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 applied in 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 described high cell concentration when submitted to GIT (11.74 and 12 log CFU.mL-1), except for 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 in the development of new probiotic products by thermal processing.

1. 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 is the status of being recognized as safe (GRAS) and of 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 capable of 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 of the gastrointestinal tract should be ensured since many microorganisms, such as *L. 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]. 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 [10]. Different

materials for encapsulation by spray drying have been used in probiotics such as dairy serums, which showed good results in the protection in addition to its nutritional value and low cost [11]. Regarding polysaccharides with prebiotic properties, inulin has been noted for increasing the survival rate of probiotics after drying and during passage to the gastrointestinal tract [12, 13].

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, as well as presenting probiotic potential, Jaskulski et al. (2020) investigated the action of *Lactococcus lactis* subsp. *lactis* R7 (*L. lactis* R7), ricotta cheese isolate, in protection against rectal colon cancer in rats. After 20 weeks *L. lactis* R7 demonstrated probiotic potential, considering that most animals had benign atypias of intestinal crypts and large lymphocytes infiltrate demonstrating 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. 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 six months of storage at the different temperatures analyzed. The authors report that microencapsulation protected *L. lactis* R7 from 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, applied in different food matrices (blueberry juice, reconstituted milk, and milk cream) and evaluated for storage viability, passage through the simulated gastrointestinal tract, and resistance to heat treatment.

2. Material And Methods

2.1 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.

2.2 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* [14].

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 buffer and kept under refrigeration at 4°C.

2.3 Microencapsulation by spray drying

Based on previous experiments [15], 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), in water as the encapsulating solution, which was dissolved in sterile distilled water resulting in a total solids 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 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 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 3.00 $m^3.min^{-1}$. The final dried product was collected in sterile vials and stored at −20°C.

2.4 Stocking of food matrices

For each 100 mL of blueberry juice, milk and milk cream was added 1 g of *L. lactis* R7 microcapsules (approximately 12 log $CFU.g^{-1}$) 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 e 28 of storage.

2.5 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 mL^{-1}.

2.6 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).

2.7 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, in 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.

2.8 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 mL of milk, of juice, and of milk cream containing free and microencapsulated cells were 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% ($w.v^{-1}$) 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 $CFU.g^{-1}$.

2.9 Statistical analysis

The data were submitted to analysis of variance (ANOVA) using the Graphpad Prism 7 program followed by the Tukey's 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.

3. Results And Discussion

3.1 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 to guarantee its activity in the digestive tract [6]. The microcapsule used in this study has previously been characterized [15], presenting adequate size (12.73μm) and morphology for application in food.

The results show that the acidity of blueberry juice (pH 3.0 ± 0.1) (Figure 1 a) 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 acidity of blueberry juice is determinant for the loss of microcapsule integrity 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 (Figure 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 *L. 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 greatest reduction in pH during 60 days of storage at 4 °C decreased significantly from 3.8 to 3.21 ($p<0.05$).

Regarding free cells incorporated into milk (Figure 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. [25] 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 log CFU.mL^{-1} 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 [11, 26].

Due to the high fat content, the milk cream (Figure 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, remaining stable during 28 days of storage. Considering that there are no reports in the literature using the matrix of the present study, the research was compared to the research for Vasile et al. [27] which used as a food matrix for soft cheese. For this, the cell viability of *Lactobacillus casei* 461 was evaluated for storage at 4 °C and a reduction of 2 cycles 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, which demonstrates that the combination of the materials used (inulin and serum) associated with the technique spray drying 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 ($p < 0.05$), being 1.68 and 0.94, respectively.

It is worth highlighting that viability data for individual food matrices are available for some probiotic strains [7, 28–30]. However, comparing multiple matrices with respect to storage-free cell viability [17, 31] under similar conditions is less frequent [32] and, the occurrence is even lower for microencapsulated cells under the same conditions [33].

3.2 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, to milk, and to milk cream matrices and then submitted to gastric simulation, as shown in Figure 2.

When added to blueberry juice (Figure 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 [13]. 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 [19, 34]. Added to this is the presence of inulin as an encapsulating material, which due to its low solubility in water may result in a longer time for powder rehydration and consequently a slower release of encapsulated bacterial cells [20]. Amakiri and Thantsha [35] noted that the addition of

inulin as encapsulating material improved *Bifidobacterium longum* Bb46 performance during gastric fluid exposure to free cells.

