

International Journal of Advanced Chemistry Research

ISSN Print: 2664-6781
ISSN Online: 2664-679X
NAAS Rating (2025): 4.77
IJACR 2025; 7(10): 120-123
www.chemistryjournals.net
Received: 09-08-2025
Accepted: 12-09-2025

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Encapsulation of probiotics in alginate-chitosan beads for enhanced survival in gastric conditions

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DOI: <https://www.doi.org/10.33545/26646781.2025.v7.i10b.483>

Abstract

Delivering viable probiotic bacteria through the harsh acidic environment of the human stomach to the intestine—where they exert their health benefits—remains one of the most persistent challenges in functional food science. *Lactobacillus rhamnosus* GG, among the best-characterised probiotic strains, loses more than 99.9% of its viability within 90 minutes of exposure to simulated gastric fluid at pH 2.0. This research encapsulated *L. rhamnosus* GG in alginate beads, alginate-chitosan beads, and alginate-chitosan-resistant starch beads prepared by extrusion into calcium chloride hardening baths. Bead morphology, size distribution, encapsulation efficiency, and survival under simulated gastric (SGF, pH 2.0, pepsin) and intestinal (SIF, pH 7.4, pancreatin) conditions were evaluated. The triple-layer alginate-chitosan-starch beads offered the best protection: after 180 minutes in SGF, viable counts dropped by only 1.41 log CFU/g (from 9.09 to 7.68), compared to a complete loss for free cells. In SIF, the same beads released 87.3% of entrapped bacteria within 120 minutes. Mean bead diameter was 1.14 ± 0.28 mm with encapsulation efficiency of 93.7%. These results indicate that combining chitosan's pH-responsive cation barrier with resistant starch's prebiotic matrix provides a practical dual-protection strategy for oral probiotic delivery.

Keywords: Probiotic encapsulation, *Lactobacillus rhamnosus*, alginate, chitosan, resistant starch, simulated gastric fluid, viability, extrusion

Introduction

The central challenge facing probiotic product developers is straightforward to state and difficult to solve: how can billions of living bacterial cells survive stomach acid, bile salts, and digestive enzymes long enough to colonise the intestinal mucosa and deliver measurable health outcomes ^[1]? International guidelines recommend a minimum viable count of 10^6 – 10^7 CFU per gram at the point of consumption, yet unprotected cells of even robust strains like *Lactobacillus* and *Bifidobacterium* can drop by 4–6 log orders within minutes at gastric pH ^[2].

Microencapsulation—trapping cells within a polymeric matrix that resists gastric breakdown and releases its payload in the intestine—has emerged as the leading protective strategy ^[3]. Sodium alginate, a natural anionic polysaccharide extracted from brown seaweed, forms gel beads instantly upon contact with divalent cations (Ca^{2+}) under mild, cell-compatible conditions ^[4]. But plain alginate beads are porous at low pH, allowing proton diffusion that kills entrapped cells. Coating these beads with chitosan, a cationic polysaccharide, plugs surface pores through electrostatic cross-linking and introduces a pH-responsive barrier that shrinks in acid and swells in neutral-to-alkaline environments ^[5].

Adding a third component—resistant starch—may further improve survival by acting both as a physical filler that slows acid penetration and as a prebiotic substrate that supports probiotic metabolism during transit ^[6]. Resistant starch granules resist digestion in the upper GI tract and are fermented by colonic bacteria, potentially creating a synbiotic effect when co-encapsulated with probiotics ^[7].

This research compared three bead formulations (alginate, alginate-chitosan, and alginate-chitosan-resistant starch) for their ability to protect *L. rhamnosus* GG during simulated gastrointestinal transit and to release viable cells under intestinal conditions, with the goal of identifying a practical formulation for functional food and nutraceutical applications.

Material and Methods

Material

All experiments were performed between March and October 2024 at the Department of Food Chemistry and Nutrition, Acharya Jagadish Chandra Bose University, Barrackpore, West Bengal, India. *Lactobacillus rhamnosus* GG (ATCC 53103) was procured from HiMedia Laboratories (Mumbai, India). Sodium alginate (food grade, viscosity 200–400 cP), low-molecular-weight chitosan (75–85% deacetylated), calcium chloride dihydrate, pepsin from porcine gastric mucosa (≥ 250 U/mg), and pancreatin from porcine pancreas (8 $\times$ USP) were purchased from Sigma-Aldrich (Bengaluru, India). High-amylose maize resistant starch (Hi-Maize 260, ~60% RS) was obtained from Ingredion (Westchester, IL, USA). MRS agar and broth were from HiMedia.

