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Fermentative behavior of native lactobacilli in goat milk and their survival under *in vitro* simulated gastrointestinal conditions

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ABSTRACT

In this study, goat fermented milk trials were prepared with six potentially probiotic autochthonous cultures – *Lactobacillus rhamnosus* EM1107, *Lactobacillus plantarum* (CNPC001, CNPC002, CNPC003 and CNPC004) and *Lactobacillus mucosae* CNPC007 – in the presence and absence of a starter culture (*Streptococcus thermophilus*, ST) and in the presence and absence of inulin (IN) or oligofructose (FOS), consisting of 36 trials. The fermented milk without the starter culture only achieved a lactic acid content above 0.6 g/100 g after 24 h, thus requiring the presence of ST culture for a faster acidification. After the fermentation, three trials with the best fermentative performance and visual firmness (EM1107 + ST + IN, CNPC003 + ST + IN and CNPC007 + ST + IN) were exposed to simulated conditions of the gastrointestinal tract (GIT) *in vitro*. All fermented milk, with *Lactobacillus* spp. population above 6 log CFU/g before GIT simulation, showed the highest viability losses in the gastric step. In fermented milk with *Streptococcus thermophilus* plus inulin, *L. rhamnosus* and *L. mucosae* performed well in the GIT assay, achieving a viability between 6.50 – 7.24 and 6.50–7.60 log CFU/g in the enteric stage, respectively. Therefore, the combination of EM1107 or CNPC007 strains with *S. thermophilus* and inulin is promising in the production of goat dairy products.

1. Introduction

Several properties make goat milk beneficial for human health, such as high digestibility and buffering capacity, lower cholesterol levels compared with cow milk, low allergenic potential, and high content of calcium. These properties also allow for manufacturing of a variety of dairy products from goat milk, such as cheese, yogurt, fermented and non-fermented dairy drinks, ice cream, butter, condensed milk, desserts, among others. Moreover, the addition of probiotics - living microorganisms that, when ingested in adequate amounts, confer benefits to the host - can add greater functional value to goat milk (de Paula et al., 2020; Pal, Dudhrejiya, & Pinto, 2017).

The search for new probiotic strains among lactic acid bacteria isolated from raw and fermented food products may reveal strains with promising functional and technological properties (Vizoso-Pinto, Franz, Schillinger, & Holzapfel, 2006). Probiotics act through various mechanisms that included alteration of the intestinal microflora, adhesion to colon cancer cells, and anti-proliferative activity. Probiotic foods must contain an adequate amount of live microorganisms, at least 10^6 CFU/g in order to provide a health impact to the consumer (Terpou et al., 2019). The beneficial effects of probiotic bacteria have been considered strain specific. As a result, different bacterial strains of the same species may induce completely different characteristics and have a different effect on the host (Mantzourani et al., 2019). In this sense, autochthonous microorganisms need to be identified and tested in a case-by-case approach and evaluate their specific characteristics and their potential positive effect on human health (Ruiz-Moyano et al., 2019). Moreover, for fermented dairy products, autochthonous cultures with good technological properties should have good growth capacity in milk, promote adequate sensory properties in the product and be stable, viable and

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functional during storage. However, neither all potentially probiotic bacteria develop well in milk nor can ferment it; thus, these are important features to be investigated. Likewise, *in vitro* tests such as resistance to simulated gastrointestinal conditions *in vitro* allow a preliminary selection of strains with probiotic potential (de Moraes et al., 2017; Vinderola et al., 2008).

Studies have been conducted to investigate some properties related to probiotic potential and technological applications of autochthonous such as *Lactobacillus mucosae* isolated from goat milk (de Moraes, dos Santos, de Barcelos, Lopes, & do Egito, 2018), *Lactobacillus plantarum* isolated from pig feces (Sirichokchatchawan et al., 2018), *Lactobacillus rhamnosus* isolated from artisanal Coalho cheese produced from bovine milk (dos Santos et al., 2015) and other lactobacilli species isolated from fish intestinal microbiota (Speranza et al., 2017).

The buffering capacity of dairy-based foods has a protective effect on probiotic bacteria when submitted to the digestion process. Other ingredients such as prebiotics may promote a more favorable environment for these microorganisms, exerting a protective effect during storage and during passage through the gastrointestinal tract (Buriti, Castro, & Saad, 2010). Prebiotics such as inulin and/or oligofructose, raffinose and polydextrose can stimulate the growth and/or activity of probiotic bacteria, increasing their viability in dairy products (Buriti, Cardarelli, & Saad, 2007; Costa et al., 2019; Marinaki, Kandylis, Dimitrellou, Zakynthinos, & Varzakas, 2016). Studies show that inulin was able to increase the survivability of *Bifidobacterium* in yogurt (Fayed, El-Sayed, Abood, Hashem, & Mehanna, 2019), *Lactobacillus paracasei* and *L. plantarum* in MRS after simulated gastrointestinal conditions (Iraporda, Rubel, Manrique, & Abraham, 2019), *L. plantarum* in fermented rice during storage (Savedboworn, Niyomrat, Naknown, & Phattayakorn, 2017) and that oligofructose increased the survival of *Lactobacillus casei* in yogurt (Costa et al., 2019). Clinical studies have shown that doses of 4–20 g/d should be ingested daily for inulin and oligofructose having a beneficial effect on intestinal microbiota (Martinez, Bedani, & Saad, 2015). Considering the last published instructions of the Brazilian Health Regulatory Agency (ANVISA), the minimum amount of inulin and oligofructose added to the serving portion of ready products with prebiotic health claim should be at least 5 g, without exceeding the limit of 30 g (Anvisa, 2016).

The objective of this study was to evaluate the fermentative behavior of potentially probiotic autochthonous cultures of *Lactobacillus* spp. in goat milk and the effect of the presence of a starter culture and prebiotics (inulin and oligofructose) on this behavior. In addition, the resistance of *Lactobacillus* spp. to *in vitro* gastrointestinal conditions was detected in the trials with better performance regarding acidification speed and culture viability.

2. Methodology

2.1. Fermentative behavior of the autochthonous cultures

2.1.1. Goat milk fermentation

The experimental design of the fermentation of goat milk, consisting of 36 trials in triplicate, is presented in Table 1.

When present, inulin Orafti® GR or oligofructose Orafti® P95 prebiotics (Beneo, Sweetmix, Brazil) were added to goat milk (Fazenda Carnaúba, Paraíba, Brazil) at a 5 g/100 mL ratio before heat treatment. For all trials, milk was heat treated for 15 min at 90 °C.

For milk fermentation, lyophilized cultures of the autochthonous strains *Lactobacillus rhamnosus* EM1107, *Lactobacillus plantarum* (CNPC001, CNPC002, CNPC003 and CNPC004) and *Lactobacillus mucosae* CNPC007 were obtained from the Culture Collection of Microorganisms of Agricultural Interests of Brazilian Agricultural Research Corporation (Embrapa). An arbitrary amount of each lyophilized strain (0.001–0.005 g) was inoculated in test tubes containing 10 mL of De Man Rogosa & Sharpe broth – MRS (Acumedia, USA) and incubated at 37 °C for 24 h (first activation). Then, 100 μL of the first culture was transferred to centrifuge tubes with 10 mL MRS broth and incubated under the same conditions as the first culture activation. After incubation, the material was centrifuged at 3500 rpm for 15 min. The obtained pellet was washed with 10 mL of 0.85% saline solution and centrifuged under the same conditions. The supernatant was discarded, and the pellet was added to the thermally treated milk cooled to 42 °C and, when present, the starter culture of *Streptococcus thermophilus* (QGE, Biotech, Brazil, 0.004 g/100 mL) was also added in this step. The inoculated milks were fermented at 42 °C or at 37 °C for those with and without *S. thermophilus*, respectively. The samples were collected in triplicate at 0, 6, 24 and 48 h of fermentation to determine the titratable acidity and viability of the autochthonous and starter cultures.

Table 1
– Experimental design of trials.

