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ENCAPSULATION OF PROBIOTICS AND THEIR APPLICATION IN BEEF PATTIES

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ABSTRACT

Background. Probiotics have been widely used in foods due to their health implications and can be used in meat products to improve physicochemical and microbiological properties.
Materials and methods. Free and encapsulated *L. plantarum* and *L. rhamnosus* were added to beef patties. The physicochemical and microbiological properties of the beef patties were analyzed during refrigerated storage (10 days). A control treatment was made without probiotic addition. The encapsulation yield, morphological characterization, and size of the capsules were also measured.
Results. The pH values of the beef patties decreased during storage in treatments with probiotic addition. Furthermore, the probiotic strains were able to maintain the traditional meat color of the products. Lipid oxidation was lower in the treatments with probiotics as free cells. The beef patties with probiotic addition presented high levels of lactic acid bacteria during the whole refrigerated storage period. The treatments with specific strain addition also had higher levels of specific probiotic counts. However, the treatments with *L. rhamnosus* showed higher specific counts than those with *L. plantarum* at the end of the storage period.
Conclusion. Both probiotic strains and strategies of incorporation proved to be suitable for use in beef patties because of their high probiotic viability. However, encapsulated *L. rhamnosus* provided higher bacterial counts at the end of the storage period of the beef patties.

Keywords: burger, extrusion, probiotic, alginate, lipid oxidation

INTRODUCTION

Beef patties are widely consumed and highly valuable for consumers due to their convenience, low price, sensorial properties, and the presence of essential nutrients, but meat products have been associated with health damage due to the presence of harmful compounds. Therefore, consumers are willing to search for healthier processed meat products with reduced sodium and fat, whose synthetic antioxidants have been replaced, and which have been fortified with minerals, vitamins, and bioactive compounds (Saldaña et al., 2021). In addition, special attention has been given to probiotics, which are living microorganisms that can exert health benefits when consumed in adequate amounts (FAO/WHO, 2022). Probiotics confer

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health benefits by altering the host commensal microbiota, modulating immunity, and enhancing intestinal barrier function (Llewellyn and Foey, 2017). Among the group of lactic acid bacteria (LAB), *Lactobacillus plantarum* and *Lactobacillus rhamnosus* are widely used in meat product related technologies (Ben Slima et al., 2018; Cavalheiro et al., 2019a; 2020; Keska and Stadnik, 2022).

Studies have investigated the addition of probiotic microorganisms to meat products, focusing on their health implications (Cavalheiro et al., 2015; Munekata et al., 2022). Probiotic strains can help to protect meat products from spoilage and pathogenic bacteria and prevent lipid oxidation (Cavalheiro et al., 2015). However, stressful processing and storage conditions decrease the probiotic viability of meat products (Khan et al., 2011). In this context, encapsulation of probiotics has emerged as an alternative to protect and improve the viability of cells in foods with unfavorable environments. Among the different techniques of probiotic encapsulation, extrusion is a simple and cheap method that causes no damage to probiotic cells. Generally, sodium alginate is used along with other compounds, such as powdered milk, inulin, chitosan, whey protein, xanthan gum, and resistant starch, as wall materials (Burgain et al., 2011).

Traditionally, probiotics have been applied as starter cultures in fermented meat products (Cavalheiro et al., 2015) because they are consumed directly after being sliced without being cooked. However, few studies can be found in the literature on the addition of probiotics to raw and cooked meat products (Ben Slima et al., 2018; De Marins et al., 2022; Jafari et al., 2017; Pérez-Chabela et al., 2013). In addition, to the best of our knowledge, no studies have used encapsulated *L. plantarum* and *L. rhamnosus* in beef patties. Thus, this study aimed to use *L. plantarum* ATCC 7469 and *L. rhamnosus* ATCC 10012, either as free cells or encapsulated in alginate beads, on beef patties and to analyze their physicochemical and microbiological characteristics during refrigerated storage (4ºC for 10 days).

MATERIALS AND METHODS

Microbial strains and growth conditions

The *L. plantarum* ATCC 7469 and *L. rhamnosus* ATCC 10012 strains were obtained from the National Institute for Quality Control in Health (INCQS) of the Oswaldo Cruz Foundation (FIOCRUZ, BRAZIL). The probiotics were grown on MRS broth (37ºC for 24 h; Merck, Darmstadt, Germany), harvested by centrifugation (4000 × g for 20 min at 4ºC; Heraeus Megafuge 40R, Braunschweig, Germany), and dissolved in 0.9% NaCl to obtain a probiotic concentration between 13 and 14 log CFU g^{-1}.

