Probiotics and Antimicrobial Proteins
https://doi.org/10.1007/s12602-025-10837-3

RESEARCH

Spray-Dried Microencapsulation of *Lacticaseibacillus Rhamnosus* GG (ATCC 53103) in Cross-Linked Alginate Enriched with Soluble Fiber for Application in Functional Flan and Soup: Viability Assessment during Processing and Storage

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Received: 24 July 2025 / Accepted: 3 November 2025

Abstract

The development of non-fermented probiotic products in powders requires strategies to ensure their viability and metabolic activity under adverse conditions. In this study, a cross-linked alginate matrix was used to encapsulate *Lacticaseibacillus rhamnosus* GG (ATCC 53103) using the spray-drying technique. The matrix was supplemented with the following encapsulating agents: chia seed mucilage (CM), flaxseed mucilage (FM) and inulin. Response surface methodology was used to evaluate the effects of the type and concentration of encapsulating agent and $CaHPO_4$ concentration on probiotic survival after spray drying. Furthermore, encapsulated *L. rhamnosus* GG viability was evaluated during storage of two supplemented powdered food products (flan dessert and soup). After encapsulation, *L. rhamnosus* GG showed reductions of 0.2 and 0.7 logarithmic cycles when the matrix was supplemented with CM (0.4%, *w/v*) and FM (0.4%, *w/v*), respectively, in combination with $CaHPO_4$ (0.5%, *w/v*). Under these conditions, *L. rhamnosus* GG survival rates reached 98% and 93%, respectively. Furthermore, microcapsules of *L. plantarum*, *B. infantis* and *B. longum* showed high survival (>90%) rates. *L. rhamnosus* GG microcapsules supplemented with FM resisted heat stress, reaching a survival rate of 78%. Regarding stability in powdered food matrices, *L. rhamnosus* GG encapsulated viability was affected by storage temperature. After 90 days at 4 °C, both products reached high counts (>9 Log CFU/g of dry product). In contrast, at 25 °C, they did not exceed 4.5 Log CFU/g of dry product. Both CM and FM can be effective alternatives as encapsulating agents in spray-drying and cold storage processes.

Keywords Probiotics · *Lacticaseibacillus Rhamnosus* GG · *Lacticaseibacillus Rhamnosus* ATCC 53103 · Cross-linked alginate matrices · Flaxseed mucilage · Chia seed mucilage · Spray drying

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Published online: 17 November 2025

Introduction

Probiotics are widely studied and applied in the cosmetic, pharmaceutical, and food industries because of their health benefits, and consumers interest in functional foods and nutraceuticals capable of preventing disease. Markets studies support this growing interest in the use and providing probiotic food products, designed for release in the small intestine and colon. The probiotic market is projected to reach USD 220.14 billion by 2030 [1]. Probiotics are associated with several human health benefits, such as action against harmful external pathogens; bacterial and viral infections; gastrointestinal tract regulation; serum cholesterol reduction; stimulation, modulation and regulation of the immune system, cancer prevention, and alleviation of lactose intolerance and allergies, as reviewed by Kerry et al. [2], Lau and Quek [3], and Maftei et al. [4]. To date, several studies have demonstrated that probiotics confer benefits in the treatment of infectious diseases in the oral cavity, atopic dermatitis, restoration of the vaginal microbiota [5–7], and can delay progression of Alzheimer's and Parkinson's disease [8]. However, no universal probiotic strain provides all of the proposed benefits, and not all strains of the same species are effective against a given health condition because the health benefits are specific to each probiotic [9].

Probiotics are generally associated with fermented foods such as yogurt or cultured milk. However, these products have limited shelf life, typically not exceeding two to three weeks under refrigeration (4 °C). Furthermore, dairy products pose a risk for people with lactose intolerance or milk protein allergies. The development of non-fermented products, such as powders, tablets, capsules, or chewable forms, requires strategies that enable probiotics to maintain their metabolic activity and viability under adverse conditions, such as those found in low-moisture products. Microencapsulation is an effective technique to enhance the survival and viability of probiotics. Complex coacervation, electrospinning, emulsion cross-linking, spray drying, and freeze-drying are some of the techniques used to encapsulate probiotics. Among them, spray drying stands out for its simplicity, ease of scale-up, and low energy consumption, high production rates (achieving a large quantity of atomized product per unit of time), and ability to produce smaller particles compared to those obtained through ionic gelation encapsulation. Furthermore, spray drying has the advantage of being ten times more economical and requiring less time to complete the process than freeze-drying [10]. Although high survival rates (> 90%) can be achieved with freeze-drying [11], microcapsules are usually porous and fragile structures [12]. High survival rates (> 95%) have also been reported for probiotic bacteria encapsulated using the electrospinning technique. However, their viability only maintained 28 days at 4 °C. Furthermore, high production costs limit their application on an industrial scale [13]. However, the high temperatures of spray drying cause cell mortality through dehydration and thermal inactivation [14], which is associated with damage to the membrane and ribosomes of probiotics [15, 16]. Despite this, the high drying speed, together with the cooling effect generated by the rapid evaporation of water and the protection provided by the encapsulating agents, contribute to reducing the thermal impact on cells [17]. In addition, other strategies such as the use of heat protectants, resistant strains, and process optimization can be employed [18–20]. Together, these factors favor higher cell viability compared to other slower drying methods or those involving direct thermal contact. Recently, spray drying has been proposed to produce powder cross-linked alginate microparticles, where particle formation, drying and alginate cross-linking occur in a single step. In this novel method, the feed solution to the spray dryer is composed of alginate, an insoluble calcium salt, an organic acid and a volatile base. Once the nozzle atomizes the fluid into small droplets, the base vaporizes in the hot air stream acidifying the atomized fluid. At low pH, the calcium salt solubilizes and cross-links with alginate. This approach was used by Strobel et al. [21] and Kawakita et al. [22] for the microencapsulation of plant-beneficial bacteria.

Probiotics that have been most studied and applied in encapsulation processes belong to the genera *Lactobacillus* and *Bifidobacterium*. The cell viability in both genera is affected by pH, processing and storage temperatures, salt and oxygen concentrations, and the presence of digestive enzymes. Each probiotic strain has its own physiological characteristics, which influence its viability, stability, and ability to reach the intestine alive [23]. Therefore, it is essential to individually evaluate each encapsulation strategy in specific strains to determine its efficacy and suitability. Probiotic survival and viability for a specific strain can be enhanced by optimizing some key factors such as exponential growth phase and pre-adaptation conditions. Additionally, it is important to analyze both the composition and solids concentration in the encapsulation solution. Each of these factors influences the formation, stability, and release of the probiotic microcapsules. Composition can alter the pH of the matrix, affecting capsule integrity and influencing the release of the microorganism at the desired target site. Similarly, solids concentration can affect parameters such as particle size, matrix density, and osmotic stress [19, 24, 25]. An appropriate encapsulation solution improves protection against adverse conditions, improving the viability of probiotics during drying, storage, and gastrointestinal transit. For this purpose, the use of protective agents such as prebiotics (inulin, galactooligosaccharides and fructooligosaccharides), as well as encapsulating agents such as soluble fiber (flaxseed and chia mucilage), maltodextrin, pectin, whey protein, and alginate, has been proposed [24, 26, 27].

The most commonly used encapsulating agents are proteins and carbohydrates; within this latter group, mucilages have gained prominence as an emerging alternative. These compounds, derived from plants or seeds, are of interest due to their function as dietary fiber and their ability to withstand high temperatures during processing. Furthermore, they are considered safe for human consumption, biodegradable, and economically viable [28, 29].

Flaxseed (*Linum usitatissimun* L.) and chia (*Salvia hispanica* L.) seed are source of bioactive phenolic compounds, polyunsaturated fatty acids, dietary fiber and proteins [30, 31]. Flaxseed mucilage (FM) is a mixture of neutral polysaccharides composed of arabinoxylan and rhamnogalacturonan I, with arabinoxylan exhibiting prebiotic properties [32]. Meanwhile, chia seed mucilage (CM) is an anionic water-soluble heteropolysaccharide, tentatively identified as β-D-xylopyranosyl, α-D-glucopyranosyl and 4-O-methyl-α-D-glucopyranosyluronic acid [33]. This mucilage is a good source of soluble fiber that forms highly aqueous viscous dispersions at low concentrations [34]. Bustamante et al. [27, 35] encapsulated *Lactobacillus acidophilus* La-05 in maltodextrin (15%, *w/v*) using spray drying at 110 °C. After the process, the survival rate was 78% when the maltodextrin was supplemented with FM (0.2%, *w/v*) and 63% when was supplemented with CM (0.2%, *w/v*). Furthermore, Bustamante et al. [27] reported high survival rates (> 85%) and high viability after 45 days of refrigerated storage for *Lactobacillus plantarum* and *Bifidobacterium infantis*, when they were encapsulated by spray drying in solutions composed of maltodextrin (15%, *w/v*) supplemented with FM (0.2%, *w/v*) or CM (0.2%, *w/v*).