Autochthonous culture	Trials	*S. thermophilus*	Inulin	Oligofructose
L. rhamnosus EM1107	T1	–	–	–
	T2	–	+	–
	T3	–	–	+
	T4	+	–	–
	T5	+	+	–
	T6	+	–	+
L. plantarum CNPC001	T7	–	–	–
	T8	–	+	–
	T9	–	–	+
	T10	+	–	–
	T11	+	+	–
	T12	+	–	+
L. plantarum CNPC002	T13	–	–	–
	T14	–	+	–
	T15	–	–	+
	T16	+	–	–
	T17	+	+	–
	T18	+	–	+
L. plantarum CNPC003	T19	–	–	–
	T20	–	+	–
	T21	–	–	+
	T22	+	–	–
	T23	+	+	–
	T24	+	–	+
L. plantarum CNPC004	T25	–	–	–
	T26	–	+	–
	T27	–	–	+
	T28	+	–	–
	T29	+	+	–
	T30	+	–	+
L. mucosae CNPC007	T31	–	–	–
	T32	–	+	–
	T33	–	–	+
	T34	+	–	–
	T35	+	+	–
	T36	+	–	+

The three trials that reached titratable acidity greater than 0.6 g lactic acid/100 g in the shortest fermentation time and that presented a visually firmer consistency were submitted to the *in vitro* simulated gastrointestinal conditions.

2.1.2. Analysis during goat milk fermentation

The titratable acidity of the samples from each sampling period were evaluated according to the procedures described in method 426/IV of the Instituto Adolfo Lutz (2008) and expressed as lactic acid, g/100 g.

To determine the populations of lactobacilli and starter cultures, 1 mL samples were added to 9 mL NaCl sterile solution (0.85 g/100 mL) and submitted to decimal dilutions using the same diluent. One mL of each dilution was pour plated in De Man, Rosa & Sharpe agar (Acumedia), acidified to a pH of 5.4 for lactobacilli determination and in M17 medium (Difco, USA) with lactose addition (Vetec, Brazil, 5 g/L) for *S. thermophilus* determination (Buriti et al., 2007). Both culture media were incubated at 37 °C for 48 h.

2.2. Resistance to in vitro gastrointestinal conditions

The evaluation of lactobacilli resistance to simulated gastrointestinal conditions was conducted using the *in vitro* methodology described by Buriti et al. (2010), at 21 days of refrigerated storage (4 °C) of fermented goat milks that reached acidity greater than 0.6 g of lactic acid/100 mL after 6 h of incubation. For this, portions of 25g 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 1N 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%, totaling 10.41 mL (3.07 g/L pepsin; 0.9 mg/L lipase) with pH between 1.5 and 2.0, 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 ($NaH_2PO_42H_2O$, 14g; NaOH 1N 150 mL; H_2O distilled q.s.p 1L) containing bile (Bovine bile, Sigma, 0.1017g) and pancreatin (Pancreatin from porcine pancreas, Sigma, 0.0101g) were added, totaling a content of 12.58 mL (9.53 g/L bile and 0.953 g/L pancreatin) with pH between 4.7 and 5.8, 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 (0.0010g) was added, totaling a final volume of 13.58 mL (9.57 g/L bile and 0.9570 g/L pancreatin), with pH between 6.2 and 6.7, which was again incubated at 37 °C and agitated at 150 rpm for 2 h, totaling 6 h of assay. Aliquots containing the simulated gastric and enteric juices with fermented milk 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. Each assay was performed in triplicate. The assays were also performed with 1 mL of the same lactobacilli cultures grown twice in MRS broth, diluted in 9 mL 0.5% NaCl solution, using the same concentration of the gastrointestinal fluids, times, incubation conditions and plating as described for goat milk.

2.3. Statistical analysis

The results along the 48 h of fermentation are shown as mean ± standard deviation. The effect of prebiotic addition was evaluated through a 6 × 2 × 3 triple factorial arrangement for the titratable acidity and *Lactobacillus* spp. viability dependent variables, and in the factorial 6 × 3 for the ST viability dependent variable. Autochthonous culture, starter culture and time effects were evaluated in the factorial 6 × 2 × 4 for the titratable acidity and *Lactobacillus* spp. viability dependent variables, and in the factorial 6 × 4 for the ST viability dependent variable.

Table 2
Titratable acidity values (mean ± standard deviation) obtained for the 36 trials during 0, 6, 24 and 48 h of fermentation.

