



Composite Alginate–Quince–Flaxseed Microcapsules for Enhanced Gastrointestinal Survival of *Lactocaseibacillus rhamnosus* GG

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Abstract

Low gastrointestinal survival of probiotic microorganisms is still a significant hurdle for the use of probiotics in functional foods. The aim of the present study was to develop composite alginate-based microcapsules of *Lactocaseibacillus rhamnosus* GG using the combination of Quince seed mucilage (QSM) with flaxseed mucilage (FSM) to enhance the stability and targeted intestinal delivery of *L. rhamnosus* GG. The four formulations were sodium alginate (SA), alginate–quince mucilage (SA-QM), alginate–flaxseed mucilage (SA-FM), and alginate–quince–flaxseed mucilage (SA-QFM), and were subjected to various physicochemical evaluations, gastrointestinal protection, storage stability, and in vitro release studies. Among the formulations, SA-QFM exhibited the highest encapsulation efficiency (96.18%) while the lowest was recorded for SA (81.42%). SA-QFM also generated the largest and most compact microcapsules (982.7µm) with excellent mechanical properties and minimum swelling index. After sequential simulated gastrointestinal exposure, the probiotic viability was maintained at 88.55% (SA-QFM) and 43.04% (free cells), with retention of 8.12 log CFU/g and 3.96 log CFU/g, respectively. Likewise, SA-QFM exhibited the maximum bile salt tolerance (8.58 log CFU/g), with the highest bile salt tolerance survival (93.57%). The maximum viability after refrigerated storage at 4 °C (8.84 log CFU/g) and the maximum cumulative release in SIF (98.4%) were observed for SA-QFM. The novelty of this study is the synergism of the natural co-encapsulating agents (quince seed mucilage and flaxseed mucilage) with alginate for the delivery of probiotics. In conclusion, the synergistic effect of the quince seed and flaxseed mucilage enabled the

1. Introduction

Lactocaseibacillus rhamnosus GG is one of the most studied probiotic strains, characterized by its excellent gastrointestinal tolerability, adhesion to intestinal cells, and immunomodulatory effects (Marco *et al.*, 2021). However, one of the biggest challenges to the successful incorporation of probiotics into functional foods is ensuring their survival throughout processing, storage, and gastrointestinal transit.

Bacterial survival can be greatly affected, and their efficacy impaired, by exposure to harsh environmental conditions such as oxygen, moisture, acidic gastric conditions, bile salts, and temperature changes (Champagne *et al.*, 2018). Hence, more advanced protection strategies are needed to ensure the viability of probiotics at the intestinal site, including delivering viable cells to the intestine.

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Protecting probiotic microorganisms by encapsulating them in biopolymeric matrices has proven an effective strategy for shielding them from unfavorable conditions. It has evolved into a method known as microencapsulation. Compared to other encapsulating materials, sodium alginate is also biocompatible, non-toxic, can be gently gelled, and can be used to form calcium-induced hydrogel networks (Cook *et al.*, 2012). However, alginate capsules have drawbacks such as poor mechanical strength, high porosity, and limited protection in the low-pH environment of the gastrointestinal tract. To reduce these limitations, the incorporation of complementary natural polymers, whose excellent hydration capacity, film-forming ability, and improved structural integrity of composite encapsulation systems are of interest, has been addressed (Nazzaro *et al.*, 2012).

Natural polysaccharide-rich hydrocolloids, such as quince seed mucilage and flaxseed mucilage, were found to have interesting functional properties for food and pharmaceutical applications. Quince seed mucilage is rich in highly hydrophilic polysaccharides with strong water-binding capacity, viscoelasticity, and film-forming ability (Jouki *et al.*, 2014). The flavor of flaxseed mucilage also consists mainly of arabinoxylans and rhamnogalacturonan-type polysaccharides, with excellent thickening, emulsifying, and stabilizing properties (Barbary *et al.*, 2009). The interaction between these plant mucilage and alginate can provide a reinforcing network for the alginate, resulting in a higher barrier and lower permeability, as well as greater protection of the probiotics than with alginate alone. The interest in encapsulating probiotics with natural polysaccharide based composite systems to enhance stability and targeted release to the gastrointestinal system has been recently emphasized. The use of alginate blends with natural biopolymers such as guar gum and cellulose has been shown to improve the encapsulation efficiency and mechanical strength of these materials, as well as their controlled intestinal release compared to alginate alone (Schofield *et al.*, 2024; Mariela *et al.*, 2025; You *et al.*, 2026). The synergistic effect of using two seed mucilage (quince seed mucilage and flaxseed mucilage) as co-encapsulating agents in alginate matrix for probiotic delivery has not been investigated to a great extent.

For this purpose, the present study aimed to develop and characterize alginate based composite microcapsules in which the quince seed mucilage and flaxseed mucilage were used for the encapsulation of *Lactocaseibacillus rhamnosus* GG. In particular, the present study investigated the effects of alginate and plant mucilage (both in single and combined) on encapsulation efficiency, physicochemical properties, mechanical properties and swelling characteristics. Also, the protection efficacy of the prepared microcapsules was evaluated under the sequential simulated

gastrointestinal conditions, bile salt stress, intestinal release and refrigerated storage conditions. The synergistic incorporation of quince seed and flaxseed mucilage in alginate matrix was hypothesized to increase probiotic encapsulation efficiency, gastrointestinal protection, controlled intestinal release and storage stability of the microcapsules when compared to alginate alone.

2. Materials and Methods

2.1. Materials

Sodium alginate (Sigma-Aldrich, St. Louis, MO, USA) was used as the primary encapsulating polymer to prepare probiotic microcapsules in a food-grade form. The seeds of mature quince (*Cydonia oblonga*) and flaxseed (*Linum usitatissimum*) were purchased from a local market of Bahawalpur, Pakistan and used to extract their mucilage. The probiotic strain *Lactocaseibacillus rhamnosus* GG (*L. rhamnosus* GG) was obtained from HiTech. BioTech Lab. Pvt. Lahore, Pakistan. The probiotic strain was activated, cultivated, and enumerated in de Man, Rogosa, and Sharpe broth and MRS agar (HiMedia Laboratories Pvt. Ltd., Mumbai, India). For ionic gelation, calcium chloride (Merck KGaA, Darmstadt, Germany) was used as the cross-linking agent. All chemicals and reagents used in the study were of analytical grade.

