum 1 (In1) was prepared by diluting 0.1 g of lyophilized *L. mucosae* CNPC007 in 10 mL of reconstituted powdered goat-milk (Caprilat®, Nova Friburgo, Rio de Janeiro, Brazil) in sterile water, with incubated for 22 h (stationary phase) at 37 °C. Final Inoculum (FIn) was prepared using Inoculum 1 (In1) at a 50:50 proportion – 10 mL of In1:10 mL powdered milk already reconstituted in sterile water, and then incubated for 22 h at 37 °C, resulting in a final count of 7–8 log CFU/g. The counts were confirmed using serial dilutions of the inoculum with sterile peptone water at a concentration of 0.1 g/100 mL (Sigma-Aldrich, St. Louis, MA, USA); 10-μL of these dilutions were poured onto MRS agar (Oxoid, Basigstoke, UK) acidified to pH 5 (IDF, 1995), using the micro-drop technique. The plates were incubated aerobically at 37 °C for 48 h, and the results were expressed in log CFU/g.

The yogurts were processed with a methodology described by Silva et al. (2017). Three formulations were prepared: (CY) control yogurt (treatment without flour), (XY1%) yogurt supplemented with 1% xique-xique flour, and (XY2%) yogurt supplemented with 2%

xique-xique flour. Pasteurized goat-milk was heat treated (90 °C/10 min), and cooled to 45 ± 1 °C, and FIn containing *L. mucosae* CNPC007, with the freeze-dried starter cultures (*Streptococcus salivarius* subsp. *thermophillus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus*, Christian Hansen®, Valinhos, Brazil, 7–8 log CFU/g) were inoculated at respective concentrations of 100 and 0.4 g/L, and fermentation was performed at 45 °C for 4 h. The yogurt samples were then cooled to 5 ± 1 °C, the clot broken using a glass rod, and the jelly added in concentrations of 15 g/100 g to the yogurts; the xique-xique flour was then added to the yogurts in concentrations of 1% and 2%. The products were then placed in high density polyethylene bottles and stored under refrigeration (4 ± 1 °C) for 28 days. The yogurts were evaluated at 1, 14, and 28 days of storage.

2.4. Technological and physical-chemical characterization of the yogurts

The yogurts were evaluated using pH (Quimis model Q400as), color (Konica Minolta colorimeter - model CR 400), titratable acidity (TA), total soluble solids (TSS), ash, protein, and lipids (AOAC, 2019). Viscosity was evaluated using a Brookfield viscometer model DV II + Pro coupled to a thermostatic bath to control the sample temperature, and analysis performed at a rotation speed of 40 rpm, and a temperature of 5 ± 1 °C, measured with a Brookfield Thermosel Spindle (SC4-27).

2.5. Sugar and acid organic profiles of the yogurts

Sugars (glucose, lactose, and galactose), and organic acids (citric, lactic, malic, and propionic) were determined as described by Ball, Bullock, Lloyd, Mapp, and Ewen (2011). The data obtained were processed using OpenLAB CDS ChemStation Edition TM software (Agilent Technologies). Glucose and lactose standards were obtained from Sigma-Aldrich; galactose was obtained from Chem Service (West Chester, USA); organic acid standards were obtained from Vetec Química Fina (Rio de Janeiro, Brazil), and all presented purity of ≥99%. Ultrapure water was obtained using a MilliQ® system (EMD Millipore), and sulfuric acid was obtained from Merck (Darmstadt, Germany).

2.6. The total phenolic and total flavonoid contents of the yogurts

For extract preparation, 2 g of each yogurt was homogenized with 80% methanol (Sigma-Aldrich) for 10 min in a mini-Turrax apparatus (Tecnal, Piracicaba, São Paulo, Brazil), kept resting for 24 h, and then filtered with a 125 mm-filter paper (Whatman®, GE Healthcare, Chicago, IL, USA). Total phenolic content was measured using the Folin-Ciocalteu method (Liu et al., 2002), and absorbance was measured at 765 nm with a spectrophotometer (BEL Photonics, Piracicaba, São Paulo, Brazil). The final phenolic content was determined using a standard curve prepared with gallic acid (Sigma-Aldrich). Results were expressed as mg equivalent of gallic acid (EGA) per 100 g of sample (mg EGA/100 g).

Total flavonoid content was measured using the procedure described by Guevara-Figueroa et al. (2010). Sample absorbance was measured at 510 nm with a spectrophotometer (BEL Photonics) against a blank (without extract). Total flavonoid content was determined using standard catechin curve (Sigma-Aldrich) equivalents (CE). Results were expressed as mg catechin equivalents (CE) per 100 g of sample (mg CE/100 g).

2.7. Phenolic profiles and antioxidant activity of the yogurts

To prepare the extract, 5 g of each yogurt formulation was homogenized with 5 mL of 80% methanol (Sigma-Aldrich), centrifuged (9000×*g*, 15 min, 4 °C) and filtered with a 0.45 μm-filter (Millex Millipore, Barueri, SP, Brazil). Identification of the phenolic compounds was performed following Padilha et al. (2018), with gradient and runtime adaptations for quantification of stylbenes, flavonols, and flavanones. Analysis was performed using an Agilent 1260 Infinity LC System liquid chromatograph (Agilent Technologies, Santa Clara - USA) coupled to a diode array detection (DAD) system (model G1315D). The column used was a Zorbax Eclipse Plus RP-C18 (100 × 4.6 mm, 3.5 μm) and a Zorbax C18 pre-column (12.6 × 4.6 mm, 5 μm) (Zorbax, USA). The data were processed using the OpenLAB CDS ChemStation Edition software (Agilent Technologies, Santa Clara - USA), and identification and quantification was performed through comparison with external standards (Sigma-Aldrich). The results were expressed as mg of phenolic per 100 g of sample (mg/100 g).

For analysis of antioxidant activity, initially, 2 g of each yogurt formulation was homogenized with 10 mL of 80% methanol (Sigma-Aldrich), for 10 min with a mini-Turrax apparatus (Tecnal), and left to rest for 24 h, and then filtered with a 125 mm-filter (Whatman®). The ability of extracts to reduce iron was measured using the FRAP (ferric reducing ability of plasma) method as previously described (Rockenbach et al., 2011). The FRAP reagent was prepared with 3 mol/L of acetate buffer (pH 3.6) + 10 mM/L of TPTZ (2,4,6-tris (2-pyridyl)-s-triazine) in a 40 mM/L HCl solution + 20 mM $FeCl_3$. A 200 μL-aliquot of the extract was added to 1800 μL of the FRAP solution, stirred with a vortex mixer (Quimis) for 30 s and placed in a water bath for 30 min at 37 °C. Absorbance was measured at 593 nm with a spectrophotometer (Bel Photonics). The standard curve was created with Trolox 1 mM, and results were expressed in micromoles of Trolox equivalent antioxidant capacity (TEAC) per 100 g (μmol TEAC/100 g).

