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FOOD MICROBIOLOGY - RESEARCH PAPER

Effect of co-encapsulation using a calcium alginate matrix and fructooligosaccharides with gelatin coating on the survival of *Lactobacillus paracasei* cells

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Abstract

The demand for functional foods is increasing each year because consumers are gaining awareness about the importance of a healthy diet in the proper functioning of the body. Probiotics are among the most commonly known, commercialized, and studied foods. However, the loss of viability of probiotic products is observed during their formulation, processing, and storage. This study aimed to investigate the co-encapsulation of two *Lactobacillus paracasei* probiotic strains (LBC81 and ELBAL) with fructooligosaccharides (FOS) in a calcium alginate matrix using extrusion technology with gelatin as a coating material. The viability of the strains under gastrointestinal conditions and in storage at low temperature was also assessed. An immobilization yield of more than 59% was observed for both bacterial strains. Exposure to 2% biliary salts led to a decrease in the viability of free cells in the two *L. paracasei* strains, whereas the viability of microencapsulated cells increased up to 47%. After 35 days of storage at 4°C, the population of free cells was reduced, but microencapsulated cells remained stable after storage at low temperature. LBC81 bacteria microencapsulated with 1.5% FOS coated with gelatin were the most resistant to the stressful environments tested. Therefore, these results showed that co-encapsulation with FOS in a calcium alginate matrix coated with gelatin improved *L. paracasei* survival and may be useful for the development of more resistant probiotics and new functional foods.

Keywords Probiotics · Microencapsulation · Cell viability · Functional food · *Lactobacillus paracasei*

Introduction

The demand for functional foods is increasing each year because of the awareness among consumers regarding the role of a healthy diet in the proper functioning of the body. Foods for health improvement provide basic nutrition and are safe to be consumed without medical supervision [1–3]. Probiotics are one of the most popular, commercialized, and researched foods.

According to Fortune Business Insight [4], the global market for probiotics was estimated at US$ 42.55 billion in 2017 and is projected to reach US$ 74.69 billion by 2025. Consumption of probiotics stimulates the growth of beneficial microorganisms in the intestine, thus eliminating potentially harmful bacteria, reinforcing the immune system, and helping in the management of lactose intolerance [5–7]. However, to prolong the beneficial effects, probiotic bacteria need to be metabolically stable and active during product consumption and need to remain alive while passing through the host gastrointestinal tract, which is a hostile environment for bacterial survival due to its low pH and presence of biliary salts [8–10].

The loss of viability of probiotic products is observed during their formulation, processing, and storage. Some factors that influence probiotic cells are pH, oxygen levels, storage temperature, inhibitors, and microbial contaminants that cause high stress to probiotic cells. Thus, technological innovations

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are extensively explored to provide solutions to problems relating to the stability and viability of probiotic foods [11, 12].

In this context, the loss of viability can be reduced using microencapsulation technology, which involves cell imprisonment in a polymeric matrix with sodium alginate. Sodium alginate is a non-toxic, low-cost, and easy-to-handle compound that dissolves in the intestine, thus releasing cells from the microcapsules. However, the use of sodium alginate has limitations because it may form a porous gel and become unstable in the presence of chelating agents. These problems can be solved by coating these capsules with other polymers, such as resistant amide, chitosan, or gelatin [8, 10, 13, 14].

Gelatin and sodium alginate can form a strong complex because of electrostatic interactions between the amide group of gelatin and the carboxylic groups of alginate. The advantages of gelatin include an excellent capability of membrane formation, probiotic biocompatibility, and non-toxicity to the consumer. Previous studies showed that an alginate matrix coated with gelatin can improve the survival of probiotic bacteria under adverse conditions [15–17].

To improve probiotic cell viability during production and storage of microcapsules, the use of prebiotics has gained increasing attention among researchers. The process of encapsulation of probiotic cells with a prebiotic compost is called co-encapsulation. The main advantage of co-encapsulation is improvement of probiotic resistance to stressful conditions in the gastrointestinal tract of the host. The use of symbiotic microcapsules in foods is an important strategy in food functionalization [9, 18, 19].

The aim of this study was to determine the effect of microencapsulation in a calcium alginate matrix with or without gelatin coating on the viability of two *Lactobacillus paracasei* strains under refrigerated storage conditions, in the presence of biliary salts, and at low pH. Moreover, the effect of co-encapsulation using different concentrations of fructooligosaccharides (FOS) on the viability of probiotics cells was assessed to determine the most protective treatment that can be applied to bacterial cells for their use in probiotic beverages.

Materials and methods

Bacterial reactivation and cellular suspension

The probiotic bacteria used were *L. paracasei* subsp. *paracasei* (LBC81) and *L. paracasei* (ELBAL). LBC81 was purchased from DuPont™, Danisco®, and ELBAL was isolated from commercial, fermented milk. Inocula were prepared by inoculating an isolated colony from each bacterium in 2 mL de Man, Rogosa, and Sharpe (MRS, Merck Whitehouse Station, EUA) broth. After 24 h of incubation at 37°C, the supernatant was discarded, and the biomass was used to inoculate 10 mL of the same broth. This process was repeated under the same conditions, and the biomass was transferred to 100 mL MRS broth. After 24 h, cell counting was performed by serial dilution and drop plating in MRS agar containing 0.1% cysteine hydrochloride [20–22].