Free and microencapsulated *L. lactis* R7 cells inserted in milk (Figure 2b) showed similar behavior ($p > 0.05$) when evaluating resistance at 30 min of gastric simulation at all conditions. However, after 120 min, there was loss of capsule integrity and microorganism exposure (> 10 log $CFU.mL^{-1}$) were observed at all pH levels, demonstrating that stress conditions (pH and enzymes) promote 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 (Figure 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 that found in the present study were reported by Martins et al. [37] who analyzed the viability of the passage to the gastrointestinal tract in vitro of free cells *Lb rhamnosus* applied to goat cheese. The authors observed that in 7 days of storage at 4 °C the cell concentration reduced 4.8 log $CFU.g^{-1}$ after 120 minutes (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 for the promotion of acid pH tolerance during gastric tract passage [32].

3.3 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 (Figure 3a) and presence of bile salts (Figure 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 its strong inhibitory for the gastrointestinal tract [38].

Still, 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 food matrix has significant influence on *in vitro* gastrointestinal tolerance of different probiotics exposed to low pH and bile salts [19, 39].

The microencapsulated microorganism when submitted to intestinal fluids in the absence of bile salts presents lower cell concentrations when compared to the presence of bile salts, especially when applied in blueberry juice. The presence of pancreatin and bile salts are determinant 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 [40].

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 [19]. 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].

3.4 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].

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 [20, 41].

Regarding the formulation and/or preparation of food products using heat, the thermotolerance of microencapsulated *L. lactis* R7 was evaluated [6] according to the 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 15 min treatment at 60 °C. After 30 min, viability was less than 6 log, showing no probiotic effect. Same behavior was observed at 65 °C, however no survival was observed after 10 minutes 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 [42].

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 encapsuling 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. [33] 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 when submitting the matrix to cooking at 180 °C by 10 min (80 °C inside the dough), a survival rate of 10% of microencapsulated cells, 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. Unlike the present study in which the highest temperature evaluated was not able to promote total disruption of the microcapsule 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 to improve this tolerance with treatments that expose these microorganisms to moderate heat, as it occurs with the microencapsulation technique [43–45]. 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. [46], 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.

Declarations

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Tables

Table 1. Effect of heat treatment on *L. lactis* R7 free and microencapsulated in food matrices

Temp. (^{0}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
60	0	10.18 ± 0.30^{aA}	NR	10.18 ± 0.30^{a}	NR	10.18 ± 0.30^{a}	NR
	5	9.00 ± 0.20^{b,D}	9.60 ± 0.15^{b,C}	9.40 ± 0.20^{b,A}	8.00 ± 0.10^{b,B}	9.50 ± 0.15^{b,A}	6.70 ± 0.21^{d,C}
	10	7.30 ± 0.12^{c,E}	9.70 ± 0.21^{b,B}	8.030 ± 0.15^{c,B}	8.18 ± 0.20^{b,B}	7.60 ± 0.10^{c,B}	7.23 ± 0.12^{c,B}
	15	6.90 ± 0.15^{c,F}	10.00 ± 0.10^{b,A}	6.90 ± 0.30^{d,D}	8.54 ± 0.10^{b,C}	7.20 ± 0.20^{c,B}	8.00 ± 0.30^{b,B}
	30	VC	10.10 ± 0.20^{b,A}	VC	9.18 ± 0.30^{a,A}	VC	9.40 ± 0.15^{a,A}
65	0	10.18 ± 0.30^{a,A}	NR	10.18 ± 0.30^{a,B}	NR	10.18 ± 0.30^{a}	NR
	5	9.48 ± 0.18^{b,A}	9.81 ± 0.15^{aA}	9.10 ± 0.18^{b,C}	8.95 ± 0.10^{c,C}	9.10 ± 0.30^{b,D}	9.48 ± 0.18^{c,D}
	10	8.30 ± 0.12^{c,B}	10.00 ± 0.22^{aA}	7.40 ± 0.10^{c,D}	9.18 ± 0.18^{c,C}	8.00 ± 0.20^{c,E}	11.30 ± 0.30^{b,C}
	15	VC	10.10 ± 0.10^{a,A}	VC	10.48 ± 0.22^{b,B}	VC	11.40 ± 0.20^{b,B}
	30	VC	10.50 ± 0.30^{a,A}	VC	11.90 ± 0.35^{a,A}	VC	12.00 ± 0.10^{a,A}
70	0	10.18 ± 0.30^{a,A}	NR	10.18 ± 0.30^{a,B}	NR	10.18±0.30^{a,A}	NR
	5	VC	10.00 ± 0.18^{b,A}	VC	10.80 ± 0.10^{a,A}	6.65 ± 0.15^{b,E}	9.78 ± 0.28^{c,A}
	10	VC	10.50 ± 0.10 ^{a,A}	VC	8.85 ± 0.10^{b,C}	VC	11.48 ± 0.10^{b,C}
	15	VC	10.10 ± 0.15^{b,A}	VC	8.00 ± 0.22^{c,D}	VC	12.10 ± 0.10^{a,B}

