Survival of Microencapsulated *Lactococcus lactis* Subsp. *lactis* R7 Applied in Different Food Matrices

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Abstract
Survival of *Lactococcus lactis* subsp. *lactis* R7, microencapsulated with whey and inulin, was analyzed when added to blueberry juice, milk, and cream. For 28 days, cell viability was evaluated for storage (4 °C), simulated gastrointestinal tract (GIT), and thermal resistance. All matrices demonstrated high cell concentration when submitted to GIT (11.74 and 12 log CFU mL⁻¹), except for the blueberry juice. The thermal resistance analysis proved the need for microencapsulation, regardless of the food matrix. The results indicate that *L. lactis* R7 microcapsules have potential for application in different matrices and development of new probiotic products by thermal processing.

Keywords  Symbiotic · Probiotics · Blueberry juice · Milk · Milk cream · Spray drying

Introduction

*Lactococcus lactis* (*L. lactis*) belongs to the group of lactic acid bacteria, often used in dairy fermentation, especially in the production of cheese, yogurt, and similar products. Besides adding flavor to the food by producing organic acids, *L. lactis* promotes the...
preservation of the product, which reinforces its role in the food industry [1]. Among its characteristics for food administration are both the status of being recognized as safe (GRAS) and having probiotic properties [2].

The use of different probiotic-carrier food matrices allows regular intake of probiotics and ensures that their beneficial health effects are maintained [3]. A large number of dairy products have been developed as delivery vehicles for probiotic bacteria, including fermented milk, dairy drinks, ice cream, desserts, cheese, and powdered milk [4]. In addition, soy-based beverages, cereals, fruits, and vegetables have been proposed as new probiotic-carrier products [5].

It should be noted that the characteristics of the food matrix in which the probiotic is inserted may affect not only the viability during processing and storage but also its functional properties, such as susceptibility to gastrointestinal tract conditions [6]. These parameters need to be known and evaluated in probiotic products and carrier matrices, as they play a vital role in process effectiveness [7].

In face of difficulties in handling these microorganisms, it should be considered that probiotic bacteria are generally mesophilic and do not survive in extreme temperatures. In addition, to be effective, the probiotic must be suitable for large-scale preparation, ensuring cell viability [8]. During use and storage, viability and stability are key factors for application in different food matrices, ensuring appropriate technological properties to the product. Survival in the gastrointestinal tract should be ensured since many microorganisms, such as \( L. \text{lactis} \), do not belong to the human intestinal microbiota and, as a consequence, there is a significant reduction in the number of viable cells reaching the colon after oral ingestion [2].

For these reasons, the use of immobilized microbial cells is an advantage when compared to the use of free cells [9]. Encapsulation or entrapment promotes protection of bioactive compounds such as probiotic cells, in addition to targeted delivery to the specific site, in this case the intestine. The particle size is suitable for application in food, so that it does not interfere with sensory characteristics. Also, encapsulation promotes thermal and/or chemical stability of the encapsulate, increases its bioavailability, and decreases the speed of deterioration and loss of functionality during storage [10, 11].

One of the most popular, simple, and economical methods is spray drying, which can be applied to a wide range of nutraceuticals, probiotics, enzymes, and peptides. It is interesting to note that the fast-drying process allows heat-sensitive ingredients to be encapsulated with this technique [12]. Different materials for encapsulation by spray drying have been used in probiotics such as dairy serums, which showed good results in protection, in addition to its nutritional value and low cost [13]. Regarding polysaccharides with prebiotic properties, inulin has been noted for increasing the survival rate of probiotics after drying and through the gastrointestinal tract [14, 15].

Considering that the choice of a new bacterial isolate can contribute not only to the improvement of palatability, processability, and nutritional value of the food, but also to probiotic potential, Jaskulski et al. [14] investigated the action of \( Lactococcus \text{lactis} \) subsp. \( L. \text{lactis} \) R7 (\( L. \text{lactis} \) R7), ricotta cheese isolate, in the prevention of rectal colon cancer in rats. After 20 weeks, \( L. \text{lactis} \) R7 demonstrated probiotic potential, considering that most animals had benign atypia of intestinal crypts and large lymphocytic infiltrates, with an improvement of host immune response. In addition, the isolate presented the ability to stabilize weight gain and reduce abdominal adiposity when compared to the control group.