A cellular suspension was prepared as described by Lima et al. [23] with modifications. Cultures were centrifuged at 4000 rpm for 15 min, and the pellet was resuspended in 0.5 mL sterile distilled water to produce a cellular suspension to be used for microencapsulation. The concentration and initial cell viability were determined as described by Adams [20].

Bacterial microencapsulation

Extrusion technology was used according to the procedure described by Krasaekoopt and Watcharapoka [2] with modifications. A cellular suspension (0.5 mL) was added to 5 mL of sterile solution containing 0%, 0.5%, 1.0%, and 1.5% of FOS and immediately mixed to 20 mL of 2.0% (m/v) sterile sodium alginate solution. The solution was dripped through a sterile insulin syringe in a 0.05 M calcium chloride solution, and the solution was slightly agitated. The distance between the syringe and calcium chloride solution was set to 25 cm [24]. Microcapsules were maintained in a static condition for 30 min to promote gelling. Later, half of the microcapsules were coated with gelatin.

Coating was performed as described previously [17]. Briefly, microcapsules were immersed in 100 mL of 4.0% (m/v) commercial, no-flavor gelatin solution (Fleischmann) and agitated at 1 g for 40 min in an orbital agitator. Subsequently, the microcapsules were washed with sterile distilled water and maintained in 0.1% peptone solution at 4°C until further use. Table 1 lists all treatments and FOS concentrations used in the production of the microcapsules.

Counting of cell population within the microcapsules was performed according to Krasaekoopt and Watcharapoka [2], with modifications. Microcapsules (0.1 g) were added to 9.9 mL phosphate buffer (pH 7.0; 0.1 M) and mechanically homogenized for 10 min at 14 g using a homogenizer (Marconi ® 440/CF). Cell counting of the suspension was performed according to Adams [20]. The immobilization yield, which combines the measures of the effectiveness of entrapment and survival of viable cells during microencapsulation was assessed as described by Trabelsi et al. [17] (Eq. 1).

$$IY = N/N_0 \times 100 \quad (1)$$

where IY refers to immobilization yield, N is the number of viable entrapped cells released from the microparticles, and N_0 is the number of free cells added during microencapsulation.

Table 1 Treatments and fructooligosaccharide (FOS) concentrations of LBC81 and ELBAL microcapsules with gelatin coating

Bacteria	Treatment	FOS concentration (%)
LBC81	Uncoated	0
		0.5
		1.0
		1.5
	Gelatin coating	0
		0.5
		1.0
		1.5
ELBAL	Uncoated	0
		0.5
		1.0
		1.5
	Gelatin coating	0
		0.5
		1.0
		1.5

Size and viability of free and encapsulated bacteria under refrigerated conditions

Microcapsule diameter was measured using a digital pachymeter. The microstructure analysis of microcapsules was performed using an optical microscope (×40) at days 1 and 35 of storage at 4°C [24]. The viability of microencapsulated bacteria after storage at 4°C was assessed by cell counting as mentioned above. The population of free cells (control) was also assessed by cell counting. Bacterial survival was measured after days 1, 14, 28, and 35 [17].

Survival analysis of free and microencapsulated bacteria subjected to low pH

Bacterial survival in low pH was analyzed according to Mandal et al. [25], with modifications. In total, 0.1 g microcapsules were added to a 1 mL of acidic solution (0.2 % NaCl; pH 1.5) and incubated at 37°C. After 90 min, microcapsules were washed, homogenized for 10 min, and plated by drop plate method [20, 21]. A suspension of 100 μL free cells was used as the control and incubated under the same conditions as those applied to the microencapsulated cells.

Survival analysis of free and microencapsulated bacteria subjected to different concentrations of biliary salts

A solution of bovine bile (Ox Bile, NutriCology®) was prepared according to Guo et al. [26], with modifications. Briefly, 1.0% or 2.0% bovine bile was added to MRS broth, and the final solution was sterilized using a 0.45-μm sterile filter (polytetrafluoroethylene membrane; non-pyrogenic and non-cytotoxic). A total of 0.1 g of microcapsules and 100 μL of free cells were separately added to 1-mL tubes containing 0%, 1.0%, and 2.0% of bovine bile and incubated at 37°C. After 3 h and 12 h, free cells were plated by drop plate method, and the microcapsules were washed twice with sterile distilled water and quantified as mentioned previously in the "Survival analysis of free and microencapsulated bacteria subjected to low pH" section [25].