The ability of the extracts to capture the $ABTS^{\bullet+}$ cation (2,2-azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid) was measured with using the ABTS method as previously described (Sariburun, Sahin, Demir, Turkben, & Uylaser, 2010). The ABTS reagent, which was prepared by mixing 5 mL of 7 mM ABTS with 88 μL of 140 mM potassium persulfate (final concentration of 2.45 mM), and $ABTS^{\bullet+}$ radical was formed after resting the ABTS reagent for 12–16 h at room temperature (25 ± 0.5 °C) under the dark. The $ABTS^{\bullet+}$ solution was diluted with distilled water to an absorbance value of 0.800–0.900 at 734 nm. Absorbance of the reaction mixture (600 μL) with 100 μL of extract and 500 μL of $ABTS^{\bullet+}$ solution was measured at 734 nm in a spectrophotometer (Bel Photonics). A control solution with 100 μL of extracting solvent +500 μL of ABTS radical was prepared. The negative control solution was the extracting solvent for each extract used to reset the spectrophotometer. The standard curve was created with Trolox 1 mM, and results were expressed in micromoles of Trolox equivalent antioxidant capacity (TEAC) per g of sample (μmol TEAC/g).

2.8. Microbiological analyses

E. coli count quality control tests, total mold and yeast counts in CFU/g, as well as detection of absence of *Salmonella* spp./25 g (APHA, 2015) were performed. Lactic bacteria viability tests included *Streptococcus salivarius* subsp. *thermophillus* (APHA, 2015), *Limosilactobacillus mucosae* CNPC007 (London et al., 2015), and *Lactobacillus* subsp. *Bulgaricus* counts (Lima et al., 2009).

2.9. Statistical analysis

All experiments were performed in triplicate and all data are presented as means ± standard deviation. Data were submitted to the Student's *t*-test or to analysis of variance (ANOVA) followed by Tukey's test using $p < 0.05$ in *Sigma-Stat* software, version 3.5 (Jandel Scientific Software, San Jose, California) (SIGMASTAT, 2006). Multivariate analysis was performed using the *prinqual* procedure in the Statistical Analysis System (SAS, 2012) software.

3. Results and discussion

3.1. Technological and physicochemical characteristics of the yogurts

Table 1 presents color parameters and physicochemical characteristic of the probiotic yogurts during storage. The visual aspect of color is one of the most significant parameters present in all food products, including fermented milk, and can be affected by spoilage, addition of ingredients, and shelf life (Pan, Liu, Luo, & Luo, 2019). In this study, we observed the influence of both storage time and of the addition of xique-xique flour relative to most of the evaluated instrumental color parameters ($p < 0.05$). An increase in luminosity (L*) during refrigerated storage for the yogurts supplemented with xique-xique flour ($p < 0.05$) was noted. Up to 14 days of storage, the samples with higher concentrations of xique-xique flour contributed to L* reductions ($p < 0.05$), as indicated by the yellow-green hue of the xique-xique flour.

Luminosity can be attributed to compression of the solid matrix, due to the growth of soluble complexes that reduce the gel's opacity during shelf life (Trigueros, Wojdylo, & Sendra, 2014). This did not occur for either XY1% or XY2%, as they displayed increased luminosity only after 28 days of storage. For lighter-colored products (such as dairy products) this behavior is interesting and luminosity is an important parameter (Pan et al., 2019) if artificial and/or natural dyes are not added.

As for the instrumental colors a* [chromaticity green (−)/red (+)], and b* [chromaticity blue (−)/yellow (+)], we observed that as the concentrations of xique-xique flour increased in the formulation, the green-yellow hue predominated ($p < 0.05$), corroborating the luminosity data, and likely due to the influence of the yellowish green color of the xique-xique flour. Similar results have also been observed by Bezerril et al. (2021a), studying yogurts supplemented with xique-xique jelly, and by Machado et al. (2021), in cookies prepared with differing xique-xique flour concentrations (*Pilosocereu gounellei*).

Neither addition of xique-xique flour, nor storage time influenced the pH and titratable acidity parameters ($p \geq 0.05$). The formulations were only slightly acidic. From the point of view of sensory acceptance by consumers, who prefer low acidity yogurts (Costa et al., 2017), low acidity is thus feasible.

Increasing the concentration of xique-xique flour in the yogurts directly influenced the total soluble solids and ash (minerals) parameters ($p < 0.05$). XY2% presented higher values of these variables for most of the times ($p < 0.05$) evaluated. The addition of different

Table 1
Color parameters and physicochemical characteristic of the probiotic yogurts over storage.

Parameter	Days	Treatment		
		CY	XY1%	XY2%
L*	1	70.91 ± 0.31^{Ba}	67.49 ± 0.06^{Bb}	68.04 ± 0.20^{Bb}
	14	82.92 ± 1.22^{Aa}	70.10 ± 1.25^{ABb}	64.82 ± 0.47^{Bc}
	28	73.99 ± 0.81^{Bab}	71.38 ± 0.75^{Ab}	77.05 ± 1.80^{Aa}
a*	1	−1.97 ± 0.04^{Aa}	−2.01 ± 0.04^{Aab}	−2.28 ± 0.02^{Ab}
	14	−1.88 ± 0.00^{Aa}	−2.03 ± 0.09^{Aab}	−2.54 ± 0.02^{Bb}
	28	−1.85 ± 0.12^{Aa}	−2.04 ± 0.08^{Ab}	−2.01 ± 0.13^{Ab}
b*	1	6.59 ± 0.04^{Ac}	8.37 ± 0.07^{Ab}	10.23 ± 0.09^{Aa}
	14	7.46 ± 0.21^{Ac}	8.72 ± 0.03^{Ab}	9.61 ± 0.01^{Ba}
	28	6.63 ± 0.48^{Ab}	8.17 ± 0.72^{Aab}	10.21 ± 0.12^{Aa}
pH	1	4,35 ± 0,07^{Aa}	4,30 ± 0,00^{Aa}	4,29 ± 0,00^{Aa}
	14	4,42 ± 0,00^{Aa}	4,43 ± 0,00^{Aa}	4,40 ± 0,00^{Aa}
	28	4,35 ± 0,00^{Aa}	4,40 ± 0,00^{Aa}	4,36 ± 0,00^{Aa}
Titratable acidity (g/100 g)	1	0,68 ± 0,00^{Ab}	0,77 ± 0,00^{Aa}	0,78 ± 0,02^{Aa}
	14	0,54 ± 0,78^{Aa}	0,79 ± 0,00^{Aa}	0,78 ± 0,01^{Aa}
	28	0,84 ± 0,05^{Aa}	0,79 ± 0,00^{Aa}	0,80 ± 0,00^{Aa}
Total Soluble Solids (g/100 g)	1	18.93 ± 0.04^{Ac}	19.93 ± 0.07^{Ab}	20.74 ± 0.01^{Aa}
	14	18.78 ± 0.35^{Ac}	19.02 ± 0.12^{Ab}	20.73 ± 0.32^{Aa}
	28	17.74 ± 0.29^{Bc}	18.72 ± 0.26^{Ab}	20.36 ± 0.01^{Aa}
Ash (g/100 g)	1	0.60 ± 0.01^{Ab}	0.63 ± 0.02^{Ab}	0.70 ± 0.01^{Aa}
	14	0.59 ± 0.01^{Ab}	0.61 ± 0.01^{Aab}	0.70 ± 0.02^{Aa}
	28	0.61 ± 0.01^{Ab}	0.62 ± 0.01^{Ab}	0.67 ± 0.01^{Aa}
Protein (g/100 g)	1	2.69 ± 0.06^{Bb}	2.79 ± 0.02^{Bab}	2.91 ± 0.03^{Aa}
	14	2.87 ± 0.14^{ABab}	2.59 ± 0.05^{Bb}	3.30 ± 0.23^{Aa}
	28	3.28 ± 0.09^{Aa}	3.24 ± 0.12^{Aa}	3.12 ± 0.08^{Aa}
Lipids (g/100 g)	1	2.60 ± 0.01^{Aa}	2.60 ± 0.01^{Aa}	2.80 ± 0.14^{Aa}
	14	2.65 ± 0.07^{Aab}	2.50 ± 0.01^{Ab}	2.70 ± 0.01^{Aa}
	28	2.80 ± 0.01^{Aa}	2.50 ± 0.01^{Aa}	2.60 ± 0.01^{Aa}