Although the spray-drying encapsulation is one of the most studied and used techniques for the protection of probiotic bacteria, few studies have addressed the optimization of the cross-linked alginate matrix by incorporating CM, FM, or inulin, to improve the survival of these bacteria. The aim of the present work was to evaluated the effect of adding each of these compounds CM, FM, or inulin to a cross-linked alginate matrix on *Lacticaseibacillus rhamnosus* GG (ATCC 53103) survival encapsulated by spray drying. Moreover, the survival of selected probiotics (*Lactiplantibacillus plantarum* ATCC 8014, *Bifidobacterium infantis* ATCC 15679 and *Bifidobacterium longum* ATCC 15707) in the optimized encapsulation solution was evaluated.

Materials and methods

Materials

Bifidobacterium infantis ATCC 15679, *Bifidobacterium longum* ATCC 15707, *Lacticaseibacillus rhamnosus* ATCC 53103 and *Lactobacillus plantarum* ATCC 8014 (American Type Culture Collection, Rockville, MD, USA). MRS broth and agar were from BD (MD, USA) [36]. Inulin was purchased from Terrium® (Chile). Chia seeds and flaxseed (Fig. 1a, e) were acquired from the local market. L-cysteine-HCl was purchased from Calbiochem® (Darmstadt, Germany). Succinic acid was purchased from Merck (Darmstadt, Germany). Low viscosity sodium alginate, $CaHPO_4$ and NH_4OH were purchased from Sigma-Aldrich (St. Louis, MO, USA). GasPaK™ EZ anaerobiosis generator system was purchased from BD (MD, USA). All the chemicals were of analytical grade.

Methods

Media and Cultivation Conditions

Each probiotic strain was sub-cultured thrice with 5% (*v/v*) inoculum in 5 mL of MRS broth and incubated (37 °C, 12 h). For strains of the *Bifidobacterium* genus, MRS broth was supplemented with L-cysteine-HCl (0.05%, *w/v*) and incubated under anaerobic conditions with an anaerobiosis generator system.

For the encapsulation assays, MRS (200 mL) was inoculated (5%, *v/v*) with the grown pre-culture and incubated (37 °C, 12 h). Then, probiotic biomass was recuperated by centrifugation (6000× *g*, 4 °C, 15 min); the probiotic biomass was washed twice with sterile distilled water and centrifuged as described above. Finally, probiotic biomass was re-suspended in sterile distilled water (2 mL) and added to the encapsulating solution.

Extraction of the Chia Seed and Flaxseed Mucilage

CM was extracted according to Bustamante et al. [19]. Briefly, seeds were extracted with hot distilled water (80 °C, pH 5.0) at a 1:40 (*w/v*) ratio for 2 h. The extraction cycle was repeated twice. FM was extracted according to Bustamante et al. [35]. Seeds were extracted with hot distilled water (90–95 °C, pH 5.0) at a 1:10 (*w/v*) ratio for 30 min. The extraction cycle was repeated three times. Both CM and FM extracts were separated from the seeds, spread on trays, dried in an air convection oven (60 °C), milled, sieved (0.425 mm mesh), and stored (–20 °C) until use.

Control Encapsulating Solution Preparation

The control encapsulation solution was formulated according to Strobel et al. [21] with some modifications. Briefly, in 120 mL of distilled water: 100 mL were prepared with sodium alginate (4.0%, *w/v*) and succinic acid (2.0%, *w/v*). The solution was then, sterilized (121 °C, 15 min), after

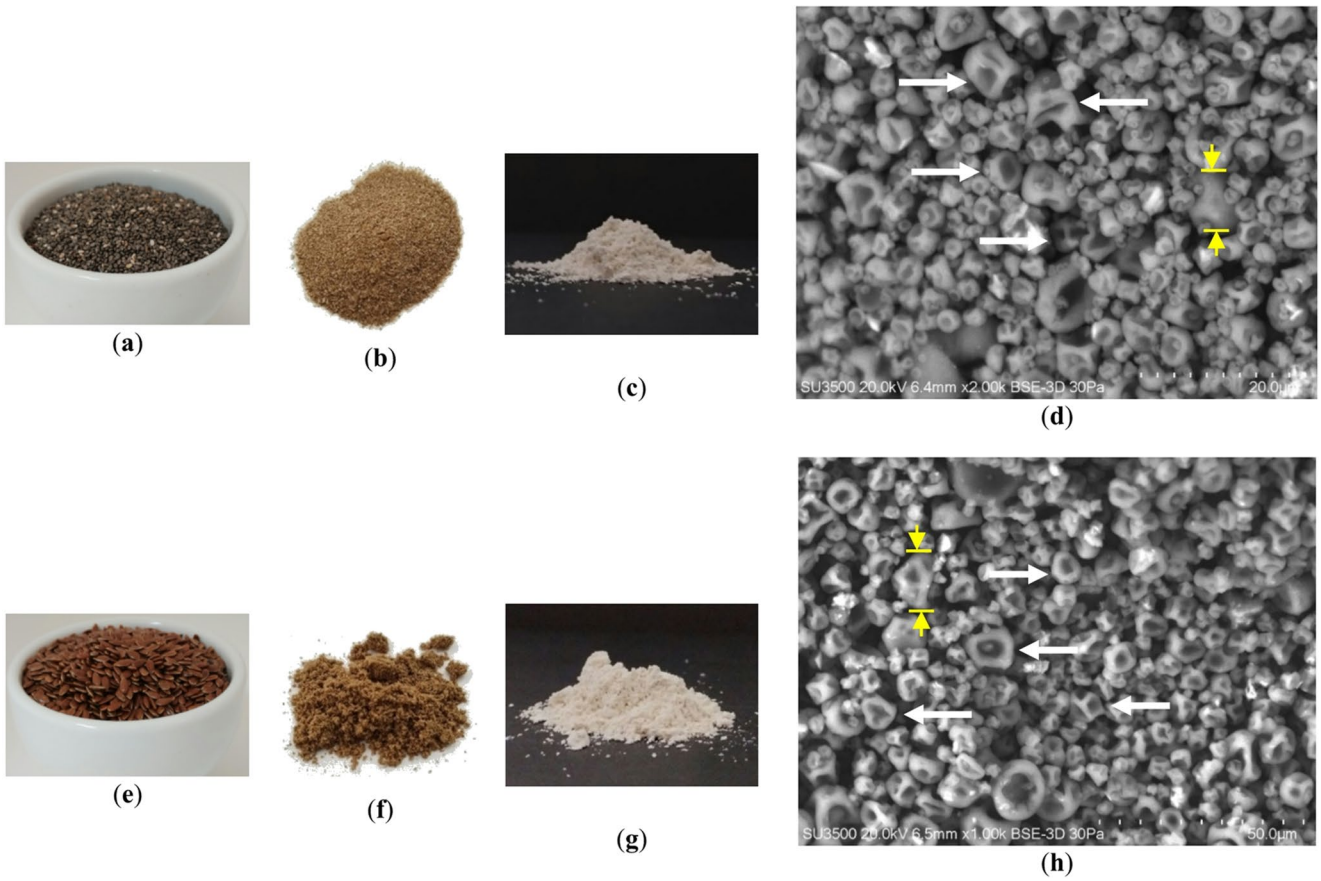

Fig. 1 (**a**) Chia seeds (*Salvia hispanica* L.), (**e**) Flaxseed (*Linum usitatissimun* L.). Mucilage powder from: (**b**) chia seed, (f) flaxseed. *L. rhamnosus* GG encapsulated in cross-linked alginate matrix supplemented with 0.4% (*w/v*) (**c**) chia seed mucilage (CM), (**g**) flaxseed mucilage (FM). Scanning electron micrographs of spray-dried, encapsulated *L. rhamnosus* GG in a cross-linked alginate matrix with: (d) CM (scale bar = 20 µm) and (h) FM (scale bar = 50 µm) after 1 day of storage at 4 °C

which the pH was adjusted to 5.6 ± 0.2 with NH_4OH. Then, 20 mL of a sterile and homogeneous suspension of $CaHPO_4$ was added to activate the calcium alginate cross-linking. The mixture was stirred for 30 min a room temperature. Afterwards, the re-suspended *L. rhamnosus* GG probiotic biomass was added to form the control encapsulation solution. Finally, the bacterial suspensions were kept under constant stirring until spray drying.

The encapsulation solution supplemented with CM, FM (Fig. 1b, f) or inulin was prepared in the same way as the control solution, with some differences. A 100 mL solution was prepared with low-viscosity sodium alginate (4.0%, *w/v*), succinic acid (2.0%, *w/v*), and CM, FM or inulin. The solution was then sterilized (121 °C, 15 min), after which the pH was adjusted to 5.6 ± 0.2 with NH_4OH. A sterile, homogeneous suspension of $CaHPO_4$ was then added. The mixture was stirred for 30 min at room temperature. Then, the re-suspended probiotic biomass was added to complete the encapsulation solution. Finally, the bacterial suspensions were kept under constant stirring until spray drying process. The compositions of the encapsulation solutions are shown in Table 1.