Alginate capsule preparation

Alginate capsules were prepared according to Cavalheiro et al. (2019a) with slight modifications. Sodium alginate (1%; Sigma-Aldrich, Steinheim, Germany), milk powder (2%; Nestlé, Araçatuba, Brazil), green banana flour (0.5%; Leve Crock, Piraí do Sul, Brazil), and xanthan gum (0.3%; Leve Crock) were used as wall materials. The obtained slurries were dropped into 0.1 M $CaCl_2$ from a height of 10 cm, and the formed capsules were left in the $CaCl_2$ for 30 min for complete hardening, washed with sterile distilled water, and then stored at 4ºC for future analysis and use in the manufacture of the beef patties.

Encapsulation yield (*EY*)

The *EY* expresses the efficiency of cells that are entrapped and the survival of viable ones during the encapsulation procedure and was calculated as follows:

$$EY, \% = (N_{capsules} / N_{suspension}) \times 100$$

where:

$N_{capsules}$ – the number of viable probiotics entrapped in the capsules,

$N_{suspension}$ – the total number of probiotics added to the solution.

Morphological characterization and size of capsules

The capsules were submitted to critical point drying and lyophilization before evaluation in a scanning electron microscope (JSM6390, JEOL, Tokyo, Japan). Capsule sizes were assessed using a caliper (150 mm-6", TramontinaPro, Carlos Barbosa, Brazil).

Beef patty manufacturing

Fresh beef (73% moisture; 24% protein; 2% fat; and pH 5.8) and pork backfat (13% moisture, 3% protein, 82% fat, and pH 5.9) were purchased from the local

market. Beef patties were prepared according to a traditional formula and replicated on different days. The meat and pork backfat were ground in a meat grinder (8 mm diameter die plate; MCR08 3.0, Arbel, São José do Rio Preto, Brazil) and divided into 5 treatments, as follows:

1. Control: with no addition of probiotic strains and composed of beef meat (71.69%), pork backfat (17.92%), water (4.48%), breadcrumbs (4.48%), and sodium chloride (1.43%)
2. LP-Free: with the addition of *L. plantarum* as free cells in 1.0% (w/w) level replacing water
3. LP-Enc: with the addition of *L. plantarum* in capsules in 1.0% (w/w) level replacing water
4. LR-Free: with the addition of *L. rhamnosus* as free cells in 1.0% (w/w) level replacing water
5. LR-Enc: with the addition of *L. rhamnosus* in capsules in 1.0% (w/w) level replacing water.

Each formulation was aseptically hand-mixed for 7 min, and then 80 g portions were shaped in a patty former (10 cm diameter and 1 cm thick), packaged under aerobic conditions in polyvinyl chloride bags, and stored for 10 days at 4ºC. During the storage period, the beef patties were periodically evaluated for their pH, instrumental color, and microbial growth on days 0, 3, 7, and 10. Lipid oxidation analyses were done on days 0 and 10 of storage.

Physicochemical analyses

The pH of the beef patties was determined on 10 g homogenate samples in 90 mL of distilled water using a digital potentiometer (model mPA-210; Tecnopon, São Paulo, Brazil) equipped with a glass electrode for direct contact with the homogenized sample and calibrated with buffer solutions at pH 4.0 and 7.0 before each reading. The instrumental color was measured after the beef patties were removed from their packaging and they had been exposed to atmospheric air for 10 min in a colorimeter (Konica Minolta, CR-400, Japan). The equipment was calibrated using a white calibration plate (L^* = 85.79, a^* = –0.45, and b^* = 3.98), with D65 standard illuminant, 10º observer, and 8 mm aperture size. Measurements were performed at 5 different points on the surface of each beef patty, and the L^* (lightness), a^* (redness), and b^* (yellowness) parameters were measured. Lipid oxidation was estimated using thiobarbituric acid reactive substances (TBARS) according to the method described by Triki et al. (2013) with slight modifications, and the results were expressed as milligrams of malondialdehyde (MDA kg^{-1}) of sample. All analyses were performed in triplicate.