Statistical analysis

All experiments were performed three times, and samples were analyzed in triplicate. Data were analyzed using analysis of variance (ANOVA), and mean values were compared using Scott-Knott test. Statistical analysis was performed using SISVAR 5.3 software [27]. Values of $p < 0.05$ were considered to indicate statistical significance.

Results and discussion

Size of microcapsules

The uncoated microcapsules of both LBC81 and ELBAL bacterial strains ranged from 1.55 to 1.75 mm in size and were significantly smaller than the microcapsules coated with gelatin, which ranged from 1.84 to 2.00 mm in size (Supplementary Table 1). This may be because of the addition of one more layer of polymer during coating, which resulted in a double protective wall. A previous study showed that microcapsule diameter produced using extrusion technology range from 500 μm to 3 mm [28]. Previous studies by Krasaekoopt and Watcharapoka [2] and Krasaekoopt [29] also reported similar microcapsule diameters results as those found in this study.

The addition of prebiotics during microencapsulation did not influence the size of the microcapsules. However, studies have shown that the addition of prebiotics can increase the size of microcapsules [2, 30]. Apart from prebiotics, other factors, such as the concentration and composition of sodium alginate, needle diameter used, and the distance between the gelling solution and the place where the drops falls, can also contribute to differences in the size of the microcapsules [10, 31].

The ideal size of microcapsules depends on bacterial growth and mechanical resistance of the capsule. Previous studies reported that microcapsules with a diameter of less than 1 mm of diameter may result in mechanical instability during long-term fermentation and may not protect probiotic bacteria against stimulated gastric juices when compared with free cells. In contrast, microcapsules with a diameter of more

than 1 mm may result in a rough texture, thereby negatively affecting the sensory acceptance of the food product [32–35].

Microencapsulation yield

Extrusion technology for the microencapsulation of probiotic microorganisms is considered the most recommended and commonly used technique because it is performed under mild conditions for the cells and provides high levels of immobilization [35–37]. The initial cell count used for immobilization process was 9.0 log CFU/mL for both *L. paracasei* strains. Immobilization yield for LBC81 ranged from 59.0 to 71.0% and for ELBAL ranged from 63.0 to 73.0%. There was no statistically significant difference in the immobilization yield among all treatments or between the two bacterial strains (Supplementary Table 2).

Similar results were described by Trabelsi et al. [17], who obtained maximum yield of 68.0% after immobilization of *L. plantarum* in alginate coating with chitosan and gelatin. Several factors, including microcapsule size, alginate concentration, cell population used, and calcium chlorate concentration, can affect microencapsulation yield [36].

To examine the viability of microencapsulated bacteria, the microcapsules were dissolved using chemical and physical methods, and the number of free bacteria were calculated. However, this method can be repetitive, demanding and may cause false results by identifying cells that are metabolically active but difficult to grow. Furthermore, disintegration of some rigid microcapsules using homogenization may affect cell viability. Hence, the development and standardization of fast and non-destructive methods for the assessment of cell viability are necessary [34, 38].

Survival of free and encapsulated bacteria at low pH

A food marketed as having high functional properties must contain probiotics that are resistant to stress and can survive in an acidic environment similar to that found in the stomach. Usually, probiotic *L. paracasei* cells are resistant to stress and remain viable in pH ranging from 2.0 to 5.0 [39–41]. The effects of acidic conditions on the viability of free and encapsulated LBC81 and ELBAL cells are shown in Fig. 1.

After exposure to an acidic solution for 3h at pH 1.5, the viability of free LBC81 and ELBAL cells decreased 82.0% and 63.7%, respectively. In co-encapsulation experiments, after exposure to an acidic solution for 3h, a reduction in the viability of microencapsulated LBC81 cells was observed, which decreased 4.0%, 2.3%, and 3.1% with the uncoated treatments using 0% FOS and 1.5% FOS and with treatment using 1% FOS coated with gelatin, respectively. Using uncoated 1.0% FOS, 0% FOS coated with gelatin, and 0.5% FOS coated with gelatin, the viability decreased 34.0%, 70.0%, and 71.0%, respectively. In contrast, using uncoated 0.5% and 1.5% FOS treatments, the cell viability increased 2.3% and 7.8%, respectively. There was a loss of viability ranging from 20.0 to 56.0% in microcapsules containing ELBAL bacteria.

Microencapsulated cells of the *L. paracasei* LBC81 strain were more resistant than those of the ELBAL strain because they remained stable at low pH, as observed with several treatments. It is known that lactic acid bacteria from the same species can present different resistance levels to low pH because of specific characteristics of their molecular structure that promote resistance against changes in pH and provide stability under acidic conditions [42]. In this study, gelatin coating did not significantly improve the viability of microencapsulated bacteria at low pH, possibly because gelatin did not act as an efficient barrier for diffusion in acidic solution diffusion. Therefore, these results suggest that only encapsulation with calcium alginate is sufficient to protect cells against acidic conditions.