Preparation of Spray-Dried Powders

The spray-dried powders were prepared according to Bustamante et al. [37]. The encapsulation solutions fed to the spray dryer contained total viable counts ranging from 10^9 to 10^{10} CFU/mL. Spray drying was performed using a laboratory-scale Mini Spray Dryer (Büchi B-290, Büchi Labortechnik AG, Flawil, Switzerland) equipped with a 0.7 mm diameter nozzle. All formulations were processed under identical operating conditions: inlet temperature was set to 130 °C, aspirator airflow rate to 84%, peristaltic pump to 15% (corresponding to a feed rate of 6 mL/min), and nozzle airflow to 45 m^3/h as indicated on the Q-flow indicator. Under these conditions, the outlet temperatures ranged from 70 to 80 °C. Spray dried powders were collected in sterile glass bottles attached to the bottom of the cyclone, cooled using an ice bath and stored at 4 °C until characterization.

Effect of CM, FM or Inulin, and $CaHPO_4$ on *L. rhamnosus* GG Survival after Encapsulation Process by Spray Drying

The effect of soluble fiber (CM, FM or inulin) and $CaHPO_4$ was evaluated on *L. rhamnosus* GG survival after spray drying. For the microencapsulation in cross-linked alginate matrices, the solution fed to the spray dryer contained sodium alginate, succinic acid, and different concentrations (0, 0.2, 0.4% *w/v*) of CM, FM or inulin. Moreover, different degrees of cross-linking with $CaHPO_4$ were evaluated. To this end, $CaHPO_4$ concentration in the encapsulation solution was set at 0.1, 0.3 or 0.5% (*w/v*). The response surface methodology (RSM) was applied as the experimental design. The assays were organized according to a face-centered central composite design with two variables (soluble fiber and $CaHPO_4$). Each drying run was carried out in duplicate except for the central point which was made in triplicate. Nine assays were carried out for each encapsulating agent (CM, FM, and inulin), resulting in a total of 27 assays (Table 1). The survival of *L. rhamnosus* GG after spray drying was then determined.

Once the optimized encapsulation solution, it was used to encapsulate three probiotic strains—*L. plantarum*, *B. infantis*, and *B. longum*— to evaluate their survival after spray drying. The bacterial suspension of each probiotic strain was prepared following the protocol previously described for *L. rhamnosus* GG, with some modifications for *Bifidobacterim*. In this case, the MRS broth was supplemented with L-cysteine-HCl (0.05%, *w/v*) and the culture was incubated under anaerobic conditions. The assay was performed in triplicate.

Effect of Heat Treatment on the Viability of *L. rhamnosus* GG Microencapsulated in Cross-Linked Alginate Matrices and CM or FM by Spray Drying

The heat tolerance of encapsulated *L. rhamnosus* GG was evaluated according to Malmo et al. [38] with some modifications. The viability was tested against several

Table 1 Effect of soluble fiber (SF) type, and of SF and $CaHPO_4$ content in a cross-linked alginate matrix on *L. rhamnosus* GG survival, the viscosity of the encapsulation solution (μ), and the moisture content of spray dried powder

T[a]	Independent variables			μ[c] (mPa · s)	Moisture[d] (%)	Experimental survival[e] (%)	Predicted survival (%)	Student residual
	SF[b]	$CaHPO_4$ (%, *w/v*)	SF Concentration (%, *w/v*)					
1	CM	0.1	0.0	3.55±0.14	17.82±0.54	80.66±2.40	79.90	0.70
2			0.2	3.85±0.02	12.45±0.50	84.22±0.69	85.42	1.05
3			0.4	3.92±0.10	18.74±0.63	92.61±0.15	90.95	1.53
4		0.3	0.0	3.87±0.12	16.01±0.53	82.77±0.04	83.32	−0.48
5			0.2	3.87±0.09	15.02±0.76	88.70±0.63	88.85	−0.12
6			0.4	3.48±0.08	18.91±0.79	92.72±0.27	94.37	−1.44
7		0.5	0.0	3.90±0.16	15.73±0.73	87.02±0.26	86.75	0.25
8			0.2	3.88±0.12	16.52±0.37	92.75±0.11	92.27	0.42
9			0.4	3.88±0.02	15.01±0.74	98.26±0.13	97.79	0.43
10	FM	0.1	0.0	3.89±0.10	15.52±0.60	79.04±0.45	78.84	0.19
11			0.2	3.86±0.08	14.94±0.52	83.59±2.14	82.33	1.21
12			0.4	3.85±0.09	15.28±0.41	85.42±0.23	85.83	−0.40
13		0.3	0.0	3.88±0.11	18.47±0.44	81.07±0.37	81.86	−0.76
14			0.2	3.88±0.05	14.94±0.50	84.67±0.68	85.36	−0.63
15			0.4	3.84±0.03	15.41±0.52	88.56±0.48	88.85	−0.28
16		0.5	0.0	3.88±0.15	14.31±0.45	85.91±0.60	84.89	0.98
17			0.2	3.87±0.12	17.88±0.71	87.29±0.07	88.38	−1.04
18			0.4	3.84±0.10	15.72±0.41	93.01±0.63	91.88	1.09
19	In	0.1	0.0	3.96±0.13	20.02±0.55	90.64±1.42	90.69	−0.01
20			0.2	3.85±0.04	16.45±0.45	90.50±0.86	96.47	−1.15
21			0.4	3.85±0.05	16.84±0.76	92.21±1.65	86.20	1.27
22		0.3	0.0	3.87±0.03	16.24±0.48	75.80±0.09	70.03	1.11
23			0.2	3.86±0.06	16.14±0.69	80.88±5.11	80.50	0.07
24			0.4	3.83±0.11	16.82±0.74	68.58±1.26	74.92	−1.22
25		0.5	0.0	3.86±0.09	14.33±0.46	68.36±0.08	74.09	−1.21
26			0.2	3.86±0.05	17.74±0.31	94.64±2.42	89.24	1.04
27			0.4	3.86±0.08	15.59±0.39	88.66±0.56	88.34	0.07

[a] T: Treatment, [b]CM: Chia seed mucilage, FM: Flaxseed mucilage, In: Inulin; [c]Mean values of three replicates; [d]Mean values of four replicates; [e]Mean values of two replicates, except in central point in which were three replicates

time-temperature combinations: 40 °C for 5 min, 50 °C for 5 min, 60 °C for 3 min, 70 °C for 3 min, 80 °C for 2 min and 5 min. Microcapsules (0.1 g) were suspended in MRS broth (4.9 mL) contained in glass tube. Samples were placed in a temperature-controlled dry bath in above-mentioned conditions. Glass tubes were rapidly cooled after incubation and the viable cell count was determined. The assay was performed in triplicate.

Effect of Storage Conditions on the Viability of *L. rhamnosus* GG Microencapsulated in Cross-Linked Alginate Matrices and CM or FM by Spray Dried Incorporated in a Powdered Food Product

This assay was designed to determine the stability of the microcapsules in a dehydrated food product. The viability of microencapsulated *L. rhamnosus* GG was evaluated immediately after mixing with food product (flan dessert and soup) followed for 90 days storage at 4 and 25 °C. The assay was performed in triplicate.

Analyses

Viscosity The viscosity of encapsulation solutions was measured by a Digital Viscometer VISCO™ − 895 Package B (Atago CO., Ltd., Tokyo, Japan) designed for low-viscosity solutions. The measurement was performed on a 16 mL sample at 21 °C and 150 rpm.

Enumeration of Viable Probiotics Survival after drying and viability during storage of probiotics were determined by the standard plate count method. Briefly, 0.1 g of powder was diluted in 4.9 mL of sterile buffered peptone water (0.1%, *w/v*); the suspension was kept at 4 °C for 30 min to release the bacterial cells. The appropriate dilution of the probiotic suspension was seeded on MRS agar and incubated at 37 °C for 48 h. MRS agar supplemented with L-cysteine-HCl (0.05%, *w/v*) was applied for *B. infantis* and *B. longum* and incubated under anaerobic conditions.

Probiotics viability during storage was expressed as colony forming units per gram (CFU/g) of dry powder and the results were expressed as Log CFU/g. The survival percent after drying was calculated from Simpson et al. [20]:

$$\text{Survival}\ (\%) = \left(\frac{N}{N_0}\right) \times 100 \quad (1)$$

where, N is Log CFU/g of the spray-dried powder immediately after drying and N_0 is Log CFU/g of dry matter in the suspension fed to the dryer. The assay was carried out in triplicate.

Physical Properties of Probiotic Microcapsules

Moisture Content The residual moisture content of probiotic microcapsules was determined by oven drying at 105 °C until a constant weight was achieved. The assay was performed in quadruplicate.

Morphology The morphology of probiotic microcapsules was observed by scanning electron microscope (SEM) SU3500 (JEOL Hitachi, Tokyo, Japan) at 20 KV, 30 Pa vacuum and Backscattered Electron signal. The sample was dispersed over the sample holder equipped with a double-sided carbon type.

Particle Size The particle size of probiotic microcapsules was determined by laser diffraction (Particle size analyzer Shimadzu model SALAD-3101, Tokyo, Japan) at 25 °C using isopropyl alcohol as the continuous phase.

Statistical Analysis

Statistical analysis was carried out using the Design Expert 6.0 statistical software (Stat-Ease, Minneapolis, Mn, USA). Analysis of variance (ANOVA) was applied to determine the significance of effects ($p<0.05$). Differences between means were detected by the general linear model procedure, using Tukey's test one-way analysis of variance (SPSS® version 23).