Results are expressed as average (n = 3) ± standard deviation.
$^{a-c}$Mean ± standard deviation with different lowercase letters on the same line differed by the Tukey test ($p < 0.05$), between treatments.
$^{A-B}$Mean ± standard deviation with different capital letters in the same column differed by Tukey's test ($p < 0.05$), over storage time.
Formulations: CY (control yogurt), XY1% (yogurt added 1% with xique-xique flour) and XY2% (yogurt added 2% with xique-xique flour).

Table 2
Sugar and acid organics profile of the probiotic yogurt over storage.

Parameter	Days	Treatment		
		CY	XY1%	XY2%
Sugar (g/100 g)				
Lactose	1	1.98 ± 0.07^{Aa}	1.36 ± 0.01^{Ab}	1.23 ± 0.07^{Ab}
	14	1.50 ± 0.01^{Ba}	1.25 ± 0.01^{ABb}	1.16 ± 0.02^{ABb}
	28	1.31 ± 0.03^{Ba}	1.00 ± 0.01^{Bb}	1.08 ± 0.01^{Bb}
Glucose	1	0.01 ± 0.01^{Bc}	0.30 ± 0.19^{Ba}	0.19 ± 0.01^{Bb}
	14	0.02 ± 0.01^{Bc}	0.61 ± 0.01^{ABa}	0.23 ± 0.01^{ABb}
	28	0.40 ± 0.01^{Ac}	0.81 ± 0.01^{Aa}	0.55 ± 0.01^{Ab}
Galactose	1	0.86 ± 0.01^{Ca}	0.79 ± 0.01^{Ba}	0.36 ± 0.01^{Bb}
	14	1.80 ± 0.01^{Ba}	0.91 ± 0.08^{ABb}	0.46 ± 0.03^{Bc}
	28	2.05 ± 0.01^{Aa}	1.16 ± 0.02^{Aab}	1.04 ± 0.01^{Ab}
Acid organics (g/100 g)				
Citric (g/100 g)	1	0.04 ± 0.01^{Aa}	0.03 ± 0.01^{Aa}	0.01 ± 0.01^{Aa}
	14	0.01 ± 0.01^{Ba}	0.02 ± 0.02^{Aa}	0.01 ± 0.01^{Aa}
	28	0.01 ± 0.01^{Ba}	0.01 ± 0.01^{Aa}	0.01 ± 0.01^{Aa}
Lactic (g/100 g)	1	0.87 ± 0.08^{ABa}	0.71 ± 0.06^{Ba}	0.64 ± 0.08^{Ba}
	14	0.79 ± 0.08^{Ba}	0.74 ± 0.03^{ABa}	0.84 ± 0.08^{ABa}
	28	1.14 ± 0.06^{Aa}	1.29 ± 0.06^{Aa}	1.21 ± 0.35^{Aa}
Malic (g/100 g)	1	0.01 ± 0.02^{Aa}	0.06 ± 0.01^{Aa}	0.02 ± 0.01^{Aa}
	14	<LOD	0.03 ± 0.04^{Aa}	0.01 ± 0.01^{Aa}
	28	<LOD	0.05 ± 0.03^{Aa}	0.01 ± 0.01^{Aa}
Propionic (g/100 g)	1	0.08 ± 0.05^{Aa}	0.11 ± 0.01^{Aa}	0.23 ± 0.02^{Aa}
	14	0.11 ± 0.01^{Aa}	0.10 ± 0.09^{Aa}	0.16 ± 0.01^{Aa}
	28	0.13 ± 0.05^{Aa}	0.15 ± 0.05^{Aa}	0.21 ± 0.01^{Aa}

Results are expressed as average (n = 3) ± standard deviation.
$^{a-c}$Mean ± standard deviation with different lowercase letters on the same line differed by the Tukey test or *t*-Student test ($p < 0.05$), between treatments.
$^{A-C}$Mean ± standard deviation with different capital letters in the same column differed by Tukey's test ($p < 0.05$), over storage time.
Formulations: CY (control yogurt), XY1% (yogurt added 1% with xique-xique flour) and XY2% (yogurt added 2% with xique-xique flour). Abbreviations: <LOD: below the limit of detection.

concentrations of xique-xique flour in cookies (Machado et al., 2021) was also associated with an increase in minerals. According to theses authors, xique-xique flour has high concentrations of minerals, which contributed to the XY2% formulation having a higher content of ash (minerals) when compared to other formulations.

In general, the xique-xique flour did not affect fat content for most of the evaluated times ($p \geq 0.05$). The data corroborated the fact that the central stem of the matrix (*Pilosocereu gounellei*) presents very low-fat values (0.77 ± 0.04 g/100 g) (Bezerril et al., 2021b), and has no significant impact on the lipid content in yogurts. For the food processing industry, lower fat content is an important aspect of food matrices. From a nutritional and technological point of view, this is true as well, since lipid oxidation is a principal problem (affecting shelf life) in production of dairy products such as yogurt (Sartori, Alencar, Bastos, Regitano, & Skibsted, 2018). In the yogurts supplemented with xique-xique flour, the data revealed both nutritional and technological improvements, and demonstrated its potential applicability in the dairy industry, especially in fermented milk.

3.2. Yogurt viscosity

Fig. 1 shows that up to the 14th day of refrigerated storage, a reduction of viscosity ($p < 0.05$) in the CY (from 481.2 to 409.4 mPa s) and in XY1% (from 734.4 to 625 mPa s). The XY2%, however, presented an increase ($p < 0.05$) of from 459.4 to 625 mPa s, demonstrating an effect of a greater concentration of xique-xique flour on this technological parameter. Even so, by the 14th day of storage, the yogurts with the xique-xique flour (XY1% and XY2%) were more viscous than the control formulation (CY) ($p < 0.05$).