There is a synergic effect between FOS and *L. paracasei* strains, as described by Al-Sheraji et al. [43]. However, in this study, microcapsules containing FOS did not significantly improve bacterial survival. This result was unexpected because several reports showed that the presence of FOS and other prebiotics as well as coating with other polymers significantly improved the survival of probiotic bacteria at low pH [2]. The presence of prebiotics improves the survival of probiotics because prebiotics can act as a carbon source for probiotic cells [2]. Possibly, the duration of exposure of microencapsulated cells to the acidic solution (3 h) was not sufficient to initiate the use of FOS by cells as a carbon source to improve the cell viability significantly compared with the viability of free bacterial cells.

Microencapsulated bacteria may suffer from stress due to environmental changes, resulting in its reduced metabolism. This may explain why FOS was unable to improve the viability of co-encapsulated cells compared with that of the microencapsulated cells without FOS [44]. However, the presence of FOS did not negatively affect bacterial survival.

Another important characteristic was that the alginate gel remained stable in a low pH solution, becoming insoluble and more compact with water loss. This characteristic provides better protection to the encapsulated material because it prevents the release of the material into the external environment [33]. In general, microencapsulation of the two *L. paracasei* strains improved bacterial resistance to an acidic environment, similar to the finding of previous studies [25, 32, 45].

Survival of free and microencapsulated bacteria subjected to biliary salts

Microcapsules in the body are exposed to an acidic environment in the stomach and neutral pH and biliary salts in the intestine. Biliary salts are a major threat to probiotic survival

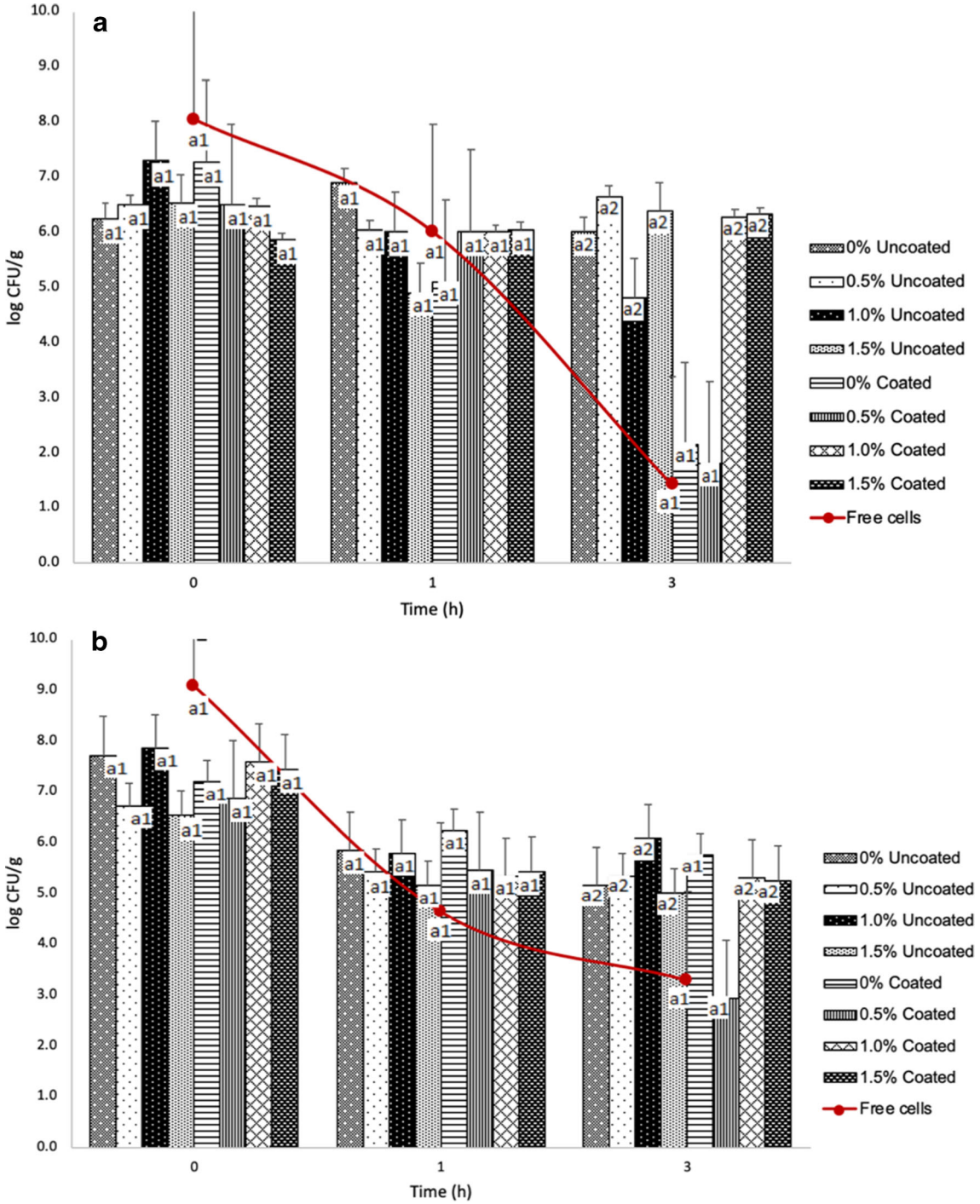

Fig. 1 Effect of pH 1.5 on free and immobilized *Lactobacillus paracasei* cells in a calcium alginate matrix (with or without gelatin coating) with different concentrations of fructooligosaccharides (FOS). **a** LBC81 and **b** ELBAL. Percentage refers to FOS concentrations in the microcapsules with or without gelatin coating. *Bars with the same letter are not significantly different