Rosolen et al. [15] evaluated symbiotic microcapsules containing \( L. \text{lactis} \) R7 using whey and inulin as encapsulating materials by spray drying. The microcapsules produced showed high cell viability (13 log CFU g\(^{-1}\)) and high encapsulation efficiency (94.61%).
In addition, the microcapsules showed stability during 6 months of storage at the different temperatures analyzed. The authors report that microencapsulation protected *L. lactis* R7 when passing through the simulated gastrointestinal tract as well as from heat treatment, demonstrating that the encapsulating materials were adequate and effective to the microencapsulation process by spray drying, with potential for application in food.

Based on these considerations, the aim of this study was to evaluate the survival of *Lactococcus lactis* subsp. *lactis* R7, microencapsulated with whey and inulin, in different food matrices (blueberry juice, reconstituted milk, and milk cream), as well as storage viability, passage through the simulated gastrointestinal tract, and resistance to heat treatment.

**Material and Methods**

**Preparation of Food Matrices**

Commercial blueberry pulp was reconstituted according to its manufacturer’s instructions (Sítio Bello, Brazil®), with a median composition of 0.4% protein, 0.3% fat, and 14.5% carbohydrate. The juice was vacuum-filtered twice (PrismaTec, Brazil) to reduce the amount of suspended solids. The commercial whole milk powder used (CCGL, Brazil®) had a median composition of 26% protein and fat and 38% carbohydrate. According to the manufacturer, it was prepared from reconstitution in 10% sterile distilled water (w v⁻¹). Both the juice and the milk were heat-treated at 65 °C for 30 min. The milk cream that was purchased (Nestlé®) was ultra-pasteurized (UHT), with a median composition of 0% protein and carbohydrate and 20% fat, according to the manufacturer. All matrices were kept refrigerated at 4 °C in light-protected vials.

**Culture Conditions of Lactococcus lactis Subsp. lactis R7**

*Lactococcus lactis* subsp. *lactis* R7 was isolated from ricotta cheese and deposited in GenBank (KF879126). The isolate had previously shown probiotic potential in preliminary tests in vivo and in vitro [16].

Cultivation was performed according to Rosolen et al. [15]. *Lactococcus lactis* subsp. *lactis* R7 (*L. lactis* R7) was reactivated in whey, which was reconstituted in sterile distilled water at 6% (w v⁻¹), previously heat-treated at 65 °C for 30 min using an on orbital shaker (CERTOMAT BS-1, Germany) at 150 rpm, 37 °C for 16 h, under anaerobiosis. Afterwards, the inoculum was added at a concentration of 3% in a bench top bioreactor (BIOSTAT B—New Brunswick, Edison, NJ, USA) to a 2-L tank containing 2 L of whey, kept at 37 °C for 16 h at 100 rpm, in anaerobic conditions. The cells were centrifuged at 2370×*g* for 10 min at 4 °C, and the pellet was washed in phosphate-buffered saline (PBS, 10 mM, pH 7.0), resuspended in 100 mL of PBS and kept under refrigeration at 4 °C.

**Microencapsulation by Spray Drying**

Based on previous experiments [17], we chose a mixture of 12% whey with a mean composition of 11% protein, 1.5% fat, 6% mineral salts, and 3% humidity, according to the manufacturer (Relat—Estação, RS, Brazil), 10% long-chain inulin fraction (DP 10–60), and 1.25% aerosil (Farma-química, Malaga, Spain), with water as the encapsulating solution,
which was dissolved in sterile distilled water resulting in a total solid content of 23.25% (w v⁻¹), followed by heat treatment at 65 °C for 30 min and dissolved in an orbital shaker at 150 rpm at 25 °C for 16 h. The L. lactis R7 cell suspension was added to the encapsulating solution and maintained homogenized by a magnetic stirrer. For the free cells, the biomass was resuspended in sterile distilled water, and both feed solutions were subjected to the drying process using a spray dryer (LabMaq—MSDi 1.0, São Paulo, SP, Brazil). The operational parameters used were 100 °C of inlet temperature and 68 °C of outlet, with feed flow of 0.25 L h⁻¹ and drying air flow of 3.00 m³ min⁻¹. The final dried product was collected in sterile vials and stored at −20 °C.