Results

Effect of CM, FM or Inulin, and $CaHPO_4$ on *L. rhamnosus* GG Survival after Encapsulation Process by Spray Drying

The composition of the encapsulating solution is an important factor, as it can determine the protection level provided to the probiotics against the spray drying process, storage and transit through the gastrointestinal tract. Therefore, we evaluated the effect of three soluble fiber (CM, FM or inulin; 0, 0.2, 0.4% *w/v*) and $CaHPO_4$ (0.1, 0.3, 0.5% *w/v*) as components of the encapsulating solution on *L. rhamnosus* GG survival after spray drying. A RSM with a face-centered central composite design was applied to determine the significance of the effects on probiotic survival after drying. The results obtained from the nine drying runs for each soluble fiber are shown in Table 1. Considering the components of the encapsulation solution—sodium alginate, succinic acid, $CaHPO_4$, and soluble fiber—the solids content in the nine drying runs ranged between 6.1 and 6.9 g of solids per 100 mL of solution.

The viscosity of the feed solution is one of the factors influencing the particle size distribution obtained from spray drying. High viscosity can obstruct the atomizer nozzle, producing larger droplets and generating dehydrated particles with high moisture due to incomplete solvent evaporation. It can also result in a low-yield process [39]. On the other hand, higher viscosity may require higher drying gas temperature, which can decrease probiotic viability. In contrast, a low-viscosity feed solution generates smaller particles with low moisture content and, therefore, a longer shelf life [40]. The viscosity of the encapsulating solution was not affected by the type and concentration of soluble fiber or by the concentration of $CaHPO_4$ (Table 1). It ranged from 3.48 mPa·s when the solution was prepared with CM (0.4%, *w/v*) and $CaHPO_4$ (0.3%, *w/v*), to 3.96 mPa·s, when inulin (0.0%, *w/v*) and $CaHPO_4$ (0.1%, *w/v*) were used.

The results of 27 experimental combinations of two independent variables (soluble fiber and $CaHPO_4$) were recorded to investigate their impact on *L. rhamnosus* GG survival (Table 1). *L. rhamnosus* GG survival in terms of CM, FM and $CaHPO_4$ concentration fits a linear relationship according to the following equations:

$$S\ (\%) = 88.85 + 3.42\ CaHPO_4 + 5.52\ CM \quad R^2 = 0.955 \tag{2}$$

$$S(\%) = 85.36 + 3.03\ CaHPO_4 + 3.49\ FM \quad R^2 = 0.926 \tag{3}$$

On the other hand, the *L. rhamnosus* GG survival in terms of inulin (In) and $CaHPO_4$ concentration fits a quadratic equation:

$$S\ (\%) = 80.50 - 3.62\ CaHPO_4 + 2.44\ In + 4.68\ CaHPO_4\ In + 12.35\ CaHPO_4^2 - 8.03\ In^2 \quad R^2 = 0.727 \tag{4}$$

The values 88.85, 85.36, and 80.50, given in Eqs. 2, 3, and 4, respectively, represent the baseline values for predicted survival (%), when the independent variables are at the central concentration levels, i.e., $CaHPO_4$ (0.3%, *w/v*) and CM, FM, and inulin (0.2%, *w/v*) (Table 1). Equation 2 shows that increasing $CaHPO_4$ by one unit increases the *L. rhamnosus* GG survival by 3.42% maintaining the CM constant. Similarly, increasing CM by one unit increases survival by 5.52% without modifying $CaHPO_4$. Therefore, $CaHPO_4$ and CM exert a positive effect on survival, with CM having the greatest impact. On the other hand, Eq. 4 indicates that the linear terms have opposite effects: increasing inulin concentration (+2.44) increases survival, while the linear term for $CaHPO_4$ (−3.62) decreases it. Furthermore, the interaction ($+4.68 \cdot CaHPO_4 \cdot In$) is synergistic: when $CaHPO_4$ and inulin are present, *L. rhamnosus* GG survival improves more than either factor would alone. As for the quadratic terms, $+12.35 \cdot CaHPO_4^2$ indicates a positive quadratic effect (upward curvature), such that at high $CaHPO_4$ levels survival improves after a certain point. In contrast, $-8.03 \cdot In^2$ shows a negative quadratic effect, such that after a threshold survival declines.

The results of analysis of variance (ANOVA) showed that the models used were significant for *L. rhamnosus* GG survival when the encapsulating solution was prepared with $CaHPO_4$ and soluble fiber (CM, FM or inulin) (Table 2).

The *p*-values for the Lack of Fit for the survival varied from 0.0001 (inulin) to 0.048 (FM), indicating a significant ($p<0.05$) Lack-of-Fit of the applied RSM models. The model's *p*-value was highly significant ($p<0.01$) for the encapsulating solution prepared with inulin, and extremely significant ($p<0.001$) for encapsulating solution with CM and FM. Furthermore, the CV ranged from 1.32 (FM) to 7.30 (inulin), and the Adeq. Precision ranged from 7.740

Table 2 Analysis of variance for the overall effects of chia seed mucilage (CM), flaxseed mucilage (FM), or inulin (In) concentration in the encapsulating solution and CaHPO4 on *L. rhamnosus* GG survival after spray drying

Factors	Model F-value (*p*-value)	Mean Square	F_{exp} [a]	*p*>F	C.V.	Adeq. precision	Adjusted R^2	Std. Dev.	F-value (Lack of Fit)	*p*-value (Lack of Fit)
CM	168.790 (<0.0001)	366.080	243.850	<0.0001	1.380	36.756	0.949	1.230	3.850	0.029
$CaHPO_4$		140.730	93.740	<0.0001						
In	6.930 (0.0023)	71.610	1.940	0.1872	7.300	7.740	0.622	6.080	21.110	0.0001
$CaHPO_4$		156.960	4.250	0.0599						
In^2		282.280	7.640	0.0161						
$CaHPO_4^2$		668.430	18.090	0.0009						
In x $CaHPO_4$		175.47	4.750	0.0483						
FM	101.060 (<0.0001)	146.450	115.510	<0.0001	1.320	29.138	0.918	1.130	3.260	0.048
$CaHPO_4$		109.810	86.610	<0.0001						

[a] F_{exp} = Fisher ratio. Test for comparing model variance with residual variance

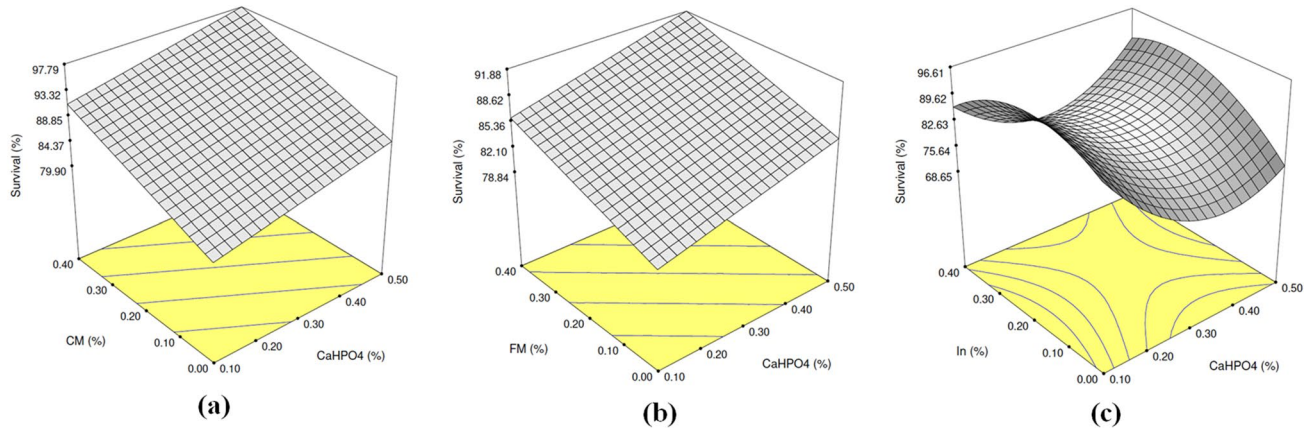

Fig. 2 Three-dimensional surface plot for spray dried *L. rhamnosus* GG survival. Encapsulating solution was prepared with: sodium alginate (4%, *w/v*), succinic acid (2%, *w/v*), $CaHPO_4$ (0.1, 0.3, 0.5%, *w/v*) and soluble fiber (0.0, 0.2, 0.4%, *w/v*): (**a**) Chia seed mucilage (CM), (**b**) Flaxseed mucilage (FM), (**c**) Inulin (In)

(inulin) to 36.756 (CM). Moreover, R^2 was in the range of 0.727 to 0.955, showing the stronger correlation for CM (0.955) and a weaker for inulin (0.727), as given in Eqs. 2 and 4, respectively. The results showed that the applied RSM models were adequate, precise and reproducible for optimizing the encapsulation solution in presence of CM and $CaHPO_4$ or FM and $CaHPO_4$.