2.2. Activation of Bacterial Culture

The *Lactocaseibacillus rhamnosus* GG freeze-dried culture was reactivated according to the method described by Zubair *et al.* (2022). The culture was briefly lyophilized, then aseptically transferred into sterile de Man, Rogosa and Sharpe (MRS) broth and cultured at 37 °C for 24 h to reactivate the bacteria. An aliquot of the activated inoculum was inoculated into 100 mL of fresh MRS medium and incubated again for 24 hours at 37 °C to obtain an actively growing culture. Next, the bacterial cells were collected by centrifuging at 5000 x g for 10 minutes at 4 °C; the supernatant was removed, and the cell pellet was resuspended in fresh sterile MRS broth. The culture was then incubated for 20 h at 37 °C to maximize cell growth. After the 2nd incubation, the cells were centrifuged under the same conditions and washed with sterile phosphate-buffered saline (PBS) to remove the growth medium. Lastly, the bacterial suspension was prepared to a viable cell count of ~10⁹ CFU/mL and immediately used to produce bacterial encapsulation matrices containing probiotics.

2.3. Preparation of Alginate-Based Composite Microcapsules

A sodium alginate (2%, w/v) solution was prepared by dissolving food-grade sodium alginate in sterile distilled water under continuous magnetic stirring until fully hydrated, then stored overnight at 4 °C to obtain a homogeneous solution. The seeds were dispersed in 20× (w/v) distilled water, and the slurry was heated at 60 °C for 20 min with stirring to extract Quince seed mucilage (QM).

The seeds were swollen, and the mucilage was released from their surfaces by gently vibrating them in a laboratory blender. The extracted mucilage was filtered through cheesecloth to separate the seeds and other insoluble materials, then dried in a hot-air oven at 45 °C, powdered, and stored in airtight containers for future use (Jouki *et al.*, 2014). The extraction of flaxseed mucilage (FM) was done by suspending flaxseeds in distilled water (1:20, w/v) and stirring at 80 °C for 2 h. The extract was filtered through glass wool, concentrated at 40 °C under reduced pressure using a rotary evaporator, and the mucilage was precipitated by adding 80% ethanol (1:4, v/v). Precipitated mucilage was obtained by standing at 4 °C for 1 h, followed by centrifugation (6000 rpm, 35 min), re-dispersed in distilled water, freeze-dried, and stored in airtight containers until used (Barbary *et al.*, 2009).

Four encapsulation matrices were then formulated: SA (2% sodium alginate), SA-QM (2% sodium alginate + 0.5% QM), SA-FM (2% sodium alginate + 0.5% FM) and SA-QFM (2% sodium alginate + 0.25% QM + 0.25% FM). The selected concentrations were preliminary optimized in the trials and based on literature research, because they gave good viscosities, bead stability and high encapsulation efficiencies. All the polymer dispersions were prepared 24 h before encapsulation and stored at 4 °C to ensure full polymer hydration and better gel properties. *L. rhamnosus* GG was grown in MRS medium at 37 °C for 24 h, centrifuged (5000 × g, 10 min, 4 °C), washed twice with sterile phosphate-buffered saline (PBS), and re-suspended at approximately 10⁹ CFU/mL. The bacterial suspension was aseptically added to each polymer formulation at a polymer-to-bacterial suspension ratio of 9:1 (v/v) and gently stirred continuously. Microencapsulation was performed by extrusion: the probiotic polymer mixture was extruded from a sterile 21-gauge syringe into a sterile 0.4 M calcium chloride solution at a fixed height to induce ionic gelation. The calcium chloride solution was used to allow the freshly prepared beads to harden for 30 min, after which the beads were filtered, washed twice with sterile distilled water to remove excess calcium ions, and stored at 4 °C in sterile 0.1% peptone water until further analyses were conducted. The control treatment was the free-cell suspension (LR), which was analyzed in the same way, but without encapsulation. The encapsulation method was modified from the extrusion method to suit the formulation used in the present study, as described by Yasmin *et al.* (2019).

2.4. Encapsulation Efficiency

The probiotic microcapsules were encapsulated, and the encapsulation efficiency (EE) was calculated using the method described by Ali *et al.* (2023). In brief, 1 g of freshly prepared microcapsules was added to 9 mL of sterile 2% (w/v) sodium citrate solution (pH 7.0) to dissolve the calcium alginate matrix and release all *L. rhamnosus* GG

cells. This suspension was then well vortexed, and suitable tenfold serial dilutions were prepared in sterile 0.1% peptone water. Aliquots of appropriate dilutions were counted by the pour plate method on de Man, Rogosa and Sharpe (MRS) agar. The inoculated plates were then incubated anaerobically at 37 °C for 48 h, and the number of viable colonies was determined by using a digital colony counter. The encapsulation efficiency was determined by dividing the number of viable cells obtained from the dissociable microcapsules (N) by the number of free bacterial suspension initially added to the encapsulation matrices (N₀) and then multiplying by 100, as follows:

$$EE (\%) = \left(\frac{N}{N_0} \right) \times 100$$

The encapsulation efficiency (EE) is the ratio of the number of viable cells recovered from the dissolved microcapsules to the number of healthy free cells originally encapsulated (N/N₀), expressed as a percentage (0-100).

2.5. Characterization of Microcapsules

2.5.1. Bead Diameter

Ali *et al.* (2024) recommended a method for determining the mean diameter of freshly prepared microcapsules. The microcapsules were randomly collected from each treatment and observed by an inverted microscope (XJP-16A, Shenzhen Pride Instrument Inc., Shenzhen, China). Images of the beads containing digital data were taken, and their diameters were measured using the microscope's image analysis software. To obtain representative data, at least 30 beads were randomly selected from each treatment, and bead diameter was measured in micrometers (μm).

2.6.2. Scanning Electron Microscopy (SEM)

The surface morphology and microstructural characteristics of the prepared microcapsules were examined using scanning electron microscopy (SEM) following the method described by Ali *et al.* (2024). Briefly, freeze-dried microcapsules were mounted on aluminum stubs, sputter-coated with a thin layer of gold, and observed under SEM at an appropriate accelerating voltage. The obtained micrographs were analyzed for surface morphology, integrity, and structural differences among the encapsulation treatments.

2.5.3. Texture Profile Analysis (TPA)

The mechanical properties of the freshly prepared probiotic microcapsules were determined using texture profile analysis (TPA) according to the procedure described by Ligarda-Samanez *et al.* (2025). The analysis was carried out using a Brookfield AMETEK Texture Analyzer (AMETEK Brookfield, Middleboro, MA, USA) fitted with a 5000 g load cell. A double compression test was carried out on approximately 2 g of microcapsules from each treatment using a cylindrical probe (TA3/1000) from the Tensile Paks. The crosshead speed was set at 1 mm/s, and the samples were compressed to 40% deformation. The TPA test

comprised two consecutive compression cycles to determine the textural properties of the microcapsules, including hardness and resilience. The three replicates were analyzed for each treatment, and the results were reported as mean $\pm$ standard deviation. Texture data were obtained and analyzed with the Texture Pro software, version 1.0.19.