	30	VC	9.90 ± 0.20^{b,A}	VC	7.40 ± 0.18^{d,E}	VC	9.18 ± 0.30^{d,D}

VC = Viable cells < 6 log CFU.g^{-1}. NR=Microcapsule not ruptured. $^{a-d}$ Means ± standard deviation with different lowercase superscript letters in the same column indicate significant differences ($p < 0.05$) for same sample in the same temperature evaluated

Figures

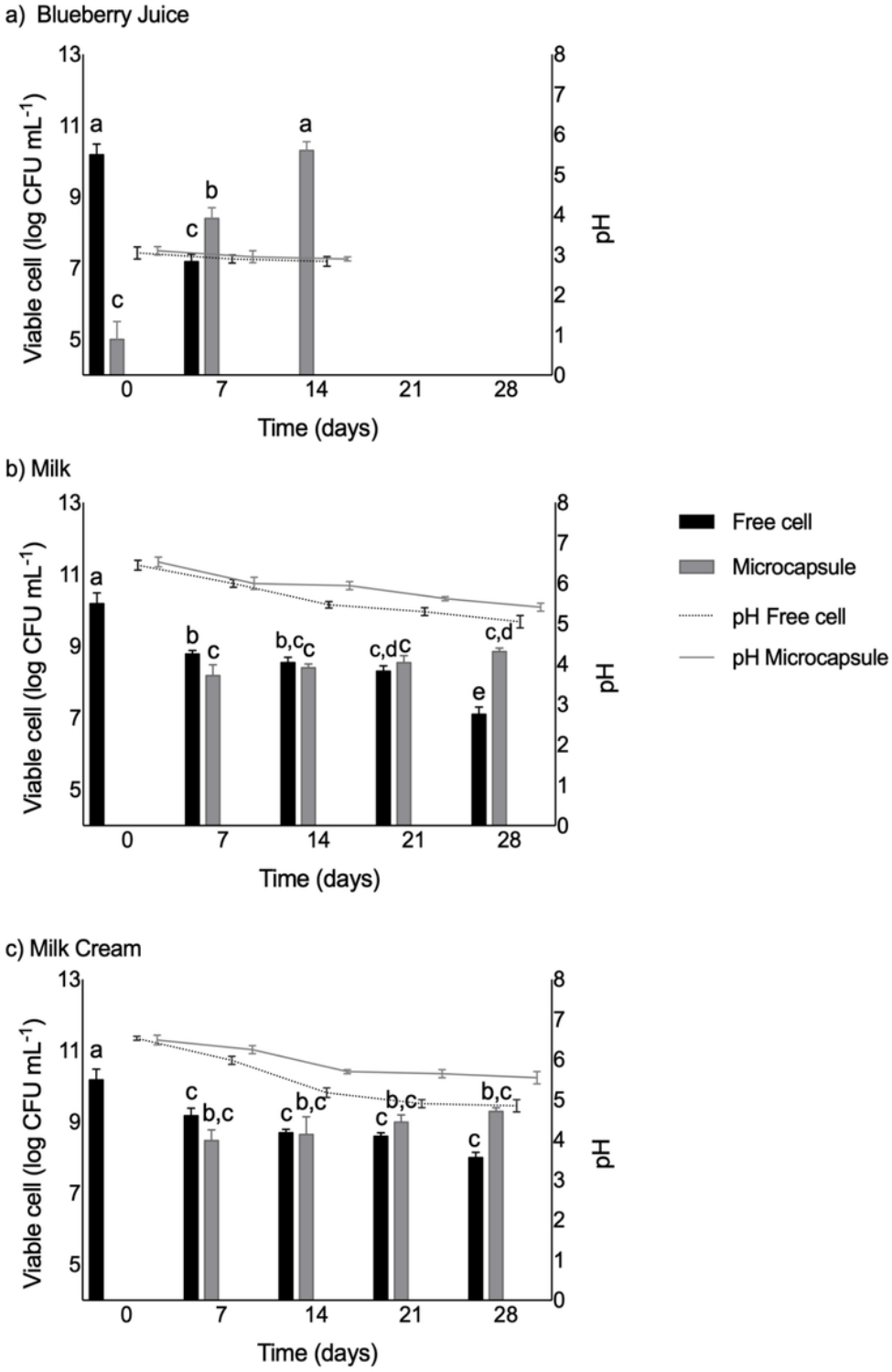

Figure 1

Cell viability of free and microencapsulated L. lactis R7 and pH changes during storage at 4 °C in (a) blueberry juice (b) milk and (c) milk cream. (a-f) Results are shown as the means and standard deviations (n=3). Different superscript letters in the same food matrices indicate significant differences ($p<0.05$).