Figure 2 shows the three-dimensional (3D) response surface plot of *L. rhamnosus* GG survival affected by soluble fiber (CM, FM or inulin) and $CaHPO_4$ concentrations. In general, CM (0.4%, *w/v*) and $CaHPO_4$ (0.5%, *w/v*) provided the highest *L. rhamnosus* GG survival (98.26%), while the lowest survival (68.36%) was observed without soluble fiber in the encapsulating solution composed of sodium alginate (4%, *w/v*), succinic acid (2%, *w/v*), and $CaHPO_4$ (0.1%, *w/v*) (Table 1). In the presence of $CaHPO_4$ (0.5%, *w/v*), *L. rhamnosus* GG survival reached 98.26%, 93.01%, and 94.64% when the encapsulation solution was supplemented with CM (0.4%, *w/v*), FM (0.4%, *w/v*), and inulin (0.2%, *w/v*), respectively, (Fig. 2a-c; Table 1).

The optimized encapsulation solution for *L. rhamnosus* GG survival in CM or FM presence was subsequently used to determine *L. plantarum*, *B. infantis*, and *B. longum* survival (Table 3). After drying process, the viable cell counts ranged from 1.5×10^{10} to 4.8×10^{11} CFU/g of dry weight. The results show that the survival rate was greater than 90% for the four probiotic strains after encapsulation by spray drying at 130 °C. In the CM (0.4%, *w/v*) presence, the survival rate varied between 93.39% (*B. infantis*) and 98.47% (*L. plantarum*), while in the FM (0.4%, *w/v*) presence it ranged from 93.28% (*L. rhamnosus* GG) and 97.91% (*L. plantarum*). The survival rate of *L. plantarum* was significantly ($p < 0.05$) higher than that achieved by *B. infantis* and *L. rhamnosus* GG when encapsulated with CM and FM, respectively.

Table 3 Survival of probiotic strains encapsulated by spray drying (130 °C) in a cross-linked alginate matrix (sodium alginate [4%, *w/v*] and succinic acid [2%, *w/v*]) supplemented with CM or FM and $CaHPO_4$ (0.5%, *w/v*)

Probiotic Strain	Survival rate of probiotic strains microcapsulated in cross-linked alginate matrices † (%)	
	CM † (0.4%, *w/v*)	FM † (0.4%, *w/v*)
L. rhamnosus GG	97.83 ± 0.87 ab	93.28 ± 0.29 b
L. plantarum	98.47 ± 1.02 a	97.91 ± 1.52 a
B. infantis	93.39 ± 3.32 b	97.75 ± 2.37 ab
B. longum	94.69 ± 1.26 ab	97.75 ± 1.93 ab

†Average of three independent experiments. Different letters in the same column indicate significant differences according to Tukey's one-way ANOVA ($p < 0.05$)

Physical Properties of Dry Probiotic Microcapsules

Moisture Content Overall, the moisture content (Table 1) of *L. rhamnosus* GG microcapsules in a cross-linked alginate matrix supplemented with CM, FM, or Inulin ranged from 12.45% to 20.02%. Inulin supplemented microcapsules exhibited the highest moisture content (14.33–20.02%). Moisture content influences the quality of dehydrated products, affecting attributes such as appearance, flavor, weight, microbial growth, and shelf life. These products are expected to have low moisture content (~ 5%) according to FAO [41]. The high moisture content can be related to the cross-linked alginate-based microencapsulation technique, as well as the presence of soluble fiber components (CM, FM, or inulin). Alginate hinders the release of moisture

from the encapsulating matrix due to its high water-binding capacity [42]. Similarly, mucilages can retain water due to the presence of hydroxyl groups [29].

Morphology SEM micrographs (Fig. 1) show microcapsules containing *L. rhamnosus* GG encapsulated in a cross-linked alginate matrix, supplemented with CM (0.4% *w/v*) (Fig. 1d) or FM (0.4% *w/v*) (Fig. 1h). The microcapsules exhibit no structural differences; they vary in size, are free of entrapped cells, fissures or cracks, and possess an external structure with cavities similar to a "deflated balloon" (indicated by a white arrow). Smaller microcapsules are trapped within the larger cavities. The morphology is similar to that observed in inulin supplemented microcapsules. Morphology of spray dried microcapsules depends on factors such as the drying temperature, presence of components, and the viscosity of the encapsulating solution. The irregular shapes of the microcapsules are probably related to the processing conditions, particularly heat penetration and water evaporation rate from the droplets [43].

Particle Size Laser diffraction particle size analysis revealed a monomodal distribution for all three microcapsules types (*L. rhamnosus* GG encapsulated in cross-linked alginate matrix: control, and those supplemented with CM, FM or inulin). The average particle sizes were 8.80, 9.83, 10.97, and 7.20 µm, respectively, ranging from 0.15 to 414.05 µm (Fig. 1d, h).

Effect of Heat Treatment on the Viability of *L. rhamnosus* GG Microencapsulated in Cross-Linked Alginate Matrices and CM or FM by Spray Drying

We investigated the heat tolerance of *L. rhamnosus* GG encapsulated in cross-linked alginate matrix, either in the control ($CaHPO_4$, 0.5% *w/v*) or supplemented with 0.4% (*w/v*) CM or FM under different combinations of time and temperature. *L. rhamnosus* GG survival rate (Table 4) ranged from 57.22% (80 °C × 5 min) to 103.98% (40 °C × 5 min) when encapsulated with CM and FM, respectively. The average viable cell count was 6.93×10^5 CFU/mL and 5.15×10^{10} CFU/mL, respectively after heat treatment. *L. rhamnosus* GG survival rate was significantly ($p < 0.05$) lower when encapsulated with CM and subjected to 70 °C for 3 min and 80 °C for 5 min, compared to control microcapsules and those supplemented with FM. In presence of FM, the survival rate was significantly ($p < 0.05$) higher at 60 °C for 3 min, reaching a value of 95.55%.

Table 4 Survival rate (%) of encapsulated *L. rhamnosus* GG after different heat treatments

Heat Treatment	Survival rate of *L. rhamnosus* GG microcapsulated in cross-linked alginate matrices † (%)		
	Control	CM	FM
40 °C × 5 min	103.42 ± 0.64 a	97.21 ± 0.80 b	103.98 ± 0.08 a
50 °C × 5 min	101.05 ± 0.85 a	97.41 ± 0.38 b	101.93 ± 0.15 a
60 °C × 3 min	90.67 ± 0.40 b	91.23 ± 0.10 b	95.55 ± 0.09 a
70 °C × 3 min	87.69 ± 0.68 a	79.79 ± 0.83 c	82.31 ± 0.18 b
80 °C × 2 min	84.02 ± 0.81 a	71.13 ± 0.08 b	82.60 ± 0.61 a
80 °C × 5 min	81.43 ± 0.59 a	57.22 ± 0.45 c	78.59 ± 0.34 b

†Average of three independent experiments. Different letters within the same heat treatment indicate significant differences according to Tukey's one-way ANOVA ($p < 0.05$)

Effect of Storage Conditions on Microencapsulated *L. rhamnosus* GG Viability in Incorporated in Food Products

L. rhamnosus GG survival rates during storage at 4 °C are shown in Table 5 and at 25 °C in Fig. 4. At this stage, three *L. rhamnosus* GG microcapsules types were used: control formulation encapsulated in a cross-linked alginate matrix, and two formulations supplemented with CM and FM. Each of microcapsule type was mixed with two powdered food products: instant soup and flan. The mixtures were then incubated at 4 °C and 25 °C, and viability was monitored over 90 days. Moreover, the rate constants for probiotic inactivation during storage were calculated according to a first-order kinetics model.

L. rhamnosus GG viability at the beginning of storage ranged from 9.66 ± 0.07 to 10.18 ± 0.06 Log CFU/g dry powder (Table 5). Thereafter, the viability decreased linearly during storage at 4 °C, both in the microcapsules added to the flan powder dessert and the powdered soup. Values ranged from 9.02 to 9.67 Log CFU/g after 90 days storage. *L. rhamnosus* GG viability showed a great reduction during storage at 25 °C compared to 4 °C storage. A 25 °C, microcapsules added to the powdered flan dessert, supplemented with CM, reached counts of 6.01 and 4.70 Log CFU/g of dry product after 21 and 90 days of storage, respectively (Fig. 3a). Meanwhile, microcapsules added to the powdered soup and supplemented with FM reached 6.92

Table 5 Viability of encapsulated *L. rhamnosus* GG in soup powder (SP) and Flan powder dessert (FPD) during storage at 4 °C for 1, 45, and 90 days

Encapsulating solution	Food Product	Viability † (Log CFU/g Powder)		
		Day 1	Day 45	Day 90
CM	FPD	9.86 ± 0.03	9.47 ± 0.21	9.02 ± 0.51
	SP	9.89 ± 0.01	9.43 ± 0.21	9.41 ± 0.28
FM	FPD	10.12 ± 0.15	9.86 ± 0.01	9.53 ± 0.11
	SP	10.18 ± 0.06	9.89 ± 0.04	9.67 ± 0.13
Control	FPD	9.66 ± 0.07	10.00 ± 0.19	9.62 ± 0.12
	SP	9.86 ± 0.07	9.63 ± 0.23	9.49 ± 0.05