Stocking of Food Matrices

For each 100 mL of blueberry juice, milk, and milk cream, 1 g of L. lactis R7 microcapsules (approximately 12 log CFU g⁻¹) was added, in accordance with Seyedain-Ardabili et al. [16]. Similarly, free cells (control) were inoculated at a concentration of 4% (w v⁻¹) in each food matrix (approximately 10 log CFU mL⁻¹) in accordance with Pimentel et al. [17] with modifications. In both conditions, the matrices were mixed manually until complete homogenization and kept under refrigeration at 4 °C ± 1 for 28 days, as well as protected from light. The matrices were subjected to cell viability analysis on days 0, 7, 14, 21, and 28 of storage.

Viability of Free and Microencapsulated L. lactis R7 During Storage

Viability of free and microencapsulated L. lactis R7 in food matrices was performed in accordance with Ortakci and Sert [18], with modifications, at 0, 7, 14, 21, and 28 days of storage. For counting, 0.1 mL aliquot of the free and microencapsulated L. lactis R7 containing matrices was serially diluted and plated on Man, Rogosa, and Sharpe (MRS) agar, incubated at 37 °C for 48 h in anaerobic conditions, and the concentration of viable cells expressed in log CFU per milliliter.

pH Analysis of Food Matrices

The pH measurements were performed at 0, 7, 14, 21, and 28 days of storage using a digital pH meter (KASVI, China).

Survival Evaluation of Free and Microencapsulated L. lactis R7 Exposed to Simulated Gastrointestinal Conditions

After 24 h of storage of free and microencapsulated L. lactis R7 in milk, juice, and in milk cream, the survival rate was assessed during 30, 60, and 120 min of exposure to simulated gastric fluids and 240 min to simulated intestinal fluids. The test was performed as proposed by Ranadheera et al. [19], with modifications. For this, 0.1 mL of each matrix was exposed to 0.9 mL of gastric and intestinal juice. Gastric simulation was prepared using 3.0 mg mL⁻¹ porcine gastric mucosal pepsin (Chem Supply, Australia) and 0.5% saline (w v⁻¹), acidified with HCl to pH 2.0, 2.5, and 3.0. Intestinal simulation was prepared with 1.0 mg mL⁻¹ pancreatin (Sigma-Aldrich, USA) and 0.5% saline solution (w v⁻¹), and the pH was adjusted to 8.0 with or without bovine bile (0.5%). Both solutions
were sterilized by 0.22-μm membrane filtration (Sartorius Stedim Biotech, GmbH, Goettingen, Germany). Viable cell counts were performed with serial dilution and plating on MRS agar, incubated at 37 °C for 48 h under anaerobic conditions.

**Thermal Resistance**

Thermal resistance of free and microencapsulated *L. lactis* R7 in the food matrices was performed according to Pinto et al. [20], with modifications. Two milliliters of milk, juice, and milk cream, containing free and microencapsulated cells, was transferred to test tubes using temperatures of 60, 65, and 70 °C for 0, 5, 10, 15, and 30 min in a thermostatic bath (QUIMIS, Brazil) and immediate cooling by immersion in an ice bath for 10 min. Aliquots were collected and the viable cell counts were determined by serial dilution in 0.1% (w/v) peptone water, plated on MRS agar, and incubated at 37 °C for 48 h in anaerobic jars. The concentration of viable cells was expressed as log CFU per gram.

**Statistical Analysis**

The data were submitted to analysis of variance (ANOVA) using the GraphPad Prism 7 program followed by the Tukey test to compare means at a level of 95% (*p* < 0.05) of significance. The analyses were performed in triplicate, and the results of the microencapsulation were calculated with the means of three independent experiments.

Results related to obtaining and testing the microcapsule used in this study were presented in another study previously published by the same research group [17].

**Results and Discussion**

**Viability and pH of Free and Microencapsulated *L. lactis* R7 in Different Food Matrices During Storage**

In view of improving the stability of probiotic bacteria, microencapsulation is presented as one of the most efficient solutions, not only for maintaining cell viability during processing and storage, but also for guaranteeing its activity in the digestive tract [6]. The microcapsule used in this study has previously been characterized [17], presenting adequate size (12.73 μm) and morphology for application in food.

