ISSN Print: 2664-844X
ISSN Online: 2664-8458
NAAS Rating (2025): 4.97
IJAFS 2025; 7(12): 619-626
www.agriculturaljournals.com
Received: 15-10-2025
Accepted: 20-11-2025

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Microencapsulation of *Lactobacillus acidophilus* Using partially hydrolysed guar gum: Bead characterization and survivability under simulated gastrointestinal and thermal stress conditions

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DOI: https://www.doi.org/10.33545/2664844X.2025.v7.i12h.1100

Abstract
The functional efficacy of probiotic foods depends on the delivery of adequate numbers of viable microorganisms to the intestine; however, probiotic bacteria such as *Lactobacillus acidophilus* are highly sensitive to heat stresses and adverse gastrointestinal conditions. Microencapsulation has therefore emerged as an effective strategy to enhance probiotic stability and targeted delivery. The present study aimed to develop a synbiotic microencapsulation system incorporating partially hydrolysed guar gum and to evaluate its protective effect on *Lactobacillus acidophilus* NCDC-15 under simulated gastric, intestinal, and thermal stress conditions. The probiotic culture was microencapsulated using a sodium alginate-corn starch matrix supplemented with partially hydrolysed guar gum at 3% and 5% (w/v) concentrations, with microcapsules formed by ionic gelation in calcium chloride solution. Survivability of encapsulated and non-encapsulated cells was assessed under simulated gastric pH conditions (pH 1.0, 1.5, and 2.0), bile salt concentrations ranging from 1.0 to 2.0%, and thermal treatments at 75 °C and 80 °C for 30 s. Microencapsulation significantly enhanced the survivability of *L. acidophilus* under all tested stress conditions, with PHGG-containing microcapsules, particularly at the 5% level, providing superior protection against acidic environments while maintaining viable counts above the recommended therapeutic threshold ($\geq 10^7$-10^8 CFU/g) even after prolonged exposure. Encapsulated cells also exhibited significantly higher tolerance to bile salts and thermal treatments compared with non-encapsulated controls, demonstrating a clear concentration-dependent protective effect of partially hydrolysed guar gum. Overall, incorporation of Partially hydrolysed guar gum into alginate-starch microencapsulation matrices markedly improves the resistance of *Lactobacillus acidophilus* NCDC-15 to gastrointestinal and thermal stresses, emphasizing the potential of this synbiotic encapsulation system for enhancing probiotic stability during food processing and digestion and supporting its application in functional foods, nutraceuticals, and sensitive formulations such as infant and medical nutrition products.

Keywords: Microencapsulation, Partially Hydrolysed Guar Gum, Synbiotic delivery system, Probiotic survivability, Simulated gastrointestinal conditions, Thermal stability

Introduction
The incorporation of probiotic microorganisms into functional foods has attracted increasing scientific, clinical, and industrial interest owing to their established roles in promoting gastrointestinal health, enhancing immune competence, and modulating host metabolic and inflammatory responses (Latif *et al*., 2023) [6]; Petrariu *et al*., 2024 [12]; Tukaram *et al*., 2025 [20]. Probiotics exert their beneficial effects through multiple mechanisms, including competitive exclusion of pathogenic microorganisms, modulation of gut microbiota composition, production of antimicrobial metabolites, enhancement of intestinal barrier integrity, and regulation of host immune signaling pathways (Huang *et al*., 2022) [5]; Zhao *et al*., 2020 [23]; Duhan *et al*., 2025 [3]. Among the diverse probiotic strains investigated to date, *Lactobacillus acidophilus* remains one of the most extensively studied and commercially relevant lactic acid bacteria, widely employed in fermented dairy products and nutraceutical formulations due to its documented safety, functional efficacy,

and physiological compatibility with the human gastrointestinal tract (Liu *et al*., 2024) [8]; Zhu *et al*., 2025 [24].

Lactobacillus acidophilus is particularly valued for its ability to adhere to intestinal epithelial cells, suppress enteric pathogens, improve lactose digestion through β-galactosidase activity, and contribute to the maintenance of intestinal homeostasis (Huang *et al*., 2022 [5]; Zhao *et al*., 2020) [23]; Liu *et al*., 2024 [8]. Despite these well-recognized health benefits, the successful delivery of viable *L. acidophilus* cells through food matrices to the site of action remains a major technological challenge (Rodrigues *et al*., 2020) [15]; Sbehat *et al*., 2022 [17]. The organism exhibits pronounced sensitivity to environmental stresses encountered during food processing, storage, and gastrointestinal transit, resulting in substantial viability losses prior to consumption or intestinal colonization (Bustos *et al*., 2025 [2]; Vivek *et al*., 2023) [21]. Consequently, ensuring probiotic stability throughout the product lifecycle is a critical prerequisite for the development of efficacious probiotic and synbiotic foods (Wang *et al*., 2022) [22]; Sin *et al*., 2025 [18].