The decrease in the viscosity of the CY and XY1% in the first 14 days of refrigerated storage could be due to degradation of the gel network by LAB and the subsequent loss of gel stability, causing them to release fluid. On the other hand, the increase in viscosity in the first 14 days of storage of XY2% yogurt occurred probably due to the post-acidification of yogurt at 4 °C. At this temperature, milk protein can form a firmer gel and hence suffer increased viscosity (Mohammadi-Gouraji, Soleimanian-Zad, & Ghiaci, 2019). Furthermore, probably the higher concentration of xique-xique flour in XY2% may have stimulated a greater exopolysaccharide (EPS) formation by the LAB in the first 14 days of storage, causing an increase in the viscosity (Parvarei et al., 2021). EPSs produced by LAB are known to interact with milk constituents and then act as gelling agents, stabilizers, texturizers, and viscosities of dairy products (Korcz & Varga, 2021; Parvarei et al., 2021).

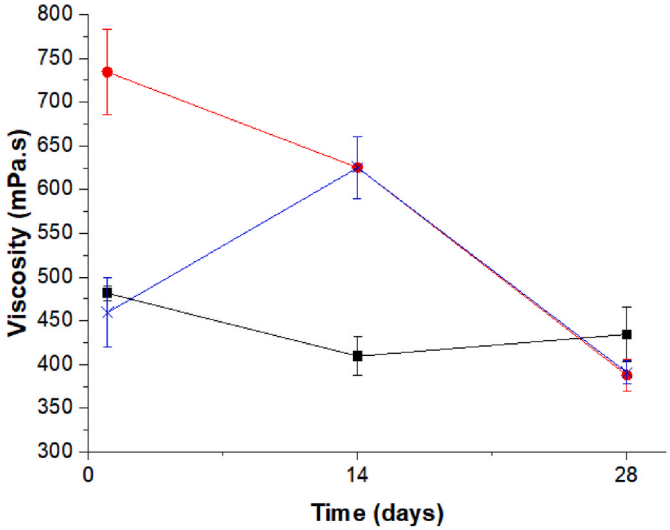

Fig. 1. Apparent viscosity (mPa.s) in CY (■), XY1% (●), and XY2% (▲) during storage. Results are expressed as averages (n = 3) ± standard deviation.

The interaction of phenolic compounds and polysaccharides of ingredients added in yogurt formulations with their protein network could lead to the rearrangement of the network, thereby increasing the viscosity of yogurt during storage (Almusallam et al., 2021; Trigueros et al., 2014; Vital et al., 2015). The xique-xique flour used in this study presented about 16.59 g/100 of total fiber (Machado et al., 2021). Table 3 shows that the yogurt supplemented with the highest concentrations of xique-xique flour (XY2%) presented higher total phenolic contents at time 1 (9.68 mg GAE/100 g) and 14 days of storage (7.88 mg GAE/100 g). This may have influenced the stability of the three-dimensional protein networks, increasing viscosity during the first 14 days of storage. A similar increase in the viscosity has been reported in yogurt fortified with phycocyanin (Mohammadi-Gouraji et al., 2019). Yogurt fortified with grape pomace during cold storage has been reported (Demirkol & Tarakci, 2018), whose ingredients were sources of fiber and/or phenolic compounds.

However, from the 14th day onward (to the end of storage), there was a considerable reduction in viscosity for both XY1% (from 625 to 387.5 mPa s) and XY2% (from 625 to 390.6 mPa s). This can be attributed to a lower yogurt protein network sustainability, which affects with viscosity. The presence of fiber in the xique-xique flour directly impacts the sustainability of the yogurt protein network, making it difficult to rearrange the proteins and establish lower protein-protein interactions, consequently decreasing the viscosity of these formulations (Lee & Lucey, 2010). In other words, the fibers might have caused the yogurt gel network to break up and, thus, reduce viscosity as a result of reduced surface tension.

Only at the end of its shelf life did the CY present a higher viscosity (434.4 mPa s) than the test samples supplemented with xique-xique flour ($p < 0.05$). Similar results have also been described in goat-milk yogurt supplemented with xique-xique jelly, with decreased apparent viscosity when compared to conventional yogurt (Bezerril et al., 2021a). Sah, Vasiljevic, McKechnie, and Donkor (2016) similarly observed that yogurts made from bovine milk and powder-fortified with pineapple peel show markedly lower viscosity values compared to non-fortified control yogurt during the storage period.

Yogurts made with goat's milk present a more open structure, consisting of larger serum pores, intermediate protein filaments, and thus fewer crosslinks between protein filaments, all of which makes the porous microstructure network weaker when compared to other dairy matrices (Nguyen et al., 2018); these are likely to suffer greater alterations when added with other ingredients, such as jellies, powders and flours. Therefore, further study of the effects of the addition of ingredients based on xique-xique to dairy derivatives is recommended.

3.3. Sugar and organic acid profiles of the yogurts

Table 2 presents the sugar and organic acid profile results for the yogurt formulations. At all storage times, the lactose content was higher ($p < 0.05$) for CY compared to the XY1% and XY2% samples. There was, however a reduction in this disaccharide occurred for all formulations ($p < 0.05$) during storage. This is related to the metabolic activities of lactic acid bacteria in the yogurt fermentation process in which there is an increased need for energy production for multiplication (Wang et al., 2020).

The other sugars also suggest bacterial fermenting activity. The presence of glucose and galactose in more significant amounts suggests lactose hydrolysis during fermentation and release of glucose and galactose in the medium (Barros, Cutrim, Costa, Conte Junior, & Cortez, 2019). The starter culture used in the present study (YF L903) shows a short lag phase, resulting in a fast fermentation process with high hydrolysis of lactose (Asensio-Vegas et al., 2016). In this study, there was an increase in levels of both glucose and galactose ($p < 0.05$) during the refrigerated storage, possibly due to the increase in lactose degradation during fermentation. In addition, the addition of xique-xique flour to XY1% and XY2% formulations had a direct impact on the behavior of the

Table 3
Contents of phenolic, flavonoids compounds and antioxidant activity of the probiotic yogurts over storage.