because of their detergent properties responsible for connections with lipids in the bacterial membrane. These connections result in alterations in the integrity and permeability of the bacterial membrane that can lead to cellular death [33, 46, 47]. Biliary salts are also responsible for the generation of free radicals, which can alter the secondary structure of RNA, induce DNA damage, and activate DNA repair enzymes. The average concentration of biliary salts in the small intestine ranges from 0.2 to 2.0% [46–49]

In this study, the cell populations before and after exposure to 0%, 1.0%, and 2.0% of biliary salts for 12 h were assessed and are listed in the Table 2. There was a decreased in the free cells population of the two bacterial strains, LBC81 and ELBAL, in the absence of biliary salts (control), but no

Table 2 Population of free and immobilized LBC81 and ELBAL cells in a calcium alginate matrix with or without gelatin coating and different concentrations of fructooligosaccharides (FOS) before and after exposure to different concentrations of biliary salts

Bacteria	Treatment (gelatin coating)	FOS (%)	Initial Population Log (UFC/g)	0% biliary salts Log (UFC/g)	1% biliary salts Log (UFC/g)	2% biliary salts Log (UFC/g)
LBC81	Control (free cells)	-	8.19 ±0.26^{a2A1}	6.82 ±1.39^{a3A1}	4.97 ±0.24^{a1A1}	4.99 ±1.13^{a2A1}
	Uncoated	0	6.01 ±1.18^{a1A1}	7.53 ±1.89^{a4A1}	6.83 ±1.31^{a3A1}	7.15 ±1.29^{a4A1}
		0.5	6.08 ±1.86^{a1A1}	7.56 ±1.70^{a4A1}	6.88 ±0.76^{a3A1}	7.22 ±0.72^{a4A1}
		1.0	5.95 ±2.04^{a1A1}	7.22 ±1.95^{a4A1}	6.82 ±1.02^{a3A1}	6.68 ±0.68^{a3A1}
		1.5	5.82 ±1.52^{a1A1}	7.34 ±1.82^{a4A2}	6.64 ±0.92^{a3A1}	6.23 ±1.01^{a3A1}
	Coated	0	6.31 ±2.24^{a1A1}	8.47 ±1.67^{a4A1}	6.91 ±0.85 ^{a3A1}	6.38 ±0.92^{a3A1}
		0.5	5.98 ±1.61^{a1A1}	7.57 ±1.69^{a4A1}	6.76 ±0.84^{a3A1}	5.80 ±1.56^{a3A1}
		1.0	5.66 ±1.07^{a1A1}	7.75 ±1.48^{a4A1}	7.13 ±0.55^{a4A2}	6.39 ±1.04^{a3A1}
		1.5	5.14 ±1.12^{a1A1}	7.60 ±1.71^{a4A2}	6.32 ±1.21^{a3A1}	6.75 ±0.61^{a3A2}
ELBAL	Control (free cells)	-	7.99 ±1.15^{a2A1}	7.34 ±0.10^{a4A1}	6.80 ±0.45 ^{a3A1}	5.09 ±0.18 ^{a2A1}
	Uncoated	0	6.13 ±2.64^{a1A1}	7.97 ±1.71^{a4A2}	7.64 ±1.14^{a4A1}	7.56 ±1.01^{a4A1}
		0.5	7.22 ±1.66^{a2A1}	7.97 ±1.76^{a4A1}	7.72 ±1.37^{a4A1}	7.61 ±1.19^{a4A1}
		1.0	7.06 ±2.02^{a2A1}	8.32 ±1.38^{a4A1}	8.21 ±1.11^{a4A1}	7.95 ±0.66^{a4A1}
		1.5	6.28 ±2.09^{a1A1}	8.12 ±1.78^{a4A1}	7.91 ±1.38^{a4A1}	7.75 ±1.17^{a4A1}
	Coated	0	5.55 ±2.95^{a1A1}	8.80 ±0.96^{a4A2}	8.42 ±0.30^{a4A2}	8.20 ±0.11^{a4A2}
		0.5	6.71 ±2.71^{a2A1}	8.34 ±1.39^{a4A1}	8.02 ±0.91^{a4A1}	7.96 ±0.83^{a4A1}
		1.0	6.41 ±2.41^{a1A1}	8.13 ±1.57^{a4A1}	7.78 ±0.96^{a4A1}	7.71 ±0.84^{a4A1}
		1.5	6.82 ±2.04^{a2A1}	8.29 ±1.53^{a4A1}	7.90 ±1.00^{a4A1}	7.87 ±0.97^{a4A1}

