

Release kinetic study of encapsulated probiotic *Lacticaseibacillus rhamnosus* strain NCDC 347 in double-layer matrix encapsulated by spray drying

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Abstract

Probiotics are increasingly used in functional foods, but maintaining their viability in the gastrointestinal tract remains a challenge. This study focused on skim milk powder (SMP)+carrageenan hydrogel for the controlled oral delivery of *Lactocaseibacillus rhamnosus* NCDC 347. Encapsulated cells showed controlled release in deionised water (OD_{600} : 0.12–0.38), whereas release in simulated intestinal fluid (SIF) reached 0.70–0.72 after 4.5 h. Under simulated gastric fluid (SGF), release was pH-dependent, reaching OD_{600} values of 0.42 at pH 2.0 and 0.75–0.78 at pH 1.0. Encapsulation reduced cumulative release after 9 h to 0.40 mg cm⁻² in water and 0.37–0.38 mg cm⁻² in SIF, compared with 0.64 and 0.60 mg cm⁻², respectively, for free cells. The release kinetics showed water uptake was predominantly Fickian ($n = 0.326–0.396$), whereas probiotic release was non-Fickian and relaxation-associated ($n > 0.85$; $K_r > K_d$), suggesting diffusion-driven hydration followed by polymer relaxation and matrix restructuring. The encapsulated cells showed reduced swelling in SGF but exhibited significant swelling in SIF, reaching 2.3 g/g of water and a swelling ratio of 2.9. The viability of encapsulated cells was also improved, with a count of 6.5 log CFU/g after 120 min of incubation in SGF at pH 2.0 and 6.3–6.5 log CFU/g after 120 min of incubation in 2% bile salts. Overall, the developed SMP+carrageenan hydrogel system can be used for the controlled delivery of probiotics and is suitable for functional food and nutraceutical applications.

1. Introduction

Probiotic bacteria are widely used in functional foods due to their beneficial effects on the gastrointestinal tract, the modulation of the immune system, and metabolic activity. However, the key challenge related to the production of such foods is the high risk of losing viability of the microorganisms throughout the process of food production, storage, and digestion. One of the methods that can address this issue is microencapsulation, which has proven to be effective at protecting the probiotic strains, improving their viability, and contributing to targeted delivery to the site of action (Gulati et al., 2019). Within the concept of microencapsulation, spray drying is a promising technique, which is scalable, less expensive, and offers excellent opportunities for obtaining stable encapsulated products (Pupa et al., 2021). However, there is a significant limitation associated with this method, including the fact that such a process imposes extreme conditions on probiotic bacteria, which leads to a deterioration of their viability. As a consequence, a specific choice of protective wall materials must be made to minimise stress during spray drying. The key factors affecting the functional properties of the produced encapsulated probiotics include temperature, pH, and osmolarity, which should be taken into account when selecting the wall material in order to ensure the best possible quality of the end product. The main challenge in this case is to ensure high bacterial viability after spray drying and to maintain it throughout the entire food production and storage process.

The efficiency of spray-dried probiotic primarily depends on the physicochemical properties of the carrier matrices used during encapsulation. Food-grade polymers such as proteins and polysaccharides are considered safe and efficient excipients due to their ability to protect the probiotic bacteria (Gulati et al.,

2019). For instance, skim milk powder (SMP) contains lactose and casein proteins that can be used to create a coating of a bacterial cell surface and prevent osmotic stress or heat damage during drying (Pupa et al., 2021). The protein component of SMP can also help stabilise the membrane structure and retain moisture during the drying process and storage. On the other hand, the sulfated linear polysaccharides extracted from red seaweed, commonly known as carrageenan, demonstrated an enhanced ability to control water affinity, form gels, and bind moisture (Kim et al., 2021). A previous study reported that the physicochemical properties of encapsulating polymers can affect encapsulation efficiency and release kinetics of active ingredients (Gulati et al., 2019).

Although the use of individual wall materials may impart certain protective functions, blended matrices are considered to be more advantageous due to their potential to combine the beneficial features of protein and polysaccharide-based systems. Specifically, the SMP+carrageenan blended matrix was suggested to provide synergistic protection to encapsulated compounds due to the combination of film-forming properties of milk proteins and viscoelastic and diffusive barriers of carrageenan (Riseh et al., 2022). Protein-polysaccharide interactions were reported to improve the structural properties of the matrix and contribute to the formation of a protective barrier that prevents the ingress of moisture and mechanical damage during drying and storage (Riseh et al., 2022). Though extensive studies have investigated protein- and polysaccharide-based matrices for probiotic encapsulation (Gulati et al., 2019), comparatively fewer studies have systematically evaluated the release kinetics and storage behaviour of spray-dried probiotics in double-layer matrix systems. Most previous studies have primarily focused on encapsulation efficiency and probiotic survival, whereas the mechanistic understanding of matrix-dependent release behaviour and stability characteristics remains comparatively limited. Quantitative kinetic modelling is particularly important because it helps explain dominant transport mechanisms, predict release performance, and enable objective comparison among encapsulation systems. A better understanding of these kinetic and stability characteristics is essential for the rational design of probiotic powders with improved storage and delivery performance.

Previous studies have demonstrated that polysaccharide-based encapsulation systems can significantly enhance probiotic stability by providing a physical barrier against environmental stress factors such as low pH, bile salts, oxygen exposure, and osmotic stress (Ko et al., 2024). Furthermore, protein- and polysaccharide-based matrices have been reported to improve the survivability of probiotics during storage and gastrointestinal transit (Nezamdoost-Sani et al., 2023). Although carrageenan- and milk protein-based systems have previously been investigated for probiotic encapsulation, limited information is available regarding the application of SMP+carrageenan double-layer spray dried matrices specifically for *Lactocaseibacillus rhamnosus*, particularly in relation to release kinetics and matrix-dependent stability behaviour. Therefore, the novelty of the present work lies not in the use of the individual encapsulating materials themselves, but in evaluating the influence of matrix architecture on probiotic protection, release behaviour, and stability characteristics.

Therefore, the present study investigated the kinetic behaviour and stability characteristics of spray-dried *L. rhamnosus* strain NCDC 347 encapsulated in double-layer (SMP+carrageenan) matrices. Specifically,

the study aimed to compare the effects of matrix composition and architecture on probiotic viability, release kinetics, and survival under simulated gastrointestinal conditions, and to establish relationships between matrix structure and functional performance.

2. Materials and methods

2.1 Procurement of microbial strain and encapsulation materials

Lactocaseibacillus rhamnosus NCDC 347 was procured from the Microbiology lab, NIFTEM-K, and used as the probiotic strain in this study. The encapsulation matrices comprised SMP, carrageenan, and a combination of SMP and carrageenan, prepared according to the methods described by Kumar et al. (2023). The De Man, Rogosa and Sharpe (MRS) agar (HiMedia, India) was used for microbial culture. All chemicals and reagents used were of analytical grade.

2.2 Preparation of a cell mixture with different wall matrix

A 24 h old grown cells of NCDC 347 (9 log CFU/mL) were harvested using centrifuge (3-18KS, Sigma, Germany) and mixed individually with SMP powder (20% w/v), carrageenan (20% w/v) and SMP+carrageenan (each 10% w/v; 1:1). The encapsulation matrices were dispersed in distilled water to achieve a core-to-wall ratio of 1:1. The mixtures were homogenized to ensure uniformity and then subjected to spray drying.

2.3 Spray Drying

The spray drying process was carried out using a laboratory-scale spray dryer (SMST 18, Equitron Medica Pvt. Ltd., India). To ensure consistency, the dryer was preheated for 20–30 min, maintaining inlet and outlet temperatures at 120 °C and 60 °C, respectively, throughout the operation. The slurry, comprising cells and an encapsulating matrix, was constantly agitated at 120–150 rpm using a magnetic bead stirrer within a feed bottle (Koh et al., 2022). This bottle was connected to a feeding pump set to 8 mL/min. The airflow rate and pressure were fixed at 30 m³/h and 0.275 MPa, respectively. The spray-dried powder was collected from the lower part of the dryer chamber and stored in laminated low-density polyethylene (LDPE) pouches at 4 °C for further analysis (Kumar et al., 2023; Salamat et al., 2026).

2.5 Kinetic cell release, swelling behaviour and cell viability under simulated gastrointestinal conditions

A known amount of the spray-dried SMP+carrageenan capsule was suspended in release media comprising simulated gastric fluid (SGF; Fisher Chemicals, Suwanee, GA), simulated intestinal fluid (SIF; Fisher Chemicals, Suwanee, GA), and deionised (DI) water following the method of Lo (2007). For comparative release studies, an equivalent concentration of free NCDC 347 cells was suspended in DI water and SIF under identical experimental conditions to evaluate the influence of encapsulation on

release behaviour (Yang et al., 2024). The suspensions were incubated under continuous agitation at 150 rpm, and 1 mL aliquots were withdrawn at predetermined intervals for analysis. After each sampling, an equal volume of fresh release medium was added to maintain a constant volume. Cell concentration in the release medium was monitored by measuring the optical density at 600 nm (OD_{600}) using a Helios spectrophotometer (Thermo Spectronic, Rochester, USA). Dilutions were performed as needed, and a previously established strain calibration curve was used to convert OD_{600} values to released cell concentration (Choudhury et al., 2021). All measurements were carried out in triplicate, and the mean values were reported.