The functional efficacy of probiotic foods is strongly dependent on the delivery of an adequate number of viable cells, generally accepted to be in the range of $\geq 10^7$-10^8 CFU per gram or millilitre, at the time of consumption and, more importantly, upon arrival in the intestine (Naissinger da Silva *et al*., 2020 [11]; Solanki *et al*., 2015) [19]. During manufacturing and storage, probiotic cells are exposed to a multitude of stressors, including oxygen exposure, moisture fluctuations, osmotic stress, acidic environments, and thermal treatments associated with food processing (Mansouripour *et al*., 2013 [9]; Bustos *et al*., 2025) [2]. Following ingestion, these stresses are further compounded by exposure to the highly acidic gastric environment (pH 1.5-3.0), digestive enzymes such as pepsin, bile salts, and intestinal peristalsis (Naissinger da Silva *et al*., 2020) [11]; Moghanjougi *et al*., 2021 [10]. Collectively, these factors can result in severe reductions in probiotic viability, thereby diminishing or nullifying the intended health benefits of probiotic-enriched products (Sbehat *et al*., 2022 [17]; Vivek *et al*., 2023) [21].

Microencapsulation has emerged as an effective and versatile technological approach to enhance probiotic stability and to facilitate targeted delivery to the intestine (Rodrigues *et al*., 2020) [15]; Wang *et al*., 2022 [22]. This technique involves entrapping probiotic cells within protective biopolymeric matrices, forming discrete microbeads or microcapsules that function as physical and biochemical barriers against environmental stresses (Moghanjougi *et al*., 2021) [10]; Sbehat *et al*., 2022 [17]. Encapsulation not only improves resistance to acidic and bile environments but also allows controlled release of viable cells in the intestinal tract (Vivek *et al*., 2023) [21]; Sin *et al*., 2025 [18]. A wide range of encapsulating materials, including polysaccharides, proteins, lipids, and composite systems, have been investigated for probiotic protection (Rodrigues *et al*., 2020 [15]; Wang *et al*., 2022) [22]. Among the critical parameters influencing encapsulation performance, bead size, matrix composition, encapsulation efficiency, and structural integrity play decisive roles in determining the protective capacity, mechanical stability, and release kinetics of encapsulated probiotics (Moghanjougi *et al*., 2021) [10]; Vivek *et al*., 2023 [21].

In recent years, increasing attention has been directed toward the incorporation of prebiotic compounds into encapsulation matrices to create synbiotic delivery systems that combine probiotic protection with selective stimulation of beneficial gut microbiota (Salaria *et al*., 2013) [16]; Ramírez-Damián *et al*., 2025 [13]. Partially hydrolyzed guar gum (PHGG), a water-soluble dietary fiber derived from guar gum, has gained prominence due to its low viscosity, high fermentability, and excellent gastrointestinal tolerance (Al-Asmari *et al*., 2025) [1]; Reider *et al*., 2020 [14]. PHGG selectively promotes the growth and metabolic activity of beneficial microorganisms, Particularly *Lactobacillus* and *Bifidobacterium* species, leading to enhanced production of short-chain fatty acids and improved intestinal function (Liu *et al*., 2022) [7]; Reider *et al*., 2020 [14]. Beyond its prebiotic effects, PHGG exhibits favourable physicochemical characteristics, including thermal stability, resistance to acidic conditions, and compatibility with a wide range of food matrices, making it a promising functional component for probiotic microencapsulation systems (Al-Asmari *et al*., 2025 [1]; Sin *et al*., 2025) [18].

The integration of PHGG into probiotic encapsulation matrices may therefore offer a dual functional advantage: enhanced physical protection of probiotic cells during processing and gastrointestinal transit, and synbiotic stimulation of probiotic growth and activity upon release in the intestine (Salaria *et al*., 2013) [16]; Ramírez-Damián *et al*., 2025 [13]. Although previous studies have reported improved probiotic survivability through the incorporation of various prebiotics into encapsulation systems, systematic investigations specifically evaluating the influence of PHGG on microbead characteristics, particularly bead size distribution, and its protective effects under simulated gastric, gastrointestinal, and thermal stress conditions remain limited (Moghanjougi *et al*., 2021) [10]; Sbehat *et al*., 2022 [17]. Furthermore, probiotic responses to encapsulation are highly strain-specific, underscoring the need for targeted evaluation of technologically and clinically important strains such as *Lactobacillus acidophilus* NCDC-15 (Bustos *et al*., 2025) [2]; Liu *et al*., 2024 [8].