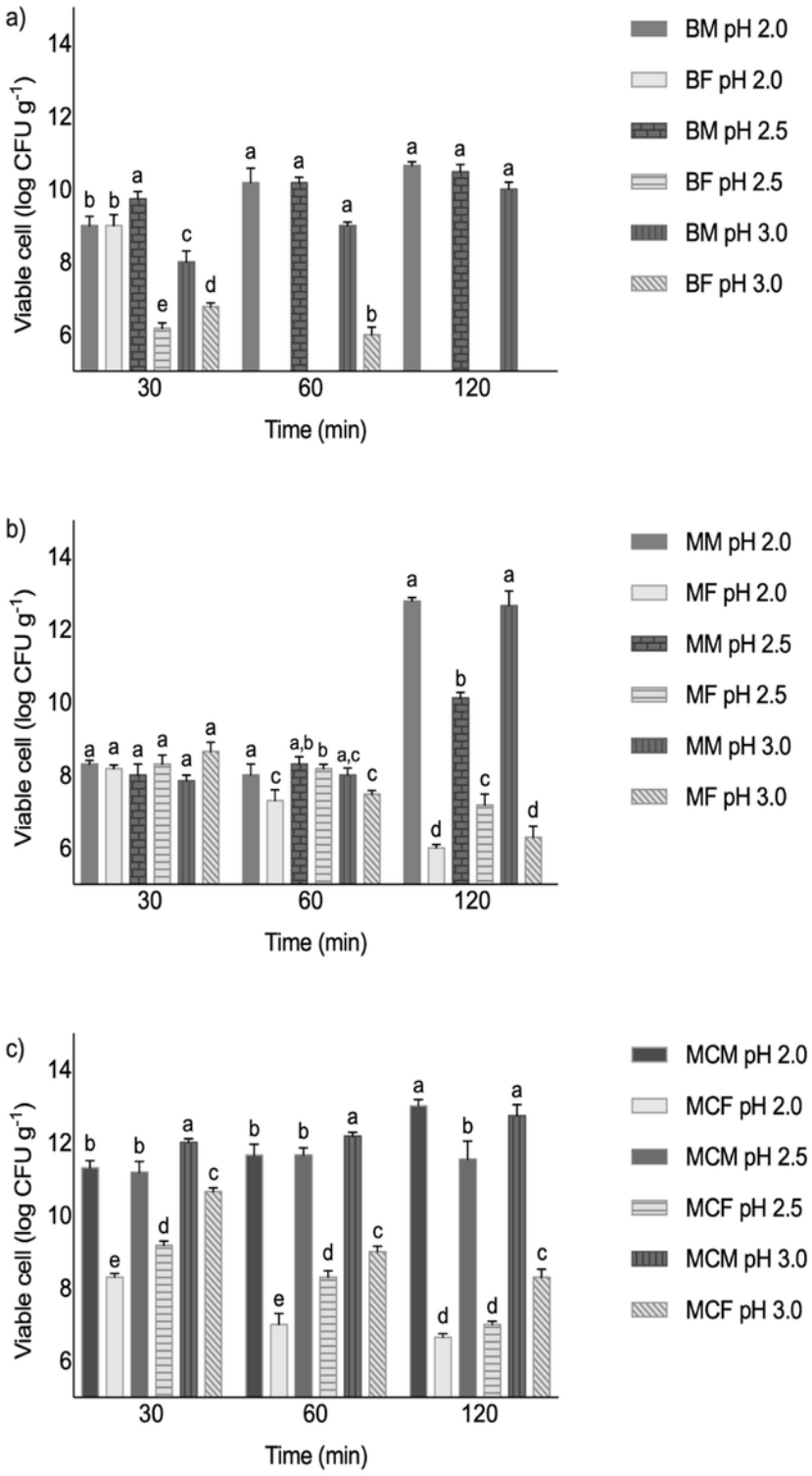

Figure 2

Viability of free cells and microencapsulated L. lactis R7 (log CFU g-1) after 7 days of storage at 4 °C, after exposure to in vitro simulated gastro conditions for 30, 60 and 120 min. (a) blueberry juice, (b) milk and (c) milk cream. a-e Results are show as the means and standard deviations (n=3). Different superscript lower-case letters in the same incubation time indicate a significant difference ($p < 0.05$). BM= Blueberry