The release kinetics of encapsulated cells were evaluated in DI water for 72 h at room temperature (150 rpm), in SIF (pancreatin, KH_2PO_4 and NaOH; pH 6.8) for 24 h at room temperature (150 rpm), and in SGF (pepsin, NaCl and HCl) at pH 1.0 and pH 2.0 at 37°C (150 rpm). Sequential gastrointestinal release was further evaluated by transferring the capsules to SIF after 2 h of exposure to SGF (pH 2.0), as described by Sun et al. (2024). However, the major changes in cell release occurred within the initial 5 h, with negligible changes thereafter; therefore, only the first 5 h release profiles were considered for subsequent analysis.

The dynamic swelling behaviour of the SMP+carrageenan encapsulation matrix was evaluated to understand its hydration and structural response under simulated gastrointestinal conditions. The NCDC 347 was incorporated into the SMP+carrageenan matrix, while a blank SMP+carrageenan matrix without probiotic cells was used as the control. The capsules were sequentially exposed to SGF (pH 2.0) and SIF (pH 6.8), and changes in water absorption and swelling ratio were monitored. Dynamic swelling and water absorption of the SMP+carrageenan capsules were determined by immersing a known weight of dried capsules in the respective test medium under the same incubation conditions. At predetermined intervals, the capsules were removed, gently blotted with filter paper to remove excess surface liquid, and weighed immediately. The swelling ratio and water absorption capacity were calculated gravimetrically based on the increase in capsule weight relative to the initial dry weight and expressed as $mg\ g^{-1}$, following previously reported procedures for hydrogel-based probiotic encapsulation (Anwar et al., 2022; Yang et al., 2024). All measurements were performed in triplicate.

The protective effect of encapsulation against SGF stress was evaluated by comparing the survival of free and encapsulated *L. rhamnosus* NCDC 347 cells under acidic and bile salt conditions. For SGF tolerance, free cells and an equivalent number of encapsulated cells were separately suspended in SGF adjusted to pH 2.0 and 3.0, respectively, and incubated at 37°C at 150 rpm. Samples were withdrawn after 0, 30, 60 and 120 min. For bile salt tolerance, samples were incubated in SIF containing 0, 1.0 and 2.0% (w/v) bile salts at 37°C, and viable counts were determined after 60- and 120-min. Encapsulated cells were recovered by dissolving the capsule matrix before enumeration. Serial dilutions were prepared in sterile saline, plated on de Man–Rogosa–Sharpe (MRS) agar, incubated at 37°C for 48 h, and viable counts were expressed as $\log\ CFU\ g^{-1}$ (Dianawati et al., 2016).

The release and swelling data corresponding to fractional release values (M_t/M_{∞}) between 0.1 and 0.6 were analysed using the Higuchi, Korsmeyer-Peppas, and Peppas-Sahlin kinetic models (Higuchi, 1961; Korsmeyer et al., 1983; Peppas and Sahlin, 1989) using MATLAB version 6 R13 (MathWorks Inc., Natick, MA). Model parameters were estimated by nonlinear least-squares fitting, as commonly employed for analysing diffusion and relaxation-controlled release mechanisms in polymeric encapsulation systems (Dash et al., 2010).

2.6 Statistical analysis

The results of experimental studies were presented as mean $\pm$ standard deviation. Statistical analysis was carried out with IBM SPSS Statistics 19 software (IBM Corp., Armonk, NY, USA). One-way analysis of variance was used to compare differences between the means of more than two independent groups. All experiments were carried out in three replications; differences were considered significant at $p < 0.05$.

3. Results

3.1 Release kinetics of NCDC 347 from SMP+carrageenan capsules under simulated gastrointestinal conditions

The release of NCDC 347 encapsulated in the SMP+carrageenan matrix was studied in DI water, SIF, and SGF (pH 1.0) and SGF (pH 2.0) (Fig. 1). The indirect determination of cell release was achieved by measuring the OD_{600} of the suspension from which the cells were released. The encapsulated cells showed a gradual and prolonged release in DI water throughout the experiment. The OD_{600} increased gradually from approximately 0.12 at the beginning to approximately 0.38 after 4.5 h. A significantly higher cell release was observed for SIF. After an initial lag phase during the first hour, the OD_{600} increased steeply to reach approximately 0.70–0.72 after 4.5 h (Fig. 1). (Fig. 1a).

The release behaviour in SGF was demonstrated in Fig. 1b. Compared to SGF with pH 2.0, where OD_{600} increased slightly during the first 2 h and then gradually climbed to 0.42 at 4.5 h, capsules subjected to SGF with pH 1.0 exhibited a rapid release of cell content. The OD_{600} values rose sharply to 0.75–0.78 at 4.5 h (Fig. 1b).

3.2 Comparative release kinetics of free and encapsulated NCDC 347

The cumulative release profiles of free and encapsulated NCDC 347 were compared in DI water and SIF to assess the release controlling ability of the SMP+carrageenan encapsulation matrix (Fig. 2). For both DI water and SIF, the free and encapsulated cells showed an increase in cumulative release with increasing incubation time (Fig. 2a). Free cells showed rapid diffusion immediately after immersion, reaching approximately 0.25 mg cm^{-2} within the first hour and increasing continuously to approximately 0.64 mg cm^{-2} after 9 h. In contrast, encapsulated cells displayed a significantly slower release

throughout the experiment, reaching only 0.19 mg cm⁻² after 1 h and approximately 0.40 mg cm⁻² after 9 h.

A similar tendency was observed for SIF (Fig. 2b). For free cells, the amount of SIF released increased rapidly to approximately 0.31 mg cm⁻² after 1 h and further to almost 0.60 mg cm⁻² after 9 h. On the other hand, encapsulated cell showed a tendency to release SIF in a more controlled manner, reaching 0.21 mg cm⁻² at 1 h and 0.37–0.38 mg cm⁻² at 9 h.

The release data corresponding to fractional release values (Mt/Mf) between 0.1 and 0.6 were analysed using the Higuchi, Korsmeyer–Peppas, and Peppas–Sahlin models. The calculated kinetic parameters are summarised in Table 1. The release profiles showed poor fitting to the Higuchi model. In contrast, the Korsmeyer–Peppas model provided a better fit for n values greater than 0.85, whereas the Peppas–Sahlin model showed higher relaxation constants (K_r) than diffusion constants (K_d) (Table 1).

Table 1
Analysis of probiotic cell release data from SMP+carrageenan encapsulated capsules using the Higuchi, Korsmeyer–Peppas, and Peppas–Sahlin kinetic equations.

Capsule formation with skim milk (10%) and carrageenan (10%)	Higuchi equation		Korsmeyer-Peppas equation			Peppas-Sahlin equation		
	K' (min ^{-0.5})	R ²	n	K (min ⁻ⁿ)	R ²	K _d (min ^{-0.43})	K _r (min ^{-0.86})	R ²
pH = 6.2; X = 0.7%	0.2374	0.7888	2.043	0.03598	0.9924	— 0.5222 ^b	0.473	0.9936
pH = 6.2; X = 1.0%	0.0432	0.8704	1.233	0.00098	0.9467	— 0.044 ^b	0.0116	0.94881
pH = 4.5; X = 0.7%	0.3304	0.9459	1.082	0.2422	0.9954	— 0.1275 ^b	0.3724	0.9946
pH = 4.5; X = 1.0%	0.1954	0.8878	1.284	0.1001	0.9959	— 0.1646 ^b	0.2624	0.9951
* K' represents the Higuchi release constant, n is the release exponent indicating the transport mechanism, K is the Korsmeyer–Peppas release constant, K _d represents the Fickian diffusion contribution, K _r denotes the polymer relaxation contribution, and R ² indicates the goodness-of-fit of the corresponding model. Values of n were used to characterise the release mechanism, where n < 0.45 indicates Fickian diffusion, 0.45 < n < 0.89 indicates anomalous transport, and n > 0.89 indicates super case-II transport behaviour.								

3.3 Water Absorption and Dynamic Swelling

Water absorption remained low during the initial SGF phase but increased substantially after exposure to SIF. In the probiotic-containing sample, water absorption increased from approximately 0.3 at 2 h to 1.0

at 3 h, 1.35 at 4 h, 1.65 at 5 h, and approximately 2.3 at 14 h, after which it increased only slightly. The control showed a comparatively faster increase in water absorption during the early intestinal phase, reaching approximately 2.5 g/g at later time points (Fig. 3). During the initial gastric phase, both the sample and the control showed limited swelling. After transfer to SIF, the swelling ratio increased progressively, with a more pronounced increase observed for the probiotic-containing sample. The swelling ratio of the sample reached approximately 1.5 at 5 h, 1.9 at 6 h, and increased further to about 2.9 by the end of the observation period. In comparison, the control showed a rapid increase during the early intestinal phase and reached a relatively stable level of approximately 2.6. The water absorption and swelling profiles are presented in Fig. 3a and Fig. 3b, respectively.