Accordingly, the present study was undertaken to develop a PHGG-assisted microencapsulation system For *Lactobacillus acidophilus* NCDC-15 and to omprehensively assess its protective efficacy under conditions relevant to food processing and gastrointestinal transit. The specific objectives of the study were to (i) prepare and microencapsulate *L. acidophilus* NCDC-15 using PHGG-based matrices, (ii) estimate and characterize microbead size, (iii) evaluate survivability under simulated gastric and gastrointestinal conditions, (iv) and assess thermal tolerance of encapsulated cells establish the significance of observed effects. The outcomes of this investigation are expected to contribute valuable insights into the design of synbiotic encapsulation systems with enhanced probiotic stability, supporting their application in functional foods and nutritionally sensitive formulations, including infant and medical nutrition products (Solanki *et al*., 2015) [19]; Wang *et al*., 2022 [22].

Materials and methods

Selection of Probiotic Organism

A probiotic strain of *Lactobacillus acidophilus* NCDC-15 was selected for the present study due to its established probiotic functionality, gastrointestinal relevance, and

frequent application in dairy-based functional foods. The culture was procured from the National Collection of Dairy Cultures (NCDC), ICAR-National Dairy Research Institute (NDRI), Karnal, India, in freeze-dried form. Upon receipt, the culture was stored under refrigerated conditions (4±1 °C) until further use.

Activation and Preparation of Probiotic Culture

The *L. acidophilus* NCDC-15 culture was activated following a two-step procedure to ensure optimal metabolic activity and cell viability prior to encapsulation (Moghanjougi *et al*., 2021) [10]; Vivek *et al*., 2023 [21]. Initially, the culture was inoculated into chalk litmus milk and incubated at 37±1 °C for 18-24 h to facilitate regeneration and confirmation of acid-producing activity (Salaria *et al*., 2013) [16]; Solanki *et al*., 2015 [19]. Following visible coagulation and acid development, the activated culture was aseptically transferred (1%, v/v) into de Man, Rogosa and Sharpe (MRS) broth and incubated at 37±1 °C for 18-24 h under anaerobic conditions (Liu *et al*., 2024) [8]; Ramírez-Damián *et al*., 2025 [13]. This secondary activation step ensured attainment of the logarithmic growth phase, which is considered optimal for microencapsulation (Rodrigues *et al*., 2020) [15]; Vivek *et al*., 2023 [21].

The activated culture was subsequently harvested by centrifugation at 5,000 × g for 10 min at 4 °C (Moghanjougi *et al*., 2021) [10]. The resulting cell pellet was washed twice with sterile phosphate-buffered saline (PBS, pH 7.0) to remove residual growth medium and resuspended in sterile PBS to obtain a concentrated probiotic suspension for encapsulation (Naissinger da Silva *et al*., 2020 [11]; Sbehat *et al*., 2022) [17].

Microencapsulation of Probiotic Organism

Preparation of Encapsulation Matrix

The encapsulation matrix was prepared using food-grade biopolymers as mentioned in **Table 1**. Sodium alginate (3%, w/v) served as the primary gelling agent, corn starch (2%, w/v) was incorporated to enhance structural rigidity and matrix density, and partially hydrolyzed guar gum (PHGG) was added at two different concentrations (3% and 5%, w/v) to evaluate its effect on bead characteristics and probiotic survivability (Salaria *et al*., 2013; Al-Asmari *et al*., 2025) [1, 16]; Sin *et al*., 2025 [1, 18].

Table 1: Composition of matrix for microencapsulation

Component	Concentration (%)
Sodium alginate	3
Corn starch	2
PHGG	3 and 5

All matrix components were dissolved in sterile distilled water with continuous stirring at ambient temperature until a homogeneous solution was obtained. The activated probiotic suspension was then aseptically mixed into the matrix solution to achieve uniform cell distribution.

Formation of Microcapsules

The activated *Lactobacillus acidophilus* NCDC-15 culture was harvested by centrifugation and the resulting cell pellet was resuspended in sterile phosphate-buffered saline to obtain a standardized cell concentration of approximately $\geq 10^{8}$-10^{9}/g, as determined by plate count analysis (Moghanjougi *et al*., 2021 [10]; Naissinger da Silva *et al*., 2020) [11]. This concentration range was selected to ensure sufficient probiotic loading within the microcapsules while maintaining matrix integrity and uniform bead formation (Rodrigues *et al*., 2020) [15]; Vivek *et al*., 2023 [21].

A calculated quantity of the standardized probiotic suspension was aseptically incorporated into a predetermined volume of the encapsulation matrix composed of sodium alginate, corn starch, and partially hydrolyzed guar gum (PHGG) at different concentration levels (Salaria *et al*., 2013) [16]; Ramírez-Damián *et al*., 2025 [13]. The probiotic-polymer mixture was homogenized under constant gentle stirring for 15-20 min at ambient temperature to achieve uniform cell distribution and to minimize air entrapment, which could otherwise compromise bead integrity and encapsulation efficiency (Wang *et al*., 2022) [22]; Sbehat *et al*., 2022 [17].