Microbiological analyses

First, the solubilization of the alginate beads was measured by adding 10 g of beef patty to 90 mL of phosphate buffer (0.1 mol L^{-1}, pH 7.5; Sheu and Marshall, 1993) followed by homogenization in a stomacher for 20 min at room temperature to completely break apart the formed polymer and completely release the encapsulated probiotics into the phosphate buffer. Then, other appropriate serial dilutions were done using 0.1% sterile peptone water (9 ml). Plate count agar (Acumedia, Lansing, USA) was used for total viable counts (TVC; 37°C for 48 h), and MRS agar (Merck, Darmstadt, Germany) was used to enumerate the lactic acid bacteria (LAB; 37°C for 48 h). LPSM agar (37ºC for 72 h) was used for selective enumeration of *L. plantarum* (Bujalance et al., 2006), and MRS agar supplemented with vancomycin (1 mg mL^{-1}; 37°C for 72 h) was used for selective enumeration of *L. rhamnosus* (Tharmaraj and Shah, 2003). The potentially pathogenic bacteria were also analyzed: coagulase-positive staphylococci, thermotolerant coliforms, *E. coli*, *Salmonella*, and reducing-sulfite clostridia (APHA, 2013). Microbiological analyses were performed in triplicate and microbial counts were converted to logarithms of colony forming units per gram (log CFU g^{-1}).

Statistical analysis

The data were subjected to a two-factor analysis of variance (ANOVA) using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) to analyze the effects of the addition of probiotics as free cells or encapsulated, as well as the refrigerated storage period of the beef patties on their physicochemical and microbiological properties. A completely randomized design included treatment groups (Control, LP-Free, LP-Enc, LR--Free, and LR-Enc) and storage time (0, 3, 7, and 10 days) as the fixed effects, and two replications as the random effect. Means were compared using Tukey's HSD test, and differences were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

Encapsulation yield

The *EY* values obtained for the capsules containing *L. plantarum* and *L. rhamnosus* were 86.17% and 83.88%, respectively. The results showed that the wall materials and the extrusion technique did not affect the viability of either probiotic, as evidenced by the higher *EY* values. As it was performed at room temperature (~25ºC) and without using organic solvents, the extrusion technique generally produced a higher *EY* (above 80%) than other studies (Cavalheiro et al., 2019b).

Morphological characterization of capsules

In the direct visualization, the obtained wet capsules were whitish and spherical, and had a smooth and uniform surface. In scanning electron microscopy, it was possible to observe that the capsules maintained their spherical shape and smooth surface when dried at the critical point (Fig. 1A). The spherical shape of beads provides a uniform mass transfer in the carrier (Krunic and Rakin, 2022) which is crucial for probiotic viability. Encapsulated bacteria cells inside beads need a substrate for growth, and the carrier must be able to release the products of metabolism to the substrate around them (Krunic and Rakin, 2022). However, the lyophilization process promoted the formation of agglomerated porous due to the matrix contact in the drying process, thus leading to the loss of spherical shape in lyophilized capsules (Fig. 1B). This was also evidenced by Etchepare et al. (2016) and could be a drawback when using dried capsules because it can expose inner probiotic bacteria to stressful environments. Nevertheless, it could be observed that the wall material was well distributed throughout the matrix (Fig. 1C) with a small presence of probiotics on the external surface of the capsules, and the extrusion technique efficiently achieved the encapsulation