2.5.4. Swelling Index

The swelling behavior of probiotic microcapsules has been determined in simulated gastric fluid (SGF) at pH 2.0 and in simulated intestinal fluid (SIF) at pH 7.2, with slight modifications to the method of Anwar *et al.* (2022). In short, a known weight (W_0) of freshly prepared microcapsules from each treatment was placed in separate 25 mL portions of SGF or SIF and shaken continuously at 100 rpm at 37 °C. The microcapsules were collected at the allotted times (30, 60, 90, and 120 min), excess surface moisture was gently removed with filter paper, and they were weighed immediately (W_t). The swelling index was determined by calculating the percentage increase in bead weight from the following equation:

$$\text{Swelling Index (\%)} = \frac{W_t - W_0}{W_0} \times 100$$

W_0 is the weight of the microcapsules at the initial stage before immersion, and W_t is the weight of the swollen microcapsules at a specific sampling time.

2.6. Evaluation of Protective Capacity of Microcapsules

2.6.1. Survival under Sequential Simulated Gastrointestinal Conditions (SGF–SIF)

Free and encapsulated *Lactocaseibacillus rhamnosus* GG were assessed for their gastrointestinal protective effects under sequential simulated gastrointestinal conditions, as described by Ali *et al.* (2023). In brief, 0.5 mL of free cells or 0.5 g of microcapsules were first shaken for 2 h at 37 °C in 4.5 mL of simulated gastric fluid (SGF; pH 2.0 with 3 g/L of pepsin). The samples were then transferred to 4.5 mL of simulated intestinal fluid (SIF; pH 7.2) and re-incubated under the same conditions for an additional 2 h. Then, strains were enumerated at 0, and 120 min intervals, using the previously described method.

2.6.2. Bile Salt Tolerance

The bile salt tolerance of free (LR) and encapsulated *L. rhamnosus* GG was assessed following the method described by Yasmin *et al.* (2019). Briefly, 0.5 mL of free-cell suspension or 0.5 g of microcapsules was added to 4.5 mL of sterile 2% (w/v) bile salt solution (pH 7.0) and incubated at 37 °C under continuous shaking (100 rpm). Viable bacterial counts were determined at 0, 120, and 240 min as described previously.

2.7. Refrigerated Storage Stability at 4 °C

The storage stability under refrigerated conditions was evaluated for free (LR) and encapsulated by *Lactocaseibacillus rhamnosus* GG as described by Saeed *et al.* (2024). Samples were kept at 4 ± 1 °C, and viable cell counts were determined on days 0, 14, and 28 in the same manner as described above.

2.8. In Vitro Release of *L. rhamnosus* GG in Simulated Intestinal Fluid (SIF)

The release behavior of encapsulated *Lactocaseibacillus rhamnosus* GG (LGG) in simulated intestinal fluid (SIF) was assessed following the procedure described by Yasmin *et al.* (2019), with a few modifications. Microcapsules (0.5 g) were added to 4.5 mL of pre-warmed SIF (pH 6.8) and shaken continuously at 100 rpm at 37 °C. The samples were removed at specific time points (30, 60, 90, 120 and 150 min) and counted for viable cells as described in Section 2.4. The release profile was presented as 'Viable bacterial count (log CFU/g) vs incubation period.

2.9. Statistical Analysis

All experiments were repeated thrice and the data presented as mean $\pm$ standard deviation. The data were analyzed using one-way analysis of variance (ANOVA) in IBM SPSS Statistics, and significant differences between treatment means were determined using Tukey's honestly significant difference (HSD) test at $p < 0.05$.

3. Results and Discussions

3.1. Encapsulation Efficiency of *Lactocaseibacillus rhamnosus* GG

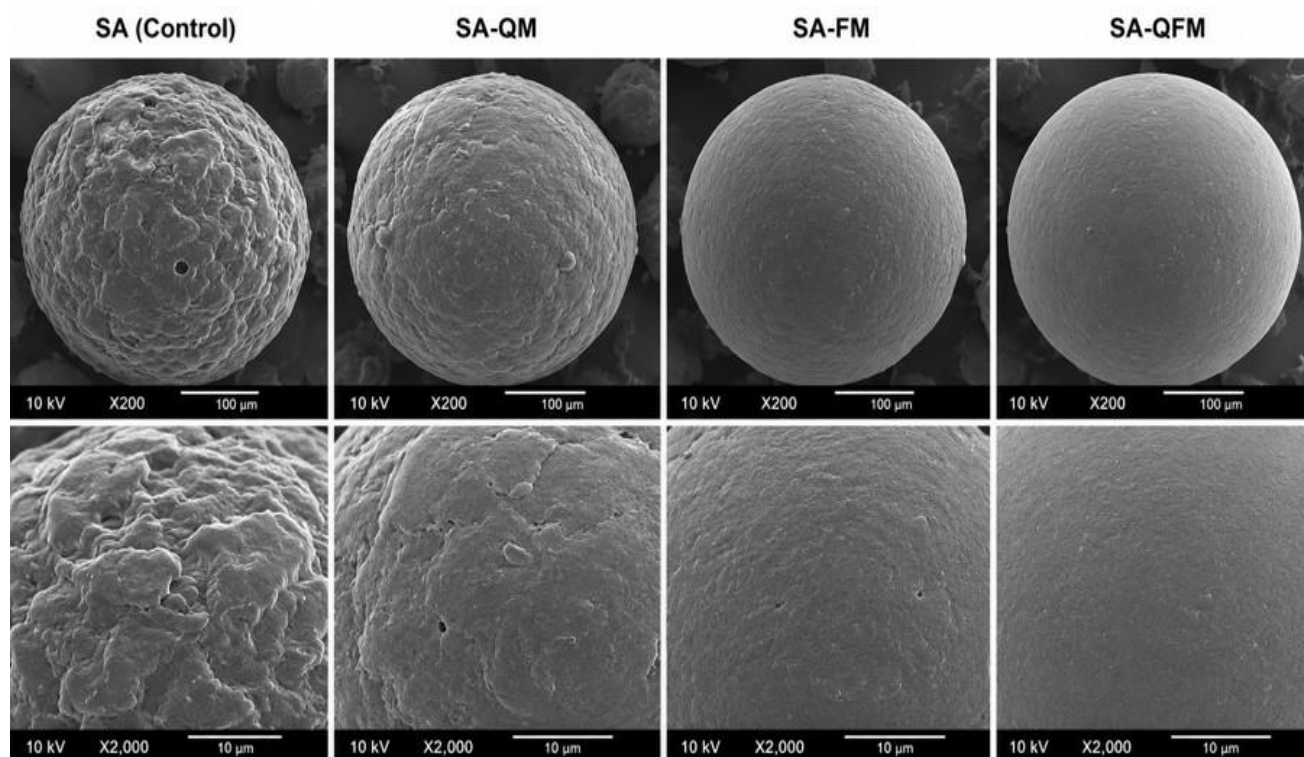


Figure 1. Morphology analysis of SA, SA-QM, SA-FM, SA-QFM composite microcapsules ($n = 3$)