† Average of three independent experiments

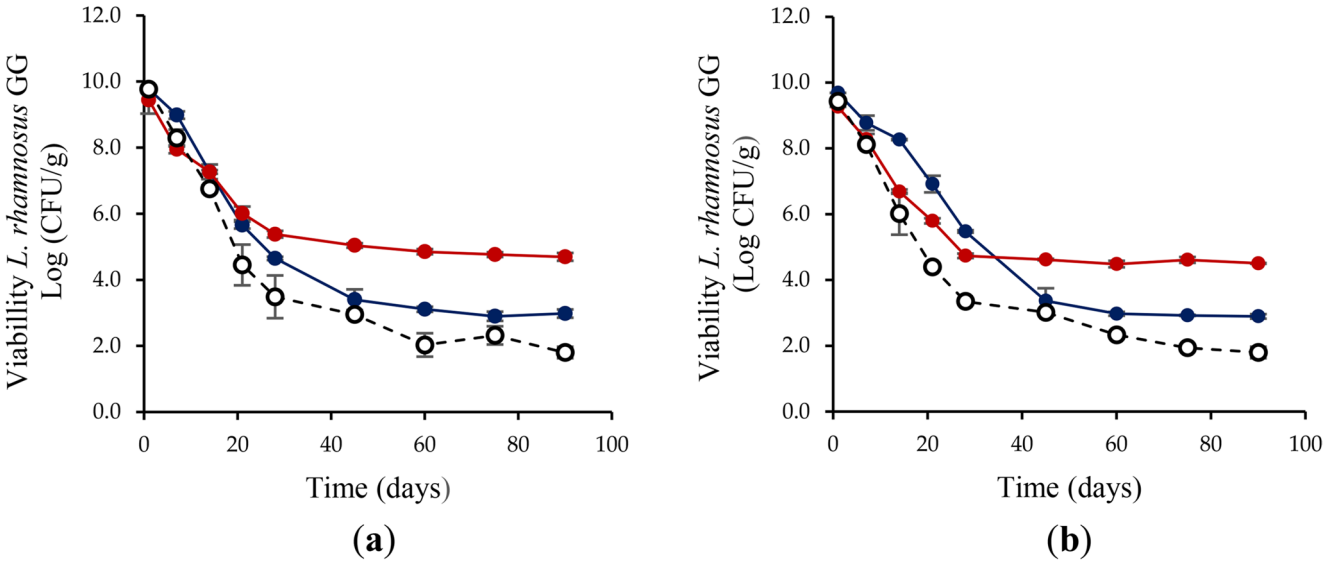

Fig. 3 Viability of encapsulated *L. rhamnosus* GG in (**a**) Flan powder dessert, (**b**) Soup powder, during storage at 25 ºC for 90 days. The encapsulation solution supplemented with 0.4% (*w/v*) of: (●) FM, (●) CM, and (○) control (without CM or FM)

Log CFU/g of dry product; and no mixture exceeded 4.51 Log CFU/g of dry product after 90 days (Fig. 3b).

Additionally, the viability reduction rate of microencapsulated *L. rhamnosus* GG was determined as a function of the encapsulation solution, storage temperature and food product. This parameter allows quantifying how rapidly its ability to survive decreases under certain conditions, such as storage, temperature, formulation, and others. The results show that the linear slope—indicator of this rate—was higher for *L. rhamnosus* GG encapsulated in the control solution (without CM or FM), both in the mixture with powdered flan dessert and in powdered soup, reaching values of 0.241 (Fig. 4a) and 0.246 (Fig. 4b), respectively.

Furthermore, to evaluate the inactivation rate of microencapsulated *L. rhamnosus* GG during storage at 25 °C, the inactivation rate constants were determined using a first-order kinetic model. This model showed a good fit, with R^2 values ranging from 0.974 to 0.993. The results showed that the encapsulation solution with CM had a lower inactivation rate ($k = 0.148\ day^{-1}$) when mixed with powdered flan dessert. Furthermore, the encapsulation solution with FM

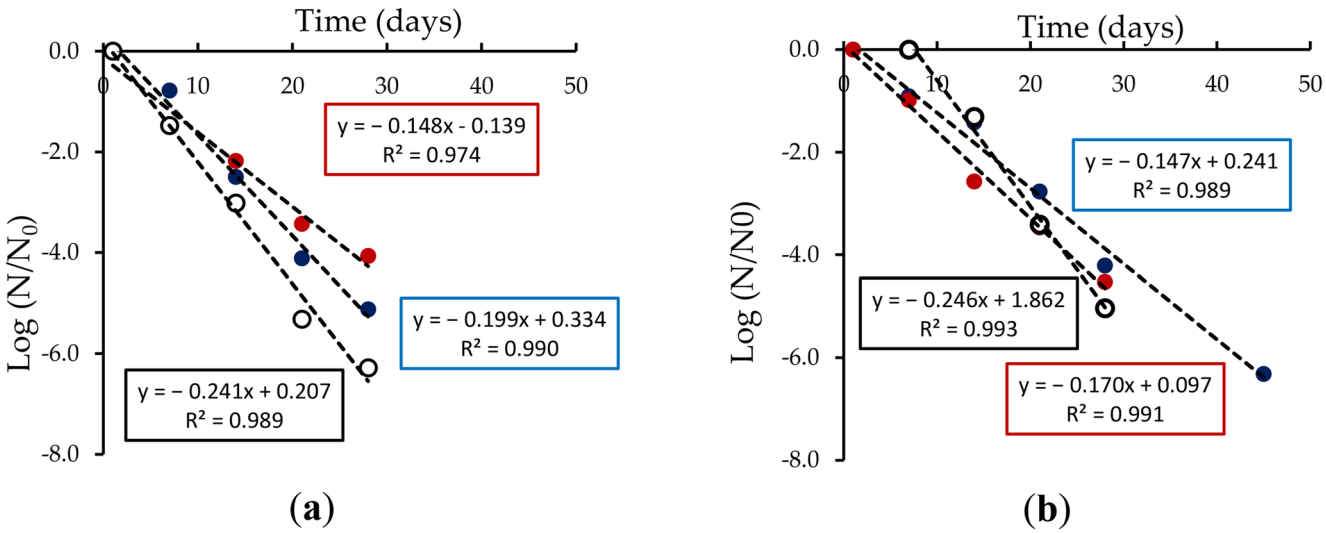

Fig. 4 Survival rates of microencapsulated *L. rhamnosus* GG as a función of the encapsularon solution, storage temperature (25 ºC), and food product. (**a**) Flan powder dessert, (**b**) Soup powder, The encapsulation solution supplemented with 0.4% (*w/v*) of: (●) FM, (●) CM, and (○) control (without CM or FM)

provided greater stability to the probiotic in powdered soup, with an inactivation rate of k = 0.147 day^{-1}.

Discussion

Spray-drying encapsulation is considered a versatile process, as it allows control over both the characteristics of the final product and the process efficiency through optimization of the encapsulation solution and operating parameters. Factors that influence final product quality and process efficiency include feed solution viscosity, inlet air temperature, feed flow rate, and drying air flow [40]. The encapsulation solution viscosity affects particle size [44], moisture content and product shelf life. The control encapsulation solution (without CM, FM, or inulin) showed viscosity increase with increasing $CaHPO_4$ concentration. This change in viscosity is associated with increase in solids concentration, due to the higher $CaHPO_4$ content in the solution. On the other hand, the encapsulation solution viscosity decreased to varying degrees in the presence of CM, FM, or inulin at the same $CaHPO_4$ concentration. This decrease could be related to $CaHPO_4$, whose divalent ions induce the aggregation and contraction of polysaccharides present in CM, FM and inulin, reducing the solution viscosity [45, 46].

We evaluated the effect of the encapsulating solution used in the new spray-drying method, in combination with CM, FM or inulin as encapsulating agents, on *L. rhamnosus* GG (ATCC 53103) survival. The encapsulation solution was optimized using RSM, a useful tool to evaluate the effects of multiple factors and their interactions on one or more response variables [47]. As shown in Figs. 2a, b *L. rhamnosus* GG survival increased with increasing CM or FM concentrations, as well as with increasing $CaHPO_4$ concentrations reaching survival rates (> 90%). On the other hand, Fig. 2c shows a saddle-point response when the encapsulation solution was supplemented with inulin and $CaHPO_4$. This point corresponds to an inflection between a relative maximum and minimum [48]. The results indicate that *L. rhamnosus* GG survival can be improved by optimizing the composition of the encapsulation solution. The higher survival observed at the highest concentration of CM or FM suggests that these components may act as thermoprotective agents during the drying process. This protective effect can be attributed to the thermal properties of CM and FM, whose degradation temperatures range from 280 to 360 °C and 200–350 °C, respectively [49, 50].

The high moisture content in *L. rhamnosus* GG microcapsules is due to the presence of alginate and seed mucilages (CM and FM), which promote water retention in the encapsulation matrix. This characteristic makes it difficult to remove water during spray-drying, resulting in a higher-than-expected final moisture content for powdered products. Furthermore, the $CaHPO_4$ inclusion creates a dense matrix that could hinder water migration during spray drying. This matrix, which can protect the encapsulated microorganisms from adverse conditions, contributes to moisture retention within the particles. High residual moisture can negatively affect probiotic stability, powder flowability, and susceptibility to undesired reactions or microbiological contamination during storage. Some strategies to improve this parameter include reducing the mucilage content and adjusting the inlet temperature and feed rate during drying.