The resulting probiotic-loaded polymer suspension was subsequently atomized through a pneumatic spray nozzle under constant operating pressure into a chilled (4±1 °C) 0.1 M calcium chloride solution. The extrusion-spray process facilitated immediate ionic crosslinking of sodium alginate with calcium ions, leading to the formation of spherical microcapsules (Moghanjougi *et al*., 2021) [10]; Wang *et al*., 2022 [22]. Rapid gelation in the chilled calcium chloride bath minimized cell leakage and thermal or mechanical stress on the probiotic cells during bead formation (Rodrigues *et al*., 2020) [15]; Vivek *et al*., 2023 [21].

The formed microcapsules were allowed to harden in the calcium chloride solution for 20 min to ensure complete gelation and structural stabilization of the encapsulation matrix (Sbehat *et al*., 2022) [17]. Following hardening, oversized or irregularly shaped beads were removed manually to obtain a uniform bead size distribution (Moghanjougi *et al*., 2021) [10]. The remaining microcapsules were collected by filtration using Whatman No. 1 filter paper, rinsed with sterile distilled water to remove residual calcium ions, and transferred into sterile containers (Rodrigues *et al*., 2020) [15]. The freshly prepared microcapsules were stored under refrigerated conditions (4±1 °C) until further analysis (Solanki *et al*., 2015) [19].

Assessment of Survivability of Microencapsulated Probiotic Organism

Survivability under Simulated Gastric pH Conditions

Simulated gastric environments were prepared using sterile buffer solutions adjusted to pH 1.0, 1.5, and 2.0 using 1 N HCl (Naissinger da Silva *et al*., 2020) [11]; Moghanjougi *et al*., 2021 [10]. Microencapsulated probiotic beads were suspended in the respective solutions and incubated at 37±1 °C for a predetermined exposure period (Sbehat *et al*., 2022) [17]. At the end of incubation, samples were withdrawn, neutralized, and the beads were disintegrated using sodium citrate solution to release entrapped cells (Rodrigues *et al*., 2020) [15]. Viable counts were determined by the standard plate count method using MRS agar, followed by incubation at 37±1 °C for 48 h (Vivek *et al*., 2023) [21].

Survivability under Simulated Intestinal Bile Salt Conditions

To simulate intestinal stress, bile salt solutions were prepared at concentrations of 1.0%, 1.5%, and 2.0% (w/v) (Naissinger da Silva *et al*., 2020 [11]; Wang *et al*., 2022) [22]. Microencapsulated probiotic beads were exposed to these

solutions at 37±1 °C (Moghanjougi *et al*., 2021) [10]. Following exposure, viable cell counts were determined using the plate count method after appropriate dilution and bead disintegration (Sbehat *et al*., 2022) [17]. The survivability of encapsulated cells was compared with non-encapsulated controls to quantify the protective effect of the encapsulation system (Vivek *et al*., 2023) [21].

Survivability during Thermal Treatment

Thermal stability of microencapsulated *L. acidophilus* NCDC-15 was assessed to simulate processing conditions commonly encountered in dairy and functional food manufacturing (Mansouripour *et al*., 2013 [9]; Rodrigues *et al*., 2020) [15]. Microcapsules were subjected to heat treatments at 75 °C for 30 s and 80 °C for 30 s using a thermostatically controlled water bath (Solanki *et al*., 2015) [19]; Vivek *et al*., 2023 [21]. Immediately after heating, samples were cooled rapidly in an ice bath to arrest thermal damage (Mansouripour *et al*., 2013) [9]. Viable counts were determined as described previously and expressed as log CFU/g (Sbehat *et al*., 2022) [17].

Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean±standard deviation. The data obtained from survivability studies under simulated gastric pH, bile salt concentrations, and thermal treatments were subjected to appropriate statistical analysis to determine the significance of observed differences. Analysis of variance (ANOVA) was performed using suitable SPSS statistical software, and mean comparisons were carried out at a significance level of $p<0.05$.

Results and discussions

Effect of PHGG on Survivability of Microencapsulated Probiotic Organism

The survivability of *Lactobacillus acidophilus* NCDC-15 under simulated gastrointestinal stress conditions was evaluated to elucidate the protective effect of partially hydrolyzed guar gum (PHGG) when incorporated into the microencapsulation matrix. Sodium alginate-starch microcapsules containing different levels of PHGG (3% and 5%) were compared with non-encapsulated control cells across a range of simulated gastric pH values (1.0, 1.5, and 2.0). The results demonstrate a clear pH-dependent decline in probiotic viability, with PHGG-containing microcapsules consistently exhibiting superior survivability relative to control cells.

Survivability of *Lactobacillus acidophilus* NCDC-15 in Simulated Gastric pH

Exposure to highly acidic gastric conditions represents one of the most critical barriers to probiotic survival following oral administration. As expected, a pronounced reduction in viable counts of *L. acidophilus* was observed with decreasing pH and increasing incubation time (Table 1). However, the magnitude of viability loss varied significantly depending on the presence and concentration of PHGG in the encapsulation matrix.