The results show that the acidity of blueberry juice (pH 3.0 ± 0.1) (Fig. 1a) had damaging effects on free cells, resulting in viability loss below the minimum value to be considered probiotic [21] after 14 days of storage at 4 °C, as well as a marked reduction in cell viability (3 log CFU mL⁻¹) observed at 7 days of storage.

When the microcapsules added to the blueberry juice were analyzed, the concentration of viable cells was 5.00 ± 0.50 log CFU mL⁻¹, indicating that the cell content was exposed from the loss of capsule integrity. This effect is perceived up to 14 days of storage at 4 °C, when it presents a cell concentration of 10.30 ± 0.25 log CFU mL⁻¹, with loss of viability only at 21 days.

Thus, this demonstrates that the exposure time to the blueberry juice acidity is determinant in the microcapsule integrity loss and microorganism exposure, corroborating the
results presented by Rosolen et al. [15]. Miranda et al. [22] showed that microencapsulation of *Lactobacillus casei* with sodium alginate had no protective effect on orange juice (pH 3.74–3.92) when compared to free cells. The pH variations were observed (Fig. 1a), and no statistical difference was noted during the analyzed period, indicating that the presence of metabolizable substrates in the blueberry juice was not sufficient to guarantee the viability of *Lactobacillus lactis* R7 free cells in an acidic environment over the long term. It needs to be emphasized that the viability of probiotics in fruit juices is affected by strain, microbial culture preparation method, inoculated cell status, storage temperature, oxygen level, and fiber presence [23]. Mokhtari et al. [24] evaluated pH changes in grape juice and found that *Lactobacillus acidophilus*–free cells had the greatest reduction in pH over 60 days of storage at 4 °C, significantly decreasing from 3.8 to 3.21 (*p* < 0.05).

Regarding free cells incorporated into milk (Fig. 1b), there was a reduction in cell concentration of 3.08 log CFU mL⁻¹ at the end of 28 days of storage, while for microcapsules, it remained stable (*p* < 0.05). The physicochemical characteristics of milk associated with
pH near neutrality were sufficient to promote the maintenance of microcapsules in the storage. Shi et al. [23] obtained similar results for storing *Lactobacillus bulgaricus* microcapsules in milk at 4 °C, with complete preservation for 30 days. A reduction of free cells was reported from 10 to 6.88 log CFU mL⁻¹ over the period, showing that microencapsulation significantly increases bacterial stability in refrigerated systems.

Free cells promoted higher acidification (*p* <0.05) in milk when compared to microcapsules, demonstrating the ability of *L. lactis* R7 to use naturally occurring disaccharides in milk [3]. The lower acidification of dairy products containing microencapsulated microorganisms suggests that the encapsulation process was effective in physically trapping the material of interest [13, 25].

Due to the high fat content, the milk cream (Fig. 1c) proved to be an adequate delivery vehicle for probiotic bacteria, given the high content of total solids, which helps to maintain cell viability. Free cells reduced 1.00 log CFU mL⁻¹ over the first 7 days and were stable at 8.70 log CFU mL⁻¹ (*p* >0.05) during 28 days of storage. Microcapsules incorporated into the milk cream had a cell concentration of 8.48 log CFU mL⁻¹ from day 7 on, remaining stable during 28 days of storage. Considering that there are no reports in the literature using the matrix of the present study, it was compared to the research by Vasile et al. [26], which used soft cheese as food matrix. For this, the cell viability of *Lactobacillus casei* 461 was evaluated for storage at 4 °C and a reduction of 2 cycle logarithmic (5.47 log CFU g⁻¹) was observed at the end of 14 days, not having minimum probiotic viability (<6 log CFU g⁻¹). The microcapsules showed an increased 0.5 cycles at the end of the same period, indicating concentrations lower than the present study. This demonstrates that the combination of the materials used (inulin and serum), associated with the spray drying technique, was effective in protecting *L. lactis* R7.

The pH near the neutrality of the milk cream, as well as the milk, was not sufficient for the total loss of microcapsule integrity and exposure of *L. lactis* R7 in the matrix. The pH values of free and microencapsulated cells were significantly different (*p* <0.05), 1.68 and 0.94, respectively.