Trial		Titratable acidity (g 100/g)			
Code	Components	Time (h)			
		0	6	24	48
T1	EM1107	0.150±0.003$^{Ac\#}$	0.166±0.005$^{Bc\#}$	0.267±0.005$^{Cb\#}$	0.520±0.013$^{Da\#}$
T2	EM1107+IN	0.165±0.005$^{Ac\#}$	0.160±0.010$^{Bc\#}$	0.337±0.016$^{Cb\#}$	0.480±0.036$^{Da\#}$
T3	EM1107+FOS	0.145±0.004$^{Ac\#}$	0.170±0.014$^{Bc\#}$	0.302 ±0.050$^{Cb\#}$	0.408±0.006$^{Da\#}$
T4	EM1107+ST	0.178±0.003$^{Ac\#}$	0.631±0.032$^{Ab\$}$	0.730±0.003$^{ABa\$}$	0.880±0.043$^{Ba\$}$
T5	EM1107+ST+IN	0.160±0.002$^{Ac\#}$	0.702±0.020$^{Ab\$}$	0.904±0.093$^{ABa\$}$	0.736±0.010$^{Ba\$}$
T6	EM1107+ST+FOS	0.188±0.010$^{Ac\#}$	0.694±0.006$^{Ab\$}$	0.790±0.080$^{ABa\$}$	0.844±0.001$^{Ba\$}$
T7	CNPC001	0.169±0.006$^{Ac\#}$	0.164±0.001$^{Bc\#}$	0.608±0.014$^{Ab\#}$	0.820±0.015$^{Ba\#}$
T8	CNPC001+IN	0.166±0.005$^{Ac\#}$	0.167±0.005$^{Bc\#}$	0.642±0.026$^{Ab\#}$	0.691±0.020$^{Ba\#}$
T9	CNPC001+FOS	0.162±0.003$^{Ac\#}$	0.172±0.002$^{Bc\#}$	0.579±0.038$^{Ab\#}$	0.686±0.044$^{Ba\#}$
T10	CNPC001+ST	0.172±0.004$^{Ac\#}$	0.652±0.011$^{ABb\$}$	0.751±0.040$^{ABa\$}$	0.767±0.034$^{Ba\$}$
T11	CNPC001+ST+IN	0.158±0.011$^{Ac\#}$	0.597±0.011$^{ABb\$}$	0.809±0.015$^{ABa\$}$	0.763±0.010$^{Ba\$}$
T12	CNPC001+ST+FOS	0.160±0.005$^{Ac\#}$	0.620±0.006$^{ABb\$}$	0.620±0.070$^{ABa\$}$	0.759±0.028$^{Ba\$}$
T13	CNPC002	0.164±0.006$^{Ac\#}$	0.193±0.017$^{Ab\#}$	0.212±0.003$^{Cb\#}$	0.542±0.004$^{Da\#}$
T14	CNPC002+IN	0.172±0.004$^{Ac\#}$	0.261±0.003$^{Ab\#}$	0.371±0.021$^{Cb\#}$	0.374±0.021$^{Da\#}$
T15	CNPC002+FOS	0.170±0.006$^{Ac\#}$	0.360±0.005$^{Ab\#}$	0.396±0.012$^{Cb\#}$	0.303±0.005$^{Da\#}$
T16	CNPC002+ST	0.159±0.003$^{Ac\#}$	0.648±0.006$^{Bb\$}$	0.692±0.091$^{Ca\$}$	0.672±0.024$^{Cb\$}$
T17	CNPC002+ST+IN	0.169±0.003$^{Ac\#}$	0.585±0.011$^{Bb\$}$	0.650±0.010$^{Ca\$}$	0.428±0.017$^{Cb\$}$
T18	CNPC002+ST+FOS	0.170±0.005$^{Ac\#}$	0.517±0.013$^{Bb\$}$	0.762±0.013$^{Ca\$}$	0.660±0.015$^{Cb\$}$
T19	CNPC003	0.138±0.001$^{Ad\#}$	0.148±0.005$^{ABc\#}$	0.382±0.001$^{Bb\#}$	1.201±0.003$^{Aa\#}$
T20	CNPC003+IN	0.131±0.009$^{Ad\#}$	0.244±0.008$^{ABc\#}$	0.505±0.010$^{Bb\#}$	1.174±0.085$^{Aa\#}$
T21	CNPC003+FOS	0.138±0.001$^{Ad\#}$	0.263±0.001$^{ABc\#}$	0.428±0.012$^{Bb\#}$	1.123±0.016$^{Aa\#}$
T22	CNPC003+ST	0.156±0.001$^{Ad\#}$	0.593±0.008$^{Bc\$}$	0.746±0.023$^{BCb\$}$	0.950±0.015$^{Aa\$}$
T23	CNPC003+ST+IN	0.153±0.008$^{Ad\#}$	0.570±0.004$^{Bc\$}$	0.737±0.042$^{BCb\$}$	0.904±0.020$^{Aa\$}$
T24	CNPC003+ST+FOS	0.150 ±0.004$^{Ad\#}$	0.602±0.006$^{Bc\$}$	0.750±0.004$^{BCb\$}$	1.058±0.046$^{Aa\$}$
T25	CNPC004	0.160±0.006$^{Ac\#}$	0.153±0.006$^{Bc\#}$	0.429±0.015$^{Cb\#}$	0.587±0.010$^{Ca\#}$
T26	CNPC004+IN	0.155±0.012$^{Ac\#}$	0.198±0.009$^{Bc\#}$	0.241±0.008$^{Cb\#}$	0.549±0.001$^{Ca\#}$
T27	CNPC004+FOS	0.152±0.004$^{Ac\#}$	0.231±0.003$^{Bc\#}$	0.390±0.005$^{Cb\#}$	0.505±0.013$^{Ca\#}$
T28	CNPC004+ST	0.172±0.004$^{Ad\#}$	0.695±0.022$^{ABc\$}$	0.815±0.084$^{Ab\$}$	0.951±0.032$^{Aa\$}$
T29	CNPC004+ST+IN	0.151±0.010$^{Ad\#}$	0.623±0.013$^{ABc\$}$	0.813±0.005$^{Ab\$}$	0.894±0.016$^{Aa\$}$
T30	CNPC004+ST+FOS	0.165±0.002$^{Ad\#}$	0.591±0.018$^{ABc\$}$	0.848±0.003$^{Ab\$}$	0.894±0.007$^{Aa\$}$
T31	CNPC007	0.152±0.006$^{Ad\#}$	0.264±0.007$^{ABc\#}$	0.744±0.009$^{Bb\#}$	1.268±0.025$^{Aa\#}$
T32	CNPC007+IN	0.131±0.007$^{Ad\#}$	0.288±0.005$^{ABc\#}$	0.970±0.026$^{Bb\#}$	1.376±0.018$^{Aa\#}$
T33	CNPC007+FOS	0.138±0.002$^{Ad\#}$	0.321±0.008$^{ABc\#}$	0.739±0.001$^{Bb\#}$	0.954±0.028$^{Aa\#}$
T34	CNPC007+ST	0.143±0.005$^{Ad\#}$	0.484±0.012$^{Bc\$}$	0.925±0.021$^{BCb\$}$	0.892±0.013$^{Aa\$}$
T35	CNPC007+ST+IN	0.157±0.001$^{Ad\#}$	0.691±0.015$^{Bc\$}$	0.776±0.005$^{BCb\$}$	0.883±0.016$^{Aa\$}$
T36	CNPC007+ST+FOS	0.156±0.005$^{Ad\#}$	0.658±0.011$^{Bc\$}$	0.705±0.011$^{BCb\$}$	0.912±0.016$^{Aa\$}$

^{A,B,C,D} Trials sharing a same uppercase letter in a column do not differ significantly at the same time ($p>0.05$).
^{a,b,c,d} A same lowercase letter in a row denote that a same trial do not differ significantly over time ($p>0.05$).
$^{\$,\#}$ A same symbol in a column denote that trials with and without ST do not differ significantly at the same time ($p> 0.05$).
IN: inulin; FOS: oligofructose; ST: *Streptococcus thermophilus*.

Statistical analysis was carried out through analysis of variance (ANOVA) with the F test of Snedecor and, when necessary, the Tukey test was applied to verify the contrasts, taking on $p < 0.05$. All analyses were conducted using R software (R Core Team, 2018), with R studio integrated development environment (IDE) for R (Rstudio Team, 2016), and with the use of GExpDes (2019) and Multicomp (Hothorn, Bretz, & Wesfall, 2008) packages.

For the survival of *Lactobacillus* spp. through the *in vitro* assay, the exact binomial test was carried out (Conover, 2001) with the null hypothesis (H_0) that the proportion of survival above 6 log CFU/g throughout the assay should be at least 25%.

3. Results and discussion

3.1. Fermentability of goat milk

Tables 2–4 show the results of the titratable acidity, viability of *Lactobacillus* spp. and *S. thermophilus* (ST) population, respectively, obtained at 0, 6, 24 and 48 h of fermentation for the 36 trials studied (six different autochthonous probiotic strains, with the presence or absence of *S. thermophilus*, inulin or oligofructose).

No significant difference ($p > 0.05$) was detected between the trials with prebiotics inulin (IN) or oligofructose (FOS) added, regardless of the presence or absence of the starter culture ST. Similar to that verified in our study, no significant differences were observed in acidification when oligofructose (Delgado-Fernandes, Corzo, Olano, Hernández-Hernández, & Moreno, 2019) or raffinose (Marinaki et al., 2016) was added to yogurt.

The trails with or without adding ST did not differ significantly ($p > 0.05$) from each other regarding the acidity (Table 2) at the initial fermentation time (0 h). Moreover, among the products with the presence of the starter culture, no significant difference on the evolution of titratable acidity values was observed ($p > 0.05$).

However, the trials without ST did not reach the acidity equal to or greater than 0.6 g lactic acid/100g after 6 h of fermentation in any of the tested combinations. This acidity was only reached after 24 h of fermentation in trials T7 (CNPC001), T8 (CNPC001 + IN), T31 (CNPC007), T32 (CNPC007 + IN), T33 (CNPC007 + FOS). Although four *L. plantarum* strains (CNPC001, CNPC002, CNPC003 and CNPC004) have been used in the trials, metabolic behavior may be different among strains of the same species (Guidone et al., 2014). Thus, after 24h, CNPC001 and CNPC007 strains showed a better fermentative performance when added as monocultures to milk. The other trials only reached this value after 48 h. Nonetheless, according to Abesinghe et al.

Table 3
Lactobacillus spp. population (mean ± standard deviation) obtained for the 36 trials during 0, 6, 24 and 48 h of fermentation.