Parameter	Days	Treatment		
		CY	XY1%	XY2%
Phenolic compounds (mg/100 g)				
Catechin	1	0.21 ± 0.00^{Bc}	0.39 ± 0.01^{Aa}	0.31 ± 0.00^{Ab}
	14	0.23 ± 0.00^{ABc}	0.38 ± 0.01^{ABa}	0.30 ± 0.01^{Ab}
	28	0.25 ± 0.01^{Ab}	0.36 ± 0.00^{Ba}	0.27 ± 0.01^{Bb}
Epigallocatechin galato	1	<LOD	0.04 ± 0.00^{Ab}	0.06 ± 0.00^{Aa}
	14	<LOD	0.04 ± 0.00^{Ab}	0.06 ± 0.00^{Aa}
	28	<LOD	0.01 ± 0.02^{Ab}	0.06 ± 0.01^{Aa}
Procyanidin B2	1	0.03 ± 0.01^{Aa}	0.03 ± 0.03^{Aa}	0.04 ± 0.01^{Aa}
	14	0.04 ± 0.01^{Aa}	0.03 ± 0.01^{Aa}	0.03 ± 0.01^{Aa}
	28	0.04 ± 0.01^{Aa}	0.03 ± 0.01^{Aa}	0.03 ± 0.01^{Aa}
Procyanidin A2	1	<LOD	<LOD	0.15 ± 0.03^{Aa}
	14	<LOD	<LOD	0.09 ± 0.00^{ABa}
	28	<LOD	<LOD	0.07 ± 0.00^{Ba}
Syringic	1	<LOD	0.01 ± 0.01^{Aa}	0.01 ± 0.01^{Aa}
	14	<LOD	0.01 ± 0.01^{Aa}	0.01 ± 0.01^{Aa}
	28	<LOD	0.01 ± 0.01^{Aa}	0.01 ± 0.01^{Aa}
Total phenolics (mg EGA/100 g)[a]	1	7.79 ± 0.00^{Ab}	8.07 ± 0.13^{Aa}	9.68 ± 0.00^{Aa}
	14	5.91 ± 0.00^{Bb}	6.00 ± 0.12^{Bb}	7.88 ± 0.13^{Ba}
	28	5.62 ± 0.13^{Bb}	6.98 ± 0.00^{Bb}	7.51 ± 0.12^{Ba}
Total flavonoids (mg EC/100 g)[b]	1	0.52 ± 0.01^{Ac}	0.58 ± 0.00^{Bb}	0.64 ± 0.00^{Aa}
	14	0.52 ± 0.01^{Ac}	0.59 ± 0.00^{Ab}	0.64 ± 0.00^{Aa}
	28	0.51 ± 0.01^{Bc}	0.58 ± 0.00^{Bb}	0.64 ± 0.00^{Aa}
FRAP (μmol TEAC/100 g)[c]	1	2.49 ± 0.01^{Ab}	2.81 ± 0.01^{Aa}	2.82 ± 0.01^{Aa}
	14	2.17 ± 0.01^{Bb}	2.34 ± 0.01^{Ba}	2.51 ± 0.01^{Ba}
	28	2.03 ± 0.01^{Cc}	2.18 ± 0.01^{Cb}	2.35 ± 0.01^{Ca}
ABTS (μmol TEAC/g)[d]	1	0.16 ± 0.01^{Ab}	0.19 ± 0.01^{Aa}	0.19 ± 0.01^{Aa}
	14	0.16 ± 0.01^{Ab}	0.16 ± 0.01^{Bb}	0.19 ± 0.01^{Aa}
	28	0.15 ± 0.01^{Bb}	0.16 ± 0.01^{Ba}	0.16 ± 0.01^{Ba}

Results are expressed as average (n = 3) ± standard deviation.
[a-c]Mean ± standard deviation with different lowercase letters on the same line differed by the Tukey test or *t*-Student test ($p < 0.05$), between treatments.
[A–C]Mean ± standard deviation with different capital letters in the same column differed by Tukey's test ($p < 0.05$), over storage time.
Formulations: CY (control yogurt), XY1% (yogurt added 1% with xique-xique flour) and XY2% (yogurt added 2% with xique-xique flour). Abbreviations: <LOD: below the limit of detection; FRAP - ferric reducing ability of plasma; $ABTS^{\bullet+}$ cation - 2.2-azino-bis (3-etilbenzo-tiazoline)-6-sulfonic acid.
[a] The results are expressed in milligram equivalents of galic acid (EGA) per hundred grams of sample (mg EGA/100 g).
[b] The results are expressed in milligram equivalents of catechin (EC) per hundred grams of sample (mg EC/100 g).
[c] The results are expressed as micromoles of Trolox equivalent antioxidant capacity (TEAC) per hundred grams of sample μmol TEAC/100 g).
[d] The results are expressed as micromoles of Trolox equivalent antioxidant capacity (TEAC) per gram of sample (μmol TEAC/g).

sugar profile during storage, with greater drops in lactose values and concomitant greater release of glucose and consumption of galactose in these formulations ($p < 0.05$). Starter cultures and/or probiotic cultures generally use sugars in their metabolism (Costa et al., 2019). First, glucose is converted into pyruvate in the Embden Meyerhoff-Parnas pathway. Then, pyruvate is used as an H-acceptor, and, forming lactate (Costa, Frasao, Lima, Rodrigues, & Conte Junior, 2016). In this study, the presence of the xique-xique flour was what probably stimulated greater multiplication and intensified the metabolism of the lactic acid bacteria, mediating a higher consumption of the sugars present in the formulations, especially lactose and galactose. The data agreed with the viable cell analyses for the strains present in the yogurt samples (Fig. 2), whose counts for most times were always higher in XY1% and XY2%, indicating a potential synergy between the starter culture, *L. mucosae*, and the xique-xique flour.

Previously, the prebiotic potential of the xique-xique was demonstrated (Ribeiro et al., 2020). The most current definition of prebiotic is that it is a substrate selectively utilized by host microorganisms to confer a health benefit (Gibson et al., 2017). These promotions of modulatory effects on human health, for their part, can be mediated by metabolites (*e.g.,* organic acids or short chain fatty acids) produced from the fermentation of these substrates by lactic acid bacteria in food and/or microbiota (Diez-Gutiérrez et al., 2020). Table 2 presents the organic acid values detected in the yogurt formulations during refrigerated storage. Citric, lactic, malic, and propionic acids were identified. Lactic acid was the principal organic acid in all the yogurt formulations, and although the addition of xique-xique flour did not influence the amount of lactic acid between formulations ($p \geq 0.05$), during storage there was an increase of this acid, possibly due to the higher fermentation of lactose ($p < 0.05$). During storage, however, the increase in lactic acid did not impact pH, as had been seen previously (Table 1). Lactic acid can result from the metabolism of starter and/or probiotic cultures during fermentation (Bezerril et al., 2021a; Costa et al., 2016), which could remove pyruvic acid, convert malic acid, and/or degrade lactose (Ozcan, Ozdemir, & Avci, 2021). These results may be associated with the higher consumption of galactose in this product (Table 2). Similar results were observed by Almusallam et al. (2021) and Öztürk et al. (2018), in yogurt supplemented with *Elaeagnus angustifolia* L. flour, in yogurt supplemented with moringa extract (Zhang et al., 2018), and in yogurt supplemented with jujube pulp (Feng et al., 2019).

In this study, production of propionic acid was observed, a short-chain fatty acid (SCFA) with an important role in stimulating the production of ATP (Singh, Vishwakarma, & Singhal, 2018). In addition, propionic acid is known to have other benefits such inhibiting cholesterol synthesis, positively helping to fight varied diseases, like diabetes, cancer, obesity, and autoimmune diseases (Diez-Gutierrez et al., 2020). Synthesis of short-chain fatty acids is characteristic of fermentation activity in probiotic strains (Nagpal et al., 2018); in this study the propionic acid probably came from the *Limosilactobacillus mucosae* CNPC007 heterofermentative metabolic pathway. Therefore, the consumption of this product could be beneficial for people who suffer from of the aforementioned health conditions.