The mean and standard deviation of the three replicates indicated with the same lower case letter in the columns (between treatments) and with a capital letter in the rows (between initial population and after 12 h) did not differ significantly by Scott-Knott test

statistical difference was found between the initial and final cell population. A significant increase was observed in microencapsulated LBC81 cells treated with 1.5% FOS with or without gelatin coating, whereas a significant increase was observed in microencapsulated ELBAL cells with 0% FOS treatment with or without gelatin coating. Thus, gelatin coating did not affect nutrient diffusion to the interior of the microcapsules.

In the presence of 1.0% biliary salts, the population of free LBC81 cells decreased significantly after 12 h, showing a decreased of 60.0% in their viability. Free ELBAL cells were more resistant than free LBC81 cells, showing a decrease of 15.0% in their viability. All microencapsulated cells showed an increase in viability with all treatments. This increase in the number of viable cells was possibly because of a carbon source provided by the prebiotic FOS in the microcapsules, resulting in growth of the co-encapsulated probiotic cells [2].

In the presence of 2.0% biliary salts, free cells from the two bacterial strains were significantly impacted, with a decrease of 39.0% and 36.0% in the LBC81 and ELBAL cell populations, respectively. Microencapsulated bacteria were protected and showed an increase in the number of viable LBC81 cells (1.0 to 31.0%). There was a slight loss of viability only in case of microencapsulated cells treated with 0.5% FOS with gelatin coating. The highest growth was observed in case of microencapsulated LBC81 cells treated with 1.5% FOS with gelatin coating. ELBAL cell population showed an increase in viability (5.0 to 47.0%) with all treatments after 12 h exposure to a solution containing 2.0% biliary salts for 12 h; the microencapsulated cell population in all treatments was significantly higher than the free cell population. The presence of FOS and gelatin coating did not significantly improve cell viability.

Several factors, such as resistance of strains to biliary salts, concentration and type of encapsulant materials, types of polymers incorporated into the encapsulation matrix, encapsulation method, and source of biliary salts, can influence the protection and survival of encapsulated probiotics [47]. Cell morphology is an important indicator of the tolerance of probiotics toward biliary salts. Šušković et al. [50] showed that rough colonies of *L. acidophilus* are more sensitive to biliary salts, suggesting that this difference is because of a phenotypic environmental response. Smooth cells are smaller and have more compact chain structures, whereas cells with a rough surface morphology are larger and more vulnerable to the surfactant effect of biliary salts.

The two *L. paracasei* strains used in this study showed smooth morphology, and this characteristic may contribute to the resistance of encapsulated cells. The number of free cells may also be favored by this morphology because they were not completely lost in the presence of biliary salts, although the final population was smaller than the initial population. Other factors such as different pH value, temperature,

and environment can also promote the presence of probiotic bacteria that are more vulnerable or resistant to biliary salts [48].

For the survival of a sufficient number of probiotic bacteria exposed to biliary salts to colonize the gastrointestinal tract, an elevated population of cells should be microencapsulated. Hence, the concentration of cells used in this study was approximately 10^9 CFU/mL. In the presence of 2.0% biliary salts, the final cell population was higher than 6.0 log CFU/g for both microencapsulated *L. paracasei* strains. According to the US Food and Drug Administration, this is the minimum bacterial concentration recommended for probiotic foods to be beneficial to organisms.

Survival of free and microencapsulated bacteria during storage at low temperature and visualization of microcapsules

During 35 days of storage at low temperature, there was no difference between the initial and final cell population in all treatments with immobilized cells, suggesting that probiotic microencapsulated bacteria were protected by microcapsules. However, the viability of free LBC81 and ELBAL cells (control) decreased to 28.0% and 35.0%, respectively (Table 3).

The stability of microencapsulated bacteria of both strains after 35 days is shown in Fig. 2. At days 1 and 35 after microencapsulation, microcapsules were visualized using an optical microscope to verify surface degradation after storage at low temperature (Fig. 3). All microcapsules, independent of bacterial and FOS concentrations, presented rounded shape. More uniform edges were observed in microcapsules coated with gelatin than in those without coating. After 35 days, microcapsules presented the same morphology as that observed at day 1 without deformations. Studies on encapsulated probiotic stability in the intestinal environment are necessary to further explore this technology and for the development of new functional probiotic foods [51].

