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Survival of *Lactobacillus rhamnosus* EM1107 in simulated gastrointestinal conditions and its inhibitory effect against pathogenic bacteria in semi-hard goat cheese

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ABSTRACT

This study evaluated the viability of *Lactobacillus rhamnosus* EM1107 incorporated in semi-hard goat cheese (Coalho) when exposed to simulated gastrointestinal conditions, as well as the inhibitory effects of this strain against pathogenic bacteria in goat Coalho cheese during refrigerated storage. After *in vitro* digestion, no change in viable cell count of *L. rhamnosus* (6.75 log CFU/g) was observed compared with the count before simulation (6.53 log CFU/g). Against *Staphylococcus aureus*, *L. rhamnosus* exhibited inhibition rates of 1.55%, 1.7% and 21.66% at 7, 14 and 21 days of storage, respectively. Furthermore, against *Salmonella* Enteritidis, the inhibition rates were 4.36%, 5.33% and 5.51% at 7, 14 and 21 days of storage, respectively and against *Listeria monocytogenes*, the inhibition rates were 2.62%, 1.57% and 10.23% at 7, 14 and 21 days of storage, respectively. Against *Escherichia coli*, *L. rhamnosus* showed inhibition rate of 7.98% at 7 days of storage and no inhibitory effects at 14 and 21 days. The results indicate that goat Coalho cheese has a protective effect on the viability of *L. rhamnosus* EM1107 during artificial digestion. In addition, this strain could be used as a protective culture to delay the growth of pathogenic bacteria, particularly *S. aureus* and *L. monocytogenes*.

1. Introduction

The growing consumer awareness of diet-related health has increased the demand for foods with distinct health-promoting effects (Saad, Delattre, Urdaci, Schmitter, & Bressollier, 2013). Food-associated probiotics are living micro-organisms that, upon the ingestion of certain quantities, exert benefits to the consumer's health beyond the inherent basic nutrition (FAO/WHO, 2002). In addition, the inclusion of probiotics in food matrices can assist in maintaining their viability during human digestion, because to exert their beneficial effects on host health, probiotic bacteria must survive through the gastrointestinal tract, tolerating acids, bile salts and gastric enzymes, and then adhere to and colonize the intestinal epithelium (Huang & Adams, 2004; Ranadheera Evans, Adams, & Baines, 2012). Fermented dairy products, such as fermented milks and fresh cheeses, have been described as vehicles of interest for the incorporation of probiotic bacteria (Figueroa-González, Quijano, Ramírez, & Cruz-Guerrero, 2011; Saxelin, 2008). Due to a higher pH than most fermented dairy products as well as a high fat content, solid consistency and higher buffering capacity, cheeses can promote the viability of these bacteria not only throughout the product shelf-life but also during their passage through the gastrointestinal tract after consumption (Coman et al., 2012).

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Goat Coalho cheese is a semi-hard cheese with intermediate to high moisture (36–45%) high yield and good acceptance among consumers (Garcia, Oliveira, Queiroga, Machado, & Souza, 2012; Oliveira, Garcia, Queiroga, & Souza, 2012). This product is produced mainly in northeastern Brazil, where goats have an important and significant role in the socioeconomic development of the region, especially in poor and semi-arid areas (Queiroga et al., 2013). Although the production, processing and marketing of the global production of goat milk and its by-products are much lower than cow's milk, goat products are widely consumed worldwide (Gerosa & Skoet, 2012; Queiroga et al., 2013). Studies have reported the efficiency of goat Coalho cheese as appropriate matrix to serve as a vehicle for probiotic bacteria from the genera *Lactobacillus* and *Bifidobacterium*. Another important finding was that the incorporation of the test strains did not cause changes in the product's quality characteristics but instead improved its sensory attributes (Oliveira et al., 2012; Santos et al., 2012).

Furthermore, the incorporation of probiotic bacteria into cheese can promote additional advantages, as some strains can inhibit the growth of pathogenic bacteria in food matrices due to their production of substances such as organic acids, bacteriocins and fat and amino acid metabolites (Chen et al., 2007). Fresh and semi-hard cheeses, such as goat Coalho cheese, are among the foods predominantly involved in food poisoning outbreaks worldwide (Almeida et al., 2013; Kousta, Mataragas, Skandamis, & Drosinos, 2010). *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp. stand out among the prevalent pathogenic bacteria that are the etiologic agents of food outbreaks involving dairy products (Cokal, Dagdelen, Cenet, & Gunsen, 2012). Therefore, the addition of probiotic lactic cultures with recognized antimicrobial activity to cheeses can contribute to the maintenance of the microbiological quality of these products during storage (Costa, Suguimoto, Miglioranza, & Gomez, 2012).