To further understand the mechanism of water uptake, the experimental data were fitted to the Higuchi, Korsmeyer–Peppas, and Peppas–Sahlin models (Table 2). The Higuchi model showed good fits, with R^2 values of 0.9772 and 0.9537 for the two formulations. The Korsmeyer–Peppas model yielded n values of 0.396 and 0.326, respectively. Since both values were below 0.45, the results indicate that water transport through the matrix was predominantly governed by Fickian diffusion. The Peppas–Sahlin model also indicated a predominant diffusional contribution, with positive K_d values and negative K_r values for both formulations.

Table 2

Analysis of dynamic swelling data for SMP+carrageenan encapsulated capsules using the Higuchi, Korsmeyer–Peppas, and Peppas–Sahlin kinetic equations.

Capsule formation with skim milk + carrageenan (10% each)	Higuchi equation		Korsmeyer-Peppas equation			Peppas-Sahlin equation		
	K'	R^2	n	K	R^2	K_d	K_r	R^2
	($\text{min}^{-0.5}$)			(min^{-n})		($\text{min}^{-0.43}$)	($\text{min}^{-0.86}$)	
pH = 6.2; X = 0.7%	0.0536	0.9792	0.396	0.0094	0.9926	0.0863	-0.007 ²	0.9971
pH = 6.2; X = 1.0%	0.0911	0.9537	0.326	0.1889	0.9467	0.158	-0.0057 ²	0.9859
* K' represents the Higuchi release constant, n is the release exponent indicating the transport mechanism, K is the Korsmeyer–Peppas release constant, K_d represents the Fickian diffusion contribution, K_r denotes the polymer relaxation contribution, and R^2 indicates the goodness-of-fit of the corresponding model. The values of n were used to characterize swelling behaviour, where $n < 0.45$ indicates Fickian diffusion-controlled swelling, $0.45 < n < 0.89$ indicates anomalous transport, and $n > 0.89$ indicates case-II transport behaviour.								

3.4 Cell Viability

3.4.1 Survival under simulated gastric conditions

Exposure to acidic gastric conditions resulted in a progressive reduction in the viability of both free and encapsulated cells; however, encapsulation significantly enhanced bacterial survival throughout the incubation period. As shown in Fig. 4a, free cells exposed to SGF at pH 2.0 exhibited a rapid decline in viability from approximately 9.0 log CFU/g at the beginning of the experiment to nearly 2.0 log CFU/g after 120 min, corresponding to a reduction of almost 7 log cycles. In contrast, encapsulated cells retained substantially higher viability, decreasing only to approximately 6.5 log CFU/g after the same exposure period.

A similar trend was observed under the less acidic condition (SGF, pH 3.0). Although both free and encapsulated cells experienced a gradual decline in viability over time, the reduction was markedly lower than that observed at pH 2.0 (Fig. 4b). Free cells decreased from approximately 9.0 to approximately 3.4 log CFU/g after 120 min, whereas encapsulated cells-maintained viability above 7.5–8.0 log CFU/g throughout the experiment.

3.4.2 Survival under bile salt stress

The tolerance of free and encapsulated NCDC 347 to bile salt stress was evaluated in SIF containing 0, 0.5, 1.0, 1.5, and 2.0% bile salts for 60 and 120 min (Fig. 4c and 4d). In both treatments, bacterial viability decreased with increasing bile salt concentration and exposure time; however, encapsulated cells consistently exhibited significantly higher viability than free cells under the conditions tested. After 60 min of exposure (Fig. 4c), the viability of free cells progressively decreased as bile salt concentration increased, reaching approximately 5.2 log CFU/g at 2.0% bile salts. In contrast, encapsulated cells maintained substantially higher viable counts of approximately 6.8–7.0 log CFU/g. The greater survival of encapsulated cells indicates that the SMP+carrageenan matrix provided protection against bile salt-induced stress by limiting the direct exposure of the entrapped cells to the bile-containing medium.

At lower bile salt concentrations (0–1.0%), the reduction in viability of encapsulated cells was relatively small, whereas free cells showed a greater decline. After 120 min of exposure (Fig. 4d), this difference became more pronounced. Free cells showed substantial viability losses, with counts decreasing to approximately 2.8–3.0 log CFU/g at 2.0% bile salts, whereas encapsulated cells retained viable counts above approximately 6.3–6.5 log CFU/g. These results demonstrate the protective effect of the SMP+carrageenan matrix against prolonged bile salt exposure.

4. Discussion

The encapsulation system plays an important role in controlling probiotic delivery. In the present study, various parameters related to the release behaviour of *L. rhamnosus* NCDC 347 from double-layered SMP+carrageenan capsules were investigated. The investigation of the release kinetics in the simulated SGF environment and DI water revealed a significant difference between the studied conditions, with almost no release observed in DI water. It could be explained by the maintenance of the matrix integrity that prevented the fast cell leakage. This tendency was similar to that of previously described protein-polysaccharide hydrogel systems, in which intermolecular interactions dictated the formation of

structures with reduced diffusivity (Flores and Kong, 2017; Saeed et al., 2024). In contrast, SIF led to a rapid release, probably due to the enhanced swelling and erosion of the matrix, enabling fast cell delivery. Moreover, higher OD values were observed for the SIF compared to DI water, suggesting the matrix's potential to specifically respond to the intestinal environment to deliver cells. Indeed, the release behaviour could be additionally explained by the pH responsiveness of the developed encapsulation system. Negative results at pH 2 could suggest that the matrix was resistant to the acidic conditions and started to erode at neutral pH, thus protecting the cells from harsh environments. Rapid release and reduced OD values at pH 1 could indicate poor cell viability and the disruption of the capsule matrix. It could be explained by the fact that highly acidic conditions damaged the bacterial membranes, leading to cell death and fast content release. In contrast, increased release rates could be explained by the enhanced acidic mediated erosion of the matrix. Nonetheless, it must be noted that these conditions were far more severe than those of the physiological gastric environment. Similar results have been reported for probiotic encapsulation systems, where extreme pH significantly affected membrane integrity (Saeed et al., 2024).

In order to determine the release characteristics of the encapsulated formulation, a comparative analysis of both free and encapsulated NCDC 347 was conducted. Significantly lower cumulative release values for encapsulated cells in both DI water and SIF further demonstrated the SMP+carrageenan matrix's ability to control the release of hydrophilic biomolecules in aqueous media. The lower release profile suggested the usefulness of such encapsulation for oral delivery, where reduced cellular release in the stomach and intestines could promote higher survival by reducing exposure to stomach acidity. Overall, the release properties of the SMP+carrageenan encapsulation system suggested its ability to remain stable in the SGF while maintaining reasonable release rates in the SIF, thus making it suitable for oral delivery.

The release kinetics of the probiotic cells from the SMP+carrageenan capsules indicated a predominantly non-Fickian release mechanism involving substantial matrix relaxation. The Higuchi model showed comparatively lower coefficients of determination ($R^2 = 0.7888\text{--}0.9459$), whereas the Korsmeyer–Peppas model provided a markedly better fit, with R^2 values of $0.9467\text{--}0.9959$. The calculated release exponent (n) ranged from 1.082 to 2.043 for the different formulation conditions. According to the Korsmeyer–Peppas model, the characteristic values of n are dependent on the geometry of the delivery system; for cylindrical systems, $n \approx 0.45$ represents Fickian diffusion, whereas $n \approx 0.89$ corresponds to Case II transport, with values exceeding this range indicating super Case II-type transport (Ritger and Peppas, 1987). Therefore, the n values obtained in the present study suggest that probiotic release was not controlled by simple Fickian diffusion but was strongly associated with polymer relaxation and structural changes within the hydrated capsule matrix (Viswanadha et al., 2024). This interpretation was further supported by the Peppas–Sahlin model, which provided high R^2 values ($0.9488\text{--}0.9951$) and indicated a greater contribution of the K_r relative to the K_d . The Peppas–Sahlin model was specifically developed to distinguish the diffusional and polymer-relaxation contributions to solute release (Peppas and Sahlin, 1989). Collectively, these findings suggest that hydration-induced swelling and relaxation of the carrageenan based matrix played a major role in facilitating probiotic cell

release, with diffusion alone being insufficient to explain the observed release behaviour. Such relaxation-dominated release may be beneficial for the targeted delivery of the probiotic cells by allowing their gradual release from the polymeric matrix rather than being dependent only on diffusion.