Conclusions

The results found in this study showed that the use of extrusion technology did not affect the viability of *L. paracasei* cells and provided a good yield after microencapsulation. Probiotic *L. paracasei* LBC81 and ELBAL cells co-encapsulated with FOS in calcium alginate matrix with gelatin coating showed an increase in viability when subjected to an environment mimicking the gastrointestinal tract and during storage at a low temperature. Free bacterial cells did not

Table 3 Population of free and immobilized LBC81 and ELBAL cells in a calcium alginate matrix with or without gelatin coating and different concentrations of fructooligosaccharides (FOS) during 35 days of storage

Bacteria	Treatment (gelatin coating)	FOS (%)	Day 1 Log (UFC/g)	Day 14 Log (UFC/g)	Day 28 Log (UFC/g)	Day 35 Log (UFC/g)
LBC81	Control	-	7.97 ± 0.29^{a2A2}	7.64 ± 0.25^{a2A2}	7.08 ± 0.37^{a1A2}	5.73 ± 1.72^{a1A1}
	Uncoated	0	6.09 ± 0.72^{a1A1}	5.76 ± 0.74^{a1A1}	5.35 ± 0.46^{a1A1}	5.86 ± 0.58^{a1A1}
		0.5	5.97 ± 0.78^{a1A1}	6.67 ± 0.12^{a2A1}	5.50 ± 0.49^{a1A1}	5.48 ± 0.64^{a1A1}
		1.0	6.78 ± 1.46^{a1A1}	5.85 ± 0.45^{a1A1}	5.63 ± 0.46^{a1A1}	5.47 ± 0.75^{a1A1}
		1.5	6.54 ± 1.38^{a1A1}	6.04 ± 1.16^{a1A1}	5.66 ± 0.66^{a1A1}	6.18 ± 1.46^{a1A1}
	Coated	0	6.56 ± 0.69^{a1A1}	5.73 ± 1.21^{a1A1}	5.56 ± 0.67^{a1A1}	5.38 ± 0.46^{a1A1}
		0.5	5.88 ± 0.29^{a1A1}	6.69 ± 1.06^{a2A1}	5.78 ± 0.79^{a1A1}	5.70 ± 1.41^{a1A1}
		1.0	6.43 ± 0.32^{a1A1}	6.00 ± 1.25^{a1A1}	5.92 ± 1.62^{a1A1}	5.44 ± 0.25^{a1A1}
		1.5	5.73 ± 0.44^{a1A1}	6.63 ± 1.34^{a2A1}	5.37 ± 1.19^{a1A1}	6.00 ± 1.69^{a1A1}
ELBAL	Control	-	9.08 ± 0.83^{a2A3}	8.13 ± 0.22^{a2A3}	5.55 ± 2.26^{a1A2}	3.74 ± 2.15^{a1A1}
	Uncoated	0	6.17 ± 0.33^{a1A1}	5.63 ± 0.68^{a1A1}	5.20 ± 0.39^{a1A1}	5.35 ± 0.12^{a1A1}
		0.5	5.57 ± 0.65^{a1A1}	5.89 ± 0.66^{a1A1}	5.11 ± 0.22^{a1A1}	4.97 ± 0.37^{a1A1}
		1.0	6.22 ± 0.42^{a1A1}	5.57 ± 0.51^{a1A1}	5.28 ± 0.33^{a1A1}	5.10 ± 0.06^{a1A1}
		1.5	5.37 ± 0.41^{a1A1}	5.60 ± 0.56^{a1A1}	5.04 ± 0.19^{a1A1}	5.36 ± 0.38^{a1A1}
	Coated	0	5.88 ± 0.82^{a1A1}	5.17 ± 0.27^{a1A1}	5.73 ± 0.11^{a1A1}	5.04 ± 0.02^{a1A1}
		0.5	5.54 ± 0.59^{a1A1}	5.86 ± 0.28^{a1A1}	5.45 ± 0.78^{a1A1}	5.24 ± 0.03^{a1A1}
		1.0	5.90 ± 0.5^{a1A1}	6.78 ± 0.72^{a2A1}	6.65 ± 0.76^{a1A1}	5.80 ± 1.15^{a1A1}
		1.5	5.81 ± 0.32^{a1A1}	5.57 ± 0.47^{a1A1}	5.26 ± 0.48^{a1A1}	5.30 ± 0.38^{a1A1}

The mean and standard deviation of the three replicates indicated with the same lower case letter in the columns (between treatments) and with a capital letter in the rows (between time) did not differ significantly by Scott-Knott test