The EE of *Lacticaseibacillus rhamnosus* GG significantly ($p < 0.05$) differed between alginate formulations (Table 3.1). The highest EE was observed in SA-QFM ($96.18 \pm 0.52\%$), followed by SA-FM ($93.84 \pm 0.59\%$), SA-QM ($86.37 \pm 0.64\%$), and SA ($81.42 \pm 0.71\%$). The results show that the plant-derived mucilage, when added to the alginate matrix, resulted in better bacterial entrapment than was obtained with alginate alone. The same enhancement in the encapsulation efficiency has been claimed with the incorporation of other hydrocolloids with alginate, as the addition of gums enhanced the polymer network and maintained better retention of the probiotics inside the beads (Ali *et al.*, 2023). This is because calcium alginate beads are highly porous and may allow partial diffusion of bacterial cells during the bead-forming and washing process, which may explain the relatively low EE observed for the SA beads. Sodium alginate is used for the encapsulation of probiotics, where it has the advantage of a mild gelation mechanism and biocompatibility; however, its poor mechanical strength and larger pore size may restrict the efficiency of bacterial entrapment (Cook *et al.*, 2012). Quince seed mucilage improved EE from 81.42% (SA) to 86.37% (SA-QM), and it might be related to the viscous polysaccharide structure and water-binding property of quince mucilage. Previous work on quince seed mucilage–

alginate microspheres showed that encapsulation efficiency varies from 73.76 to 85.63%, indicating the potential of quince mucilage to improve polymer matrix formation and enhance the retention of entrapped compounds (Ghumman *et al.*, 2022).

The EE of the SA-FM formulation was found to be much higher than that of SA-QM (93.84%), which indicated that flaxseed mucilage contributed to better reinforcement of the alginate network. This improved property may be attributed to the high molecular weight polysaccharides, excellent hydration ability, and film-forming property of flaxseed mucilage, which reduces bacterial cell leakage during ionic gelation and increases viscosity. However, recent studies have found that using flaxseed mucilage in alginate-based matrices improves probiotic encapsulation efficiency and protects the bacteria by strengthening the composite polymer structure. The maximum EE achieved with SA-QFM (96.18%) validated the synergistic effect of using a compound that contains quince seed with flaxseed mucilage and alginate (Bustamante *et al.*, 2025). The combined mucilage system may have increased the viscosity of the encapsulation mixture, decreased the porosity of the polymer network, and created additional physical barriers for bacterial retention. For a probiotic encapsulation system using alginate, similar synergistic improvement has been

Table 1. Encapsulation efficiency and beads diameter of different encapsulated microbeads ($n = 3$)

Treatment	Cells before encapsulation (No) (log CFU/mL)	Cells after encapsulation (N) (log CFU/g)	Encapsulation Efficiency (%)	Bead diameter (μm)
SA	9.18 ± 0.03	9.08 ± 0.02	81.42 ± 0.71^c	862.4 ± 2.8^c
SA-QM	9.26 ± 0.03	9.20 ± 0.03	86.37 ± 0.64^b	918.6 ± 3.2^b
SA-FM	9.21 ± 0.03	9.19 ± 0.02	93.84 ± 0.59^a	954.2 ± 3.7^{ab}
SA-QFM	9.18 ± 0.03	9.17 ± 0.03	96.18 ± 0.52^a	982.7 ± 3.4^a

SA = Sodium alginate (2%); SA-QM = Sodium alginate (2%) + Quince mucilage (0.5%); SA-FM = Sodium alginate (2%) + Flaxseed mucilage (0.5%); SA-QFM = Sodium alginate (2%) + Quince mucilage (0.25%) + Flaxseed mucilage (0.25%)

reported when alginate is combined with natural mucilage or gums, and the combined matrices present higher encapsulation efficiency compared with single-polymer systems. Thus, the use of quince seed and flaxseed mucilage as ingredients seemed to be a promising approach for enhancing the delivery system of the alginate-based *L. rhamnosus* GG. For gastrointestinal protection, Thoudam *et al.* (2026) mentioned that an alginate–skim milk–flaxseed mucilage matrix had an encapsulation efficiency of 95.54%, which was higher than that of the other matrices tested. Similarly, Bustamante *et al.* (2025) showed that the alginate matrices supplemented with flaxseed mucilage were effective in protecting the probiotics during processing and storage, with survival rates of 87–97%.

3.2. Physicochemical Characterization of Alginate-Based Composite Microcapsules

3.2.1. Bead Diameter

There were significant ($p < 0.05$) differences in bead diameter among the different formulations of *L. rhamnosus* GG-encapsulated microcapsules (Table 1). The sodium alginate beads (SA) showed the lowest bead diameter of $862.4 \pm 2.8 \mu\text{m}$. In comparison, the beads made with sodium alginate and quince seed mucilage (SA-QM) significantly increased the bead diameter to $918.6 \pm 3.2 \mu\text{m}$. The SA-FM formulation showed an additional increase ($954.2 \pm 3.7 \mu\text{m}$), while the largest beads were obtained with the composite formulation (SA-QFM) ($982.7 \pm 3.4 \mu\text{m}$); however, the difference between SA-FM and SA-QFM was not statistically significant. The progressive increase in bead diameter after adding plant mucilage can be explained by increased viscosity of the polymeric solutions, which leads

to larger droplets during extrusion prior to ionic gelation with calcium ions. Flaxseed mucilage has a very high molecular weight and has excellent water-binding capacity, while quince seed mucilage adds further viscosity and gel-forming ability. The combination of these likely caused the formation of a denser and more viscous polymer matrix and led to larger, more structurally stable microcapsules (Jouki *et al.*, 2014; Barbary *et al.*, 2009). Similar increases in bead size were observed for alginate-based probiotic microcapsules supplemented with natural hydrocolloids, as the addition of polysaccharides increased solution viscosity and thus bead size (Yasmin *et al.*, 2019; Ali *et al.*, 2023). The greater size of the beads in the composite formulation is beneficial, as it could aid in the entrapment of desired cells and provide better protection for the probiotics from environmental and gastrointestinal stresses, without hindering the extrusion process.