At pH 1.0, the most severe condition evaluated, non-encapsulated control cells exhibited a rapid decline in viability, with counts decreasing from an initial value of 8.71 log CFU/g to 5.26, 5.11, and 4.49 log CFU/g after 1, 2, and 3 h of incubation, respectively. This sharp reduction reflects the extreme sensitivity of *L. acidophilus* to strong acid stress, which disrupts membrane integrity, denatures intracellular proteins, and impairs enzymatic activity. In contrast, microcapsules containing 3% and 5% PHGG showed significantly improved resistance to acidic stress, retaining 5.49 and 6.82 log CFU/g, respectively, even after 3 h of exposure. The higher PHGG concentration consistently conferred greater protection, indicating a dose-dependent effect.

A similar but less severe trend was observed at pH 1.5, where control cells showed a gradual reduction in viability from 8.71 log CFU/g to 5.62 log CFU/g after 3 h of incubation. In comparison, encapsulated cells containing 3% and 5% PHGG maintained substantially higher viable counts (6.86 and 7.10 log CFU/g, respectively) over the same exposure period. The improved survivability at pH 1.5 compared with pH 1.0 emphasizes the strong influence of gastric acidity on probiotic stability, while reinforcing the protective role of PHGG-based encapsulation.

At pH 2.0, which more closely resembles postprandial gastric conditions, the decline in viability was comparatively moderate. Control cells showed a reduction from 8.71 to 6.10 log CFU/g after 3 h, whereas encapsulated cells containing 5% PHGG retained 7.30 log CFU/g. Notably, microcapsules with 5% PHGG demonstrated minimal loss in viability during the initial 1-2 h of exposure, suggesting that the encapsulation matrix effectively buffered acid diffusion and delayed proton penetration into the microcapsules.

The enhanced protective effect of PHGG is further substantiated by percent survivability data (Table 2, Figure 1). At pH 1.0, only 60.39% of non-encapsulated cells survived after 1 h, decreasing to 51.55% after 3 h. In contrast, survivability of encapsulated cells containing 5% PHGG remained as high as 78.94% after 3 h, representing a substantial improvement over both the control and the 3% PHGG formulation. Similar trends were observed at pH 1.5 and 2.0, where PHGG-containing microcapsules consistently exhibited higher survivability across all incubation periods.

The improved acid tolerance observed in PHGG-containing microcapsules can be attributed to multiple synergistic mechanisms such as the incorporation of PHGG likely increased matrix density and viscosity, thereby reducing acid diffusion into the microcapsules (Salaria *et al*., 2013) [16]; Wang *et al*., 2022 [22]; Sin *et al*., 2025 [18]. Moreover, PHGG may exert a buffering effect within the microcapsule microenvironment, moderating the internal pH of entrapped cells (Reider *et al*., 2020) [14]; Liu *et al*., 2022 [7]. In addition, PHGG may act as a prebiotic fiber that interacts with the bacterial cell surface, contributing to stabilization of membrane structures, preservation of protein functionality, and enhancement of cellular stress tolerance under acidic conditions (Bustos *et al*., 2025 [2]; Al-Asmari *et al*., 2025) [1].

The results clearly demonstrate that PHGG-assisted microencapsulation significantly enhances the survival of *L. acidophilus* NCDC-15 under simulated gastric conditions, with the 5% PHGG formulation showing the highest protective efficacy (Moghanjougi *et al*., 2021 [10]; Vivek *et al*., 2023) [21]. Importantly, viable counts remained above the minimum therapeutic threshold ($\geq 10^6$-10^7 CFU/g) even

after prolonged exposure to acidic environments, indicating the potential of the encapsulation system for effective probiotic delivery and functional performance in acidic food matrices and gastrointestinal transit (Naissinger da Silva *et al.*, 2020) [11]; Solanki *et al.*, 2015 [19]; Sbehat *et al.*, 2022 [17].

Table 1: Effect of PHGG level on survivability (log CFU/g) of *Lactobacillus acidophilus* NCDC-15 under simulated gastric pH conditions