The optimized encapsulation formulation, added with CM, would have a thermoprotective effect, offering protection against the thermal conditions present in production processes such as spray drying. The high survival rates (> 90%) achieved by the four probiotics evaluated suggest that CM and FM do not negatively affect cell viability and would preserve their functionality. Furthermore, FM can also offer protection against thermal damage during the spray drying. The higher survival rate of *L. plantarum* microencapsulated with CM and FM suggest that this strain has greater resistance to high temperatures.

Although *L. rhamnosus* GG is a mesophile, it grows over a wide range of temperatures [51], which would explain its high survival rates under mild heat treatments (Table 4). Valík et al. [52] described the growth dynamics of *L. rhamnosus* GG in milk using the cardinal temperature model. The results showed that *L. rhamnosus* GG had T(min) = 2.7 °C, T(opt) = 44.4 °C, and T(max) = 52.0 °C. Another factor to consider is the medium in which the test was performed. MRS broth is an optimal culture medium for its development, with an appropriate composition that promotes growth. On the other hand, a 10 °C increase resulted in a decrease in viability; however, this decrease was not significant. These results suggest a greater protective effect of the control encapsulation solution and the one supplemented with FM against heat stress. Malmo et al. [38] evaluated the heat stress tolerance of microencapsulated *Lactobacillus reuteri* DSM 17938 by spray drying in a chitosan-coated alginate matrix. The results showed that *L. reuteri* did not survive at 80 °C for 5 min. In contrast, *L. rhamnosus* GG exhibited survival rates above 57% under a similar heat treatment. These findings suggest a protective effect of the encapsulation solutions, both in the control and in those supplemented with CM or FM. They also suggest that the microcapsules did not disintegrate or collapse after exposure to heat treatment. Furthermore, the results show that *L. rhamnosus* GG has greater heat resistance compared to *L. reuteri* DSM 17938.

The viability of encapsulated *L. rhamnosus* GG and mixed with flan or powdered soup at two storage temperatures (4 °C and 25 °C) was monitored. Viability depended

on both the storage temperature and the product with which the microcapsules were mixed. At 4 °C, viability remained stable (> 9 Log CFU/g dry product) for 90 days (Table 5). In contrast, at 25 °C viability decreased in all tested combinations (Fig. 3). Only two samples-maintained values above 6 Log CFU/g after 21 days: microcapsules of CM mixed with flan, and microcapsules of FM mixed with dehydrated soup. In relative terms, *L. rhamnosus* GG showed the lowest viability loss when encapsulated with FM and mixed with soup, achieving a 3% reduction. In contrast, the viability loss in other combinations was greater than 20%. Furthermore, Bustamante et al. [37] reported that *L. rhamnosus* GG encapsulated in the control solution and in formulations with CM (0.4% *w/v*) or FM (0.4% *w/v*) maintained viability levels greater than 7.1 Log CFU/g of dry product, after 21 days of storage at 25 °C. This loss of viability could be associated with the composition and quality of the products, including factors such as pH, lipid content, colorants, salts, and other ingredients present in the dehydrated soup and dessert flan formulations. However, these findings suggest that cold storage remains an essential factor for product stability.

Jeoh et al. [53] point out that the extent of cross-linking of alginate influences the barrier properties of particles, such that a higher cross-linking extent can improve oxidative stability and limit diffusion. The cross-linking extents can be controlled by varying the calcium: alginate ratio (between 0.125 and 0.25). However, the choice of alginates also influences the extent crosslinking. Jeoh et al. [53] found that the gelation extent of microcapsules obtained by spray drying depends on the alginate molecular weight at saturating concentrations of the calcium salt. Furthermore, they determined that a low-viscosity alginate achieves 75% crosslinking, while a higher molecular weight alginate, and therefore higher viscosity, achieves 95%. Since molecular weight alginate is not always known, it can be correlated with viscosity, the latter being used as a selection criterion. Therefore, the *L. rhamnosus* GG encapsulated and stored at 25 °C could be improved by using a higher molecular weight alginate. Therefore, a denser cross-linked network would be expected to provide a more stable structure, capable of more effectively protecting the probiotic from variations in temperature, humidity or pH. Furthermore, greater cross-linking can reduce the probiotics premature release, favoring their survival both during storage and gastrointestinal transit.

Conclusions

In this study, the effect of adding of three soluble fibers to a cross-linked alginate matrix on *L. rhamnosus* GG survival encapsulated by spray drying was evaluated. The results demonstrated that the addition of CM and FM to a cross-linked alginate matrix improved the *L. rhamnosus* GG survival, with CM being the component with the greatest impact. Furthermore, microcapsules of *L. plantarum*, *L. rhamnosus* GG, *B. infantis* and *B. longum* showed high survival rates when the cross-linked alginate matrix was supplemented with CM and FM. Therefore, both fibers do not present adverse effects on bacterial viability and could contribute to preserving their stability.

Additionally, *L. rhamnosus* GG microcapsules showed resistance to heat stress when the cross-linked alginate matrix was supplemented with CM and FM. Regarding stability in powdered food matrices, *L. rhamnosus* GG encapsulated viability was affected by storage temperature, where cold storage remains an essential factor for product stability. The lowest inactivation rates were obtained in the presence of CM and FM.

Taken together, these findings suggest that both CM and FM could be effective alternatives as encapsulating agents in spray-drying processes. Nevertheless, the cross-linked alginate matrix supplemented with CM or FM still needs to be optimized to decrease the moisture content of the microcapsules and maintain the viability of the probiotic strains during long periods of storage at room temperature. Moreover, it is necessary to evaluate the degree of protection that a cross-linked alginate matrix, enriched with CM or FM, can offer to probiotic strains when exposed to simulated conditions of the gastrointestinal system in vitro, in order to determine their viability and efficacy in digestive environments.

Acknowledgements We acknowledge ANID for their support through FONDECYT-REGULAR project No. 1211211. We also extended our gratitude to the ANID-Milenio-NCN2023_054 project and BIOREN-UFRO for their technical assistance.

Author Contributions All authors have contributed equally in Methodology, Investigation, Formal analysis, Writing – original draft, Conceptualization.

Funding This research was supported by ANID (Agencia Nacional de Investigación y Desarrollo, Chile) through FONDECYT-REGULAR project No. 1211211.

Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

Informed Consent Not applicable." for studies not involving humans.

References

1. Research and Markest. Probiotics market size, share & trends analysis report by product, by ingredient (bacteria, yeast), by distribution channel, by end use (human probiotics, animal probiotics), by