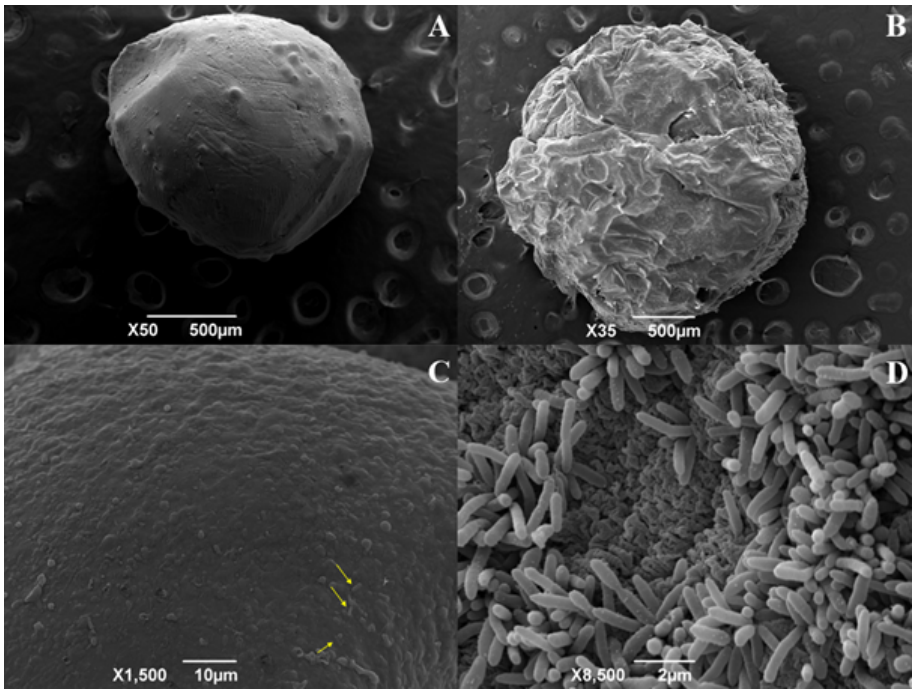

Fig. 1. Scanning electron micrograph of probiotic capsules produced with sodium alginate, milk powder, green banana flour, and xanthan gum: A – aspect of critical point dried capsules, B – aspect of lyophilized capsules, C – external surface of capsules (yellow arrows indicate the presence of probiotics), D – internal view of capsules showing the abundant presence of probiotics

of probiotics, as the abundant presence of probiotics inside the capsule was observed (Fig. 1D).

The size of the particles containing *L. plantarum* and *L. rhamnosus* were 3.53 and 3.34 mm, respectively. The viability of the probiotics is directly related to the size of the particles, since the larger the particles, the better the protection. Similar results (between 3.4 and 3.8 mm) were reported by Cavalheiro et al. (2019b) in alginate capsules. In another study, Krunic and Rakin (2022) had alginate beads that were spherical in shape and about 0.7–0.8 mm in diameter. Both shape and size are often critically controlled, and the solution's viscosity has an impact on the size and shape of the beads (Krunic and Rakin, 2022). However, the obtained capsule sizes could be in the range of micrometers up to millimeters, and sizes of 70 μm to more than 3 mm using the extrusion technique have previously been reported (Burgain et al., 2011).

Physicochemical properties of beef patties

The initial pH values of the beef patties were between 5.57 and 5.66 (Table 1) and declined in the treatments with free and encapsulated probiotic addition on day 3 of storage due to the accumulation of lactic acid via LAB activity (Zhao et al., 2011). The decrease in pH values contributes to the reduction of spoilage microorganisms, which then contributes to the product's safety. However, the pH values increased throughout the storage period in the control treatment. From day 3 onwards, the pH values were stable in the treatments with *L. rhamnosus* addition. Meanwhile, an increase was observed in the LP-Free and LP-Enc treatments (Table 1). The results in our study showed that the metabolism of both probiotics was intense, even when in encapsulated forms, and the lactic acid produced was able to pass through the capsules to the meat matrix. In addition, other LAB naturally present in the meat matrix may lead to a pH reduction (De Marins et al., 2022). At the end of the storage period (10 days), the pH values of the beef patties ranged from 5.30 to 6.15, with the control treatment the highest. A reduction in pH values during storage was also reported by Ben Slima et al. (2018) in fresh beef sausage and minced beef meat with *L. plantarum* TN8 and *P. acidilactici* inoculation and by De Marins et al. (2022) in raw and cooked beef patties with encapsulated *Lactiplantibacillus plantarum*, *Bifidobacterium lactis*, and *Lactobacillus acidophilus.*

Color is the first attribute which affects consumer acceptance of meat products. In that sense, stability of color during the storage period is important for meat products (Cavalheiro et al., 2020). In this study, the color properties of the beef patties were measured during the storage period and are presented in Table 1. Regarding lightness (L^*) values, at the beginning of storage, the values were between 44.00 and 45.22. Although the alginate capsules were whitish in color, LP--Enc had lower L^* values than LP-Free on day 0 of storage, and no differences between LR-Free and LP--Enc treatments were found (Table 1). Similar behavior was reported by De Marins et al. (2022), who reported L^* values between 41.22 and 48.46 at the beginning of the storage of raw beef patties with different probiotic addition. The control treatment showed a slight decrease in L^* values on days 3 and 7 of storage, indicating that the product became darker. However, an increase was observed on day 10, and no differences were found from those values at the beginning of storage for the control treatment (Table 1). At the end of the storage period, the L^* values of the beef patties were between 42.95 and 47.53, with LR-Enc being the highest.