It is worth highlighting that viability data for individual food matrices are available for some probiotic strains [7, 27–29]. However, comparing multiple matrices with respect to storage-free cell viability [19, 30] under similar conditions is less frequent [31], and the occurrence is even lower for microencapsulated cells under the same conditions [32].

**Survival Assessment of Free and Microencapsulated L. lactis R7 Applied in Blueberry Juice, Milk, and Milk Cream When Exposed to Simulated Gastric Fluid**

Different behaviors were observed among *L. lactis* R7 free and microencapsulated cells when added to blueberry juice, milk, and milk cream matrices and then submitted to gastric simulation, as shown in Fig. 2.

When added to blueberry juice (Fig. 2a), free cells do not show probiotic viability [21] after 60 min of gastric simulation at pH 2.0 and 2.5, not being detected at all conditions from 120 min on, suggesting their sensitivity to HCl and pepsin in gastric juice [15]. However, the microcapsules showed high cell concentration after 120 min of gastric juice, evidencing the protection conferred by the encapsulating material. Different studies report that whey and milk proteins have technological properties such as buffering capacity, good emulsification, and ability to form networks even at low concentrations, ensuring good survival during digestion [33, 34]. The study shows that the
polyphenols present in the pineapple used in their research, also present in blueberries in the present study, can form complex compounds with dairy proteins, i.e., whey through hydrogen bonds and hydrophobic interaction, thus increasing the stability of the pepsin-induced process [35].

Inulin as an encapsulating material is also added. Due to its low solubility in water, the result may be a longer period of time for powder rehydration and consequently a slower release of encapsulated bacterial cells [22]. Amakiri and Thantsha [32] noted that the addition of inulin as encapsulating material improved *Bifidobacterium longum* Bb46 performance during gastric fluid exposure to free cells.

When evaluating resistance, free and microencapsulated *L. lactis* R7 cells inserted in milk (Fig. 2b) showed similar behavior ($p > 0.05$) at 30 min of gastric simulation at all conditions. However, after 120 min, there was loss of capsule integrity and microorganism
exposure (> 10 log CFU mL⁻¹) observed at all pH levels, demonstrating that stress conditions (pH and enzymes) result in loss of microcapsule integrity.

The ability to tolerate digestive stress is one of the important properties for incorporating probiotics into food matrices, as food can protect the microorganism from gastric fluids [36]. With the milk cream (Fig. 2c) as carrier matrix, it was observed that the microcapsule showed high viability (> 11.65 log CFU mL⁻¹) when compared to the free cell (> 7.00 log CFU mL⁻¹) (p < 0.05) at all times and pH values analyzed. Values below the ones found in the present study were reported by Martins et al. [35], who analyzed the viability of passage through in vitro gastrointestinal tract of cell-free *Lb rhamnosus* in goat cheese. The authors observed that, in 7 days of storage at 4 °C, cell concentration reduced 4.8 log CFU g⁻¹ after 120 min (pH 2.33), no minimum probiotic count.

The results support the hypothesis that the application of microencapsulated probiotics in food matrices may represent a strategy to promote acid pH tolerance through gastric tract passage [31].

**Survival Assessment of Free and Microencapsulated *L. lactis* R7 Exposed to Simulated Intestinal Fluid**

The survival of *L. lactis* R7 free and microencapsulated cells in the different food matrices was analyzed during 4 h of exposure to intestinal fluids in the absence (Fig. 3a) and presence of bile salts (Fig. 3b).

Free cells presented the lowest cell concentrations when compared to microencapsulated cells in all food matrices (p < 0.05), showing the highest sensitivity in the presence of bile salts. The antimicrobial nature of bile salts is related to its detergent property, which dissolves microorganism membranes, and its amphiphilic nature makes it strongly inhibitory for the gastrointestinal tract [37].

Still, they exhibited the best performance as a carrier for free *L. lactis* R7, with cell concentration in the absence and presence of bile salts of 9.3 ± 0.30 and 8.18 ± 0.18 log CFU mL⁻¹, respectively. The other matrices had lower viability as free cell carriers, but still had minimum probiotic value (< 6 log CFU mL⁻¹). Different studies have shown that the food matrix has significant influence on in vitro gastrointestinal tolerance of different probiotics exposed to low pH and bile salts [33, 38].