Trial		*Lactobacillus* spp. (log CFU/g)			
Code.	Components	Time (h)			
		0	6	24	48
T1	EM1107	$8.11\pm0.10^{ABab\#}$	$8.45\pm0.04^{Aa\#}$	$8.04\pm0.25^{Ab\#}$	$8.79\pm0.20^{Dc\#}$
T2	EM1107+IN	$8.17\pm0.04^{ABab\#}$	$8.40\pm0.15^{Aa\#}$	$7.90\pm0.03^{Ab\#}$	$8.41\pm0.10^{Dc\#}$
T3	EM1107+FOS	$8.01\pm0.60^{ABab\#}$	$8.60\pm0.35^{Aa\#}$	$7.67\pm0.06^{Ab\#}$	$8.32\pm0.04^{Dc\#}$
T4	EM1107+ST	$8.30\pm0.07^{Ab\#}$	$8.16\pm0.20^{ABb\$}$	$10.25\pm0.25^{Aa\$}$	$6.12\pm0.20^{Bc\#}$
T5	EM1107+ST+IN	$8.25\pm0.16^{Ab\#}$	$8.17\pm0.25^{ABb\$}$	$10.35\pm0.40^{Aa\$}$	$6.40\pm0.09^{Bc\#}$
T6	EM1107+ST+FOS	$8.30\pm0.10^{Ab\#}$	$8.03\pm0.22^{ABb\$}$	$9.85\pm0.75^{Aa\$}$	$7.33\pm0.03^{Bc\#}$
T7	CNPC001	$8.02\pm0.13^{ABa\#}$	$8.11\pm0.07^{ABa\#}$	$8.06\pm0.10^{Aa\#}$	$8.01\pm0.27^{BCa\#}$
T8	CNPC001+IN	$8.01\pm0.08^{ABa\#}$	$8.00\pm0.10^{ABa\#}$	$8.18\pm0.09^{Aa\#}$	$8.17\pm0.18^{BCa\#}$
T9	CNPC001+FOS	$7.80\pm0.25^{ABa\#}$	$7.98\pm0.13^{ABa\#}$	$8.41\pm0.11^{Aa\#}$	$8.05\pm0.07^{BCa\#}$
T10	CNPC001+ST	$8.10\pm0.23^{Aa\#}$	$8.42\pm0.15^{Aa\$}$	$7.85\pm0.36^{Bb\$}$	$5.90\pm0.12^{Cc\$}$
T11	CNPC001+ST+IN	$8.15\pm0.21^{Aa\#}$	$8.45\pm0.50^{Aa\$}$	$5.92\pm0.06^{Bb\$}$	$6.01\pm0.21^{Cc\$}$
T12	CNPC001+ST+FOS	$8.46\pm0.08^{Aa\#}$	$8.50\pm0.17^{Aa\$}$	$6.29\pm0.03^{Bb\$}$	$5.63\pm0.14^{Cc\$}$
T13	CNPC002	$8.68\pm0.51^{Aab\#}$	$8.13\pm0.22^{ABb\#}$	$7.01\pm0.29^{Bc\#}$	$8.80\pm0.72^{ABa\#}$
T14	CNPC002+IN	$8.71\pm0.68^{Aab\#}$	$8.04\pm0.09^{ABb\#}$	$6.74\pm0.13^{Bc\#}$	$8.65\pm0.41^{ABa\#}$
T15	CNPC002+FOS	$8.71\pm0.66^{Aab\#}$	$7.83\pm0.08^{ABb\#}$	$6.70\pm0.47^{Bc\#}$	$8.92\pm0.62^{ABa\#}$
T16	CNPC002+ST	$8.13\pm0.22^{ABa\$}$	$7.87\pm0.23^{Ba\#}$	$5.55\pm0.13^{Db\$}$	$7.80\pm0.10^{Aa\$}$
T17	CNPC002+ST+IN	$8.04\pm0.09^{ABa\$}$	$7.64\pm0.13^{Ba\#}$	$5.13\pm0.16^{Db\$}$	$8.03\pm0.11^{Aa\$}$
T18	CNPC002+ST+FOS	$7.83\pm0.08^{ABa\$}$	$7.62\pm0.11^{Ba\#}$	$5.40\pm0.10^{Db\$}$	$7.00\pm0.04^{Aa\$}$
T19	CNPC003	$8.34\pm0.52^{ABb\#}$	$7.82\pm0.09^{Bb\#}$	$7.88\pm0.04^{Ab\#}$	$9.01\pm0.07^{Aa\#}$
T20	CNPC003+IN	$8.41\pm0.60^{ABb\#}$	$7.70\pm0.08^{Bb\#}$	$8.07\pm0.07^{Ab\#}$	$8.91\pm0.11^{Aa\#}$
T21	CNPC003+FOS	$8.47\pm0.56^{ABb\#}$	$7.80\pm0.22^{Bb\#}$	$7.86\pm0.13^{Ab\#}$	$8.59\pm0.06^{Aa\#}$
T22	CNPC003+ST	$7.83\pm0.03^{Aba\#}$	$7.76\pm0.08^{Ba\#}$	$7.85\pm0.37^{Bb\$}$	$4.90\pm0.12^{Dc\$}$
T23	CNPC003+ST+IN	$8.03\pm0.12^{ABa\#}$	$7.60\pm0.16^{Ba\#}$	$5.92\pm0.06^{Bb\$}$	$5.36\pm0.16^{Dc\$}$
T24	CNPC003+ST+FOS	$8.05\pm0.12^{ABa\#}$	$7.67\pm0.14^{Ba\#}$	$6.29\pm0.03^{Bb\$}$	$4.73\pm0.04^{Dc\$}$
T25	CNPC004	$7.92\pm0.30^{Ba\#}$	$7.61\pm0.10^{Ba\#}$	$7.51\pm0.11^{Aa\#}$	$7.98\pm0.34^{Ca\#}$
T26	CNPC004+IN	$8.09\pm0.09^{Ba\#}$	$7.84\pm0.08^{Ba\#}$	$7.64\pm0.19^{Aa\#}$	$8.05\pm0.18^{Ca\#}$
T27	CNPC004+FOS	$7.82\pm0.12^{Ba\#}$	$7.86\pm0.13^{Ba\#}$	$8.03\pm0.19^{Aa\#}$	$7.90\pm0.16^{Ca\#}$
T28	CNPC004+ST	$7.46\pm0.33^{Ba\#}$	$7.50\pm0.35^{Ba\#}$	$6.56\pm0.08^{Cc\$}$	$7.26\pm0.05^{Bb\$}$
T29	CNPC004+ST+IN	$7.87\pm0.22^{Ba\#}$	$7.82\pm0.18^{Ba\#}$	$4.97\pm0.13^{Cc\$}$	$6.88\pm0.06^{Bb\$}$
T30	CNPC004+ST+FOS	$7.60\pm0.08^{Ba\#}$	$7.82\pm0.12^{Ba\#}$	$6.85\pm0.23^{Cc\$}$	$5.78\pm0.12^{Bb\$}$
T31	CNPC007	$7.52\pm0.24^{ABb\#}$	$8.30\pm0.24^{Bb\#}$	$8.35\pm0.36^{Ab\#}$	$8.86\pm0.13^{Aa\#}$
T32	CNPC007+IN	$7.08\pm0.13^{ABb\#}$	$8.30\pm0.24^{Bb\#}$	$8.27\pm0.47^{Ab\#}$	$8.80\pm0.12^{Aa\#}$
T33	CNPC007+FOS	$7.20\pm0.20^{ABb\#}$	$8.32\pm0.20^{Bb\#}$	$8.38\pm0.40^{Ab\#}$	$7.70\pm1.70^{Aa\#}$
T34	CNPC007+ST	$7.95\pm0.17^{ABa\#}$	$7.88\pm0.10^{Ba\#}$	$6.92\pm0.17^{Bb\$}$	$5.15\pm0.27^{Dc\$}$
T35	CNPC007+ST+IN	$8.24\pm0.25^{ABa\#}$	$7.90\pm0.07^{Ba\#}$	$6.80\pm0.04^{Bb\$}$	$4.98\pm0.62^{Dc\$}$
T36	CNPC007+ST+FOS	$8.07\pm0.15^{ABa\#}$	$7.92\pm0.04^{Ba\#}$	$6.94\pm0.17^{Bb\$}$	$4.53\pm0.50^{Dc\$}$

^{A,B,C,D} Trials sharing a same uppercase letter in a column do not differ significantly at the same time ($p>0.05$).
^{a,b,c} A same lowercase letter in a row denote that a same trial do not differ significantly over time ($p>0.05$).
$^{\$,\#}$ A same symbol in a column denote that trials with and without ST do not differ significantly at the same time ($p>0.05$).
IN: inulin; FOS: oligofructose; ST: *Streptococcus thermophilus*.

Table 4
– Population of *Streptococcus thermophilus* (mean ± standard deviation) in coculture trials after 0, 6, 24 and 48 h of fermentation.