In addition, the water uptake capacity and the dynamic swelling behaviour of the SMP+carrageenan capsules in SGF and SIF solutions provided valuable information about the mechanisms involved in the release of the probiotic bacteria. The increase in water absorption during SIF exposure indicates progressive hydration of the SMP+carrageenan matrix. The corresponding increase in dynamic swelling suggests that water uptake was accompanied by expansion of the matrix. The different hydration profiles of the sample and control suggest that incorporation of the probiotic cells altered the water absorption kinetics of the encapsulation matrix. Additionally, the continued swelling of the sample during SIF exposure indicates progressive hydration and expansion of the encapsulation matrix. The greater swelling observed under SIF conditions may be related to pH-dependent changes in the interactions among carrageenan, skim milk proteins, and water within the matrix. This behaviour can be attributed to ionization of functional groups within the polymer matrix, resulting in increased electrostatic repulsion and greater hydration of the network. Similar pH-responsive swelling patterns have been reported for protein–polysaccharide systems designed for oral delivery applications (Nezamdoost-Sani et al., 2023). The swelling kinetics further confirmed that the water uptake mechanism of the prepared SMP+carrageenan capsules were primarily driven by diffusion. Specifically, the best fit of the Higuchi model to the experimental data was achieved with the highest R^2 values of 0.9792 for the pH 6.2 0.7% solution and 0.9537 for the pH 6.2 1.0% solution. Moreover, the Korsmeyer–Peppas model revealed that the n values for the pH 6.2, 0.7% and pH 6.2, 1.0% solutions were 0.396 and 0.326, respectively, which were lower than the theoretical value of 0.45, characteristic of a Fickian (diffusion-controlled) mechanism (Ritger and Peppas, 1987). In addition, the Peppas–Sahlin model provided further support for the diffusion-controlled water-absorption mechanism in the SMP+carrageenan capsules. According to the positive K_d and negative K_r values, the contribution of the relaxation of the polymeric chains to the overall water absorption by the capsules was less than the contribution from water diffusion. Taken together, the data suggest that initial matrix hydration was primarily driven by water diffusion, with a minor contribution from relaxation of the SMP+carrageenan polymer. The diffusive water absorption mechanism may facilitate further matrix swelling through hydration, which in turn can contribute to the controlled release of the encapsulated probiotic bacterial cells.

The ability of encapsulated probiotic cells to withstand SGF conditions can be considered an indicator of the SMP+carrageenan matrix's protective capacity and its potential for effective probiotic delivery. Exposure of free and encapsulated NCDC 347 cells to acidic conditions of SGF led to a gradual decrease in cell viability, but this decrease was significantly lower for encapsulated cells compared to free ones. The results indicate that the protective effect of the SMP+carrageenan matrix becomes more pronounced with longer exposure to acidic conditions. A similar trend was reported for previously studied bacteria encapsulated in carrageenan- and milk-based matrices under SGF conditions (Krasaekoopt et al., 2009; Singh et al., 2019). A similar trend was observed when cells were exposed to

bile salt in SIF conditions. For both free and encapsulated cells, increasing the exposure time and the concentration of bile salts led to a decrease in viability. However, the decrease was significantly slighter for encapsulated NCDC 347 cells than for free cells. It is possible that the protective properties of the SMP+carrageenan matrix are mediated by inhibiting direct contact between the cells and the stress factor. Previously studied bacteria encapsulated in carrageenan-based matrices showed similar resistance (Krasaekoopt et al., 2009), which indicates the high efficiency of this type of encapsulation. The high viability of NCDC 347 cells treated with SGF and SIF may be associated with the combined action of skim milk and carrageenan. On the one hand, milk can be used to form a protective coating suitable for withstanding the effects of gastric juice. On the other hand, encapsulation in a polysaccharide-based matrix such as carrageenan can reduce the damaging effects of bile salts by reducing the interaction of the probiotic strain with the stress factor. The protective effect of milk on the probiotic strain was also noted in previous studies, which demonstrated that encapsulation of NCDC 347 in milk-based matrices significantly reduced the negative effects of the SGF environment on cell viability (Singh et al., 2019). Therefore, the results described in this study indicate that the SMP+carrageenan matrix effectively protected the probiotic strain from the effects of SGF and SIF conditions. These effects are most likely manifested synergistically due to the properties of milk and carrageenan, inhibiting the damaging effects of both acidic and bile salt environments. Thus, the presented results confirm the high potential of using this type of encapsulation to preserve and deliver viable probiotic cells to the intestinal tract.

5. Conclusion

The present study revealed that the SMP+carrageenan matrix exhibited pH-dependent release of probiotics. Probiotic release was lower in the SGF and higher in the SIF, which indicates effective targeted delivery of NCDC 347. The kinetic analysis suggested that the mechanism of drug release was non-Fickian/super Case II transport due to polymer relaxation being the dominant factor, while water uptake followed Fickian diffusion. The encapsulation of probiotics with a protective layer of SMP+carrageenan increased cells viability during exposure to acidic environment and bile salts. In conclusion, this SMP-based system could be considered as a promising targeted delivery vehicle to control the release of probiotics into the gastrointestinal tract through the development of functional food and nutraceutical products.

Declarations

Author Contribution

KC: conceptualisation, writing–review and editing; RB: writing–original draft, software, monitoring; AK: writing–original draft, methodology; VK: investigation, writing–review and editing; NKT: Resources, writing–review and editing; HSO: Resources, writing–review and editing. All authors have read and approved the manuscript for submission.

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Figures

Fig. 1

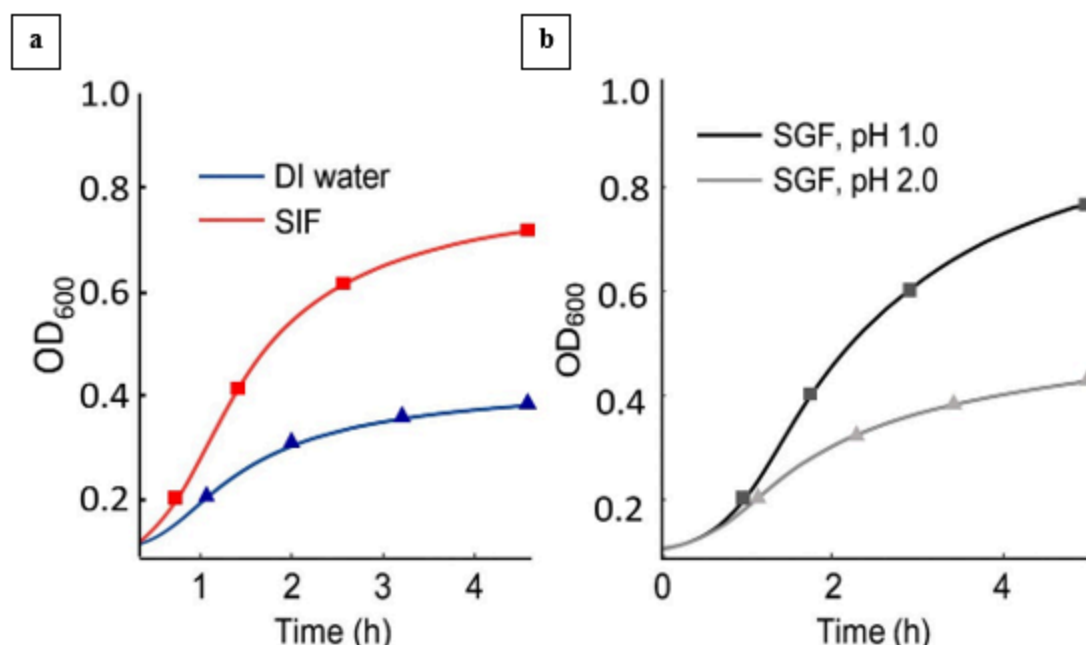


Figure 1

Release profile of *Lactaseibacillus rhamnosus* NCDC 347 encapsulated in skim milk (SMP)–carrageenan matrix capsules (carrageenan: 10% w/v, pH 6.2; skim milk: 1.0% w/v). **(a)** Time-dependent release of cells in deionised (DI) water and simulated intestinal fluid (SIF), showing enhanced release under SIF conditions. **(b)** Release behaviour in simulated gastric fluid (SGF) at pH 1.0 and pH 2.0, indicating pH-dependent protection and gradual cell release under acidic environments. Optical density (OD₆₀₀) was used as an indicator of released cell concentration over time.