When submitted to intestinal fluids in the absence of bile salts, the microencapsulated microorganism presents lower cell concentrations if compared to the presence of bile salts, especially when applied in blueberry juice. Pancreatin and bile salts are determinants for the loss of capsule integrity and exposure of *L. lactis* R7.

The action of enzymes and bile salts is controlled by the ability to identify emulsion interfaces, which is controlled by the size of the emulsion and interfacial composition, that is, its structure, thus impacting the type of food matrix in which the probiotic is inserted and its viability [39].

Although *L. lactis* R7 did not have intestinal origin, the high survival can be explained by the potential of some bacteria as antagonists to specific adverse environments. Bacteria can respond to changes in the environment via metabolic reprogramming, leading to increased resistance [33].

The findings of the study showed that microencapsulation with whey and inulin may increase the protection of *L. lactis* R7 when submitted to gastric and intestinal fluid
survival tests, as well as ensure better cell protection regardless of the physicochemical characteristics of the carrier matrix [34].

**Evaluation of the Thermal Resistance of Free and Microencapsulated L. lactis R7 in Milk Cream, Milk, and Blueberry Juice**

For probiotic cells to be effective and remain viable in food and beverages, they must withstand the recommended pasteurization temperatures and/or other industrial processing parameters [4]. The search for suitable materials that increase the thermal resistance of probiotics is also considered important, in order to facilitate their incorporation in food matrices. In addition, the developed encapsulation system should act as an isolation environment for probiotic cells [9].
Table 1  Effect of heat treatment on *L. lactis* R7 free and microencapsulated in food matrices

| Temp. (°C) | Time (min) | Number of viable cells (log CFU mL⁻¹) | Blueberry juice | Milk | Milk cream |
|------------|------------|---------------------------------------|-----------------|------|------------|
|            |            |                                       | Free cell       | Microcapsule | Free cell | Microcapsule | Free cell | Microcapsule | Free cell | Microcapsule |
| 60         | 0          | 10.18±0.30 aA                         | NR              | 10.18±0.30 a | NR       | 10.18±0.30 a |
|            | 5          | 9.00±0.20 bD                          | 9.60±0.15 bC    | 9.40±0.20 bA | 8.00±0.10 bB | 9.50±0.15 bA |
|            | 10         | 7.30±0.12 cE                          | 9.70±0.21 bB    | 8.030±0.15 cB | 8.18±0.20 bB | 7.60±0.10 bB |
|            | 15         | 6.90±0.15 cF                          | 10.00±0.10 bA   | 6.90±0.30 dD  | 8.54±0.10 bC | 7.20±0.20 bB |
|            | 30         | VC                                    | 10.10±0.20 bA   | VC             | 9.18±0.30 aA | VC         |
| 65         | 0          | 10.18±0.30 aA                         | NR              | 10.18±0.30 aB | NR       | 10.18±0.30 a |
|            | 5          | 9.48±0.18 bA                          | 9.81±0.15 bA    | 9.10±0.18 bC  | 8.95±0.10 cC | 9.10±0.30 bD |
|            | 10         | 8.30±0.12 cB                          | 10.00±0.22 bA   | 7.40±0.10 cD  | 9.18±0.18 cC | 8.00±0.20 bE |
|            | 15         | VC                                    | 10.10±0.10 bA   | VC             | 10.48±0.22 bB | VC         |
|            | 30         | VC                                    | 10.50±0.30 bA   | VC             | 11.90±0.35 aA | VC         |
| 70         | 0          | 10.18±0.30 aA                         | NR              | 10.18±0.30 aB | NR       | 10.18±0.30 a |
|            | 5          | VC                                    | 10.00±0.18 bA   | VC             | 10.80±0.10 bA | 6.65±0.15 bE |
|            | 10         | VC                                    | 10.50±0.10 bA   | VC             | 8.85±0.10 bC  | 9.78±0.28 bA |
|            | 15         | VC                                    | 10.10±0.15 bA   | VC             | 8.00±0.22 bC  | VC         |
|            | 30         | VC                                    | 9.90±0.20 bA    | VC             | 7.40±0.18 bD  | VC         |

VC, viable cells < 6 log CFU g⁻¹; NR, microcapsule not ruptured. a–dMeans± standard deviation with different lowercase superscript letters in the same column indicate significant differences (p<0.05) for the same sample in the same temperature evaluated.
Microencapsulation use in the protection of probiotics applied to heat-treated food matrices has been described by different authors as an indispensable technique for application in the food industry [22, 40].