Trial		*S. thermophilus* (log CFU/g)			
Code.	Components	Time (hours)			
		0	6	24	48
T4	EM1107 + ST	8.33 ± 0.20^{Ab}	8.40 ± 0.14^{ABCb}	9.50 ± 1.17^{Aa}	5.11 ± 0.20^{Abc}
T5	EM1107 + ST + IN	8.23 ± 0.15^{Ab}	8.37 ± 0.17^{ABCb}	9.70 ± 0.40^{Aa}	6.52 ± 0.12^{Abc}
T6	EM1107 + ST + FOS	8.33 ± 0.10^{Ab}	8.18 ± 0.10^{ABCb}	9.64 ± 1.10^{Aa}	7.26 ± 0.14^{Abc}
T10	CNPC001 + ST	8.22 ± 0.39^{Aa}	8.63 ± 0.30^{Aa}	8.05 ± 0.04^{BCb}	5.99 ± 0.27^{Bc}
T11	CNPC001 + ST + IN	8.25 ± 0.17^{Aa}	8.72 ± 0.48^{Aa}	6.56 ± 0.15^{BCb}	5.69 ± 0.08^{Bc}
T12	CNPC001 + ST + FOS	8.33 ± 0.08^{Aa}	8.81 ± 0.22^{Aa}	6.96 ± 0.15^{BCb}	5.60 ± 0.11^{Bc}
T16	CNPC002 + ST	7.83 ± 0.20^{Aa}	8.25 ± 0.09^{BCa}	7.16 ± 0.13^{Bb}	6.36 ± 0.33^{Ab}
T17	CNPC002 + ST + IN	7.95 ± 0.23^{Aa}	7.98 ± 0.10^{BCa}	6.82 ± 0.04^{Bb}	7.90 ± 0.14^{Ab}
T18	CNPC002 + ST + FOS	7.84 ± 0.30^{Aa}	7.64 ± 0.23^{BCa}	6.61 ± 0.18^{Bb}	6.64 ± 0.67^{Ab}
T22	CNPC003 + ST	8.05 ± 0.17^{Aa}	7.75 ± 0.10^{Ca}	8.05 ± 0.03^{BCb}	4.99 ± 0.27^{Cc}
T23	CNPC003 + ST + IN	7.90 ± 0.32^{Aa}	7.77 ± 0.15^{Ca}	6.56 ± 0.15^{BCb}	5.60 ± 0.26^{Cc}
T24	CNPC003 + ST + FOS	8.02 ± 0.06^{Aa}	7.93 ± 0.10^{Ca}	6.97 ± 0.15^{BCb}	4.60 ± 0.11^{Cc}
T28	CNPC004 + ST	7.60 ± 0.17^{Ab}	8.62 ± 0.07^{ABa}	8.11 ± 0.08^{Bb}	7.14 ± 0.09^{Ac}
T29	CNPC004 + ST + IN	7.65 ± 0.26^{Ab}	8.57 ± 0.07^{ABa}	7.87 ± 0.38^{Bb}	6.74 ± 0.18^{Ac}
T30	CNPC004 + ST + FOS	7.70 ± 0.19^{Ab}	8.48 ± 0.08^{ABa}	7.30 ± 0.43^{Bb}	5.74 ± 0.13^{Ac}
T34	CNPC007 + ST	7.60 ± 0.17^{Aa}	8.62 ± 0.07^{Ca}	8.11 ± 0.08^{BCb}	7.14 ± 0.09^{Cc}
T35	CNPC007 + ST + IN	7.65 ± 0.26^{Aa}	8.57 ± 0.07^{Ca}	7.87 ± 0.38^{BCb}	6.74 ± 0.18^{Cc}
T36	CNPC007 + ST + FOS	7.70 ± 0.19^{Aa}	8.48 ± 0.08^{Ca}	7.30 ± 0.43^{BCb}	5.74 ± 0.13^{Cc}

^{A,B,C} Trials sharing a same uppercase letter in a column do not differ significantly at the same time ($p > 0.05$).
^{a,b,c} A same lowercase letter in a row denote that a same trial do not differ significantly over time ($p > 0.05$).
ST: *Streptococcus thermophilus*; IN: inulin; FOS: oligofructose.

(2019), a shorter fermentation time helps to decrease the time and cost of production, so a 24-h or higher fermentation process is not interesting for the dairy industry. Milk acidification is also responsible for the texture and aroma of fermented milk due to the formation of organic acids such as lactic acid during fermentation (Costa, Frasão, Lima, Rodrigue & Conte Júnior, 2016). According to Dimitrellou, Salamoura, et al. (2019), goat milk achieves higher acidity content in relation to cow milk when fermented. In this sense, studies on the fermentability of goat milk, as the present one, deserve attention since this milk does not follow the pattern of cow milk fermentation.

On the other hand, in the trials with ST milk acidification was faster compared to those with only probiotic cultures, and more than half of the trials containing starter obtained acidity values greater than 0.6 g/100g lactic acid in 6 h. The highest acidity values at that time were observed for trials with the EM1107-ST co-culture, which differed significantly from trials with ST in co-culture with CNPC002, CNPC003 and CNPC007, with the lowest average acidity ($p < 0.05$).

For *Lactobacillus* spp. populations (Table 3), there were significant differences between trials before fermentation and in other sampling periods ($p < 0.05$). Furthermore, there was a significantly lower lactobacilli population in ST trials when compared to those without starter culture ($p < 0.05$). Other studies report that the viability of probiotic microorganisms may be affected by products of the fermentation metabolism of the starter, such as lactic acid (Buriti et al., 2007; Vinderola, Mocchiutti, & Reinheimer, 2002). Vinderola et al. (2002) reported four types of behavior between species of adjuvant potentially probiotics and starter cultures: stimulation between them to reduce fermentation time; competition between them delaying the fermentation process; competition between them with complete inhibition of the fermentation process; and the absence of interaction between them with maintenance of the fermentation process, originally performed by the starter culture. According to the authors, *S. thermophilus* generally stimulates the growth of lactic cultures during fermentation, and the symbiotic relationship between *S. thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, used in the manufacture of yogurt, is well investigated. In that study, however, Vinderola et al. (2002) also verified that not all lactobacilli species have this characteristic. Particularly concerning the inhibitory effect, those authors observed in the same study that the probiotic bacteria tested (strains of *Bifidobacterium*, *Lactobacillus acidophilus* and *Lactobacillus casei*) were more inhibitory toward starter culture than *vice versa* since they were able to inhibit some extent the growth of *S. thermophilus* and *Lactococcus lactis*. In another study, Tian, Shen, Yu, and Chen (2017) found that *S. thermophilus* and *L. bulgaricus* showed lower growth capacity during fermentation and viability throughout storage of cow milk yogurt when cocultured with the probiotics *L. casei* LC2W, *L. plantarum* ST-III, or *L. rhamnosus* GG than in the absence of these probiotics. The same authors verified that the *L. plantarum* ST-III and *L. rhamnosus* GG strains had a lower growth during fermentation and that *L. casei* LC2W, besides decreasing about 1 log cycle during 28 days of storage, was the one that most reduced the viability of *L. bulgaricus* and *S. thermophilus* within this period. The same effect was verified by Dimitrellou, Kandylis, and Kourkoutas (2019) in which free and immobilized *L. casei* ATCC 393 affected the viability of *S. thermophilus* and *L. bulgaricus* in cow milk also during 28 days of storage.

In the present study, at 6 h of fermentation for monoculture trials, the highest viability was found for EM1107, which differed significantly from CNPC003, CNPC007 and CNPC004 ($p < 0.05$). After 24 h the monocultures did not differ from each other, except for CNPC002, which presented a smaller lactobacilli population ($p < 0.05$). In 48 h, the trials containing the CNPC003 and CNPC007 strains differed significantly from the others ($p < 0.05$), presenting a higher viability.