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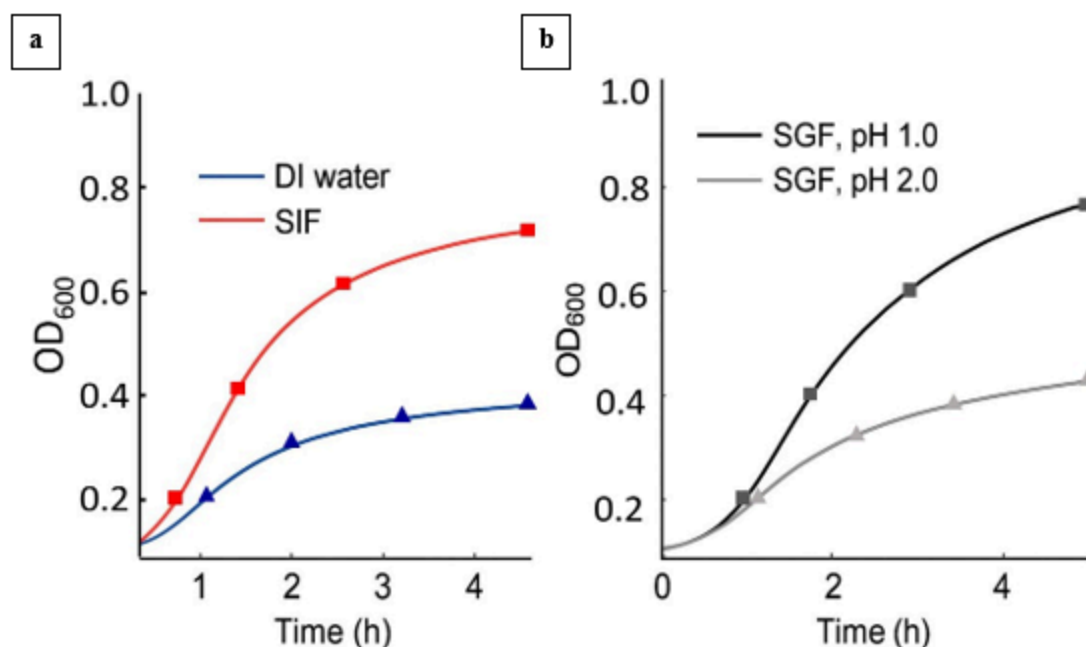


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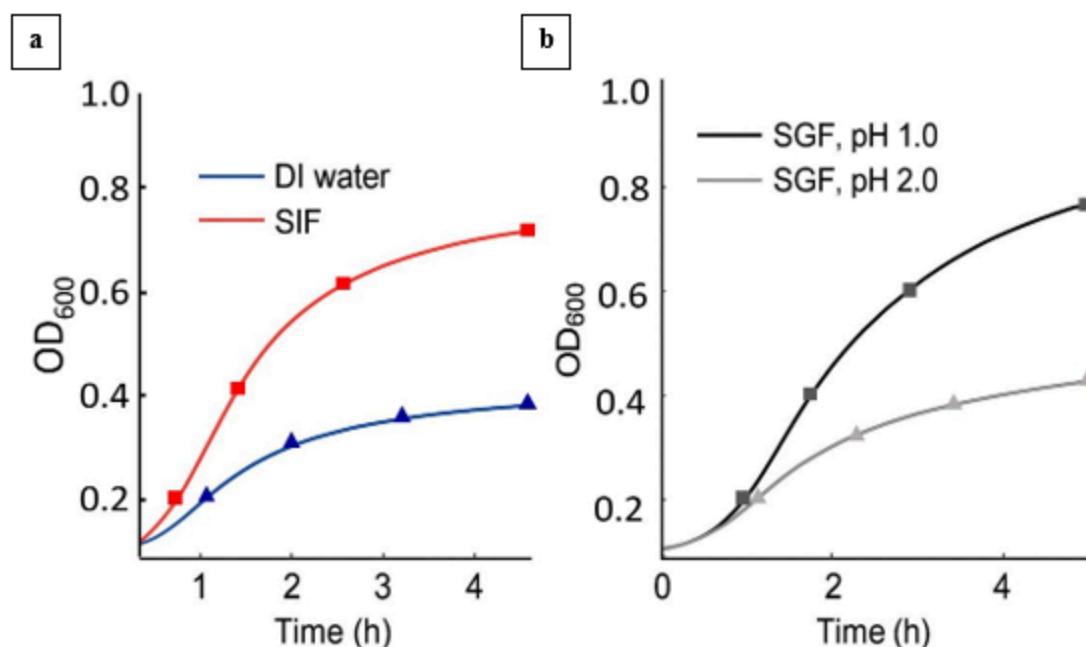


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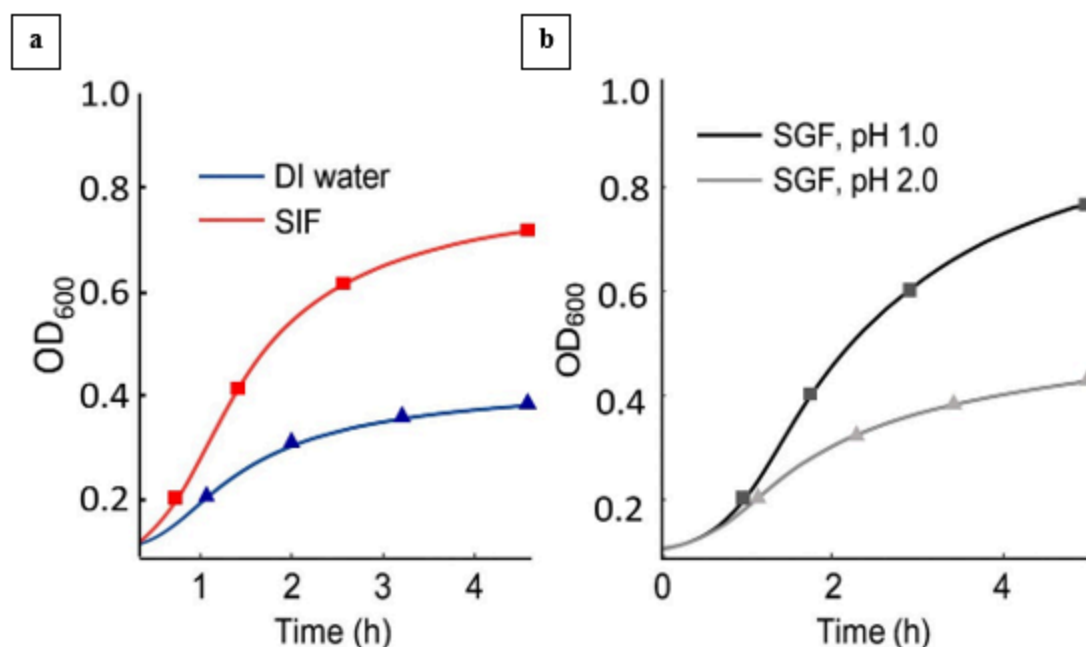


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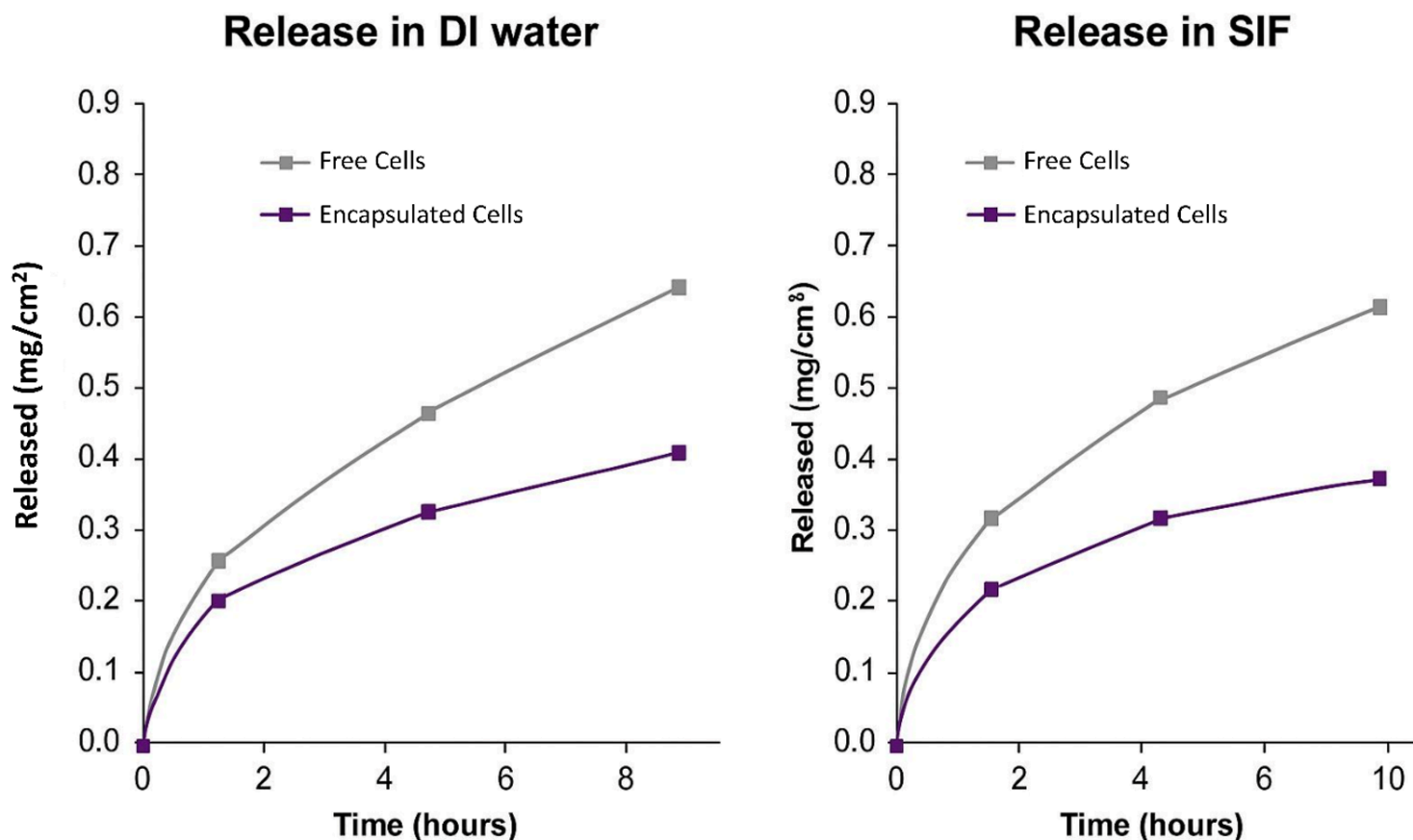