Moreover, studies have investigated new strains exhibiting resistance to the adverse conditions of the human gastrointestinal tract that have physiological characteristics compatible with probiotic properties and technological relevance for use in food products (Ugarte, Guglielmotti, Giraffa, Reinheimer, & Hynes, 2006). In a previous study, the new *Lactobacillus rhamnosus* EM1107 strain, isolated from bovine Coalho cheese was evaluated for technological, safety and functionality aspects and proven to be a promising probiotic candidate for use in fermented dairy products (Santos et al., 2014). However, other analyses are mandatory to assess the viability of the strain as a probiotic micro-organism for these types of food matrices. Included among the requisite tests are the survivability of this strain incorporated into the product during simulated gastrointestinal conditions and its inhibitory effect against possible product contamination (FAO/WHO, 2002). Thus, the present study was developed to evaluate the survival of *L. rhamnosus* EM1107 in goat Coalho cheese exposed to simulated gastrointestinal conditions, and the inhibition potential of *L. rhamnosus* EM1107 against *S. aureus*, *Salmonella enterica* serovar Enteritidis, *E. coli* and *L. monocytogenes* pathogenic bacteria in goat Coalho cheese was also evaluated over 21 days of refrigerated storage.

2. Material and methods

2.1. Coalho cheese

The cheeses used in the experiment were produced at Embrapa Goats and Sheep/Sobral, Brazil using simple manufacture according previously described by Santos et al. (2012), with exception of addition of starter lactic culture R-704 composed by *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*, considering the starter potential previously identified for *L. rhamnosus* EM1107 (Santos et al., 2014). After the removal of around 90% of whey, the cheese curd was salted with 0.8 g/L NaCl, based on the initial milk volume. Cheeses were packed in Styrofoam containers and kept refrigerated at 10 °C during transport to perform *in vitro* analyses. For the simulation of gastrointestinal conditions, cheese containing 6.53 log CFU/g viable cell count of *L. rhamnosus* EM1107 was used. *L. rhamnosus* counts in cheeses was determined by viable cell counts procedure using MRS agar (Sigma–Aldrich) supplemented with cysteine–HCl (0.05 g/100 mL), followed by incubation under anaerobic conditions (BD GasPakTM EZ anaerobe container system, Becton, Dickinson and Company, USA) for 48 h at 37 °C (Oliveira et al., 2014). This strain also served as the starter culture for cheese fermentation. To test the inhibition of pathogenic bacteria, fresh cheese manufactured without the addition of a test bacterial culture was used. Cheese were evaluated during 21 days at different time points (1, 7, 14 and 21 days) to assess the protective effects on *L. rhamnosus* EM1107 viability as previously described by Oliveira et al. (2014). The pH of the cheese immediately after manufacture, after day 1, day 7, day 14 and day 21 were: immediately after manufacture: 6.0 (±0.2); day 1: 6.21 (±0.25); day 7: 6.03 (±0.42); day 14: 6.05 (±0.20); day 21: 6.06 (±0.16).

2.2. Test strains and preparation of inocula

The freeze-dried probiotic *L. rhamnosus* EM1107 culture was obtained from the "Collection of Micro-organisms of Interest for Tropical Agroindustry", Embrapa Tropical Agroindustry/Fortaleza, Ceará, Brazil. *S. aureus* ATCC 25923 and *L. monocytogenes* ATCC 7644 were acquired from the Collection of Reference Micro-organisms of the National Institute of Quality Control in Health (FIOCRUZ, Rio de Janeiro, Brazil). *S. enterica* serovar Enteritidis UFPEDA 414 and *E. coli* UFPEDA 224 were obtained from the Department of Antibiotics at the Federal University of Pernambuco.