3.4. Phenolic and flavonoid contents, and antioxidant activity of the yogurts

Table 3 presents both the phenolic and flavonoid contents, and the antioxidant activity of the yogurt samples. The yogurt samples supplemented with xique-xique flour presented at least 5 phenolic compounds: flavonols (catechin and epigallocatechin gallate), anthocyanins (procyanidin B2 and procyanidin A2) or hydroxybenzoic acids (syringic). Catechin was the principal phenolic compound present, followed by Procyanidin A2 and Epigallocatechin gallate.

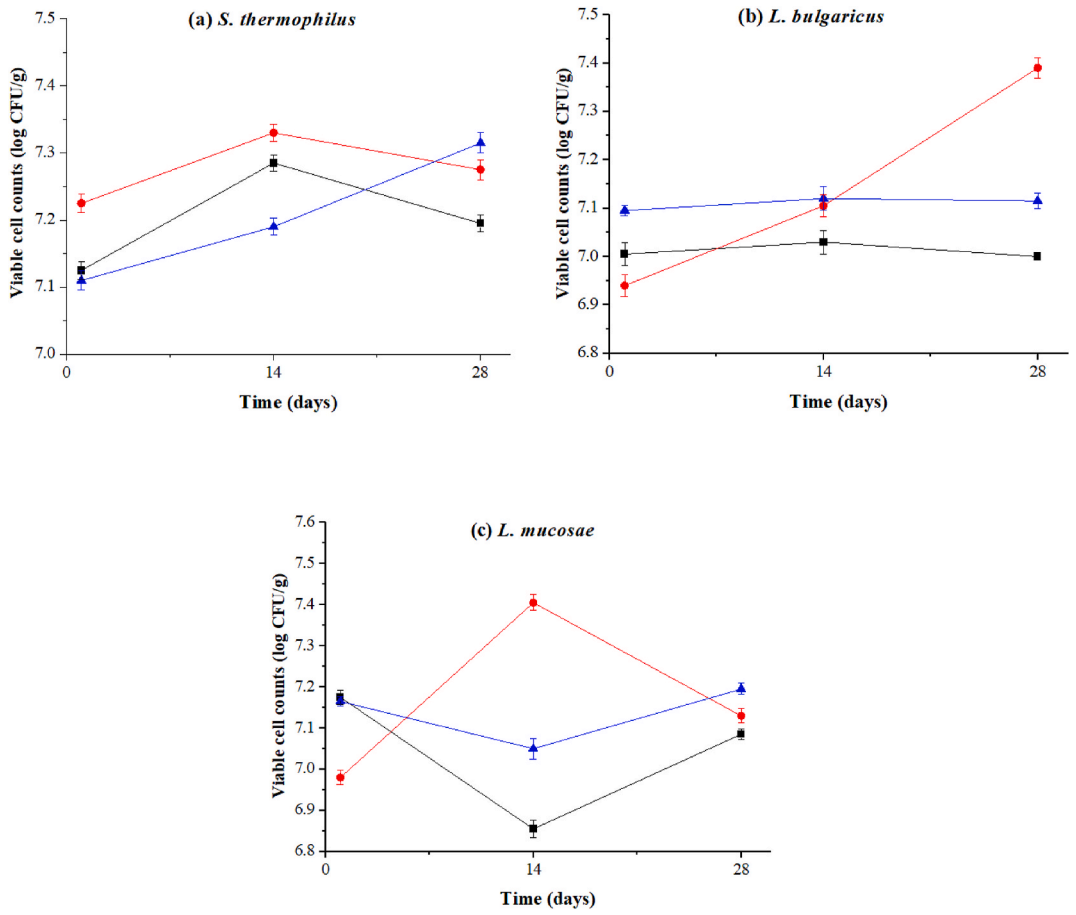

Fig. 2. Viable cell counts (log CFU/g) of lactic acid bacteria in CY (■), XY1% (●), and XY2% (▲) during storage. Results are expressed as averages (n = 3) ± standard deviation.

In general, storage time had little effect on the amounts of phenolic compounds detected ($p \geq 0.05$); except for catechin, whose content decreased after 28 days of refrigerated storage in XY1% and XY2% and increased in CY ($p < 0.05$). Interestingly, only the phenolic compounds epigallocatechin gallate, procyanidin A2 and syringic were detected in the XY1% and XY2% formulations, indicating that the xique-xique flour was the main source of these bioactive compounds. In fact, previously Bezerril et al. (2021b) evaluated the physicochemical characteristics and bioactive compounds of the xique-xique (*Pilosocereus gounellei*) and verified that the vascular cylinder of this cactus had higher amounts of the aforementioned phenolic compounds, corroborating our results.

The phenolic compounds detected for having antioxidant activity are beneficial to the body, reducing the incidence of chronic non-communicable diseases, diabetes, cardiovascular disease, cancer, and even neurological disease (Bezerril et al., 2021b; Marković et al., 2017). Besides promoting health, the presence of these natural antioxidant components in dairy derivatives can influence sensory acceptance. These compounds have been identified in goat-milk yogurt supplemented with xique-xique jelly (Bezerril et al., 2021a).

The increasing concentrations of xique-xique flour in each yogurt sample significantly increased ($p < 0.05$) total phenolic compound and total flavonoid levels, and potentially promoted the measured increase in antioxidant activities (ABTS and FRAP assays), corroborating the results of the profile of phenolic compounds detected in these formulations. The greatest increase ($p < 0.05$) was mainly observed for XY2% (Table 3). Similar results were obtained by Almusallam et al. (2021), who observed increased antioxidant activity when date palm extract was added to yogurt; by Feng et al. (2019), when phenolic compounds were studied in yogurt supplemented with jujube pulp; and by Vital et al. (2015), when low fat yogurts supplemented with *Pleurotus ostreatus* aqueous extract were evaluated.

In our study, we saw that there was a reduction in total phenolic compounds and antioxidant activity (FRAP and ABTS) during storage for all formulations ($p < 0.05$). However, these reductions were smaller ($p < 0.05$) in the formulations supplemented with flour from xique-xique, probably due to the richness of phenolic compounds in this matrix, and consequent antioxidant activity (Bezerril et al., 2021b; Maciel et al., 2016). Reductions in phenolic compounds and, consequently, in antioxidant activity in the formulations, suggest an association of different complexes of milk proteins with phenolic compounds, affecting phenolic maintenance, and causing a consequent reduction in antioxidant activity during storage (Almusallam et al., 2021; Feng et al., 2021; Vital et al., 2015).