At 6 h of fermentation for the trials with ST, the lactobacilli strain that presented the highest viability was CNPC001, differing significantly from the other trials ($p < 0.05$), except for the one with EM1107 that did not differ from the others. On the other hand, trials with EM1107 after 24 h and with CNPC002 after 48 h had the highest lactobacilli population, which differed significantly from the others in each time ($p < 0.05$).

When comparing the lactobacilli population of each strain over time, a significant difference between each trial was also observed ($p < 0.05$). For the trials added of *L. plantarum* CNPC001 and CNPC004 strains without ST starter culture no significant difference over time was detected; however for the respective trials with ST added there was a significant difference ($p < 0.05$) between 24 and 48 h, and a decrease in the lactobacilli population was observed in relation to the times of 0 and 6 h, which did not differ significantly between them ($p > 0.05$).

The trials with the CNPC002 culture with or without ST differed significantly ($p < 0.05$) only at 24 h, showing a smaller population in relation to the other times.

For the trials with only *L. plantarum* CNPC003 or *L. mucosae*

CNPC007 cultures at 48 h, lactobacilli populations differed significantly ($p < 0.05$) from the other sampling times; however, this population was higher in relation to 0 h for both strains. In contrast, for trials with the same autochthonous strains in co-culture with ST there was a smaller lactobacilli population at 24 and 48 h, differing significantly between each other and between 0 and 6 h ($p < 0.05$).

The trials with *L. rhamnosus* EM1107 strain in monoculture differed significantly at 48 h of fermentation ($p < 0.05$), presenting a smaller lactobacilli population in relation to the other times. Similarly, for the trials with CNPC003 and CNPC007 cultures with ST added, the milks containing EM1107 plus ST also presented a smaller lactobacilli population in 48 h, differing significantly from the other times ($p < 0,05$).

The initial ST populations did not differ between all co-culture trials (Table 4). After 6 h the trials with a higher ST population were those with *L. plantarum* CNPC001 added, which differed significantly from the trials with CNPC002 and CNPC007 ($p < 0.05$). In this sampling period all trials showed a ST population greater than 7 log CFU; however, a decrease in this population was observed in all trials, except for the fermented milk with *L. rhamnosus* EM1107 that achieved a larger ST population, differing significantly from the other lactobacilli strains ($p < 0.05$). According to Oliveira, Perego, Oliveira, and Converti (2012), a hypothesis to explain this result is that, while *S. thermophilus* produces small amounts of formic acid and CO_2, *L. rhamnosus* is able to release peptides through a serine protease of the subtilisin family (known as PrtR) that stimulates the growth of *S. thermophilus*. In their study with fermented skimmed milk, the authors reported that they obtained biomass values of 15.5% and 44% lower for pure cultures of *S. thermophilus* and *L. rhamnosus*, respectively, when compared with the biomass values of the same bacteria in co-cultures.

Over time, there was no significant difference in the ST population of all trials between 0 and 6 h of fermentation ($p > 0.05$), except for that added to the CNPC004 culture. In the other trials there was only significant difference after 24 h of fermentation ($p < 0.05$).

3.2. Resistance to simulated gastrointestinal conditions

Based on the analysis of the acidity, pH and lactobacilli population, the trials chosen to be submitted to the *in vitro* simulation of gastrointestinal conditions were T5 (EM1107 + ST + IN), T23 (CNPC003 + ST + IN) and T35 (CNPC007 + ST + IN), with ST added, IN and the potentially probiotic cultures EM1107, CNPC003 and CNPC007, respectively. Although the presence of inulin did not significantly interfere in these parameters, in those trials the resulting fermented milks showed to be visually more consistent and firmer. Images of T4 (EM1107 + ST) and of T5 (EM1107 + ST + IN) trials to exemplify the visual aspect of goat milk fermented without and with inulin are included in Supplementary Material 1. Moreover, based on other studies, inulin is also described as being able to mask off *flavors* (Buriti, Cardarelli, & Saad, 2008; Silveira et al., 2015), which could be useful in the development of a product with goat milk. Other trials also obtained a lactic acid content of 0.6 g/100 g in 6 h; however, they exhibited an inadequate visual texture, with clots easily dismembered, such as the trials T11 (CNPC001 + ST + IN), T17 (CNPC002 + ST + IN), and T29 (CNPC004 + ST + IN).

Table 5 shows the results of the *in vitro* assay obtained for the lactobacilli population of the three selected trials of fermented milk (T5, T23 and T35) at 21 days of storage and of the same cultures in MRS broth.

Before the assay, there was no significant difference in lactobacilli populations between trials in fermented milk and in MRS broth trials ($p > 0.05$). Throughout the assay, however, it was detected that the growth conditions did not allow for the recovery of CNPC003 culture in the plates above 10^3 CFU/g of the simulated gastrointestinal fluid containing fermented milk. Differently from the present study, Ribeiro et al. (2020) verified that *L. plantarum* CNPC003 was able to survive with population above 10^6 CFU/mL in all phases of *in vitro* gastrointestinal simulation (pH ranging from 2.3 to 2.6 in gastric, phase 5.4–5.7 in enteric phase I and 6.8–7.2 in enteric phase II) in an unfermented beverage of mixed fruit pulp juice (banana, juçara and strawberry) after 30 days of storage, although with a significant decrease after 60 and 90 days of storage, with a survival below 10^4 CFU/mL and 10^2 CFU/mL, respectively. Although those authors studied a different product, the gastric conditions used by them were less harmful than that employed in the present study (pH 1.5–2.0), suggesting that *L. plantarum* CNPC003 could tolerate higher pH values in the gastric phase, however, without resistance to more acidic and aggressive environments.

One of the main characteristics for a strain to be considered probiotic is its ability to survive through the gastrointestinal tract and reach sufficient amounts in the intestine to exert its beneficial effect (Liserre, Ré, & Franco, 2007; dos Santos et al., 2015; de Moraes et al., 2017). Resistance to gastric passage is a rare property among lactic bacteria (Cotter, Hill, & Ross, 2005) and therefore it is a usual practice to increase it by using prebiotics or whey proteins to protect them (Buriti et al., 2010) or by encapsulation (Dimitrellou et al., 2016; Feng et al., 2020; Gu et al., 2019). The bile salts secreted in the small intestine also challenge the bacterial survival in the gastrointestinal tract (Ouwehand, Derrien, De Bos, Tiihonen, & Rautonen, 2005). In the present study, the highest reduction in viable cell counts was observed after the gastric phase, and a recovery of lactobacilli counts was registered after the last enteric phase.

For the *L. rhamnosus* EM1107, after the gastric phase a decrease between 2.03 and 2.68 log cycles was observed in fermented milk, and between 2.89 and 2.24 log cycles in MRS broth. In the same phase

Table 5
– Survival of *Lactobacillus* spp. (minimum and maximum values, log CFU/g) to gastrointestinal conditions simulated *in vitro* in selected fermented milks and MRS broth.

Phases of simulated digestion	Item	Trials					
		EM1107 + ST + IN (milk)	EM1107 (MRS broth)	CNPC003 + ST + IN (milk)	CNPC003 (MRS broth)	CNPC007 + ST + IN (milk)	CNPC007 (MRS broth)
Initial	Population (log CFU/g)	7.30–7.83	8.21–8.52	5.85–6.03	8.36 ± 8.61	7.38–7.50	9.02–9.34
	% of samples above 6 log CFU/g	100 (4/4)^{A}	100 (4/4)^{A}	25 (1/4)^{A}	100 (4/4)^{A}	100 (4/4)^{A}	100 (4/4)^{A}
GP 30 min	Population (log CFU/g)	5.22–7.52	4.72–6.13	<3.00	5.36–8.13	<3.00	7. 10–8.10
GP 2 h		4.62–5.80	5.32–6.28	<3.00	<3.00	5.24–7.50	5.92–7.22
EPI		6.12–6.50	5.61–6.10	<3.00	<3.00	<3.00	<3.00
EPII (final)		6.50–7.24	5.95–6.25	<3.00	4.14–5.34	6.50–7.60	6.80–7.80
Final	% of samples above 6 log CFU/g	54 (13/24)^{A}	38 (9/24)^{A}	0 (0/24)^{B}	17 (4/24)^{B}	25 (6/24)^{A}	60 (14/24)^{A}

^{A,B} different superscript uppercase letters in a row denote that trials differ significantly in the exact binomial test ($p < 0.05$) for the viability ratio above 6 log CFU/g, the lower value obtained before the start of the simulation, considering the null hypothesis (H_0) that such proportion over the simulation to gastrointestinal conditions should be at least 25%. ST = *Streptococcus thermophilus*, IN = inulin, GP = gastric phase, EPI = enteric phase I, EPII = enteric phase II.