pH	Incubation time (h)	Control	3% PHGG	5% PHGG	F-value	CD ($p<0.05$)
1.0	0	8.71±0.13	8.51±0.14	8.64±0.11	0.65	NS
	1	7.51±0.03^{a}	7.78±0.07^{b}	8.02±0.02^{c}	28.74*	0.17
	2	5.10±0.10^{a}	6.10±0.11^{b}	7.10±0.12^{c}	100.00*	0.35
	3	4.49±0.12^{a}	5.49±0.12^{b}	6.82±0.06^{c}	134.17*	0.35
1.5	0	8.71±0.13	8.51±0.14	8.64±0.11	0.65	NS
	1	6.49±0.12^{a}	7.58±0.16^{b}	7.64±0.17^{c}	18.34*	0.52
	2	6.00±0.08^{a}	7.00±0.08^{b}	7.26±0.14^{c}	41.92*	0.36
	3	5.62±0.09^{a}	6.86±0.05^{b}	7.13±0.09^{c}	109.55*	0.27
2.0	0	8.71±0.13	8.51±0.14	8.64±0.11	0.65	NS
	1	6.56±0.14^{a}	6.71±0.14^{b}	7.83±0.07^{c}	33.54*	0.415
	2	6.52±0.14^{a}	6.52±0.14^{a}	7.67±0.11^{b}	26.36*	0.449
	3	6.10±0.10^{a}	6.30±0.17^{b}	7.30±0.17^{c}	17.71*	0.528

Values expressed as mean±standard error (SE), n = 3; Superscripts ($^{a-c}$) indicate significant differences within the same pH and incubation time ($p<0.05$); NS = not significant; PHGG: Partially Hydrolyzed Guar Gum

Table 2: Percent survivability (%) of *Lactobacillus acidophilus* NCDC-15 as affected by PHGG level under simulated gastric pH conditions

pH	PHGG level (%)	1 h	2 h	3 h
1.0	Control (unencapsulated)	60.39^{c}	58.67^{c}	51.55^{c}
	3% PHGG	84.84^{b}	71.45^{b}	64.51^{b}
	5% PHGG	85.64^{a}	82.18^{a}	78.94^{a}
1.5	Control (unencapsulated)	74.54^{c}	68.89^{c}	64.51^{c}
	3% PHGG	88.44^{b}	82.26^{b}	80.60^{b}
	5% PHGG	89.02^{a}	84.02^{a}	82.18^{a}
2.0	Control (unencapsulated)	75.32^{c}	74.84^{c}	70.04^{c}
	3% PHGG	78.85^{b}	76.60^{b}	74.04^{b}
	5% PHGG	90.66^{a}	88.82^{a}	84.50^{a}

Values represent mean percent survivability calculated relative to initial viable counts (0 h). Different superscripts ($^{a-c}$) within the same pH and incubation time indicate significant differences ($p<0.05$).

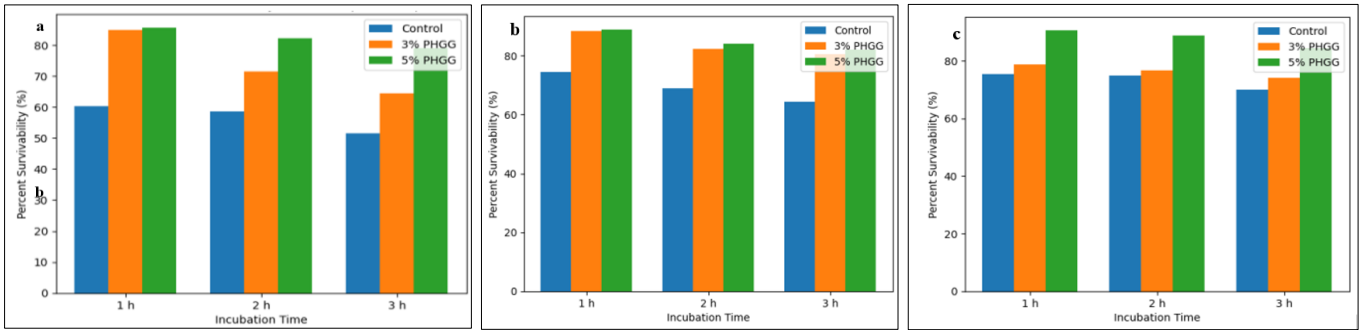

Fig 1: Effect of partially hydrolyzed guar gum concentration on percent survivability of microencapsulated *Lactobacillus acidophilus* NCDC-15 under simulated gastric conditions at (a) pH 1.0, (b) pH 1.5, and (c) pH 2.0 after 1, 2, and 3 h of incubation at 37 °C. Values represent mean percent survivability relative to initial counts.

Survival of *Lactobacillus acidophilus* NCDC-15 under Simulated Gastrointestinal Conditions

Survival in the intestinal environment is a critical determinant of probiotic efficacy, as bile salts exert strong detergent-like effects on bacterial cell membranes, leading to membrane destabilization, leakage of intracellular constituents, and subsequent loss of cell viability. The survivability of *L. acidophilus*, expressed as log CFU/ml, is provided in Table 3. Across all bile salt concentrations, a pronounced time- and concentration-dependent decline in viable counts was observed. However, the magnitude of viability loss differed markedly among treatments, with PHGG-containing microcapsules consistently exhibiting superior survivability compared with the control.