region, and segment forecasts, 2024–2030. In Probiotics Market 2024 (2024) https://www.researchandmarkets.com. Accessed 02 June 2025
2. Kerry RG, Patra JK, Gouda s, Park Y, Shin H-S, Das G (2018) Benefaction of probiotics for human health: A review. J Food Drug Anal 26:927–939
3. Lau LYJ, Quek SY (2024) Probiotics: health benefits, food application, and colonization in the human Gastrointestinal tract. Food Bioeng 3:41–64
4. Maftei NM, Raileanu CR, Balta AA, Ambrose L, Boev M, Marin DB, Lisa EL (2024) The potential impact of probiotics on human health: an update on their health-promoting properties. Microorganisms 12:234
5. Çaglar E, Cildir SK, Ergeneli S, Sandalli N, Twetman S (2006) *Salivarius mutans* Streptococci and lactobacilli levels after ingestión of the probiotic bacterium *Lactobacillus reuteri* ATCC 55739 by straws or tables. Acta Odontol Scand 64:314–318
6. Bustamante M, Oomah BD, Oliveira WP, Burgos-Díaz C, Rubilar M, Shene C (2019) Probiotics and prebiotics potential for the care of skin, female urogenital tract, and respiratory tract. Folia Microbiologica
7. Bustamante M, Oomah BD, Mosi-Roa Y, Rubilar M, Burgos-Díaz C (2019) Probiotics as an adjunct therapy for the treatment of halitosis, dental caries and periodontitis. Probiotics Antimicrob Proteins
8. Reiriz M, Beltrán-Velasco AI, Echeverry-Alzate V, Martínez-Miguel E, Gómez-Senent S, Uceda S, Clemente-Suárez VJ (2025) *Bifidobacterium infantis* and *Bifidobacterium Breve* improve symptomatology and neuronal damage in neurodegenerative disease: A systematic review. Nutrients 17:391
9. Vasiljevic T, Shah N (2008) Probiotics—from Metchnikoff to bioactives. Int Dairy J 18:714–728
10. Meena KK, Taneja N, Ojha A, Meena S (2023) Application of spray-drying and freeze-drying for microencapsulation of lactic acid bacteria: A review. Ann Phytomed 12:706–716
11. Hossain MN, Ranadheera CS, Fang Z, Ajlouni S (2021) Impact of encapsulating probiotics with cocoa powder on the viability of probiotics during chocolate processing, storage, and in vitro Gastrointestinal digestion. J Food Sci 2021:1–13
12. Albadran HA, Chatzifragkou A, Khutoryanskiy VV, Charalampopoulos D (2015) Stability of probiotic *Lactobacillus plantarum* in dry microcapsules under accelerated storage conditions. Food Res Int 74:208–216
13. Ma J, Xu C, Yu H, Feng Z, Yu W, Gu L, Liu Z, Chen L, Jiang Z, Hou J (2021) Electro-encapsulation of probiotics in gum Arabic-pullulan blend nanofibres using electrospinning technology. Food Hydrocolloids 111:106381
14. Kakuda L, Jaramillo Y, Niño-Arias FC, Souza MF, Conceição EC, Alves VF, Almeida OGG, De Martinis ECP, Oliveira WP (2023) Process development for the spray-drying of probiotic bacteria and evaluation of the product quality. J. Vis. Exp 7
15. Ananta E, Volkert M, Knorr D (2005) Cellular injuries and storage stability of spray-dried *Lactobacillus rhamnosus* GG. Int Dairy J 15:399–409
16. Anal AK, Singh H (2007) Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. Trends Food Sci Technol 18:240–251
17. Sosnik A, Seremeta KP (2015) Advantages and challenges of the spray-drying technology for the production of pure drug particles and drug-loaded polymeric carriers. Adv Colloids Interface Sci. https://doi.org/10.1016/j.cis.2015.05.003
18. Liu B, Fu N, Wai M, Chen XD (2018) Heat stability of *Lactobacillus rhamnosus* GG and its cellular membrane during droplet drying and heat treatment. Food Res Int 112:56–65
19. Bustamante M, Laurie-Martínez L, Vergara D, Campos-Vega R, Rubilar M, Shene C (2020) Effect of three polysaccharides (inulin, and mucilage from Chia and flax seeds) on the survival of probiotic bacteria encapsulated by spray drying. Appl Sci 10:4623
20. Simpson PJ, Stanton C, Fitzgerald GF, Ross RP (2005) Intrinsic tolerance of *Bifidobacterium* species to heat and oxygen and survival following spray drying and storage. J Appl Microbiol 99:493–501
21. Strobel SA, Allen K, Roberts C, Jimenez D, Scher HB, Jeoh T (2018) Industrially-scalable microencapsulation of plant beneficial bacteria in dry cross-linked alginate matrix. Ind Biotechnol 14:138–147
22. Kawakita R, Leveau JHJ, Jeoh T (2021) Optimizing viability and yield and improving stability of Gram-negative, non-spore forming plant-beneficial bacteria encapsulated by spray-drying. Bioprocess Biosyst Eng
23. 4, Goderska K, Kozłowski P (2021) Evaluation of microencapsulated synbiotic preparations containing lactobionic acid. Appl Biochem Biotechnol 193:3483–3495
24. Fritzen-Freire CB, Prudêncio ES, Amboni RDMC, Pinto SS, Negrão-Murakami AN, Murakami FS (2012) Microencapsulation of bifidobacterial by spray drying in the presence of prebiotics. Food Res Int 45:306–312
25. Lobel BT, Baiocco D, Al-Sharabi M, Routh AF, Zhang Z, Cayre OJ (2024) Current challenges in microcapsule designs and microencapsulation processes: A review. ACS Appl Mater Interfaces 16:40326–40355
26. Lapsiri W, Bhandari B, Wanchaitanawong P (2012) Viability of *Lactobacillus plantarum* TISTR 2075 in different protectants during spray drying and storage. Dry Technol 30:1407–1412
27. Bustamante M, Oomah BD, Rubilar M, Shene C (2017) Effective *Lactobacillus plantarum* and *Bifidobacterium infantis* encapsulation with Chia seed (*Salvia Hispanica* L.) and flaxseed (*Linum usitatissimum* L.) mucilage and soluble protein by spray drying. Food Chem 216:97–105
28. Waghmare R, Preethi R, Moses JA, Anandharamakrishnan C (2022) Mucilages: sources, extraction methods, and characteristics for their use as encapsulation agents. Crit Rev Food Sci Nut 62:4186–4207
29. Tosif MM, Najda A, Bains A, Kaushik R, Dhull SB, Chawla P, Walasek-Janusz M (2021) A comprehensive review on plant-derived mucilage: Characterization, functional properties, applications, and its utilization for nanocarrier fabrication. Polymers 13:1066
30. Oomah BD (2001) Flaxseed as a functional food source. J Sci Food Agric 81:889–894
31. Sandoval-Oliveros MR, Paredes-López O (2013) Isolation and characterization of proteins from Chia seeds (*Salvia Hispanica* L). J Agric Food Chem 61:193–201
32. Naran R, Chen G, Carpita NC (2008) Novel rhamnogalacturonan I and arabinoxylan polysaccharides of flax seed mucilage. Plant Physiol 148:132–141
33. Lin KY, Daniel JR, Whistler RL (1994) Structure of Chia seed polysaccharide exudate. Carbohydr Polym 23:13–18
34. Tamargo A, Cueva C, Laguna L, Moreno-Arribas MV, Muñoz LA (2018) Understanding the impact of Chia seed mucilage on human gut microbiota by using the dynamic Gastrointestinal model simgi®. J Funct Foods 50:104–111
35. Bustamante M, Villarroel M, Rubilar M, Shene C (2015) *Lactobacillus acidophilus* La-05 encapsulated by spray drying: effect of mucilage and protein from flaxseed (*Linum usitatissimum* L). LWT Food Sci Technol 62:1162–1168
36. de Man JC, Rogosa M, Sharpe ME (1960) A medium for the cultivation of *Lactobacilli*. J App Bacteriol 60:130–135
37. Bustamante M, Oomah BD, Burgos-Diaz C, Vergara D, Flores L, Shene C (2025) Viability of microencapsulated probiotics in

cross-linked alginate matrices and Chia seed or flaxseed mucilage during spray-drying and storage. Microorganisms 13:1457
38. Malmo C, La Storia A, Mauriello G (2011) Microencapsulation of *Lactobacillus reuteri* DSM 17938 cells coated in alginate beads with Chitosan by spray drying to use as a probiotic cell in a chocolate soufflé. Food Bioprocess Technol 6:795–805
39. Sansone F, Mencherini T, Picerno P, d' Amore M, Aquino RP, Lauro MR (2011) Maltodextrin/pectin microparticles by spray drying as carrier for nutraceutical extracts. J Food Eng 105:468–476
40. Arpagaus C, Andreas C, David R, Elham A, Seid MJ (2018) Nano spray drying for encapsulation of pharmaceuticals. Int J Pharma 546:194–214
41. Food and Agriculture Organization (FAO) (2018) Standard for milk powders and cream Powder, CXS 207–1999. Codex Alimentarius International Food Standards
42. Singha P, Kasiviswanathan M, Padmanaban K (2018) Influence of processing conditions of apparent viscosity and system parameters during extrusion of distiller's dried grains-based snacks. Food Sci Nutr 6:101–110
43. Benkirane G, Ananou S, Agsuti G, Manni L, Chihib NE, Dumas E, Gharsallaoui A (2024) Effect of microencapsulation on the bio-preservative and probiotic properties of *Enterococcus Durans* F21. Food Biosci 60:104312
44. Permanadewi I, Kumoro AC, Wardhani DH, Aryanti N (2022) Effect of viscosity on iron encapsulation using alginate as a carrying agent in a controlled spray drying process. Food Res 6:5667
45. Capitani MI, Corzo-Rios LJ, Chel-Guerrero LA, Betancur-Ancona DA, Nolasco SM, Tomás MC (2015) Rheological properties of aqueous dispersions of Chia (*Salvia Hispanica* L.) mucilage. J Food Eng 149:70–77
46. Roulard R, Petit E, Mesnard F, Rhazi L (2016) Molecular investigations of flaxseed mucilage polysaccharides. Int J Biol Macromol 86:840–847
47. Xu Q, Shen YY, Wang HF, Zhang NP, Xu S, Zhang L (2013) Application of response surface methodology to optimize extraction of flavonoids from *Fructus sophorae*. Food Chem 138:2122–2129
48. Nair AT, Makwana AR, Ahammed MM (2013) The use of response surface methodology for modelling and analysis of water and wastewater treatment processes: a review. Water Sci Technol 69:464–478
49. Timilsena YP, Adhikari R, Kasapis S, Adhikari B (2016) Molecular and functional characteristics of purified gum from Australian Chia seeds. Carbohydr Polym 136:128–136
50. Dubois F, Musa C, Duponchel B, Tidahy L, Sécordel X, Mallard I, Delattre F (2020) Nuclear magnetic resonance and calorimetric investigations of extraction mode on flaxseed gum composition. Polymers 12:2654
51. Liew SL, Arrif AB, Raha AR, Ho YW (2005) Optimization of medium composition for the production of a probiotic microrganism, *Lactobacillus rhamnosus*, using response surface methodology. Int J Food Microbiol 102:137–142
52. Valík L, Medveďová A, Čižniar M, Lipt.kov D (2013) Evaluation of temperature effect on growth rate of *Lactobacillus rhamnosus* GG in milk using secondary models. Chem Pap 67:737–742
53. Jeoh T, Wong DE, Strobel SA, Hudnall K, Pereira NR, Williams KA, Arbaugh BM, Cunniffe JC, Scher HB (2021) How alginate properties influence in situ internal gelation in crosslinked alginate microcapsules (CLAMs) formed by spray drying. PLoS ONE 16:e0247171