To prepare the inocula, subcultures were performed in MRS agar supplemented with cysteine–HCl (0.05 g/100 mL) (Sigma–Aldrich, St. Louis, MO, USA) for the probiotic strain and in BHI agar (Sigma–Aldrich) for the pathogenic bacteria, and these cultures were incubated for 48 h and 24 h at 37 °C, respectively, in anaerobic and aerobic conditions. Then, probiotic and pathogenic strains were inoculated into MRS (Sigma–Aldrich) and BHI broth (Sigma–Aldrich), respectively, to grow for 18 h at 37 °C. After this growth period, the strains were centrifuged (4500 g, 15 min, 4 °C), washed twice in sterile saline solution (0.85% NaCl) and resuspended in sterile saline solution. Strains (pathogenic or probiotic) were serially diluted (10^{-1}–10^{-8}) and the optical density at a wavelength of 625 nm (OD_{625}) was measured for each dilution using a spectrophotometer (SF200DM-UV-Vís, Bel Engineering, Monza, Italy) and saline solution as blank. To estimate CFU/mL in each OD_{625} reading, 0.1 mL of the each dilution was plate on MRS agar supplemented with cysteine–HCl (0.05 g/100 mL) (Sigma–Aldrich, St. Louis, MO, USA) for the probiotic strain and in BHI agar (Sigma–Aldrich) for the pathogenic bacteria, followed by incubation for 48 h and 24 h at 37 °C, respectively, in anaerobic (probiotic strain) or aerobic conditions (pathogenic strains). To obtain the desire levels of *L. rhamnosus* (8 log CFU/mL) and pathogenic bacteria (*S. aureus*, *S.* Enteritidis, *L. monocytogenes* and *E. coli*; 6 log CFU/mL) OD_{625} used was 0.8 and 0.1, respectively.

2.3. Survival of L. rhamnosus in simulated gastrointestinal conditions

The experimental design for assessing survival in simulated gastrointestinal conditions consisted of 4 groups. Two of these groups were the cheese samples with *L. rhamnosus* EM1107: Q1 and

Q2 (6 log CFU/g), and the other two groups were *L. rhamnosus* EM1107 inoculum (8 log CFU/mL) in MRS broth supplemented with 5% cysteine: C1 and C2. Q1 and C1 were not exposed to simulated gastrointestinal conditions and Q2 and C2 were exposed to these conditions. Samples of the aforementioned groups were placed in sterilized glass jars (340 mL). In Q1 and Q2, the cheeses with the potential probiotic strain were macerated and distributed in 25 g amounts, and in C1 and C2, 1 mL of inoculum at an initial count of 8 log CFU/mL was distributed in 25 mL of MRS broth.

All steps of the simulated gastrointestinal conditions are described in Table 1, including the enzymes, compartment pH values, time intervals and agitation intensities that were used to simulate peristaltic movements. Before the simulation commenced, the pH was adjusted in all digestion stages using cheese (25 g) and bacterium samples (1 mL of inoculum containing 8 log CFU/mL) in MRS broth (25 mL of broth). The pH of each sample was measured at each stage of the simulated gastrointestinal conditions using a potentiometer (Model 021/15; Quimis, Sao Paulo, Brazil), which was periodically disinfected with ethanol (90% v/v). After conditioning the samples in glass jars, all groups were kept under refrigerated storage at 7 °C ± 2 °C for 7 days, which corresponds to the minimum shelf-life of Coalho cheese. This simulation consisted of the following 10 steps (Madureira, Amorim, Gomes, Pintado, & Malcata, 2011):

- Step 1 (before simulation): cheese was evaluated under the conditions present before ingestion.
- Step 2 (mouth): chewing was simulated using saliva solution prepared with 100 U/mL of α-amylase 86250 (Sigma, St. Louis MO, USA) diluted with 1 mM $CaCl_2$. The saliva solution was added to 25 g of sample at a rate of 0.6 mL/min for 2 min. The pH was adjusted to 6.9 using a 0.1 M $NaHCO_3$ solution.
- Steps 3 to 8 (esophagus-stomach): a pepsin solution was added equal-sized aliquots at each step throughout this gastric phase at a rate of 0.05 mL/mL for a total of 90 min. The pepsin solution P7000 (Sigma, St. Louis MO, USA) was prepared in 0.1 N HCl at a concentration of 25 mg/mL. The pH values at these stages were adjusted according to Table 1 using a 1 M HCl solution (Aura, 2005).
- Step 9 (duodenum): intestinal solution was added at the beginning of this step at a rate of 0.25 mL/mL (Laurent, Besançon, & Caporiccio, 2007). This solution was prepared using 2 g/L pancreatin P7545 (Sigma, St. Louis MO, USA) and 12 g/L bile salts BS 48305 (Sigma, St. Louis MO, USA) and then diluted with a 0.1 M $NaHCO_3$ solution. For pH adjustment, 0.1 M $NaHCO_3$ was added.
- Step 10 (ileum): a solution of 0.1 M $NaHCO_3$ was added to increase the pH to 6.5.