At 1.0% bile salt, the viable count of control cells decreased significantly ($p<0.05$) from an initial value of 8.71 log CFU/ml to 5.44 log CFU/ml after 3 h of incubation. In contrast, microcapsules containing 3% and 5% PHGG retained significantly higher counts of 6.10 and 6.46 log CFU/ml, respectively, at the same exposure time. Statistical analysis revealed significant differences among treatments after 1, 2, and 3 h of incubation, as indicated by elevated F-values and distinct superscripts, confirming the protective effect of PHGG against bile-induced stress.

A similar trend was observed at 1.5% bile salt, where bile toxicity was more pronounced. After 3 h of incubation, control cells showed a drastic reduction in viability to 4.62 log CFU/ml, whereas microencapsulated cells containing 3% and 5% PHGG maintained significantly higher counts of

5.86 and 6.10 log CFU/ml, respectively. At the highest bile salt concentration (2.0%), representing severe intestinal stress conditions, probiotic survivability declined substantially in all treatments; nevertheless, PHGG-containing microcapsules continued to confer significant protection. After 3 h of exposure, viable counts in the control declined to 4.36 log CFU/ml, whereas 3% and 5% PHGG microcapsules retained 4.67 and 5.74 log CFU/ml, respectively. The consistently higher survivability observed at 5% PHGG across all incubation periods indicates a clear concentration-dependent protective effect of PHGG under extreme bile stress.

No significant differences were observed among treatments at 0 h for any bile salt concentration, confirming uniform initial probiotic loading. However, with increasing incubation time, significant differences ($p<0.05$) emerged between control and PHGG-containing microcapsules, particularly at higher bile salt concentrations. In some cases, differences between control and 3% PHGG were not significant during early incubation periods at lower bile concentrations, suggesting that higher PHGG levels are required to impart optimal protection under more severe bile concentrations.

The enhanced bile tolerance observed in PHGG-containing microcapsules can be attributed to multiple synergistic mechanisms. Incorporation of PHGG likely increased the density and compactness of the encapsulation matrix, thereby limiting bile salt diffusion into the microcapsule core (Salaria *et al*., 2013) [16]; Wang *et al*., 2022 [22]; Sin *et al*., 2025 [18]. Additionally, PHGG may interact with bile salts, reducing their effective detergent activity within the microenvironment surrounding the encapsulated cells (Reider *et al*., 2020 [14]; Liu *et al*., 2022) [7]. As a fermentable prebiotic fiber, PHGG may also contribute to stabilization of bacterial cell membranes and enhancement of stress resistance through modulation of cellular physiology and energy metabolism, collectively improving probiotic survivability under bile stress conditions (Bustos *et al*., 2025) [2]; Al-Asmari *et al*., 2025 [1]; Vivek *et al*., 2023 [21].

Table 3: Effect of PHGG concentration on survivability (log CFU/ml) of *Lactobacillus acidophilus* NCDC-15 under simulated intestinal bile salt conditions

Bile salt (%)	Incubation time (h)	Control	3% PHGG	5% PHGG	F-value	CD ($p<0.05$)
1.0	0	8.71±0.13	8.51±0.14	8.64±0.11	0.65	NS
	1	6.43±0.15^{a}	6.59±0.13^{a}	7.44±0.25^{b}	8.80*	0.633
	2	6.26±0.17^{a}	6.30±0.14^{a}	7.30±0.17^{b}	13.08*	0.564
	3	5.44±0.24^{a}	6.10±0.10^{b}	6.46±0.08^{c}	10.20*	0.558
1.5	0	8.71±0.13	8.51±0.14	8.64±0.11	0.65	NS
	1	6.33±0.20^{a}	6.58±0.16^{b}	7.36±0.23^{c}	7.28*	0.686
	2	6.10±0.10^{a}	6.26±0.14^{b}	6.91±0.04^{c}	17.81*	0.355
	3	4.62±0.08^{a}	5.86±0.05^{b}	6.10±0.03^{c}	171.03*	0.209
2.0	0	8.71±0.13	8.51±0.14	8.64±0.11	0.65	NS
	1	5.94±0.09^{a}	6.23±0.03^{b}	7.10±0.10^{c}	54.42*	0.282
	2	4.89±0.15^{a}	5.36±0.18^{b}	6.20±0.03^{c}	23.34*	0.475
	3	4.36±0.06^{a}	4.67±0.10^{b}	5.74±0.07^{c}	76.16*	0.287

Survival of *Lactobacillus acidophilus* NCDC-15 during Thermal Treatment

Heating is a common unit operation in the manufacture of several dairy products, including milk powders, and represents a major challenge to the survival of probiotic microorganisms (Mansouripour *et al*., 2013) [9]; Rodrigues *et al*., 2020 [15]. High temperatures can induce irreversible damage to bacterial cell membranes, denature intracellular proteins, disrupt enzymatic systems, and impair metabolic activity, leading to substantial losses in probiotic viability (Bustos *et al*., 2025 [2]; Vivek *et al*., 2023) [21]. To mitigate these adverse effects, microencapsulation has been proposed as an effective strategy to enhance the thermal stability of probiotic cultures during processing by providing a physical barrier against heat transfer and dehydration stress (Mansouripour *et al*., 2013) [9]; Wang *et al*., 2022 [22].