Regarding redness (a^*), the values were between 19.35 and 22.11 (day 0), and the addition of free or encapsulated probiotics seems to have had a protective effect on color stability during the storage period (Table 1). A decrease in a^* values was observed for all treatments during the storage period, but in the control treatment that reduction was more intense. This means that the control treatment lost its reddish color faster than the other treatments. According to Sousa et al. (2020), the decline in the a^* parameter may be related to oxidation of the myoglobin pigment during storage. On day 3 of storage, the control treatment showed an a^* value of 7.77, while the values were between 19.45 and 21.72 in the treatments with probiotic inoculation. A significant reduction in the a^* values was noted up to day 7 of storage, when the control treatment showed values of 4.66 and the probiotic-treated patties showed values between 17.10 and 18.31 (Table 1). The higher a^* values observed on the treatments with probiotic inoculation were probably related to the antioxidant properties associated with probiotics (Ge

Table 1. pH and color parameters of beef patties during refrigerated storage

Treatments	Days of storage			
	0	3	7	10
		pH		
Control	5.64 ±0.02^{bc,AB}	5.54 ±0.04^{c,A}	5.69 ±0.02^{b,A}	6.15 ±0.09^{a,A}
LP-Free	5.65 ±0.01^{a,A}	5.24 ±0.04^{b,B}	5.59 ±0.13^{a,A}	5.69 ±0.18^{a,B}
LP-Enc	5.66 ±0.04^{a,A}	5.34 ±0.02^{b,B}	5.63 ±0.10^{a,A}	5.61 ±0.02^{a,BC}
LR-Free	5.62 ±0.01^{a,AB}	5.28 ±0.04^{b,B}	5.35 ±0.04^{b,B}	5.38 ±0.02^{b,CD}
LR-Enc	5.57 ±0.02^{a,B}	5.31 ±0.03^{b,B}	5.32 ±0.04^{b,B}	5.30 ±0.01^{b,D}
		Lightness (L^*)		
Control	44.83 ±0.25^{a,AB}	42.03 ±0.16^{bc,C}	40.57 ±0.97^{c,B}	42.95 ±1.14^{ab,B}
LP-Free	45.22 ±0.50^{a,A}	45.08 ±0.22^{a,AB}	45.78 ±0.16^{a,A}	45.21 ±0.45^{a,AB}
LP-Enc	44.00 ±0.47^{b,B}	45.39 ±0.34^{ab,A}	45.92 ±0.05^{ab,A}	46.48 ±1.56^{a,A}
LR-Free	44.61 ±0.28^{c,AB}	44.78 ±0.65^{c,AB}	45.79 ±0.16^{b,A}	47.08 ±0.32^{a,A}
LR-Enc	44.36 ±0.27^{c,AB}	45.15 ±0.17^{bc,AB}	46.28 ±0.79^{ab,A}	47.53 ±1.02^{a,A}
		Redness (a^*)		
Control	19.35 ±0.05^{a,C}	7.77 ±0.21^{b,C}	4.66 ±0.08^{c,C}	4.56 ±0.21^{c,C}
LP-Free	21.90 ±0.34^{a,AB}	20.04 ±1.62^{a,AB}	17.20 ±0.30^{b,B}	14.99 ±0.89^{b,B}
LP-Enc	22.11 ±0.65^{a,A}	19.45 ±0.48^{b,B}	17.10 ±0.09^{c,B}	16.15 ±0.60^{c,B}
LR-Free	20.46 ±0.55^{a,C}	20.71 ±0.14^{a,AB}	17.52 ±0.21^{b,B}	16.10 ±0.24^{c,B}
LR-Enc	20.52 ±0.52^{a,BC}	21.72 ±0.56^{a,A}	18.31 ±0.16^{b,A}	18.20 ±0.48^{b,A}
		Yellowness (b^*)		
Control	8.62 ±0.20^{a,B}	5.75 ±0.16^{c,B}	7.50 ±0.24^{b,A}	7.18 ±0.35^{b,B}
LP-Free	9.57 ±0.28^{a,A}	8.50 ±0.90^{a,A}	6.27 ±0.34^{b,B}	8.43 ±0.11^{a,A}
LP-Enc	9.78 ±0.28^{a,A}	8.57 ±0.17^{a,A}	6.32 ±0.14^{b,B}	8.93 ±0.37^{a,A}
LR-Free	10.03 ±0.11^{a,A}	9.00 ±0.33^{b,A}	6.40 ±0.32^{c,B}	8.56 ±0.50^{b,A}
LR-Enc	9.49 ±0.08^{ab,A}	9.58 ±0.38^{a,A}	6.46 ±0.43^{c,B}	8.64 ±0.38^{b,A}

Means ±standard deviations. Different superscript letters in the rows (a–c) and columns (A–D) of each parameter examined indicate statistically significant differences ($P < 0.05$) as a function of treatment and storage process.
Control – no probiotic strain addition, LP-Free – addition of *L. plantarum* as free cells, LP-Enc – addition of *L. plantarum* in capsules, LR-Free – addition of *L. rhamnosus* as free cells, LR-Enc – addition of *L. rhamnosus* in capsules.