The simulation was continuous; thus, the total working volume increased at each stage (as occurs during real digestion) reaching at final volume of 110.2 mL. All enzyme solutions were prepared in flasks and sterilized by filtration using a 0.22 μm membrane filter (Millipore, Billerica, MA, USA) before use. After sterilization, all solutions were maintained on an ice bath throughout the simulation period. A 37 °C incubation chamber with mechanical stirring (TE-424 TECNAL, Orbital Shaker Incubadora, São Paulo, SP, Brazil) was used to simulate both the body temperature and the intestinal peristaltic movements similar to those achieved in each digestive compartment.

At the end of each stage of exposure to artificial digestion, the samples were diluted with 225 mL of peptone water [0.1 g/100 mL (Sigma, St. Louis, MO, USA)] (dilution 1), and then 1 mL aliquots of the contents, representing each condition in the gastrointestinal tract, were aseptically collected for subsequent serial dilution and subjected to viable cell counting of the probiotic bacteria using the method of Miles, Misra, and Irwin (1938). *L. rhamnosus* was plated on MRS agar (Sigma–Aldrich) supplemented with cysteine-HCl (0.05 g/100 mL) and incubated under anaerobic conditions (BD GasPakTM EZ Anaerobe container system, Becton, Dickinson and Company, USA) at 37 °C for 48 h. The results were expressed as the log of colony forming units per gram of cheese (log CFU/g). Viable cell counts were performed using the microdrop technique, as described by Oliveira et al. (2014).

2.4. Inhibitory effect of L. rhamnosus against pathogenic bacteria

Approximately 25 g of cheese were divided into sterile glass jars (340 mL). For the control groups, the positive control cheese was prepared by adding only inoculum containing the isolated pathogenic bacteria, the negative control cheese contained only the isolated probiotic bacteria, and a control sample lacked any added bacteria, totaling 6 control samples. The pathogenic and probiotic bacteria were inoculated in a 1 mL inoculum for each 25 g of cheese to provide a system containing 6 log CFU/g and 8 log CFU/g, respectively. The inoculum of pathogenic bacteria was defined considering the real contamination level previously reported in coalho samples (Aragon-Alegro et al., 2007; Meneses et al., 2012). The experimental cheeses consisted of cheeses with mixed cultures added: 1 mL of probiotic bacteria inoculum and 1 mL of isolated contaminant bacteria inoculum at the same bacterial concentration as previously mentioned, resulting in four samples (probiotic strain x 4 pathogenic strains). After inoculation, all inoculated cheese samples were shaken in an electric mixer (Kenwood, UK) for 5 min and stored at 7 °C for 21 days, which corresponded to 40 samples (10 samples x 4 storage times).

Each sample was subjected to a viable cell count of probiotic bacteria and contaminants at 1, 7, 14 and 21 days of refrigerated storage using the microdrop technique. For the viable cell counts of *L. rhamnosus*, MRS agar (Sigma–Aldrich) supplemented with cysteine-HCl (0.05 g/100 mL) was used, which was incubated under anaerobic conditions (BD container system GasPakTM EZ Anaerobe,

Table 1
The conditions used during each stage of the simulated digestion.

Steps	Compartment	Conditions/Volume	Stirring (rpm)	Final pH	Time of exposure (min)
1	Before simulation	–	–	–	–
2	Mouth	1.2 mL of saliva solution + 2.0 mL of 0.1 M $NaHCO_3$	200	6.9	2
3	Esophagus–stomach	1.2 mL of pepsin solution + 1.2 mL of 1 M HCl	130	5.5	10
4		1.9 mL of 1 M HCl		4.6	10
5		3.0 mL of 1 M HCl		3.8	10
6		4.0 mL of 1 M HCl		2.8	20
7		1.8 mL of 1 M HCl		2.3	20
8		1.9 mL of 1 M HCl		2.0	20
9	Duodenum	6 mL of intestinal solution + 32 mL of 0.1 M $NaHCO_3$	45	5.0	30
10	Ileum	54 mL of 0.1 M $NaHCO_3$	45	6.5	60