Thermal treatment resulted in a marked reduction in viable counts across all treatments; however, the magnitude of reduction varied significantly depending on the presence and concentration of PHGG in the encapsulation matrix, indicating a concentration-dependent protective effect of PHGG-assisted microencapsulation under heat stress conditions (Salaria *et al*., 2013) [16]; Solanki *et al*., 2015 [19]; Vivek *et al*., 2023 [21].

In control samples, the initial viable count of 8.71 log CFU/ml declined sharply to 4.22 and 4.14 log CFU/ml following exposure to 75 °C and 80 °C, respectively, indicating high sensitivity of free *L. acidophilus* cells to heat shock. In contrast, microencapsulated cells exhibited significantly higher thermal tolerance. Capsules containing 3% PHGG retained 5.26 and 5.07 log CFU/ml after treatment at 75 °C and 80 °C, respectively, whereas capsules containing 5% PHGG showed the highest protection, with viable counts of 6.20 and 5.62 log CFU/ml under the same conditions (Table 4).

At 75 °C, survivability of control cells was limited to 48.45%, while capsules containing 3% and 5% PHGG exhibited survivability of 61.60% and 71.77%, respectively. A similar trend was observed at 80 °C, where PHGG-based microcapsules consistently maintained higher survivability compared with the control. These results clearly demonstrate a concentration-dependent protective effect of PHGG against thermal stress, with the 5% PHGG formulation providing the most effective protection.

Statistical analysis of the thermal survivability data (Table 4) revealed no significant differences among treatments at the initial stage, confirming uniform initial probiotic loading. However, following thermal exposure, highly significant differences ($p<0.05$) were observed between control and PHGG-containing microcapsules at both 75 °C and 80 °C.

The improved thermal stability of *L. acidophilus* observed in PHGG-containing microcapsules can be attributed to several complementary mechanisms such as the encapsulation matrix acting as a physical barrier, reducing

direct heat transfer to the entrapped cells and thereby moderating the rate of temperature increase within the microcapsule core (Mansouripour *et al*., 2013) [9]; Rodrigues *et al*., 2020 [15]; Wang *et al*., 2022 [22]. In addition, the incorporation of PHGG likely enhances matrix cohesiveness and moisture retention, thereby reducing dehydration-induced cellular damage during heat exposure (Salaria *et al*., 2013) [16]; Solanki *et al*., 2015 [19]; Vivek *et al*., 2023 [21]. Moreover, PHGG may stabilize bacterial cell membranes and intracellular proteins by creating a protective microenvironment that limits thermal denaturation and oxidative stress, collectively contributing to improved probiotic survival under thermal processing conditions (Bustos *et al*., 2025) [2]; Al-Asmari *et al*., 2025 [1].

Table 4: Survivability (log CFU/ml) of *Lactobacillus acidophilus*NCDC-15 during thermal treatment

Thermal treatment (30 s)	Control	3% PHGG	5% PHGG	F-value	CD (p<0.05)
Initial count	8.71±0.13	8.51±0.14	8.64±0.11	0.65	NS
75 °C	4.22±0.03^{a}	5.26±0.14^{b}	6.20±0.10^{c}	96.67*	0.349
80 °C	4.14±0.05^{a}	5.07±0.05^{b}	5.62±0.07^{c}	148.71*	0.212

Values are expressed as mean±standard error (SE), n = 3; Means with different superscripts ($^{a-c}$) within the same thermal treatment differ significantly (p<0.05); NS = not significant; PHGG: Partially hydrolyzed guar gum

Conclusion

The present study demonstrates that microencapsulation using a sodium alginate-corn starch matrix supplemented with partially hydrolysed guar gum is an effective strategy for enhancing the stability of *Lactobacillus acidophilus* NCDC-15 under conditions relevant to food processing and gastrointestinal transit. Incorporation ofpartially hydrolysed guar gum significantly improved probiotic survivability against severe gastric acidity, bile salt stress, and thermal treatments, with the 5% partially hydrolysed guar gum formulation consistently providing superior protection in a concentration-dependent manner. Importantly, encapsulated probiotic cells maintained viable counts above the recommended therapeutic threshold, indicating their potential to exert functional health benefits upon consumption. The combined protective and prebiotic functionality of partially hydrolysed guar gum highlights the value of this synbiotic microencapsulation system for improving probiotic delivery and efficacy. Overall, the findings support the application of partially hydrolysed guar gum-based microencapsulation in the development of stable probiotic and synbiotic foods, nutraceuticals, and specialized products such as infant and medical nutrition formulations, where probiotic viability is critical to product performance.

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