3.2.2. Scanning Electron Microscopy (SEM)

Surface morphology of the alginate-based composite microcapsules was analyzed using scanning electron microscopy (SEM) (Figure 1). Significant morphological variations among formulations were observed, suggesting that the use of quince seed and flaxseed mucilage had a significant effect on the microcapsule structure. In general, all formulations resulted in the formation of mainly spherical beads, but with increasing amounts of plant mucilage, the surface morphology of the beads gradually smoothed and compacted. The surface of the SA microcapsules was relatively rough and irregular, with noticeable wrinkles and a few shallow pores, typical features of calcium alginate beads subjected to dehydration

Table 2. Effect of different formulations on textural properties of microbeads ($n = 3$)

Treatment	Hardness (g)	Springiness	Cohesiveness	Resilience
SA	118.6 ± 4.3^c	0.792 ± 0.018^c	0.602 ± 0.015^c	0.286 ± 0.011^c
SA-QM	147.8 ± 5.1^b	0.851 ± 0.017^b	0.673 ± 0.014^b	0.348 ± 0.012^b
SA-FM	139.5 ± 4.8^b	0.872 ± 0.016^{ab}	0.691 ± 0.013^{ab}	0.366 ± 0.010^{ab}
SA-QFM	162.7 ± 5.4^a	0.901 ± 0.014^a	0.724 ± 0.012^a	0.392 ± 0.009^a

SA = Sodium alginate (2%); SA-QM = Sodium alginate (2%) + Quince mucilage (0.5%); SA-FM = Sodium alginate (2%) + Flaxseed mucilage (0.5%); SA-QFM = Sodium alginate (2%) + Quince mucilage (0.25%) + Flaxseed mucilage (0.25%)

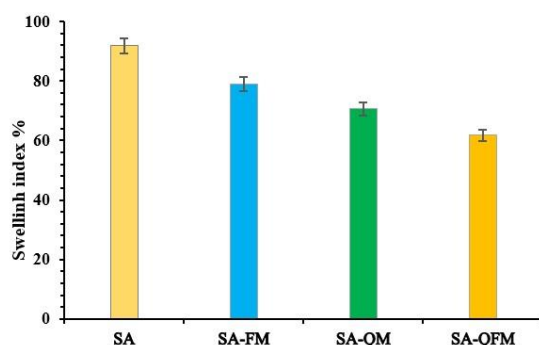


Figure 2: Swelling behavior of encapsulated *Lactocaseibacillus rhamnosus* GG

during sample preparation. These morphological characteristics have been commonly observed in alginate microcapsules and are associated with water loss during drying, leading to surface shrinkage and minor structural collapse (Allan-Wojtas *et al.*, 2008; Cook *et al.*, 2012).

The incorporation of quince seed mucilage (SA-QM) was found to improve surface uniformity and reduce surface irregularities in beads compared to SA. The improved morphology could be explained by the high water-binding capacity and gel-forming property of quince seed polysaccharides, which leads to the reinforcement of the alginate matrix and minimizes structural deformation during the drying process (Jouki *et al.*, 2014). The surface of the SA-FM formulation was found to be even denser and more homogeneous. At the same time, it too had very few visible pores, indicating the excellent film-forming and thickening properties of flaxseed mucilage. The higher viscosity of the polymer solution probably enhanced the formation of a more connected calcium-crosslinked network, which resulted in better bead integrity (Barbary *et al.*, 2009). SA-QFM showed a uniform size and shape and exhibited a smooth surface without significant cracks or pores among all the formulations. The enhanced structural stability indicates that alginate and quince seed mucilage, as well as flaxseed mucilage, have a synergistic effect and form a dense composite polymer network capable of efficiently entrapping probiotics. Few studies have also investigated the surface compactness and bead integrity of composite hydrocolloid formulations for encapsulating probiotics and found that the addition of natural gums decreased formulation porosity and increased mechanical stability (Yasmin *et al.*, 2019; Ali *et al.*, 2023). The small microstructure of SA-QFM, which was observed in the present study, is related to high encapsulation efficiency, low swelling ratio, and gastrointestinal protection of the probiotic; thus, structural reinforcement of the polymer matrix is an important factor in improving probiotic stability.

3.2.3. Texture Profile Analysis

Quince seed mucilage and flaxseed mucilage markedly ($p < 0.05$) enhanced the mechanical properties of the microcapsules based on alginate (). The SA-QFM formulation exhibited the highest hardness (162.7 ± 5.4 g), springiness (0.901 ± 0.014), cohesiveness (0.724 ± 0.012), and resilience (0.392 ± 0.009), whereas SA showed the lowest values for all parameters. The mixed use of natural mucilage strengthens the calcium alginate network, leading to enhanced textural properties of microcapsules, namely, higher resistance to compression and better structural recovery after deformation. The higher hardness of the composite formulations is due to more interactions between alginate molecules and more entanglements of the mucilage polysaccharides, leading to a denser gel network after calcium-induced crosslinking. Incorporation of plant polysaccharides into alginate probiotic capsules has recently been shown to lead to similar enhancement of these properties, with reinforcement of the polymer matrix leading to enhanced mechanical stability of the beads (You *et al.*, 2026). The flaxseed mucilage added to the plant-based formulation (SA-FM) resulted in numerically higher scores than SA-QM, with higher scores in springiness, cohesiveness, and resilience, indicating that flaxseed mucilage increased the elasticity of the formulation without compromising structural strength. This behavior is related to hydrogel mechanics, in which highly hydrated polysaccharides promote good elastic recovery but do not necessarily lead to the greatest resistance to compression. Wang *et al.* (2025) noted that modified alginate gels showed improved springiness, cohesiveness, and resilience, where better cross-linked network formation led to stronger, more flexible networks. The better mechanical properties of SA-QFM also contribute to the synergistic effect of the combination of quince seed and flaxseed mucilage with alginate. The dense composite network found in the SEM images is expected to withstand mechanical deformation better, reducing disruption of the structure during gastrointestinal transit. Zhao *et al.* (2017) established that the mechanical strength of alginate microcapsules has a positive correlation with probiotic survival during gastric and bile stress, meaning that harder and more resistant microcapsules would be able to withstand harsher conditions.

3.2.4. Swelling Behavior

The microcapsules' swelling behavior significantly ($p < 0.05$) varied between the different formulations (Figure 2). The highest swelling index ($91.8 \pm 2.6\%$) was observed for sodium alginate beads (SA), while the addition of plant mucilage gradually decreased the water uptake. The lowest swelling index was observed for the composite formulation SA-QFM ($61.7 \pm 1.9\%$), followed by SA-QM ($70.6 \pm 2.1\%$) and SA-FM ($78.9 \pm 2.3\%$). The results show that the alginate-quince seed mucilage/flaxseed mucilage (QS:FS)