Becton, Dickinson and Company, USA) for 48 h at 37 °C. For *S. aureus*, mannitol-sodium chloride-phenol red agar (Merck) was used; for *S.* Enteritidis, Salmonella-Shigella Agar (HIMEDIA Laboratories, India); for *L. monocytogenes*, Listeria Selective Base Agar supplemented with Listeria Selective Supplement (HIMEDIA Laboratories, India); and for *E. coli*, EMB Levine Agar was used (HIMEDIA Laboratories, India). All agar plates were incubated for 24 h at 37 °C (Vanderzant & Spilttstoesser, 1992). All analyses were performed in duplicate and the results were expressed in log CFU. For each storage period, the inhibition degree (inhibition rate) was calculated as:

$$\text{Inhibition rate} = [(\text{N control} - \text{N pathogen})/\text{N control}]*100,$$

where N control is the log CFU/g of the pathogenic bacteria added in isolation and N pathogen is the log CFU/g of the pathogenic bacteria added in the presence of the probiotic bacteria (Madureira, Pintado, Gomes, & Malcata, 2011).

2.5. Data analysis

The Statistical Analysis System software (SAS, 1999) was used for statistical analyses. The results were evaluated using analysis of variance (ANOVA) and Tukey test, both considering a significance level of $p < 0.05$.

3. Results and discussion

3.1. Survival in simulated gastrointestinal conditions

The viable cell counts of *L. rhamnosus* EM1107 in goat Coalho cheese (groups Q1 and Q2) and in MRS broth (groups C1 and C2), when either not exposed or exposed to simulated gastrointestinal conditions are shown in Fig. 1. In the groups not exposed to simulated gastrointestinal conditions, *L. rhamnosus* EM1107 showed viable cell counts in cheese (Q1) and MRS broth (C1) of 6.7 and 8.4 log CFU/mL, respectively. In the groups exposed to the simulation, a difference ($p < 0.05$) was observed in the viable cell count of this strain in the goat Coalho cheese (Q2) when compared with step 1 (before simulation; 6.53 log CFU/g) for the other digestion steps (between 6.1 and 7.68 log CFU/mL), except for the last step (ileum; 6.75 log CFU/g), where no difference was observed ($p > 0.05$). Thus, at the end of cheese digestion, the probiotic strain maintained its viability. These counts corroborate literature, indicating that to achieve their beneficial effects, the viable probiotics should be present in the food at a minimum level ranging from 6 to 9 log CFU/g (Plessas, Bosnea, Alexopoulos, & Bezirtzoglou, 2012; Vinderola et al., 2008).

When *L. rhamnosus* EM1107 was added to MRS broth (C2) and subjected to simulated gastrointestinal conditions, a reduction in the counts ($p < 0.05$) after step 6, or after 52 min of *in vitro* simulation (pH 2.8), was observed, which corresponded to a reduction of 2.2 log CFU/g compared with initial counts. After 72 min of stomach simulation (step 7), when the strain was exposed to pH 2.3, cell death was observed. In a previous study that evaluated the survival of *L. rhamnosus* 1107 under simulated gastric and intestinal conditions, where the strain cultivated overnight in MRS broth was exposed to pH 2.5 for 60 min and to an artificial duodenal secretion for 3 h, and Santos et al. (2014) observed a reduction of 2.46 log CFU/mL at the end of the assay, which started from an initial inoculum of 9.10 log CFU/mL. The difference between the results obtained by these authors and those of this study for the same bacterial strains can be explained by the different models for simulating the digestive steps, particularly the step including the mouth that used α-amylase for the saliva solution in the model tested herein. Another factor that may have influenced this difference was the exposure of *L. rhamnosus* M1107 to a gradual pH reduction in the stages of the simulated stomach conditions. Thus,