Figure 2

Comparative release kinetics of *Lactocaseibacillus rhamnosus* NCDC 347 were evaluated from SMP-carrageenan based capsules under (a) deionised (DI) water and (b) simulated intestinal fluid (SIF, pH 6.8) conditions. Encapsulation matrices consisted of 10% w/v skim milk and 10% w/v carrageenan. The cumulative release (mg/cm²) was monitored over time, showing a slower and more controlled release from encapsulated cells than from free cells, particularly under SIF conditions.

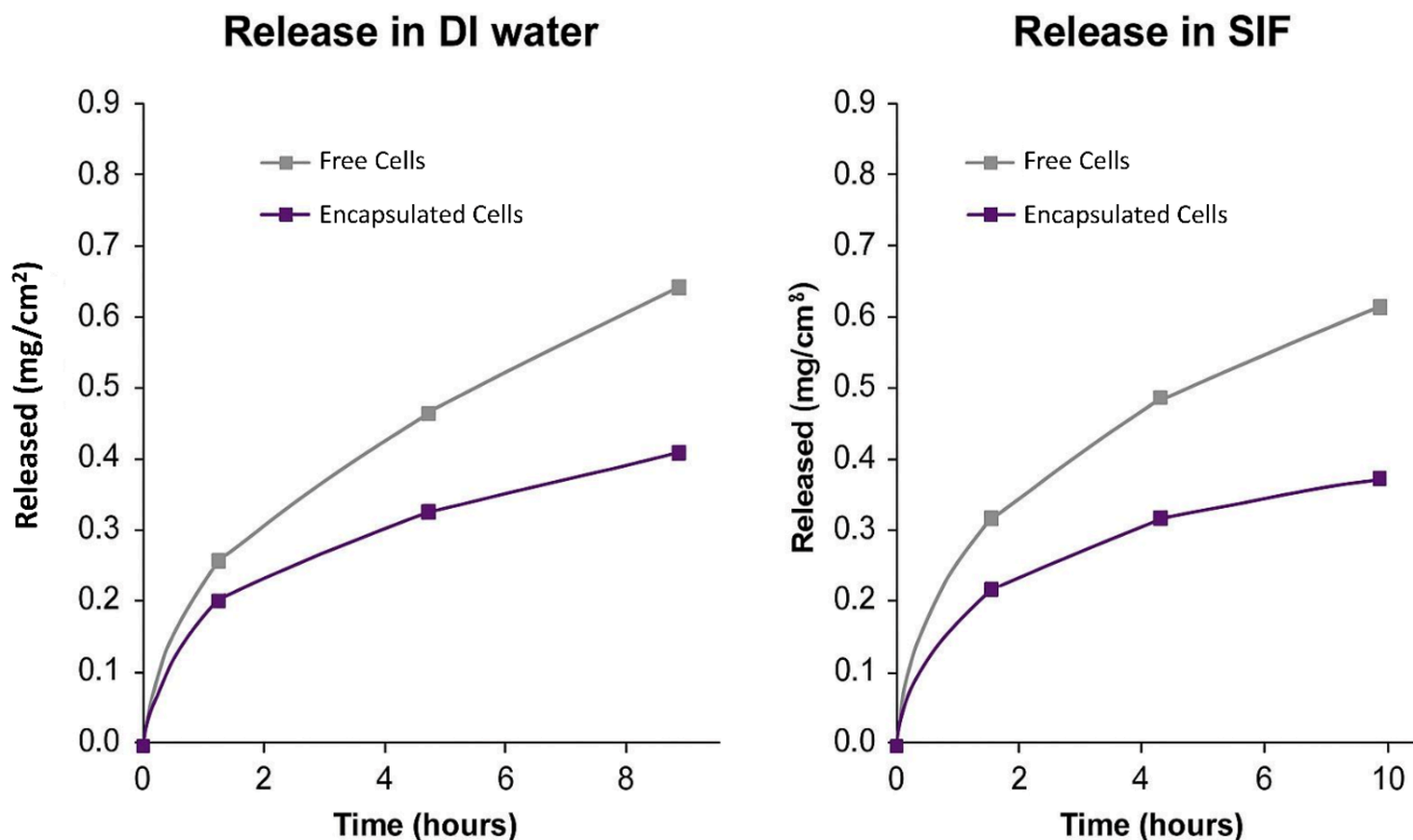


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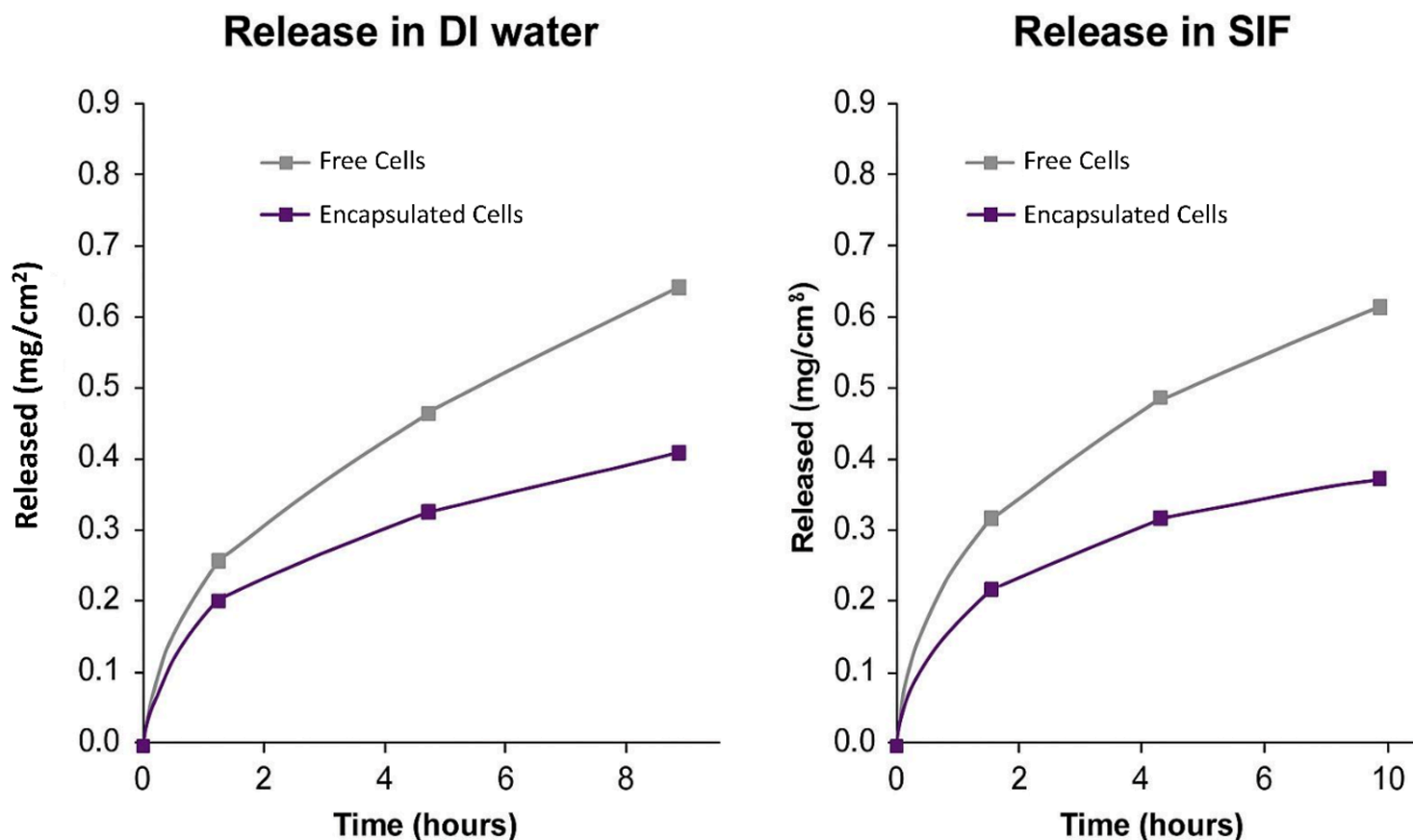