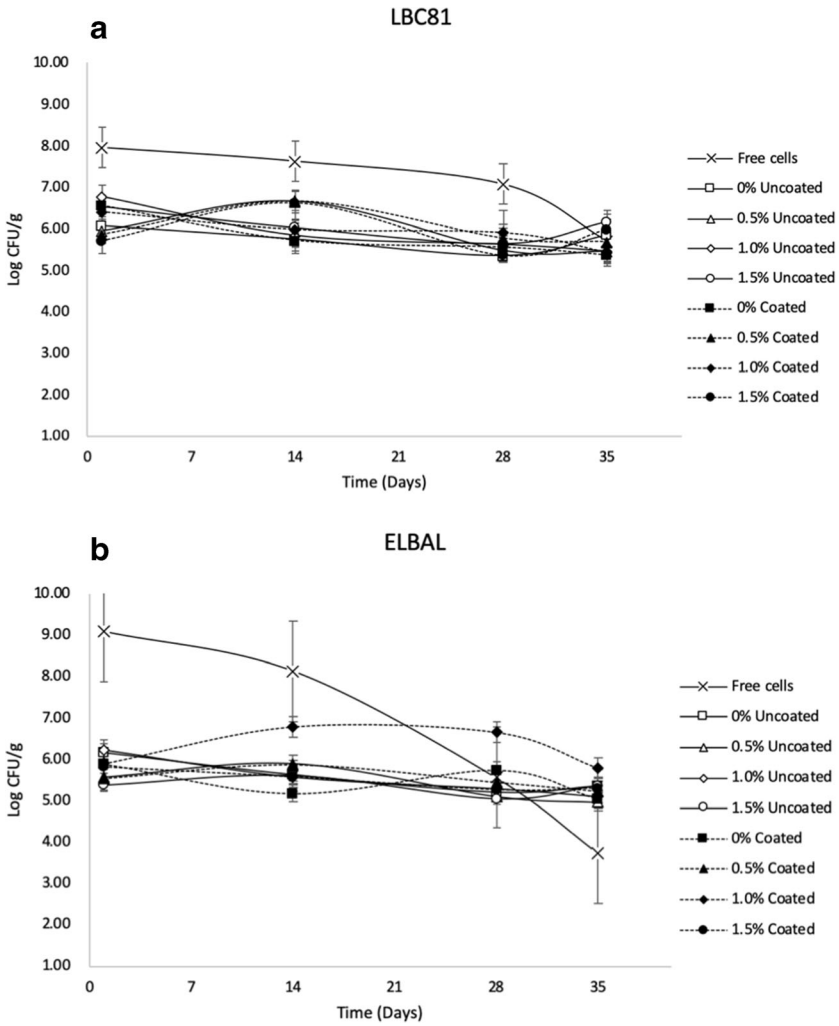

Fig. 2 Viability of *Lactobacillus paracasei* cells during 35 days of storage at 4°C. **a** LBC81 and **b** ELBAL. Percentage refers to fructooligosaccharide (FOS) concentrations in microcapsules with or without gelatin coating

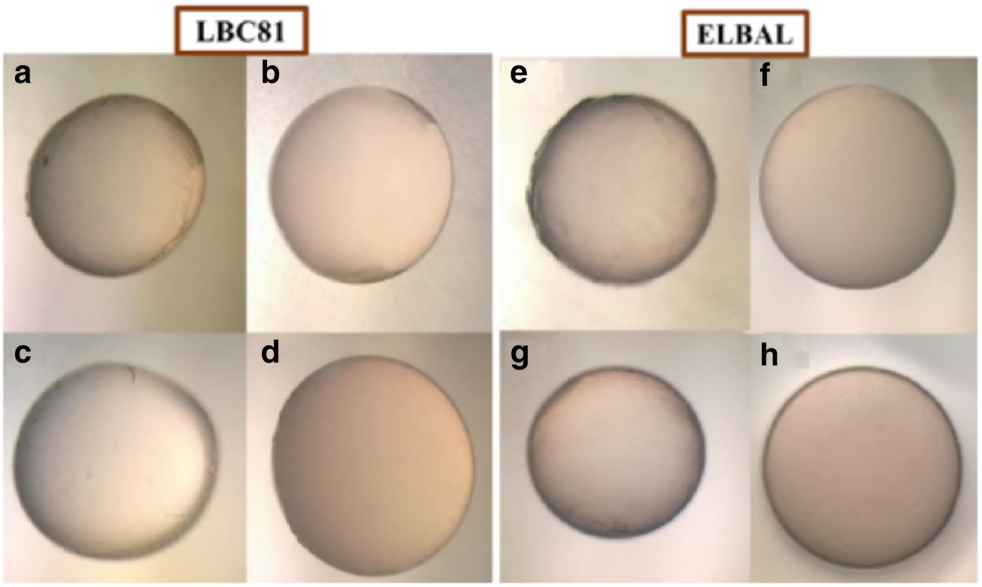

Fig. 3 Optical microscopy (×40) of microcapsules after 1 and 35 days of storage at 4°C. **a**, **e** Microcapsules without coating after 1 day; **b**, **f** microcapsules coated with gelatin after 1 day; **c**, **g** uncoated microcapsules after 35 days; and **d**, **h** microcapsules coated with gelatin after 35 days. Images show microcapsules co-encapsulated with 1.5% fructooligosaccharides (FOS)

survive without the protection provided by microencapsulation to the cells. LBC81 cells treated with 1.5% FOS with gelatin coating were the most resistant cells to the stressful conditions tested in this study. Therefore, these results showed that co-encapsulation of *L. paracasei* cells with a calcium alginate matrix coated with gelatin improved cell survival. This co-encapsulation method has immense potential for the development of more resistant probiotics, new functional foods, and probiotic beverages.

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