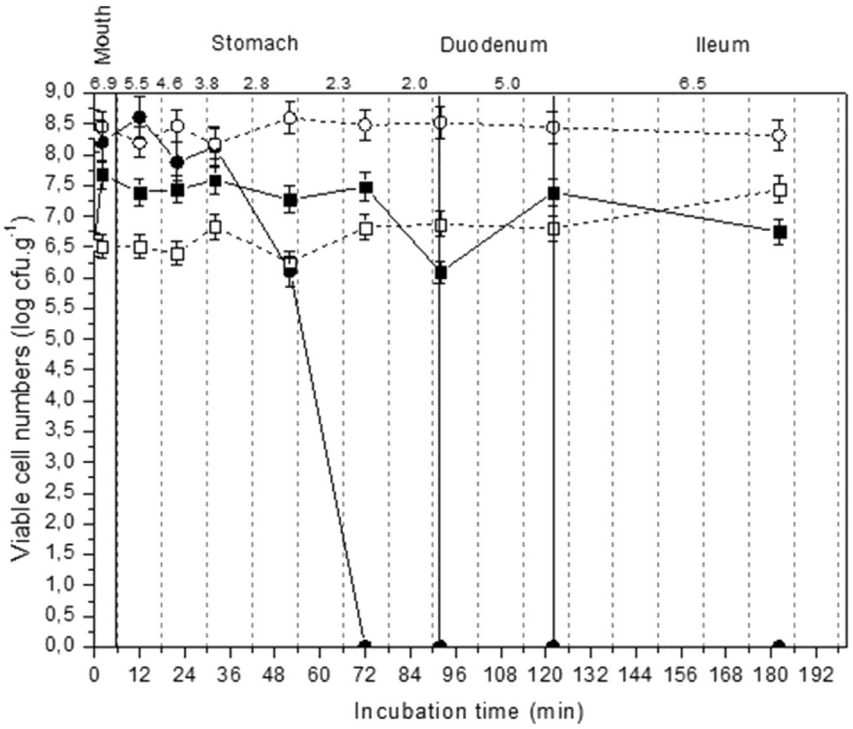

Fig. 1. The viable cell counts (average ± standard deviation) of *L. rhamnosus* EM1107, when inoculated in MRS broth (○,●) or in cheese (□,■), and either exposed (●,■) or not (○,□) the simulated gastrointestinal conditions over different incubation times. The pH values that the bacteria were exposed to are indicated in the upper left corner.

the model used in this study more realistically reflects the conditions faced by cells during digestion, because it involves all compartments of the gastrointestinal tract (from mouth to ileum) and uses different pH gradients in the stomach, which typically occurs in digestion (Madureira, Amorim, Gomes, Pintado, & Malcata, 2011).

After the exposure of cheese containing *L. rhamnosus* EM1107 (Q2) to simulated mouth conditions (step 2), there was a 1.15 log CFU/g increase ($p < 0.05$) compared with the original counts (step 1). Similar behavior was reported by Oliveira et al. (2014) and Madureira Amorim, Gomes, Pintado and Malcata (2011), who evaluated the survival of *Bifidobacterium lactis* (BB12) in goat Coalho cheese and *Bifidobacterium animalis* Bo in a cheese whey matrix, respectively, using the same digestion model that was used in this study. The simulated esophagus-stomach conditions of the present study were characterized by a steady pH reduction. However, the *L. rhamnosus* EM1107 added to goat Coalho cheese maintained its number of viable cells through step 8, i.e., after 92 min in contact with the gastric juice (pH 2.0). This same result was observed in a study by Oliveira et al. (2014), who used goat Coalho cheese as a vehicle for *B. lactis* (BB12), *Lactobacillus acidophilus* (LA-5) and *L. casei* subsp. *paracasei* (*L. casei* 01). This viability is most likely due to the high buffering capacity of goat Coalho cheese, which kept the bacterial cells protected from the low stomach pH in these steps.

In step 9 (duodenum), the goat Coalho cheese samples were exposed to an intestinal solution composed of pancreatin and bile salts at pH 5, and at the end of this step (after 122 min), there was no decrease in the *L. rhamnosus* EM1107 initial viable cell counts (6.09 log CFU/g) compared with the counts when the microorganism reached this compartment (7.3 log CFU/g). Otherwise, an increase ($p < 0.05$) of approximately 1.3 log CFU/g was observed. However, an increasing ($p < 0.05$) trend was observed. This increase may be due to the deconjugation ability of the bile salts in the tested strain (Santos et al., 2014), as this feature can improve the survival of probiotic bacteria in the intestinal environment (Begley, Hill, & Gahan, 2006). After simulated digestion of the ileum (step 10), following 182 min of digestion, the viable cell counts of *L. rhamnosus* were maintained, most likely due to the neutral pH (6.5) of this compartment. Similar results in the ileum stage were reported by Madureira, Amorim, Gomes, Pintado, and Malcata (2011) for multiple strains (*L. casei* LAFTI® L26, *L. acidophilus* LAFTI® L10 and *B. animalis* Bo) added to cheese from milk whey.