Microcapsule, BF=Blueberry Free, MM=Milk Microcapsule, MF=Milk Free, MCM= Milk Cream Microcapsule, MCF= Milk Cream Free

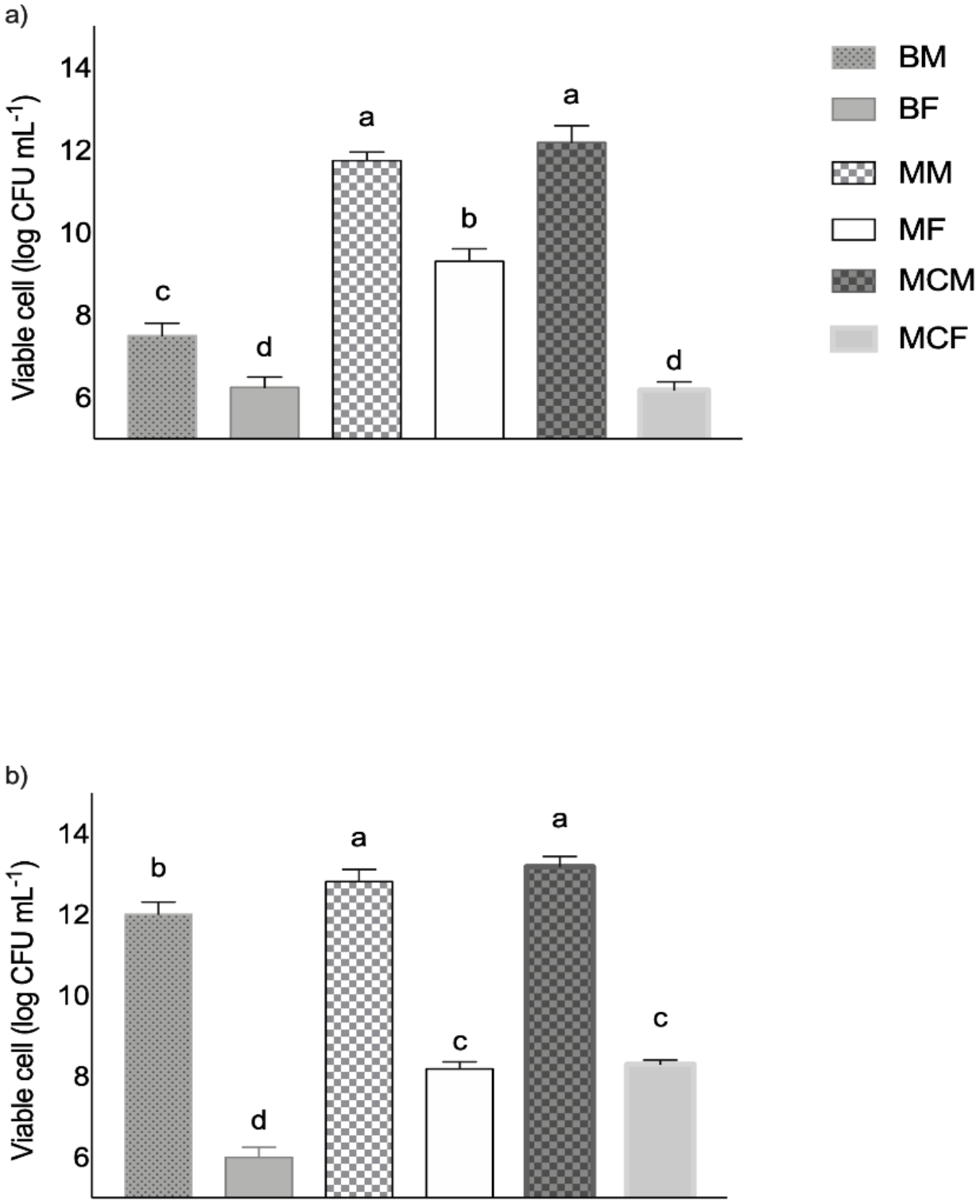

Figure 3

Viability of L. lactis R7 (log CFU mL -1) during exposure to simulated intestinal juice for 240 min at 7 days of storage at 4 °C: (a) without bile salt and (b) with 0.5% (w.v-1) bile salt. a-d Results are show as the means and standard deviations (n=3). Different superscript lower-case letters in the same storage period

indicate a significant difference ($p < 0.05$). BM= Blueberry Microcapsule, BF=Blueberry Free, MM=Milk Microcapsule, MF=Milk Free, MCM= Milk Cream Microcapsule, MCF= Milk Cream Fr

Supplementary Files

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