Furthermore, phenolic compounds may exert a prebiotic function and increase the population of beneficial bacteria, including probiotics, suggesting a mutual relationship between phenolic compounds and probiotics (Gibson et al., 2017; Llano et al., 2017; Ozdal et al., 2016; Succi et al., 2017). Selected probiotic strains have been shown capable of improving the metabolism and bioavailability of phenolic compounds. In turn, phenolic compounds may positively modulate the gut microbiota composition and protect probiotic bacteria from the conditions found during gastrointestinal passage and during the storage of different foods (Souza, Albuquerque, Santos, Massa, & Brito Alves, 2018). These properties are due to the bioconversion of the original phenolic compounds into secondary metabolites by lactic acid bacteria in food and/or intestinal microbiota. Therefore, the biotransformation of phenolic compounds by the starter and probiotic bacteria presents in yogurt formulations may have contributed to the reduction of these

bioactive compounds and, consequently, of the antioxidant properties, without major losses in these properties, mainly for the XY2% formulation.

3.5. Microbiological analyses

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. There were no counts for *E. coli*, molds and yeasts and absence of *Salmonella* spp., indicating good manufacturing practices.

The viability count of lactic acid bacteria, especially from probiotic cultures, in fermented milk becomes an important indicator of functional quality for this type of product (Feng et al., 2019). The cell viable counts of the starter culture (*S. thermophilus* and *L. bulgaricus*) and the probiotic *Limosilactobacillus mucosae* CNPC007 are shown in Fig. 1.

At the beginning of storage, counts ranged from 6.95 to 7.23 log CFU/g for the starter culture, and from 6.98 to 7.18 log CFU/g for *L. mucosae*. There was an increase in the number of viable cells of *S. thermophilus* ($p < 0.05$) up to 14 days of refrigerated storage for all formulations (CY - 7.29 ± 0.01 log CFU/g; XY1% - 7.33 ± 0.01 log CFU/g and XY2% - 7.19 ± 0.01 log CFU/g), and as the storage time approached 28 days, only the XY2% showed an increase ($p < 0.05$) in the counts of *S. thermophilus* (7.32 ± 0.01 log CFU/g). While CY and XY1% showed a decrease in viable cell counts for this strain (7.20 ± 0.01 log CFU/g and 7.28 ± 0.01 log CFU/g, respectively). The increase in viable counts of this microorganism in the formulations during the first 14 days of storage may be due to high bacterial metabolic activity under favorable conditions of pH and acidity (Almusallam et al., 2021).

Only the XY1% had a continuous increase in the number of viable cells of *L. bulgaricus* over the 28 days of storage ($p < 0.05$), with final counts of 7.39 ± 0.02 log CFU/g. Counts in formulations of the CY (7.00 ± 0.02 to 7.01 ± 0.01 log CFU/g) and XY2% (7.10 ± 0.01 to 7.12 ± 0.01 log CFU/g) did not vary over time ($p \geq 0.05$). The increase in viable counts of the starter culture in the formulation XY1% can be attributed to a prebiotic effect that the flour promoted at this concentration, considering the total fiber content in this matrix of 16.59 ± 0.09 g/100 g (Machado et al., 2021).

A previous study had already demonstrated the prebiotic potential of freeze-dried xique-xique juice, stimulating the multiplication and metabolism of different *Lactobacillus* isolates, similarly to fructooligosaccharides (FOS, a proven prebiotic ingredient) (Ribeiro et al., 2020). Greater amounts of flour can stimulate the multiplication of *S. thermophilus*, generating antimicrobial components and organic acids that interfere with the multiplication of *L. bulgaricus* (Feng et al., 2019). Therefore, the increased tolerance to acids resulting from the fermentation of sugars (Feng et al., 2019), could justify the differences in the counts detected between the microorganisms in the starter culture in each formulation.

Viable cell counts of *L. mucosae* CNPC007 decreased between the 1st and 14th day of refrigerated storage ($p < 0.05$) in CY samples (from 7.18 ± 0.02 to 6.86 ± 0.02 log CFU/g) and in XY2% (from 7.17 ± 0.01 to 7.05 ± 0.02 log CFU/g), and increased ($p < 0.05$) in XY1% (from 6.98 ± 0.02 to 7.41 ± 0.02 log CFU/g). At the end of the 28th day of refrigerated storage, XY2% presented a highest count ($p < 0.05$) of *L. mucosae* CNPC007 (7.20 ± 0.01 log CFU/g) when compared to formulations CY (7.09 ± 0.01 log CFU/g) and XY1% (7.13 ± 0.02 log CFU/g).

Antioxidant compounds such as catechin and rutin, present in plant components, can reduce the growth of LAB in fortified yogurts, through inhibition of nucleic acid synthesis, enzymatic activity and aggression to the cytoplasmic membrane (Almusallam et al., 2021; Joung et al., 2016). In this study, the formulation with greater addition of xique-xique flour promoted a greater multiplication of the probiotic strain, seemingly without interference from the phenolic compounds in the flour. In addition, the fat and fat-soluble vitamin contents found in goat milk (Verruck, Dantas, & Prudencio 2019Verruck et al., 2019) may have played a protective role, acting in synergy with the flour and its bioactive constituents, principally the fibers, which are stimulants of the multiplication of lactic acid bacteria. Synergies enable an improvement in the bioactive effect, increasing functionality and improving the multiplicity of the food matrix (Jacobs, Tapsell & Temple, 2011; Alongi & Anesi, 2021).

Still, the viable cell counts of *L. mucosae* in all yogurt formulations were above the minimum count recommended for beneficial health effects, corresponding to 6.0 log CFU/g (Terpou et al., 2019), up to the last day of refrigerated storage. This result suggests that the addition of xique-xique flour can offer satisfactory conditions for the multiplication of this strain in yogurts with probiotic potential, an extremely important characteristic that guarantees the bioactivity of the product, providing beneficial effects for consumer health (Diez-Gutierrez et al., 2020).

3.6. Principal component analysis (PCA)

Principal component analysis (PCA) enabled the characterization and exploration of the effects of the treatments investigated. In this study, PCA explained 88.69% of the total structure of variance and covariance [PC1 (62.68%), as well as PC2 (26.01%)], as shown in Table 4. To observe the effects of xique-xique flour supplementation or storage time on the physical-chemical, microbiological, and functionality properties of the different formulations of goat milk yogurt, an HJ-biplot was constructed by comparing the CY, XY1%, and XY2% eigenvalues (Fig. 3).

In the biplot, similarity among the yogurt samples during storage is reflected by plot distances. Correlation between the lines is indicated by the cosine of the angles between the vectors, where acute, obtuse and straight angles reveal correlations, positive, negative, or absent, respectively (Almusallam et al., 2021). The influences of treatment and storage time on the functional, physicochemical, and microbiological properties are observed in group formations between biplot quadrants. Group 1, the control yogurt, is clearly positioned in the first quadrant of storage days, characterized by more expressive values of lactose and galactose, especially on the 1st and 14th days. These values reflect evidence of fermenting behavior in the strains present in the control yogurt, which in this case tended to ferment less than in the formulations containing xique-xique flour. Luminosity (L*) was also representative of the CY on the 1st and 14th days, which was lighter compared to the xique-xique flour formulations. We note that the CY, after 28 days of storage, was positioned close to the centroid of the biplot, suggesting balanced PC1 and PC2 values, and but lacking stronger characteristic features in its profile than the xique-xique flour treated yogurts.