et al., 2019). De Marins et al. (2022) reported higher a^* values in beef patties with the addition of encapsulated *L. acidophilus.*

The yellowness (b^*) of the beef patties slightly decreased during the storage period (Table 1). At the beginning of the storage period, the values ranged from 8.62 to 10.03, with the LR-Free treatment the highest. However, a decrease was observed for the control (5.75) and LR-Free (9.00) treatments on day 3 of storage (Table 1). In the LP-Free, LP-Enc, and LR-Enc

treatments, a reduction in the *b** values was observed on day 7 (6.27, 6.32, and 6.46, respectively) when compared to the beginning of storage. The probiotic strains and the strategy of probiotic inoculation did not interfere with the yellowness of the beef patties throughout the whole storage period. No differences were found for this parameter between the treated beef patties during all the analyzed periods. However, the control treatment was different from the others during the whole storage period (Table 1). Similar results have been reported by Ben Slima et al. (2018) and Trabelsi et al. (2019) in beef sausages and beef minced meat with probiotic addition.

Lipid oxidation

Lipid oxidation is an important parameter related to the quality of meat products since it can lead to color changes and the presence of undesirable odors and flavors (De Marins et al., 2022). The effects of probiotic strains, the strategy of addition, and the storage period on lipid oxidation in beef patties are shown in Figure 2. At the beginning of storage, the LP-Free treatment showed the highest TBARS values (0.101 mg MDA kg^{-1}). However, at the beginning of storage, no differences were found between the treatments with the addition of free or encapsulated *L. rhamnosus.* At the end of the storage period, the strategy of probiotic incorporation strongly influenced the TBARS values of the beef patties. The TBARS values were between 0.360 and 0.801 mg MDA kg^{-1} (Fig. 2) and were lower in the treatments with the addition of probiotics as free cells than in the control and the treatments with encapsulated probiotics. The antioxidant properties of probiotic strains in meat products were reported by Trabelsi et al. (2019). In another study, Yadav (2017) reported the antioxidant properties of *L. plantarum* in chicken sausages prepared after fermentation of minced chicken meat. In addition, as can be observed in Figure 2, the encapsulation process seems to be a drawback for probiotic antioxidant action. However, according to Roghayeh et al. (2015), TBARS values higher than 2.0 mg MDA kg^{-1} are the threshold for meat rancidity. In this sense, the beef patties in this study showed TBARS values below 0.80 mg MDA kg^{-1} throughout the storage period (10 days), which are far below this limit.

Microbiological properties of beef patties

The microbiological properties of the beef patties with the addition of free and encapsulated probiotics were measured during the storage period, and the results are presented in Table 2. The addition of *L. plantarum* and *L. rhamnosus* resulted in higher initial TVC

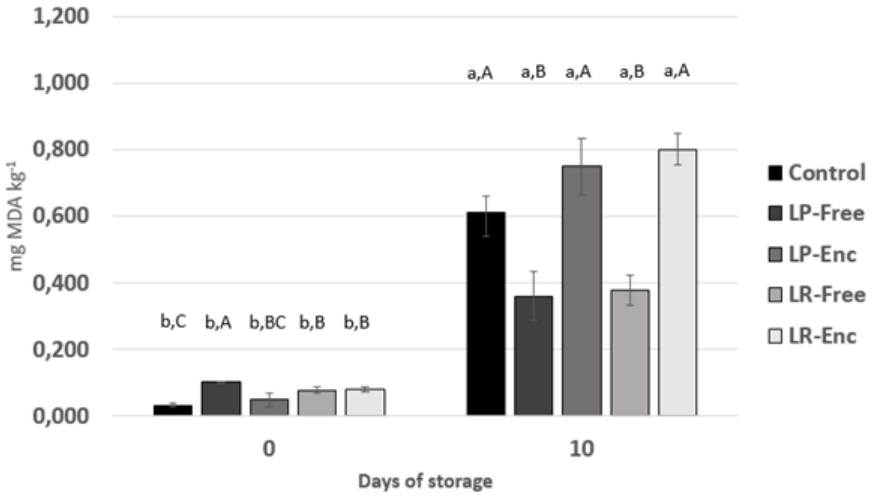

Fig. 2. Lipid oxidation during refrigerated storage of beef patties. Bars represent standard deviation. Different superscript letters indicate statistically significant differences ($P < 0.05$) as a function of storage (a–b) and treatment (A–C)