blend effectively limited matrix expansion and stabilized the structural integrity of the hydrogel network. This is because calcium alginate is highly hydrophilic, and the three-dimensional network is relatively porous, allowing water to diffuse rapidly into the polymer matrix and causing the SA to swell. The addition of quince seed mucilage significantly decreased swelling, indicating that intermolecular interactions were enhanced and cross-link density increased with the addition of mucilage, which eventually decreased the relaxation of the polymer chains and water penetration. In alginate–quince seed mucilage microspheres, the addition of quince mucilage was found to be effective in controlling swelling by enhancing gel stability and reinforcing the polymer network (Ghumman *et al.*, 2022). The swelling index of flaxseed mucilage was also lower than the swelling index of alginate alone, but still higher than the swelling index of SA-QM. This behavior corresponds to the water-holding capability and hydrated polysaccharide structure of flaxseed mucilage that readily absorbs water and undergoes chain relaxation in water. Previous studies have demonstrated the excellent hydration properties of flaxseed mucilage, which facilitate water uptake while preserving the integrity of the hydrogel matrix. The lowest swelling index of the SA-QFM illustrates the synergistic effect of QS and FSM in the alginate matrix. The composite hydrogel seemed to have less pore volume and would have formed a more homogeneous calcium-crosslinked network, thereby limiting solvent diffusion into the beads (Kandekar *et al.*, 2024). Analogous findings have been reported by Nezamdoost-Sani *et al.* (2023) for composite alginate hydrogels, where the addition of more than one natural polysaccharide resulted in the development of more robust polymeric networks, with decreased swelling, improved mechanical properties, and increased encapsulation capabilities. The lower swelling found for SA-QFM is consistent with its higher hardness, SEM morphology, and better encapsulation efficiency, indicating that the water absorption ability of the product was limited, which may lead to better structural integrity and is believed to improve the protection of the probiotics during gastrointestinal transit.

3.3. Evaluation of Protective Capacity of Microcapsules

3.3.1. Survival under Sequential Simulated Gastrointestinal Conditions

The viability of free and encapsulated *L. rhamnosus* GG was significantly ($p < 0.05$) reduced after sequential exposure to simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) (Figure 3 and 3a, $n = 3$). No more than 43.04% of free cells (LR) survived sequential digestion, with a loss from 9.20 ± 0.03 to 3.96 ± 0.05 log CFU/g. By contrast, all encapsulated treatments offered good protection from the harsh gut environment. Among them, SA-QFM maintained the highest viable count (8.12 ± 0.03 log CFU/g) with a survival of 88.55%, followed by SA-FM (85.85%),

SA-QM (82.83%) and SA (73.57%). The results clearly showed that adding quince seed and flaxseed mucilage to the alginate matrix greatly enhanced the resistance of *L. rhamnosus* GG to sequential gastrointestinal stress. The significant decrease seen in free cells is due to a combination of factors, including gastric acidity, pepsin activity, and subsequent exposure to intestinal salts, which can disrupt bacterial cell membranes and negatively affect cellular metabolism. The presence of the encapsulation significantly reduced these negative effects by creating a barrier around the probiotic cells, reducing the possibility of diffusion of hydrogen ions, digestive enzymes, and bile components into microcapsules. In a similar study, Ali *et al.* (2023) found that alginate–xanthan encapsulated *L. rhamnosus* GG had a significantly higher survival rate compared to free cells under simulated gastrointestinal conditions, and the viability of encapsulated cells remained above the therapeutic level of 10^7 CFU/g.

All the encapsulated formulations showed better protection in the case of SA-QFM, which implies that alginate, quince seed mucilage, and flaxseed mucilage have a synergistic effect. The high acid resistance of the gastrointestinal tract may be attributable to the development of a thicker and less porous polymeric network, which prevented acid

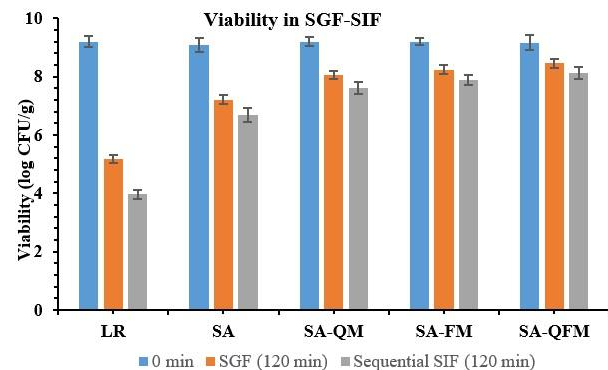


Figure 3: Survivability of free and encapsulated *L. rhamnosus* under Sequential Simulated Gastrointestinal Conditions

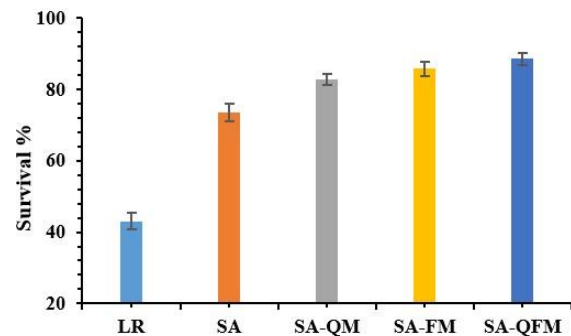


Figure 3a: Survival % of free and encapsulated *L. rhamnosus* GG under Sequential Simulated Gastrointestinal Conditions

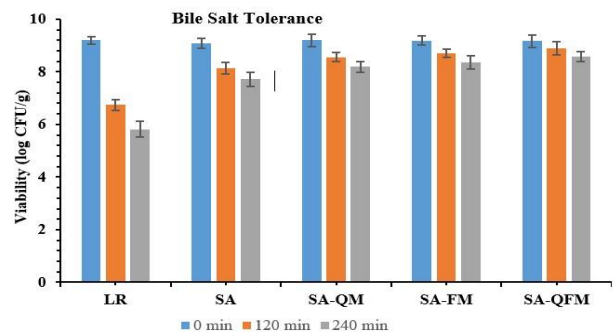


Figure 4: Survivability of free and encapsulated *L. rhamnosus* in bile salt for different time exposure

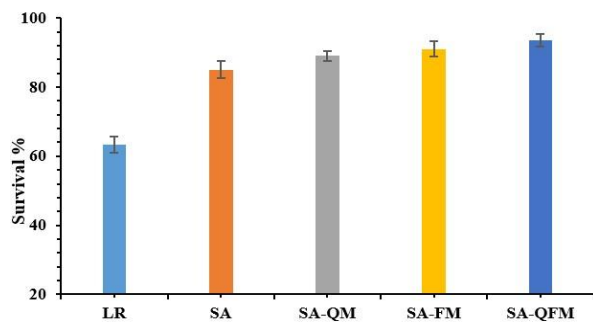


Figure 4a: Survival % of free and encapsulated *L. rhamnosus* GG under bile salt conditions

penetration and slowed structural degradation during digestion. This is in line with the findings of lower swelling and higher hardness, and the lower SEM morphology observed for SA-QFM in the present study. Similarly, microcapsules with a higher degree of cross-linking density were reported to have higher resistance to gastrointestinal digestion, as higher cross-linking density decreases probiotic leakage and ensures cell viability during prolonged exposure to SGF and SIF (Anwar *et al.*, 2022). The better survival recorded in SA-FM vs. SA-QM could be attributed to the best hydration and film-forming properties of flaxseed mucilage, which ensure matrix integrity through the change in gastric pH to the intestinal environment. However, the greatest overall protection was obtained by combining both mucilage, which indicates that interactions occurred between the two polysaccharides within the calcium-alginate network. This was also observed for composite