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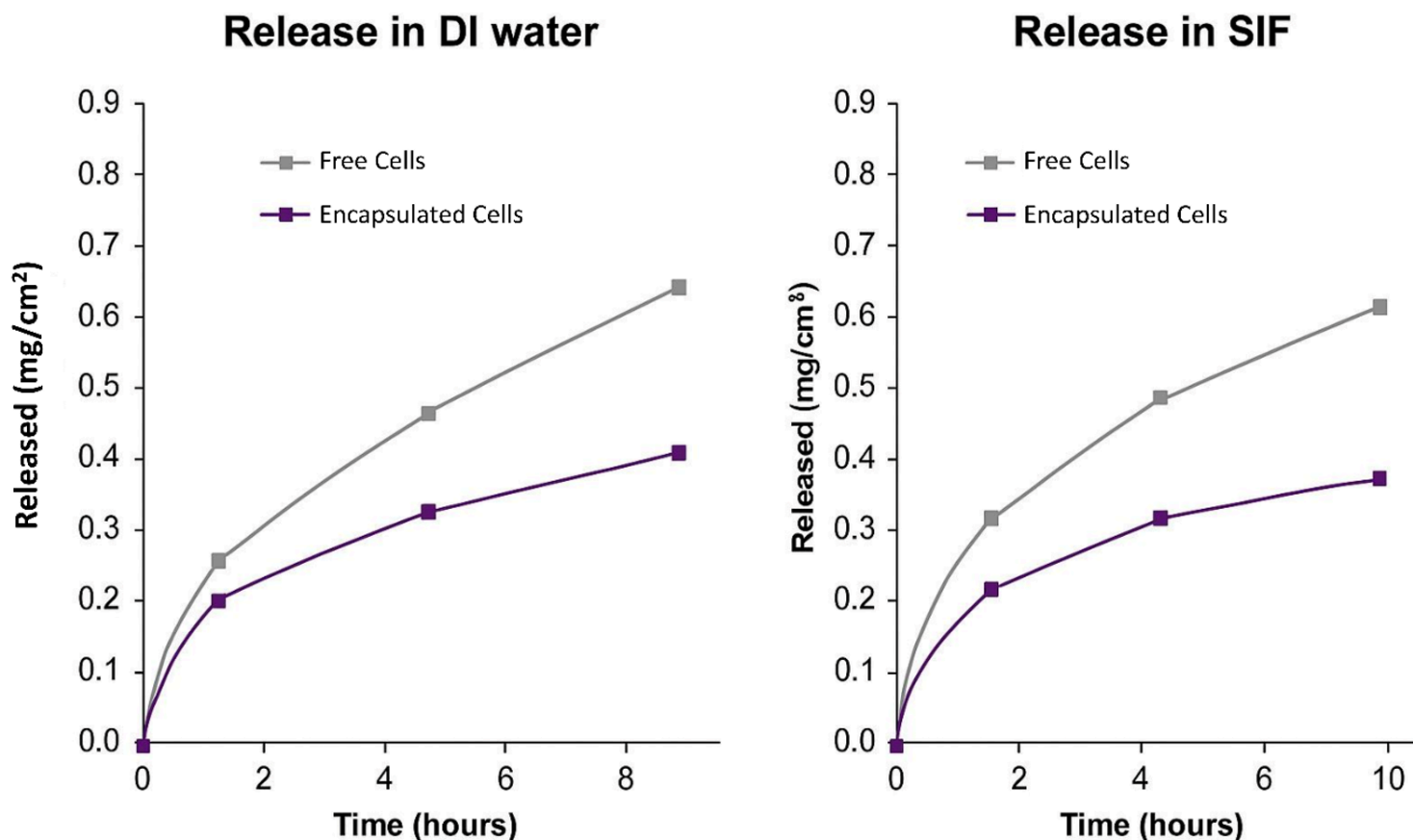


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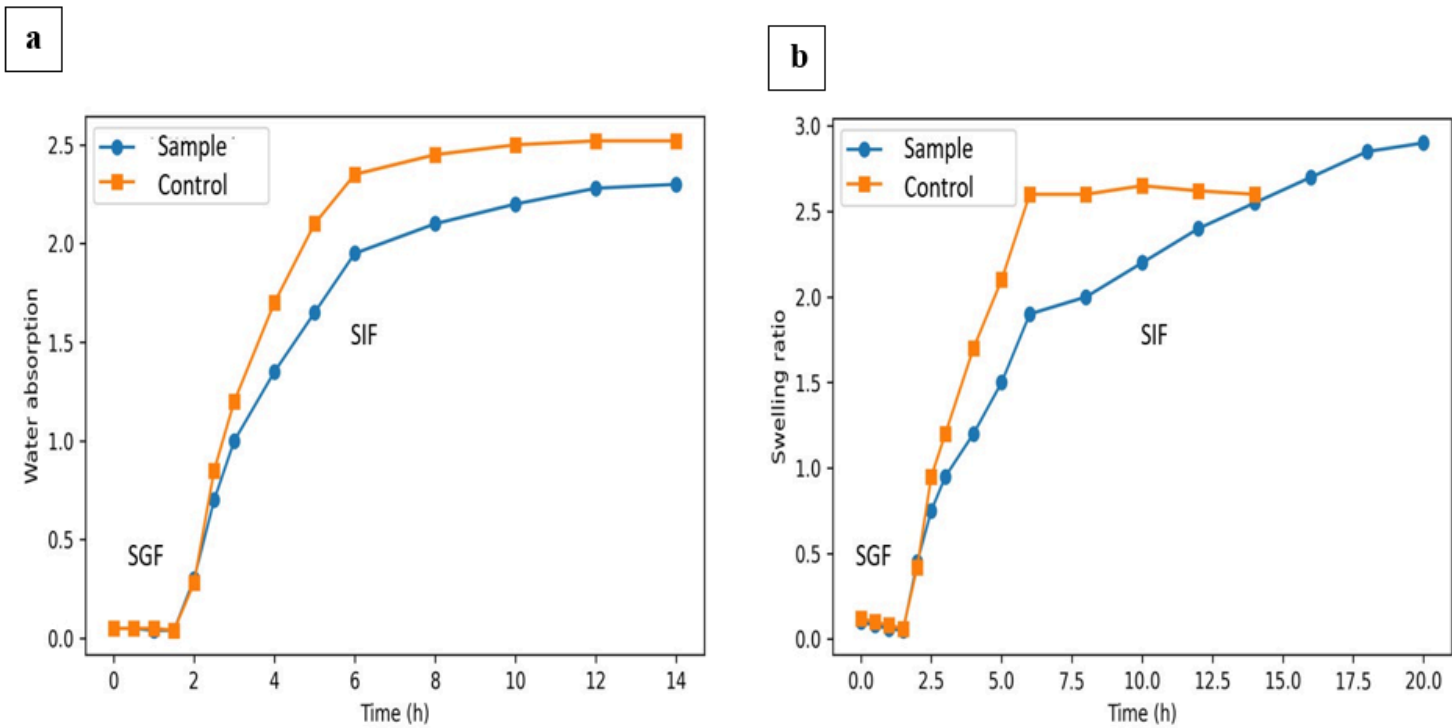


Figure 3

Dynamic water absorption and swelling behaviour of SMP-carrageenan encapsulated capsules containing *Lactacaseibacillus rhamnosus* NCDC 347 and the corresponding blank control under sequential simulated gastrointestinal conditions. **(a)** Water absorption and **(b)** swelling ratio as a function of time during exposure to simulated gastric fluid (SGF) followed by simulated intestinal fluid (SIF).