Table 2. Microbiological parameters during refrigerated storage of beef patties, log CFU g^{-1}

Treatments	Days of storage			
	0	3	7	10
	Total viable counts			
Control	5.67 ±0.03^{a,D}	5.05 ±0.04^{b,E}	4.58 ±0.03^{c,E}	4.46 ±0.03^{d,E}
LP-Free	10.48 ±0.00^{b,C}	10.96 ±0.02^{a,D}	9.08 ±0.03^{c,D}	8.77 ±0.04^{d,D}
LP-Enc	10.43 ±0.02^{c,C}	11.65 ±0.03^{b,C}	11.94 ±0.05^{a,C}	10.52 ±0.05^{c,C}
LR-Free	11.24 ±0.04^{d,B}	11.81 ±0.00^{b,B}	12.80 ±0.02^{a,B}	11.72 ±0.02^{c,B}
LR-Enc	11.66 ±0.01^{d,A}	12.83 ±0.01^{b,A}	13.72 ±0.01^{a,A}	12.40 ±0.01^{c,A}
	Lactic acid bacteria count			
Control	6.17 ±0.05^{a,E}	5.88 ±0.03^{b,E}	5.86 ±0.05^{b,E}	4.77 ±0.07^{c,D}
LP-Free	10.06 ±0.03^{a,D}	9.44 ±0.03^{c,D}	9.95 ±0.02^{b,D}	8.71 ±0.04^{d,C}
LP-Enc	10.86 ±0.04^{a,C}	10.46 ±0.03^{b,B}	10.77 ±0.05^{a,C}	10.25 ±0.03^{c,B}
LR-Free	12.28 ±0.03^{a,A}	9.65 ±0.03^{d,C}	11.83 ±0.03^{b,B}	10.26 ±0.01^{c,B}
LR-Enc	11.84 ±0.03^{c,B}	10.68 ±0.03^{d,A}	12.91 ±0.04^{a,A}	12.27 ±0.01^{b,A}
	Selective *L. plantarum* counts			
Control	3.88 ±0.01^{a,C}	3.28 ±0.04^{b,C}	2.76 ±0.05^{c,C}	2.12 ±0.04^{d,C}
LP-Free	10.26 ±0.02^{a,A}	8.14 ±0.03^{b,B}	6.07 ±0.02^{c,B}	5.93 ±0.02^{d,B}
LP-Enc	9.47 ±0.01^{a,B}	9.13 ±0.02^{b,A}	8.13 ±0.02^{c,A}	8.06 ±0.02^{d,A}
LR-Free	3.27 ±0.02^{a,D}	3.25 ±0.03^{a,C}	2.53 ±0.04^{b,D}	2.10 ±0.01^{c,C}
LR-Enc	2.29 ±0.01^{ab,E}	2.14 ±0.15^{ab,D}	2.35 ±0.05^{a,E}	2.09 ±0.08^{b,C}
	Selective *L. rhamnosus* counts			
Control	4.89 ±0.03^{a,C}	4.86 ±0.02^{a,D}	4.82 ±0.04^{a,C}	3.53 ±0.03^{b,C}
LP-Free	4.83 ±0.04^{a,C}	4.37 ±0.07^{b,C}	4.41 ±0.08^{b,D}	3.56 ±0.07^{c,C}
LP-Enc	4.10 ±0.02^{c,D}	4.46 ±0.04^{b,C}	4.75 ±0.02^{a,C}	3.38 ±0.03^{d,D}
LR-Free	12.40 ±0.02^{a,A}	10.71 ±0.04^{c,B}	11.06 ±0.03^{b,B}	9.96 ±0.02^{d,B}
LR-Enc	11.10 ±0.03^{d,B}	11.97 ±0.02^{b,A}	13.86 ±0.01^{a,A}	11.71 ±0.01^{c,A}

Means ±standard deviations. Different superscript letters in the rows (a–c) and columns (A–E) of each parameter examined indicate statistically significant differences ($P < 0.05$) as a function of treatment and storage process.
Control – no probiotic strains addition, LP-Free – addition of *L. plantarum* as free cells, LP-Enc – addition of *L. plantarum* in capsules, LR-Free – addition of *L. rhamnosus* as free cells, LR-Enc – addition of *L. rhamnosus* in capsules.

values in the probiotic-treated beef patties than in the control treatment. The higher values of TVC in those treatments are a consequence of the direct addition of probiotic strains as free cells or encapsulated forms. This was also reflected in the higher LAB counts when compared to the control treatment (Table 2). The initial LAB counts were between 6.17 and 12.28 log CFU g^{-1}, with the LR-Free treatment showing higher counts. However, even with no direct addition of probiotic strains, counts of approximately 6 log CFU g^{-1}

were observed in the control treatment, indicating that LAB may be part of the microbiota of beef patties.