hydrogel systems with the addition of other natural biopolymer materials that increased the integrity of the encapsulating matrix and have been reported to improve the survival of probiotics during simulated gastrointestinal digestion greatly (Ali *et al.*, 2024). In conclusion, the sequential gastrointestinal assay revealed that the alginate–quince seed–flaxseed composite microcapsules were able to withstand the sequential gastric and intestinal stresses and preserve viable counts above the minimum therapeutic level for probiotic functionality. The results above reflect the promise of the composite encapsulation system as an effective oral delivery system for probiotics. For gastrointestinal protection, Thoudam *et al.* (2026) mentioned that an alginate–skim milk–flaxseed mucilage matrix had an encapsulation efficiency of 95.54%, which was higher than that of the other matrices tested

3.3.2. Bile Salt Tolerance

All treatments significantly reduced ($p < 0.05$) the viable cell counts after 240 min exposure to 2% bile salt solution, but the cell survival was significantly improved by encapsulating the bacteria (and 4a, $n = 3$). The highest loss in viability was observed among free cells (LR), where the number declined from 9.20 ± 0.03 to 5.82 ± 0.04 log CFU/g after 240 min (63.26%). All encapsulated formulations, however, had viable counts above 7.7 log CFU/g, indicating that the polymeric matrices protect from bile stress. The encapsulated treatments showed that SA-QFM had the highest bile tolerance, with 8.58 ± 0.03 log CFU/g and 93.57% survival, while SA-FM, SA-QM, and SA had 91.05%, 89.02%, and 85.02% survival, respectively. Bile salts are amphiphilic molecules that can disrupt phospholipid membranes, increase membrane permeability and leakage of intracellular components and thus decreasing probiotic viability. These effects are minimized with encapsulation by reducing direct contact between bile salts and bacterial cells and by reducing the diffusion of bile molecules through the hydrogel matrix. Cook *et al.* (2012) showed that alginate encapsulation also greatly protected probiotic bacteria against the toxic effect of bile salts by creating a good physical diffusion barrier. Similarly, Gbassi and Vandamme (2012) found that a better encapsulating matrix resulted in greater resistance of the bacteria to bile stress, keeping the microcapsules intact in the intestinal simulation.

The superior bile resistance found for SA-QFM indicates that the formulation that combines the two (quince seed and flaxseed mucilage) generates a more compact and less porous network of hydrogels compared to alginate alone or with a single mucilage. This interpretation was confirmed by the increased encapsulation efficiency and hardness and the decreased swelling index of the SA-QFM, which showed that the polymeric structure of the SA was denser and thus effective at preventing bile from penetrating microcapsules. Similar results were found by Nazzaro *et al.* (2012), who reported that a more compact structural matrix led to better probiotic survival under intestinal conditions due to less matrix erosion and increased barrier characteristics. Similarly, de Prisco and Mauriello (2016) found that mechanically sturdier hydrogel systems provided more sustained protection against bile-induced membrane damage, resulting in increased delivery efficiency of viable probiotics into the intestine.

The comparatively lower survival of SA compared to the composite formulations revealed that calcium alginate alone was not enough to provide maximum resistance against prolonged bile exposure. However, incorporation of plant-derived mucilage enhanced the resistance of the resulting hydrogel to bile diffusion and strengthened the hydrogel matrix, leading to significant bacterial viability. In conclusion, the excellent performance of SA-QFM demonstrates the synergistic effect of the alginate–quince seed–flaxseed mucilage composite encapsulation system and supports its usefulness as an efficient carrier for delivering viable *L. rhamnosus* GG through the upper gastrointestinal tract.

3.4. Refrigerated Storage Stability at 4 °C

Free and encapsulated *L. rhamnosus* GG were significantly ($p < 0.05$) less viable after 28 days of refrigerated storage (Figure 5, $n = 3$). However, there were significant differences in the extent to which the treatments reduced viability. All encapsulated formulations retained viable counts above 7 log CFU/g after 28 days, while free cells (LR) had the highest reduction (9.20 ± 0.03 to 6.21 ± 0.06 log CFU/g). Among the encapsulated treatments, SA-QFM exhibited the highest storage stability, retaining 8.84 ± 0.03 log CFU/g after 28 days, followed by SA-FM (8.66 ± 0.03 log CFU/g), SA-QM (8.46 ± 0.04 log CFU/g), and SA (7.96 ± 0.05 log CFU/g). The obtained results showed that the use of quince seed mucilage and flaxseed mucilage significantly improved the viability of probiotics in alginate microcapsules during cold storage. Mainly cold-induced physiological stress, oxidative damage, and gradual loss of membrane integrity during extended refrigeration are thought to be responsible for the rapid decline observed in free cells. At the same time, the microenvironment of the encapsulated cells reduced direct exposure to external stressors and their metabolic activity, protecting their

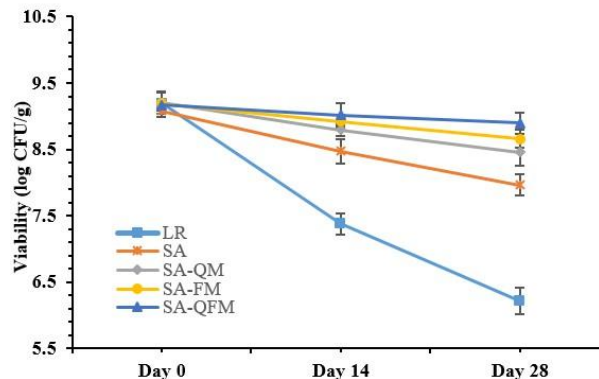


Figure 5: Storage stability free and encapsulated *L. rhamnosus* GG during refrigeration (4°C)

integrity. A similar enhancement in refrigerated storage was observed by Ni *et al.* (2023), who showed that alginate–gelatin (AG) hydrogel beads had significantly better storage stability for encapsulated *Lactobacillus plantarum*, achieved by creating a stronger polymeric network.