Thus, at the end of *in vitro* digestion, *L. rhamnosus* exhibited the same number of viable cells as were present at the beginning of digestion. In contrast, when in MRS broth, the strain lost viability at the end of digestion. These results indicated that goat Coalho cheese had a protective effect during the exposure of *L. rhamnosus* to simulated gastrointestinal conditions. Previous studies have reported that dairy matrices such as fermented milks and semi-hard fresh cheeses are good vehicles for probiotic bacteria to protect the cells during the stress imposed by gastrointestinal conditions, allowing these bacteria to reach the intestine and exert beneficial effects on the host (Oliveira et al., 2014; Pitino et al., 2012). The most convincing arguments refer to the physical and chemical characteristics of these matrices, such as higher pH value, higher buffering capacity, better nutrient availability, low oxygen content and high fat content in combination with protein density, which makes the matrix more solid (Karimi, Mortazavian, & Cruz, 2011; Pitino et al., 2012).

3.2. Inhibitory effect of *L. rhamnosus* against pathogenic bacteria

When combined with pathogenic bacteria, *L. rhamnosus* EM1107 counts from 5.8 to 6.6 log CFU/g during the 21 days of refrigerated storage (data not shown). This indicates that the probiotic strain

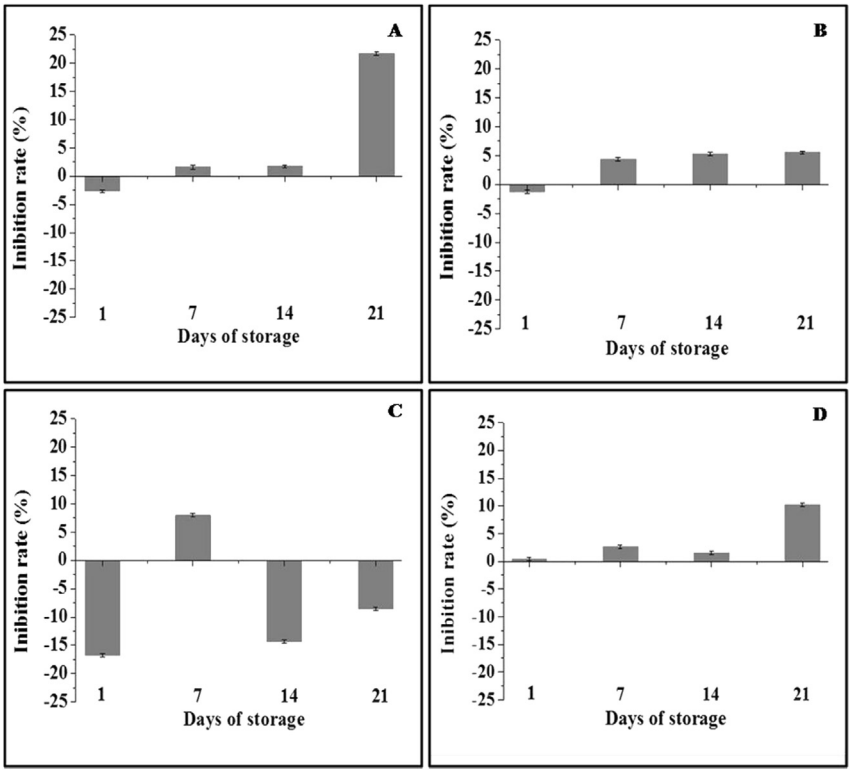

Fig. 2. Time course of inhibition (inhibition rate expressed as average ± standard deviation) of *S. aureus* (A), *S.* Enteritidis (B), *E. coli* (C) and *L. monocytogenes* (D) brought about by *L. rhamnosus* EM1107, in goat coalho cheese, during 21 days of storage at 7 °C.