L. mucosae CNPC007 populations showed a decrease up to 2.14 log cycles in fermented milk and from 2.14 to 3.11 log cycles in MRS broth. After the end of the enteric phase II (pH 6.2–6.7) populations were, in general, higher than that observed in the previous phases from the second hour of assay.

This decrease between gastric and enteric phases occurs due to temporary stress caused by pH variation in these phases. The pH of the gastric phase causes injury in the cells of microorganisms, which can be repaired in the enteric phase (Ribeiro et al., 2020). This fact was also observed by Buriti et al. (2010) and Liserre, Re, and Franco (2007). In general, tolerance to the acidic environment by lactic bacteria depends on the enzymatic profile and composition of the cytoplasmic membrane, which depends on the type of bacteria and extrinsic conditions, including the growth medium and incubation conditions (Madureira, Amorim, Gomes, Pintado, & Malcata, 2011).

Moreover, in the enteric phase II, populations of both *L. rhamnosus* EM1107 and *L. mucosae* CNPC007 reduced less in goat milk when compared to the MRS broth. It is also important to highlight that, in the study conducted by Buriti et al. (2010) with synbiotic guava mousses, all formulations in which there was recovery of probiotic (*Lactobacillus acidophilus* LA-5) after enteric phase II presented inulin in their composition (from 1.33 to 4 g/100 g). Inulin probably also contributed to the protection of lactobacilli in fermented milks during the *in vitro* assay in the present study.

Based on these results, fermented milk matrix exerted a protective effect on probiotic cultures EM1107 and CNPC007 throughout the *in vitro* assay, since survival was higher than that observed in MRS broth. It is possible that inulin also offered protection to the survival of probiotics during the *in vitro* gastrointestinal simulation, considering that MRS does not contain this ingredient. Regarding *L. plantarum* CNPC003, further studies with this strain are necessary to elucidate its behavior in food matrices submitted to stresses under different pHs, particularly in the *in vitro* simulation of the gastric phase.

4. Conclusion

The prebiotics inulin and oligofructose did not influence the fermentation time of the autochthonous strains used, either solely or in co-culture with *S. thermophilus*; however, inulin changed the texture and improved the visual aspect of the products at the end of fermentation. The monocultures of the autochthonous strains showed low acidification potential in goat milk and, therefore, the simultaneous use of *S. thermophilus* was necessary to reach, in a shorter time interval, the acidity required for fermented milks, so that the process could be industrially efficient. Although the greatest viability losses of the three autochthonous strains submitted to the *in vitro* simulated gastrointestinal conditions occurred in the gastric phase, fermented goat milk with inulin was more protective to the cultures than the MRS broth, particularly for *L. rhamnosus* EM1107 and *L. mucosae* CNPC007. Thus, the combination of the *Streptococcus thermophilus* starter culture with one of these two autochthonous strains in the presence of inulin is very promising either to improve the fermentative performance in goat milk or to allow higher survival capacity of these lactobacilli to the simulated gastrointestinal conditions, thus way increasing the functional potential of fermented goat milk.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

Not applicable.

CRediT authorship contribution statement

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Declaration of competing interest

All the authors declare that he/she has no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2020.109905.

References

Abesinghe, A. M. N. L., Islam, N., Vidanarachchi, J. K., Prakash, S., Silva, K. F. S. T., & Karim, M. A. (2019). Effects of ultrasound on the fermentation profile of fermented milk products incorporated with lactic acid bacteria. *International Dairy Journal, 90*, 1–14.

Anvisa. (2016). *Alimentos com alegações de propriedades funcionais e ou de saúde*. Brasília: Agência Nacional de Vigilância Sanitária. Download from http://portal.anvisa.gov.br/alimentos/alegacoes. (Accessed 10 June 2018).

Buriti, F. C. A., Cardarelli, H. R., & Saad, S. M. I. (2007). Biopreservation by *Lactobacillus paracasei* in coculture with *Streptococcus thermophilus* in potentially probiotic and synbiotic fresh cream cheeses. *Journal of Food Protection, 70*(1), 228–235.

Buriti, F. C. A., Cardarelli, H. R., & Saad, S. M. I. (2008). Influence of *Lactobacillus paracasei* and inulin on instrumental texture and sensory evaluation of fresh cream cheese. *Revista Brasileira de Ciencias Farmaceuticas, 44*(1), 75–84.

Buriti, F. C. A., Castro, I. A., & Saad, S. M. I. (2010). Viability of *Lactobacillus acidophilus* in synbiotic guava mousses and survival under *in vitro* simulated gastrointestinal conditions. *International Journal of Food Microbiology, 137*, 121–129.

Conover, W. J. (2001). *Practical nonparametric statistics* (3rd ed.). New York: Wiley.

Costa, M. P., Frasao, B. S., Lima, B. R. C. C., Rodrigues, B. L., & Conte Junior, C. A. (2016). Simultaneous analysis of carbohydrates and organic acids by HPLC-DAD-RI for monitoring goat's milk yogurts fermentation. *Talanta, 152*, 162–170.

Costa, G. M., Paula, M. M., Barão, C. E., Klosoki, S. J., Bonafé, E. G., Visentainer, J. V., et al. (2019). Yoghurt added with *Lactobacillus casei* and sweetened with natural sweeteners and/or prebiotics: Implications on quality parameters and probiotic survival. *International Dairy Journal, 97*, 139–148.

Cotter, P. D., Hill, C., & Ross, R. P. (2005). Bacteriocins: Developing innate immunity for food. *Nature Reviews Microbiology, 3*, 777–788.

Delgado-Fernández, P., Corzo, N., Olano, A., Hernández-Hernández, O., & Moreno, F. J. (2019). Effect of selected prebiotics on the growth of lactic acid bacteria and physicochemical properties of yoghurts. *International Dairy Journal, 89*, 77–85.

Dimitrellou, D., Kandylis, P., & Kourkoutas, Y. (2019). Assessment of freeze-dried immobilized *Lactobacillus casei* as probiotic adjunct culture in yogurts. *Foods, 8*(9), 374.

Dimitrellou, D., Kandylis, P., Petrović, T., Dimitrijević-Branković, S., Lević, S., Nedović, V., et al. (2016). Survival of spray dried microencapsulated *Lactobacillus casei* ATCC 393 in simulated gastrointestinal conditions and fermented milk. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 71*, 169–174.

Dimitrellou, D., Salamoura, C., Kontogianni, A., Ktsipi, D., Kandylis, P., Zakynthinos, G., et al. (2019). Effect of milk type on the microbiological, physicochemical and sensory characteristics of probiotic fermented milk. *Microorganisms, 7*(9), 274.

Fayed, B., El-Sayed, H. S., Abood, A., Hashem, A. M., & Mehanna, N. S. H. (2019). The application of multi-particulate microcapsule containing probiotic bacteria and inulin nanoparticles in enhancing the probiotic survivability in yoghurt. *Biocatalysis and Agricultural Biotechnology, 22*, 101391.

Feng, K., Huang, R., Wu, R., Wei, Y., Zong, M., Linhardt, R. J., et al. (2020). A novel route for double-layered encapsulation of probiotics with improved viability under adverse conditions. *Food Chemistry, 310*, 125977.

GExpDes. (2019). *GExpDes: Interface gráfica do ExpDes. Brazil: UFSC*. UNIFAL.