The selective counts of *L. plantarum* (LPSM) were between 2.29 and 10.26 log CFU g^{-1} on day 0 of refrigerated storage (Table 2). As expected, the LP-Free and LP-Enc treatments showed higher counts of *L. plantarum* when compared to the control, LR--Free, and LR-Enc treatments. Furthermore, the addition of free *L. plantarum* to the beef patties resulted in higher initial counts of that strain when compared to LP-Enc (Table 2). However, both the LP-Free and LP-Enc treatments showed a decrease in *L. plantarum* during storage, and on day 10, the selective counts were between 2.09 and 8.06 log CFU g^{-1}. Nevertheless, the reduction in the *L. plantarum* counts was more pronounced when the addition of free cells was performed, evidencing the protective effect of capsules containing sodium alginate, milk powder, green banana flour, and xanthan gum as wall materials during the refrigerated storage of beef patties.

Regarding the selective counts of *L. rhamnosus*, initial counts were between 4.10 and 12.40 log CFU g^{-1} (Table 2) and, as expected, were higher in the treatments with the addition of that strain. Between days 0 and 3 of storage, LR-Free showed a reduction in *L. rhamnosus* counts (from 12.40 to 10.71 log CFU g^{-1}, respectively), followed by an increase to 11.06 log CFU g^{-1} on day 7, and another reduction to 9.96 log CFU g^{-1} on day 10 (Table 3). The specific *L. rhamnosus* count increased in LR-Enc up to day 7 of storage, but a reduction was also observed on day 10 with counts of 11.71 log CFU g^{-1}. At the end of storage, the counts of *L. rhamnosus* were higher in the LR-Enc treatment, which indicates the protective effect of encapsulation to maintain probiotic viability during the refrigerated storage of beef patties.

Some studies have found high selective probiotic counts (higher than 7 log CFU g^{-1}) in meat products, with a focus on dry-fermented sausages because they are not cooked before consumption (Cavalheiro et al., 2019a; 2020; 2021; Pavli et al., 2020). It is well known that heat is considered a great challenge for the survival of probiotics. However, the use of thermotolerant LAB cultures may improve probiotic viability after heat treatments. In addition, probiotic encapsulation can also help to protect probiotics during stress treatments (Cavalheiro et al., 2019a). In their study, De Marins et al. (2022) related probiotic concentrations higher than 7 log CFU g^{-1}, even after the thermal processing of beef patties with microencapsulated *B. lactis*, *L. plantarum* and *L. acidophilus* addition. These results indicate that, when probiotics are encapsulated, they could be considered a probiotic after thermal processing.

The levels of coagulase-positive staphylococci, thermotolerant coliforms, *E. coli*, *Salmonella*, and reducing-sulfite clostridia were absent or lower than those established by microbiological standards (Brasil, 2019), suggesting good hygienic and sanitary conditions for the manufacture and storage of the beef patties.

CONCLUSION

In this study, we analyzed the use of *L. plantarum* ATCC 7469 and *L. rhamnosus* ATCC 10012 as free cells and in encapsulated forms during the refrigerated storage (10 days) of beef patties. We found that both probiotic strains and strategies of incorporation proved to be suitable for use in beef patties because high probiotic viability was observed, as evidenced by the high LAB and selective *L. plantarum* and *L. rhamnosus* counts. Furthermore, physicochemical properties during the whole storage period were adequated. The probiotic strains also affected the pH of the beef patties and values decreased during the storage period. Regarding the color properties, the addition of probiotics had an impact on the maintenance of the lightness and redness of the beef patties. The control treatment became darker due to lipid oxidation processes. Lipid oxidation was lower in the treatments with probiotics added as free cells. All the beef patties with probiotic addition presented high levels of LAB during the whole refrigerated storage period, and the treatments with specific strain addition also had higher levels of specific probiotic counts. However, at the end of the storage period, the treatments with *L. rhamnosus* addition showed higher specific counts than those with *L. plantarum*. New studies are in progress at our laboratory aiming to improve the thermoresistance of encapsulated probiotics so that the viability of free and encapsulated probiotics can be assessed during the passage of simulated gastrointestinal conditions, as can their viability during the frozen storage of beef patties.

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