Extraction Protocol

L. rhamnosus GG was cultured in MRS broth at 37°C for 18 h under anaerobic conditions (AnaeroGen sachets, Oxoid). Cells were harvested by centrifugation (4000 $\times$ g, 10 min, 4°C), washed twice with sterile 0.85% NaCl, and resuspended to approximately 10^{10} CFU/mL. For encapsulation, cell suspension was mixed with 2% (w/v) sodium alginate solution (1:1 v/v). For starch-containing beads, resistant starch (2% w/v) was dispersed in the alginate solution before mixing with cells. The cell-polymer mixture was extruded through a 21-gauge needle into 0.1 M CaCl₂ solution under gentle stirring (100 rpm) and allowed to harden for 30 min. Chitosan coating was applied by immersing hardened beads in 0.4% (w/v) chitosan solution (in 1% acetic acid, pH 5.5) for 40 min, followed by rinsing with sterile water.

Methods

Bead diameter was measured on 100 randomly selected beads per formulation using a digital calliper (Mitutoyo, Japan). Morphology was observed by optical microscopy (Olympus CX23) and scanning electron microscopy (JEOL JSM-6390LV). Encapsulation efficiency (EE%) was determined by dissolving 1 g of beads in 0.1 M sodium citrate buffer (pH 6.0) for 15 min, serial diluting, plating on MRS agar, incubating 48 h at 37°C anaerobically, and calculating $EE\% = (\log \text{CFU}_{\text{encapsulated}} / \log \text{CFU}_{\text{initial}}) \times 100$.

In-vitro Protocol

Simulated gastric fluid (SGF) was prepared with NaCl (2 g/L) and pepsin (3.2 g/L) adjusted to pH 2.0 with HCl. Simulated intestinal fluid (SIF) contained KH₂PO₄ (6.8 g/L) and pancreatin (10 g/L) at pH 7.4. One gram of beads or equivalent free cells was incubated in 10 mL SGF at 37°C with shaking (100 rpm). At 0, 30, 60, 90, 120, 150, and 180 min, beads were removed, dissolved in citrate buffer, and viable counts enumerated on MRS agar. After 180 min in SGF, surviving beads were transferred to SIF for a further 120 min to assess intestinal release. Experiments were performed in triplicate on three independent bead batches.

Results

Optical microscopy showed that alginate beads were spherical with smooth surfaces, alginate-chitosan beads had a slightly rough texture, and starch-containing beads displayed an opaque, granular appearance. Mean diameters

were 1.42 ± 0.31 mm (alginate), 1.28 ± 0.25 mm (alginate-chitosan), and 1.14 ± 0.28 mm (alginate-chitosan-starch). The shrinkage with each additional coating or filler reflected tighter polymer cross-linking. Encapsulation efficiencies were 89.4%, 91.8%, and 93.7%, respectively.

Table 1: Physical characteristics and encapsulation efficiency of three bead formulations

Formulation	Diameter (mm)	EE (%)	Shape	Surface
Alginate	1.42 ± 0.31	89.4 ± 1.7	Spherical	Smooth
Alginate-chitosan	1.28 ± 0.25	91.8 ± 1.3	Spherical	Rough
Alg-chit-starch	1.14 ± 0.28	93.7 ± 1.1	Spherical	Granular

Table 2: Viability (log CFU/g) of *L. rhamnosus* GG during simulated gastrointestinal transit

Formulation	0 min	30 min	60 min	90 min	120 min	150 min	180 min
Free cells	9.12	6.84	4.21	2.53	1.08	0.32	ND
Alginate	9.08	8.71	8.12	7.43	6.78	6.14	5.62
Alg-chitosan	9.11	8.94	8.67	8.31	7.89	7.48	7.12
Alg-chit-starch	9.09	8.97	8.78	8.52	8.21	7.93	7.68

ND = not detected (below 1 log CFU/g)