Guidone, A., Zotta, T., Ross, R. P., Stanton, C., Rea, M. C., Parente, E., et al. (2014). Functional properties of *Lactobacillus plantarum* strains: A multivariate screening study. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 56*(1), 69–76.

Gu, M., Zhang, Z., Pan, C., Goulettea, T. R., Zhanga, R., Hendricksb, G., et al. (2019). Encapsulation of *Bifidobacterium pseudocatenulatum* G7 in gastroprotective microgels: Improvement of the bacterial viability under simulated gastrointestinal conditions. *Food Hydrocolloids, 91*, 283–289.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal, 50*(3), 346–363.

IAL. (2008). *Métodos físico-químicos para análise de alimentos* (4th ed.). São Paulo: Instituto Adolfo Lutz.

Iraporda, C., Rubel, I. A., Manrique, G. D., & Abraham, A. G. (2019). Influence of inulin rich carbohydrates from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers on probiotic properties of *Lactobacillus* strains. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 101*, 738–746.

Liserre, A. M., Re, M. I., & Franco, B. D. G. M. (2007). Microencapsulation of *Bifidobacterium animalis* subsp. *lactis* in modified alginate-chitosan beads and evaluation of survival in simulated gastrointestinal conditions. *Food Biotechnology, 21*, 1–6.

Madureira, A. R., Amorim, M., Gomes, A. M., Pintado, M. E., & Malcata, F. X. (2011). Protective effect of whey cheese matrix on probiotic strains exposed to simulated gastrointestinal conditions. *Food Research International, 44*, 465–470.

Mantzourani, I., Chondrou, P., Bontsidis, C., Karolidou, K., Terpou, A., Alexopoulos, A., et al. (2019). Assessment of the probiotic potential of lactic acid bacteria isolated from kefir grains: Evaluation of adhesion and antiproliferative properties in in vitro experimental systems. *Annals of Microbiology, 69*(7), 751–763.

Marinaki, E., Kandylis, P., Dimitrellou, D., Zakynthinos, G., & Varzakas, T. (2016). Probiotic yogurt production with Lactobacillus casei and prebiotics. *Current Research in Nutrition and Food Science Journal, 4*, 48–53. Special Issue Nutrition in Conference October 2016).

Martinez, R. C. R., Bedani, R., & Saad, S. M. I. (2015). Scientific evidence for health effects attributed to the consumption of probiotics and prebiotics: An update for current perspective and future challenges. *British Journal of Nutrition, 114*, 1993–2015.

de Moraes, G. M. D., Abreu, L. R., Egito, A. S., Salles, H. O., Silva, L. M. F., Nero, L. A., et al. (2017). Functional properties of *Lactobacillus mucosae* strains isolated from Brazilian goat milk. *Probiotics and Antimicrobial Proteins, 9*(3), 235–245.

de Moraes, G. M. D., dos Santos, K. M. O., de Barcelos, S. C., Lopes, S. A., & do Egito, A. S. (2018). Potentially probiotic goat cheese produced with autochthonous adjunct culture of *Lactobacillus mucosae*: Microbiological, physicochemical and sensory attributes. *LWT-Food Science and Technology, 94*, 57–63.

Oliveira, R. P. S., Perego, P., Oliveira, M. N., & Converti, A. (2012). Effect of inulin on the growth and metabolism of a probiotic strain of *Lactobacillus rhamnosus* in co-culture with *Streptococcus thermophilus*. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 47*, 358–363.

Ouwehand, C., Derrien, M., De Vos, W., Tiihonen, K., & Rautonen, N. (2005). Prebiotics and other microbial substrates for gut functionality. *Current Opinion in Biotechnology, 16*, 212–217.

Pal, M., Dudhrejiya, P., & Pinto, S. (2017). Goat milk products and their significance. *Beverage & Food World, 44*(7), 21–25.

de Paula, C. M., dos Santos, K. M. O., Oliveira, L. S., da Silva Oliveira, J., Alonso Buriti, F. C., & Saad, S. M. I. (2020). Fat substitution by inulin in goat milk ice cream produced with cajá (*Spondias mombin*) pulp and probiotic cultures: Influence on composition, texture, and acceptability among consumers of two Brazilian regions. *Emirates Journal of Food and Agriculture, 32*(2), 140–149.

R Core Team. (2018). *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Download from https://www.R-project.org/. (Accessed 20 June 2019).

Ribeiro, A. P. O., Gomes, F. S., dos Santos, K. M. O., da Matta, V. M., de Sá, D. G. C. F., Santiago, M. C. P. A., et al. (2020). Development of probiotic non-fermented blend beverage with juçara fruit: Effect of the matrix on probiotic viability and survival to the gastrointestinal tract. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 118*, 108756.

Rstudio Team. (2016). *RStudio: Integrated development for R*. Boston, MA: RStudio, Inc. Download from. http://www.rstudio.com. (Accessed 20 June 2019).

Ruiz-Moyano, S., Gonçalves dos Santos, M. T. P., Galván, A. I., Merchán, A. V., González, E., et al. (2019). Screening of autochthonous lactic acid bacteria strains from artisanal soft cheese: Probiotic characteristics and prebiotic metabolism. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 114*, 108388.

dos Santos, K. M. O., Vieira, A. D. S., Buriti, F. C. A., Nascimento, J. C. F., Melo, M. E. S., Bruno, L. M., et al. (2015). Artisanal coalho cheeses as source of beneficial *Lactobacillus plantarum* and *rhamnosus* strains. *Dairy Science & Technology, 95*, 209–230.

Savedboworn, W., Niyomrat, S., Naknovn, J., & Phattayakorn, K. (2017). Impact of inulin on viability and storage stability of probiotic *Lactobacillus plantarum* TISTR 2075 in fermented rice extract. *Agriculture and Natural Resources, 51*(6), 463–469.

Silveira, E. O., Lopes Neto, J. H., Silva, L. A., Raposo, A. E. S., Magnani, M., & Cardarelli, H. R. (2015). The effects of inulin combined with oligofructose and goat cheeses whey on the physicochemical properties and sensory acceptance of a probiotic chocolate goat dairy beverage. *Lebensmittel-Wissenschaft und -Technologie-Food Science and Technology, 62*, 445–451.

Sirichokchatchawan, W., Pupa, P., Praechansri, P., Am-in, N., Tanasupawat, S., Sonthayanon, P., & Prapasarakul, N. (2018). Autochthonous lactic acid bacteria isolated from pig faeces in Thailand show probiotic properties and antibacterial activity against enteric pathogenic bacteria. *Microbial Pathogenesis, 119*, 208–215.

Speranza, B., Racioppo, A., Beneduce, L., Bevilacqua, A., Sinigaglia, M., & Corbo, M. R. (2017). Autochthonous lactic acid bacteria with probiotic aptitudes as starter cultures for fish-based products. *Food Microbiology, 65*, 244–253.

Terpou, A., Papadaki, A., Lappa, I. K., Kachrimanidou, V., Bosnea, L. A., & Kopsahelis, N. (2019). Probiotics in food systems: Significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. *Nutrients, 11*(7), 1591.

Tian, H., Shen, Y., Yu, H., & Chen, C. (2017). Effects of 4 probiotic strains in coculture with traditional starters on the flavor profile of yogurt. *Journal of Food Science, 82*(7), 1693–1701.

Vinderola, G., Capellini, B., Villareal, F., Suárez, V., Quiberoni, A., & Reinheimer, J. (2008). Usefulness of a set of simple *in vitro* tests for the screening and identification of probiotic candidate strains for dairy use. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 41*, 1678–1688.

Vinderola, C. G., Mocchiutti, P., & Reinheimer, J. A. (2002). Interactions among lactic acid starter and probiotic bacteria uses for fermented dairy products. *Journal of Dairy Science, 85*, 721–729.

Vizoso-Pinto, M. G., Franz, C. M. A. P., Schillinger, U., & Holzapfel, W. H. (2006). *Lactobacillus* spp. with *in vitro* probiotic properties from human faeces and traditional fermented products. *International Journal of Food Microbiology, 109*, 205–214.