Table 4
Coefficients and total variation explained by each principal component (PC).

Parameter	Principal Component	
	PC1	PC2
Lactose	−0.429	- 0.155
Galactose	−0.204	0.351
Glucose	0.380	0.253
Lactic	0.160	0.313
Propionic	0.322	−0.029
Malic	0.198	−0.280
Acidity	0.419	−0.129
Viscosity	0.044	−0.288
pH	−0.053	0.343
L*	−0.239	−0.239
a*	−0.127	−0.127
b*	0.342	0.342
S. thermophilus	0.116	0.116
L. bulgaricus	0.133	0.133
L. mucosae	0.244	0.244
Eigenvalue	9.400	3.901
Variation (%)	62.680	26.010
Accumulated Variation (%)	62.680	88.690

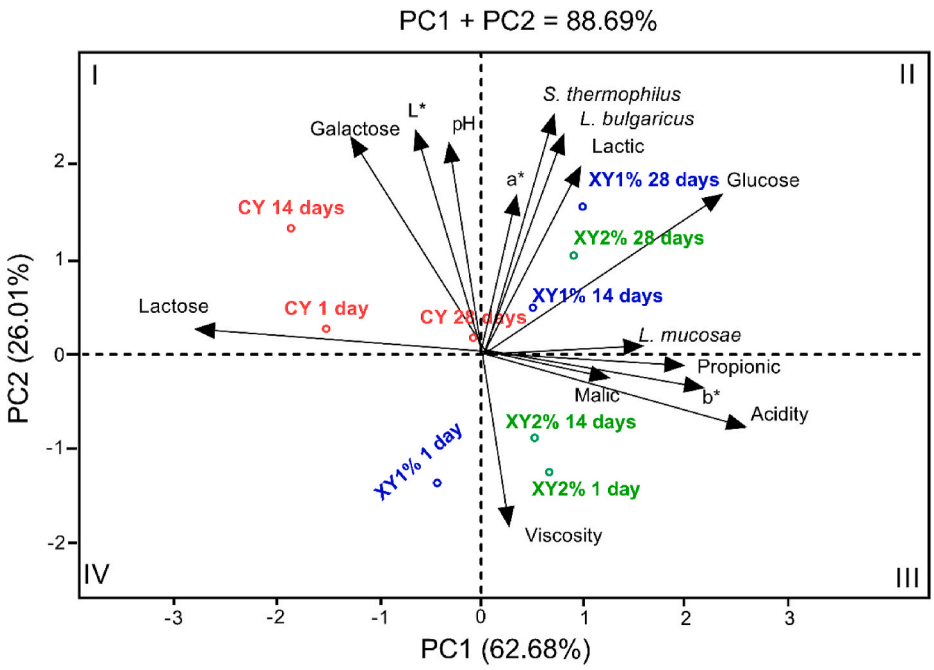

Fig. 3. HJ-biplot based on principle component analyses of control yogurt (●), and samples with xique-xique flour added at 1.0% (●), and 2.0% (●), as affected by treatment and storage time.

In the second quadrant, there is another group formed by the XY1% (on the 14th and 28th days) and XY2% (at 28 days) treatments. This group was characterized by greenish color (a*), and a greater number of viable LAB cells (*L. bulgaricus, S. thermophilus,* and *Limosilactobacillus mucosae* CNPC007), consequently increasing both sugar production (glucose) and lactic acid. The behavior was evident from the 14th day forward in the XY1% samples, and on the 28th day in both the XY1% and XY2% samples. The results are interesting because this multiplication of the microorganism reveals the influence of both sugar fermentation (lactose), and metabolite production (monosaccharides and organic acids).

In the third quadrant, the group formed by XY2% (1st and 14th days) is observed, and is characterized by higher (b*) values, a yellow tone, and especially by higher values of acidity and viscosity associated with low brightness (L*) values. Nevertheless, there is evidence of greater probiotic strain activity, due to the higher malic and propionic acid values. The group formed by the XY1% treatment on the 1st day of storage in quadrant four is characterized by lower LAB activity for the *L. bulgaricus*, and *Limosilactobacillus mucosae* CNPC007 strains, and by lower values of glucose in association with higher viscosity values.

4. Conclusions

This study concludes that the incorporation of xique-xique flour in yogurt modifies the nutritional, bioactive and technological properties of the product. Compared to the control yogurt, addition of xique-xique flour produced yogurts with a more green-yellow hue and with lower viscosity after 28 days of storage, which was attributed to the fibers present in the xique-xique flour, causing the rupture of the yogurt gel network and, thus, reducing viscosity. The addition of xique-xique flour also resulted in yogurt with higher concentrations of minerals. The fermentation process mediated by the synergy between starter bacteria, the probiotic strain *L. mucosae,* and xique-xique flour led to an increase in lactose degradation, with a consequent increased production of lactic acid during storage. These conditions were likely influenced by multiplication of the lactic acid bacteria during storage, without affecting the total viable cell counts of the probiotic strain *L. mucosae*, which after 28 days of storage presented counts >7 log CFU/g (specifically XY2%). Although during storage there was a reduction in total phenolic compounds and antioxidant activity, these reductions were less in yogurts with xique-xique flour added, probably due to the higher content of bioactive compounds detected in these formulations, mainly catechin, epigallocatechin gallate, procyanidin A2 and syringic, which are major phenolic compounds in xique-xique flour. This study may help promote the beneficial use of xique-xique flour in probiotic goat milk yogurt formulations, and result in a product with great nutritional, bioactive and technological potential for the functional food industry.

Conflict of interest

Authors declare no conflict of interest.

CRediT authorship contribution statement

Dalyane Laís da Silva Dantas: Conceptualization, Methodology, Formal analysis, Writing – original draft, Preparation, Writing – review & editing. **Vanessa Bordin Viera:** Methodology, Formal analysis, Supervision. **Juliana Késsia Barbosa Soares:** Methodology, Formal analysis. **Karina Maria Olbrich dos Santos:** Methodology, Formal analysis, Resources. **Antônio Silvio do Egito:** Methodology, Formal analysis, Resources. **Rossana Maria Feitosa de Figueirêdo:** Methodology, Formal analysis. **Marcos dos Santos Lima:** Methodology, Formal analysis. **Nítalo André Farias Machado:** Methodology, Formal analysis. **Maria de Fátima Vanderlei de Souza:** Conceptualization, Writing – review & editing. **Maria Lúcia da Conceição:** Methodology, Formal analysis. **Rita de Cássia Ramos do Egypto Queiroga:** Conceptualization, Data curation, Writing – original draft, Preparation, Writing – review & editing, Supervision. **Maria Elieidy Gomes de Oliveira:** Conceptualization, Data curation, Resources, Writing – original draft, Preparation, Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

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