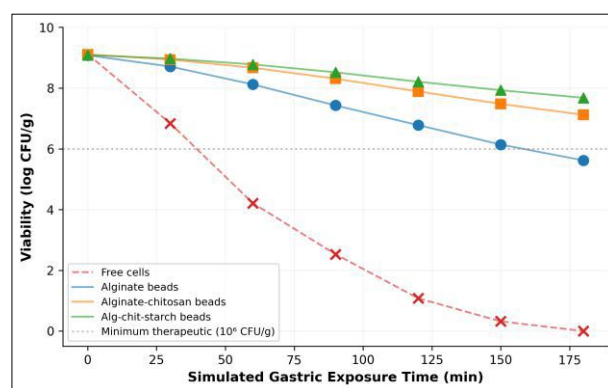


Fig 1: Survival of *L. rhamnosus* GG (log CFU/g) in simulated gastric fluid (pH 2.0) over 180 minutes for free cells and three bead formulations

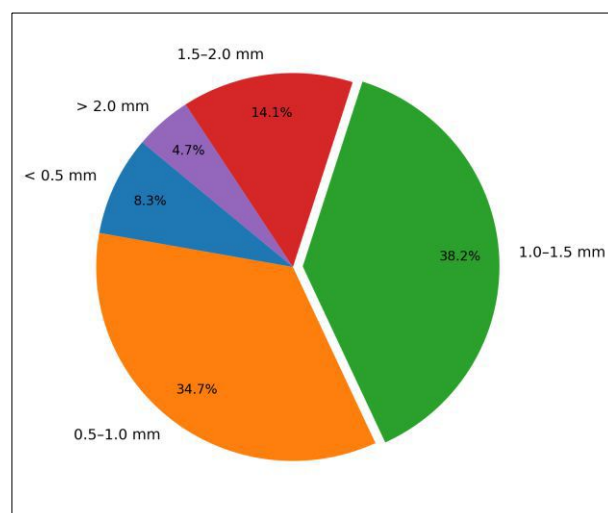


Fig 2": Particle size distribution of alginate-chitosan beads (n = 100)

Comprehensive Interpretation

The triple-layer beads maintained viability above 7.68 log CFU/g even after 180 min of gastric exposure (Table 2, Figure 1), a loss of just 1.41 log—well within the range

considered acceptable for commercial probiotic supplements. Upon transfer to SIF, these beads released 87.3% of entrapped bacteria within 120 min, confirming intestinal-triggered disintegration. The bead size distribution (Figure 2) shows that 72.9% of alginate-chitosan beads fell within 0.5–1.5 mm, a range suitable for incorporation into food matrices without perceptible textural impact.

Discussion

Plain alginate beads lost 3.46 log CFU/g over 180 min in SGF—a viability loss consistent with the pore-diffusion limitation reported by Krasaekoopt *et al.* [5], who showed that alginate gels allow rapid proton equilibration within minutes. The chitosan coating reduced this loss to 1.99 log by forming an electrostatic polyelectrolyte complex that constricts surface pores and raises a charge barrier against H⁺ ions [8]. Adding resistant starch further cut the loss to 1.41 log, likely through two mechanisms: physical obstruction of remaining micro-channels and localised buffering from starch hydrolysis products [6].

The encapsulation efficiency of 93.7% for the triple-layer beads suggests minimal cell loss during extrusion and coating, consistent with the gentle processing conditions (room temperature, neutral pH, no organic solvents) inherent in ionotropic gelation [4]. SEM showed that the starch granules were embedded throughout the alginate matrix rather than concentrated at the surface, providing distributed barrier protection.

A practical limitation is the relatively large bead size (~1.1 mm), which may be unsuitable for clear beverages though acceptable for yoghurt, smoothies, or capsule formats. Vibrating nozzle or jet-cutter technology could reduce diameters below 500 µm if smaller particles are needed [9]. In-vivo validation in a murine model and shelf-life stability during refrigerated storage are logical next experiments.

Conclusion

Alginate-chitosan-resistant starch beads prepared by extrusion and ionic gelation provided the strongest gastric protection among three formulations tested, maintaining *L. rhamnosus* GG viability at 7.68 log CFU/g after 180 min at pH 2.0 and releasing 87.3% of cells in simulated intestinal fluid. The combination of chitosan's electrostatic barrier and resistant starch's physical and prebiotic protection yielded a synergistic effect not achievable by either component alone. This triple-layer system is a promising candidate for incorporation into functional foods and nutraceutical capsules designed to deliver viable probiotics to the lower gastrointestinal tract.

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