remained viable to exert its inhibitory effect against pathogenic bacteria during the storage period. The inhibition times (inhibition rates) caused by *L. rhamnosus* against *S. aureus*, *S.* Enteritidis, *E. coli* and *L. monocytogenes* are shown in Fig. 2. *L. rhamnosus* EM1107 showed inhibition rates against *S. aureus* of only 1.55% (±0.31) and 1.7% (±0.26) after 7 and 14 days of storage ($p > 0.05$), respectively (Fig. 2A). However, after 21 days of refrigerated storage, the inhibition rate was 21.66% (±0.25) ($p < 0.05$) for this pathogenic microorganism. Against *S.* Enteritidis, the inhibition rates were 4.36% (±0.30) ($p > 0.05$), 5.33% (±0.30) ($p < 0.05$) and 5.51% (±0.30) ($p < 0.05$) after 7, 14 and 21 days of storage, respectively (Fig. 2B). Against *E. coli*, *L. rhamnosus* showed an inhibition rate of 7.98% (±0.32) ($p < 0.05$) after 7 days of storage, however no inhibitory effect were observed on the remainder storage time assessed (Fig. 2C). At day 1, day 14 and day 21 of storage, the viability of *E. coli* was not affected by the presence of *L. rhamnosus* in the same environment. Against *L. monocytogenes*, the inhibition rates on days 7, 14 and 21 were 2.62% (±0.32) ($p > 0.05$), 1.57% (±0.31) ($p > 0.05$) and 10.23% (±0.32) ($p < 0.05$), respectively (Fig. 2D). Based on these results, *L. rhamnosus* EM1107 is more effective against *S. aureus* and *L. monocytogenes* on day 21 of refrigerated storage compared to the other pathogenic bacteria studied. Early study reported that *L. casei* subsp. *paracasei* exhibited inhibition rates of 7.87% and 23.63% against *S. aureus* on the 14th and 21st days of storage, respectively, in goat coalho cheese. Against *L. monocytogenes* the inhibition rates reported were 12.96% and 32.99% on the 14th and 21st days of storage, respectively (Oliveira et al., 2014).

The ability of *L. rhamnosus* to inhibit pathogenic bacteria was described in a previous study in which Douillard et al. (2013) evaluated 100 *L. rhamnosus* strains isolated from Universities and Hospitals and observed that most strains showed inhibitory effects on the growth of *E. coli*, *Yersinia enterocolitica* and *L. monocytogenes*. The exclusion or reduction capacity of enteropathogens is one of the most important characteristics attributed to certain lactic acid bacteria (LAB) strains. In general, this effect is due to the production of various compounds during the fermentative metabolism of LAB, such as organic acids, antibacterial peptides and active proteins (Chen et al., 2007; Marianelli, Cifani, & Pasquali, 2010). However, the nature of the compounds produced by LAB effective against pathogenic bacteria could vary among the LAB strains, as well as the efficacy of these compounds against different pathogens (Douillard et al., 2013). This could partially explain why *L. rhamnosus* EM1107 was not effective against *E. coli* in all times assessed, as well as the higher efficacy of *L. casei* subsp. *paracasei* previously reported by Oliveira et al. (2014) in inhibition of the same pathogenic bacteria assayed in the present study. However, even the lowest inhibitory rates of *L. rhamnosus* EM1107 against the pathogenic bacteria assayed could be considered biologically relevant because we used a bacterial inoculum containing large amounts of cells (10^6 log CFU/g) and in food samples these pathogens rarely exceeds 10^4 CFU/g (Oliveira et al., 2014; Sergelidis et al., 2012).

Overall, probiotic strains that exhibit antimicrobial activity against spoilage or pathogenic bacteria within the matrix in which they are incorporated are of interest for industrial application, because in addition to performing their probiotic effect, may contribute to an increase in product shelf life (Costa et al., 2012).

4. Conclusions

Goat Coalho cheese proved to be an effective matrix to carry *L. rhamnosus* EM1107 along the gastrointestinal tract, ensuring that this strain was supplied in satisfactory amounts to the intestine (6.75 log CFU/g). Furthermore, this strain delayed the growth of *S. aureus*, *S.* Enteritidis, *E. coli* and *L. monocytogenes* in goat Coalho cheese during refrigerated storage for 21 days. Therefore, this study demonstrated the potential of this strain of *L. rhamnosus* to be used as a protective culture, delaying the growth of pathogens commonly associated with goat Coalho cheese, particularly *S. aureus* and *L. monocytogenes*.

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