Regarding the formulation and/or preparation of food products using heat, the thermo-tolerance of microencapsulated *L. lactis* R7 was evaluated [6] according to Table 1. Results showed the protective effect of microencapsulation when analyzing the thermal resistance, independent of the food matrix, compared to free cells. Free cells showed a reduction in viable cell count of 3.28 log cycles for blueberry juice and milk and of 2.98 log cycles for milk cream after a 15-min treatment at 60 °C. After 30 min, viability was less than 6 log, showing no probiotic effect. The same behavior was observed at 65 °C. However, no survival was observed after 10 min of exposure. It is noteworthy that *L. lactis* R7 free, at 70 °C, did not present viability at all times analyzed. This corroborates the results obtained by Pinto et al. [20], who found a decrease in *Bifidobacterium* BB-12 free cells by 2.57 log cycles after 5 min of heat treatment at 60 °C. The excessive heat affects the structure of macromolecules such as proteins and nucleic acids of bacterial cells, causing the breakdown of the bond between monomeric units and destruction of monomers, leading to cell death [41].

When applied to *L. lactis* R7 microcapsules in blueberry juice, after 30 min of heat treatment at different temperatures, increased cell viability was observed, demonstrating that the time/temperature binomial was effective in disintegrating encapsulating material and exposure of the microorganism, but without cellular damage. For milk, it was observed that at 60 °C, there was no disruption and total exposure of the microorganism after 30 min, since the concentration of viable cells increased as a function of exposure time. At 65 °C, high cell viability was observed after 30 min, unlike the temperature of 70 °C, at which cell viability decreased as exposure time increased.

In milk cream, high cell concentration was observed after 30 min at 65 °C and after 15 min at 70 °C. As far as we know, there are no reports in the literature of microencapsulated probiotics applied to food matrix and tested for thermal resistance. For comparative purposes, the study by Malmo et al. [30] microencapsulated *Lactobacillus reuteri* DSM17938 by spray drying and used alginate and chitosan as wall materials, applying free and microencapsulated cells in chocolate soufflé. The authors observed a survival rate of 10% of microencapsulated cells when submitting the matrix to cooking at 180 °C for 10 min (80 °C inside the dough), thus not obtaining a probiotic product. The same authors report that the disintegration/collapse of microcapsules after treatment at 80 °C led to the release of cells with their consequent cell death. It was different in the present study, in which the highest temperature evaluated was not able to promote total microcapsule disruption and exposure of the microorganism, since high probiotic concentrations were observed in juice and milk cream.

Some specific genes and/or proteins are related to the tolerance of heat probiotics. It has been reported in the literature the improvement of this tolerance with treatments that expose these microorganisms to moderate heat, as it occurs with the microencapsulation technique [42–44]. The results obtained in the present study indicate the need for *L. lactis* R7 microencapsulation when the food is subjected to heat treatment, such as the pasteurization process applied to various foods. According to Tárrega et al. [42], the high polymerization inulin, used in the present study, is thermally stable and poorly soluble in water, thus offering greater protection to heat treatment.

In conclusion, food matrices such as blueberry juice, reconstituted milk, and milk cream are suitable for maintaining the viability of microencapsulated *L. lactis* R7, using inulin and whey encapsulants stored at 4 °C. Furthermore, regardless of the food matrix, the
encapsulating material influenced the protection of microcapsules under the conditions of the simulated gastrointestinal tract and thermal resistance. Therefore, the results show that *L. lactis* R7 microcapsules have potential for application in different matrices and in the development of new probiotic products using thermal processing.

**Author Contribution**  MR, WS, and SP conceived and designed this research. AF, PO, and FC contributed new reagents or analytical tools. MR, GL, and FB performed the experiments. All authors analyzed the data. MR and FB wrote the manuscript. All authors read and approved the final version of the manuscript.

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**Data Availability**  Not applicable.

**Code Availability**  Not applicable.

**Declarations**

**Ethics Approval**  Not applicable.

**Consent to Participate**  The authors declare that they consent to participate.

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**Conflict of Interest**  The authors declare no competing interests.

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