The most stable hydrogel matrix was SA-QFM, showing the lowest loss of viability during storage compared to the other composite formulations. This increased storage performance may be due to the denser microstructure seen in SEM, the higher hardness, lower swelling index, and higher encapsulation efficiency, all of which decreased the diffusion of oxygen and moisture into the microcapsules. Micheli *et al.* (2021) also found that the alginate-coated *L. rhamnosus* GG exhibited <1 log reduction after 35 days of refrigerated storage, possibly due to the protective effect of the encapsulation matrix. Moreover, Tan *et al.* (2023) showed that the addition of alginate-encapsulated *L. rhamnosus* GG resulted in significant improvements in the refrigerated stability of the probiotic in acidic beverage systems, where the inactivation of *L. rhamnosus* GG cells was decreased, and the probiotic remained viable during the storage period. The relatively weaker stability of SA compared to composite formulations suggests that calcium alginate alone provides a less compact hydrogel network, which allows higher diffusion of water and oxygen, resulting in higher reduction of bacterial viability. Conformable behavior has been revealed by Li *et al.* (2023), who demonstrated that the use of natural polymeric fillers in calcium alginate microgels significantly enhanced the survival of probiotics during refrigerated storage by strengthening the microgel network and mitigating degradation. The excellent performance of the SA-QFM thus indicates simultaneous use of two complementary plant mucilage with alginate results in a mechanically strong encapsulation matrix that can preserve probiotic viability during refrigerated storage, suggesting it is a good carrier for functional food applications.

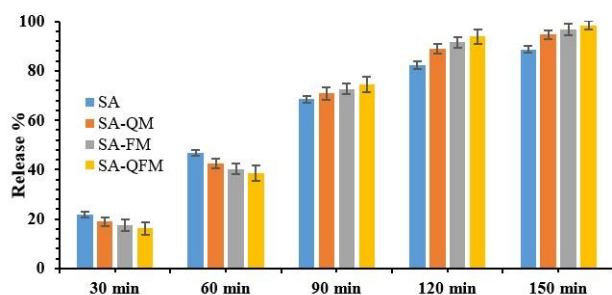


Figure 6: Release profile encapsulated *L. rhamnosus* GG in different formulations for different time exposure

3.5. In Vitro Release of *Lacticaseibacillus rhamnosus* GG in Simulated Intestinal Fluid

The release profiles of *L. rhamnosus* GG from the various alginate-based microcapsules in simulated intestinal fluid (SIF) are shown in Figure 6 ($n = 3$). A statistically significant ($p < 0.05$) increase in cumulative probiotic release was found as incubation time increased for all formulations. Among the formulations, the highest cumulative release ($98.4 \pm 0.8\%$) after 150 min was achieved for formulation SA-QFM, while formulation SA-FM ($96.8 \pm 0.9\%$), SA-QM ($94.6 \pm 1.1\%$), and SA ($88.6 \pm 1.2\%$) showed comparable release. The slower release during the first stage is explained by the integrity of the calcium-crosslinked alginate matrix, which initially limited the diffusion of entrapped probiotic cells. The result of the incubation in SIF was the progressive replacement of Ca^{2+} ions by the arrival of phosphate ions, which led to relaxation of the alginate's polymer network, enlargement of the pores, and finally to the complete dissolution of the alginate hydrogel matrix. This resulted in gradual release of bacteria into the culture medium. This was the same finding observed by Chehreh *et al.* (2022), as only a limited amount of *L. rhamnosus* was released during the initial phase of the intestinal digestion, while a large portion was released rapidly after the alginate-based multilayer capsules underwent structural disintegration under simulated intestinal conditions.

The enhanced release efficiency of SA-QFM suggests that incorporation of both quince seed mucilage and flaxseed mucilage resulted in a composite matrix that can maintain structural integrity in the stomach but readily disintegrates in the intestine under acidic conditions. This behavior is similar to the desirable properties of an oral probiotic delivery system (an encapsulating matrix that protects the bacteria from the gastric environment and rapidly releases them in the intestine). The results were also corroborated by Zhang *et al.* (2021), who showed that biofortification of calcium alginate capsules improved protection against the gastrointestinal environment and subsequent probiotic release by controlling matrix swelling and degradation in gastrointestinal media. Despite the low cumulative release,

this formulation was also revealed to have the lowest encapsulation efficiency and mechanical properties, indicating that the alginate formulation was a less responsive delivery matrix than the composite formulations. However, the improved release of SA-QFM was in good agreement with its improved encapsulation efficiency, smaller SEM size, hardness, and swelling index, suggesting that the composite network remained intact during protection but dissociated in a controlled manner under intestinal conditions. Similar release characteristics have been reported for alginate-based probiotic delivery systems, which demonstrated the ability to achieve efficient release in simulated intestinal fluid while also shielding the bacteria, and optimized composite matrices were achieved. In addition, Sbehat *et al.* (2022) reported that varying the structural properties of the encapsulation system improved the recovery of the bacteria, *L. rhamnosus*, after sequential gastrointestinal digestion, highlighting the potential of optimized encapsulation matrices for protective release of bacteria in the intestine. In conclusion, the release kinetics developed in the present study indicate that the SA-QFM formulation was a good, targeted delivery system for probiotics to the colon, with almost complete release in the SIF and negligible leakage in the stomach. Controlled-release properties are critical to ensure that sufficient living cells reach the intestine to have beneficial functional effects in food and nutraceutical products.

Conclusion:

The use of quince seed and flaxseed mucilage in alginate matrix significantly enhanced the encapsulation and delivery of *Lacticaseibacillus rhamnosus* GG in this study. The composite microcapsules had higher encapsulation efficiency, better simulated gastrointestinal and bile salt resistance, better mechanical strength, better refrigerated storage stability, and more efficient probiotic release in simulated enteric fluid than alginate alone. The alginate–quince–flaxseed composite (SA-QFM) achieved the best overall formulation, reflecting the synergistic effect of the two plant-derived mucilages. The developed composite microcapsules have the potential to be used in functional foods and nutraceuticals as a natural delivery system to enhance the stability and targeted delivery of probiotics in the intestine, showing great potential for this application.

Ethical Approval

Not applicable

Conflict of Interest

The authors declare no conflict of interest.

Funding

Not applicable

Author Contributions

Muhammad Salman contributed to conceptualization, methodology, investigation, data collection, laboratory analysis, data interpretation, and manuscript drafting. Abdul

Rehman Ayub contributed to conceptualization, study supervision, methodology, data interpretation, manuscript review, and overall coordination of the research work. Shafiud Din contributed to methodology, investigation, laboratory analysis, data collection, and critical review of the manuscript. Umama Hayat contributed to data collection, laboratory analysis, investigation, data organization, and manuscript review. Zahida Qadeer contributed to investigation, laboratory analysis, data interpretation, and critical revision of the manuscript. Asma Aslam contributed to methodology, investigation, data analysis, interpretation of results, and manuscript review. Muhammad Areeb Safdar contributed to data collection, laboratory analysis, investigation, data organization, and manuscript preparation. Muhammad Waqas Hussain contributed to data analysis, statistical interpretation, methodology, critical review, and revision of the manuscript. Umair Ali contributed to supervision, conceptualization, validation, interpretation of results, critical revision, and final approval of the manuscript. All authors critically reviewed the manuscript, approved the final version, and agreed to be accountable for all aspects of the work.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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