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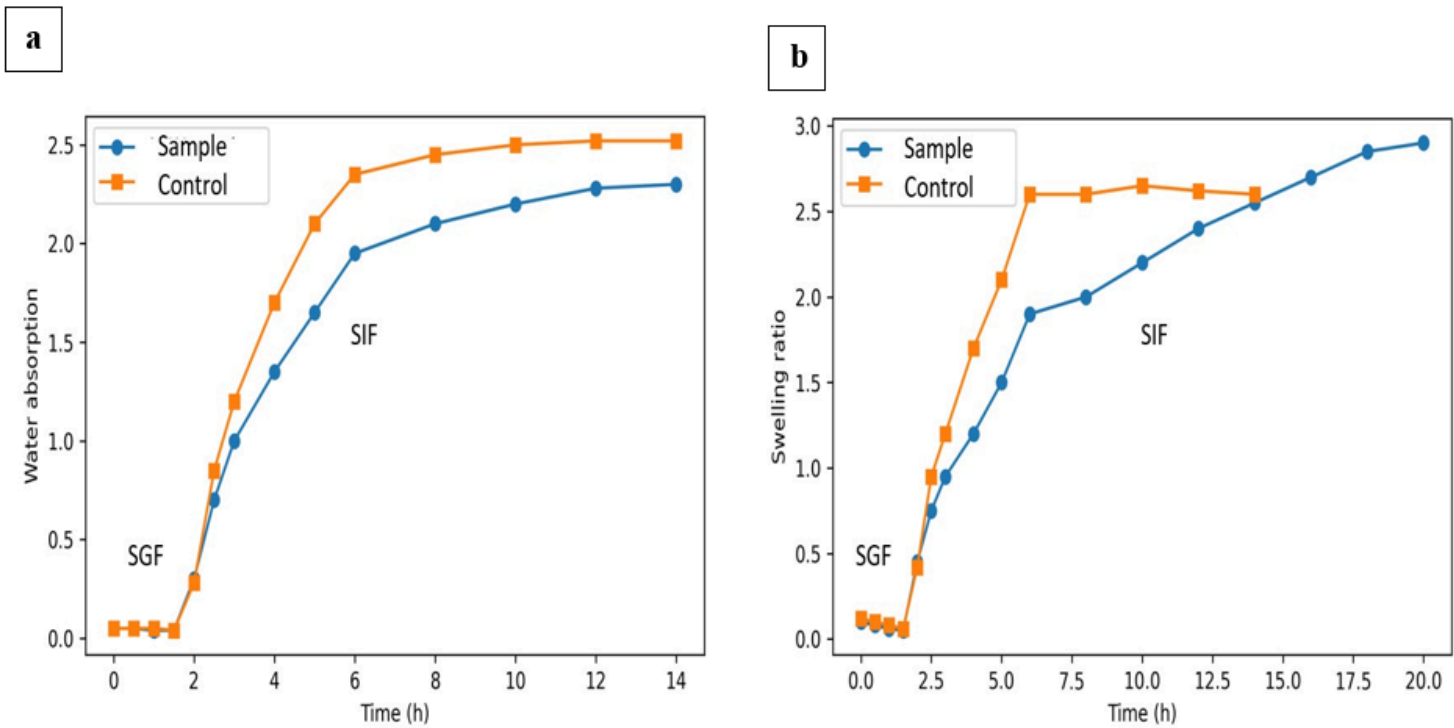


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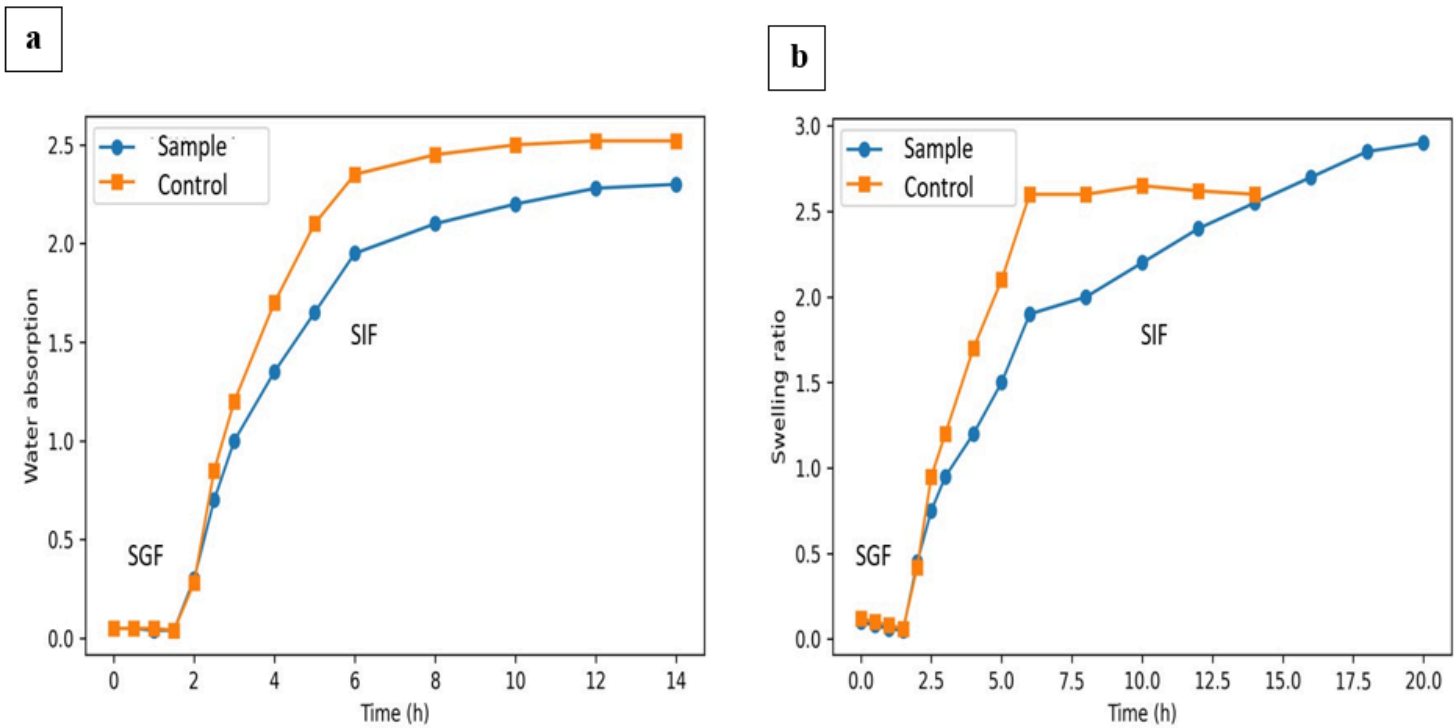


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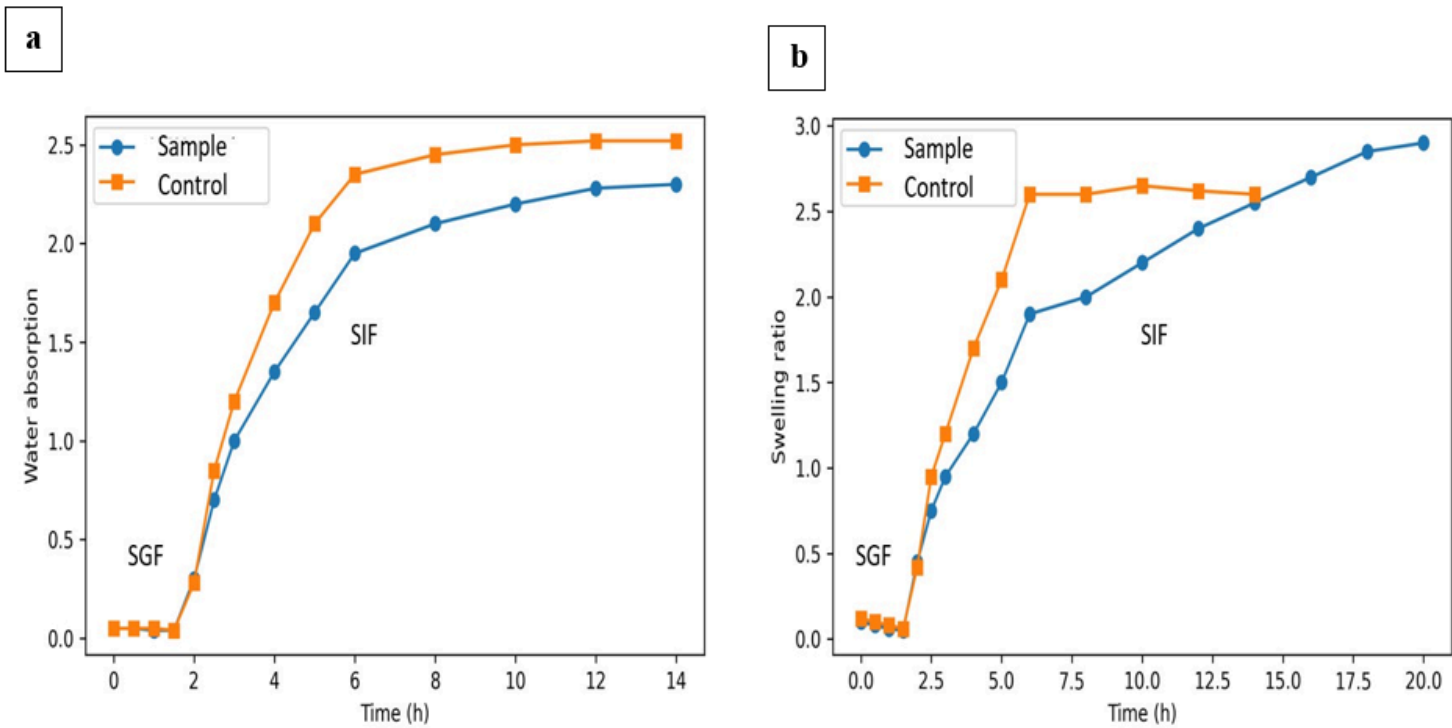


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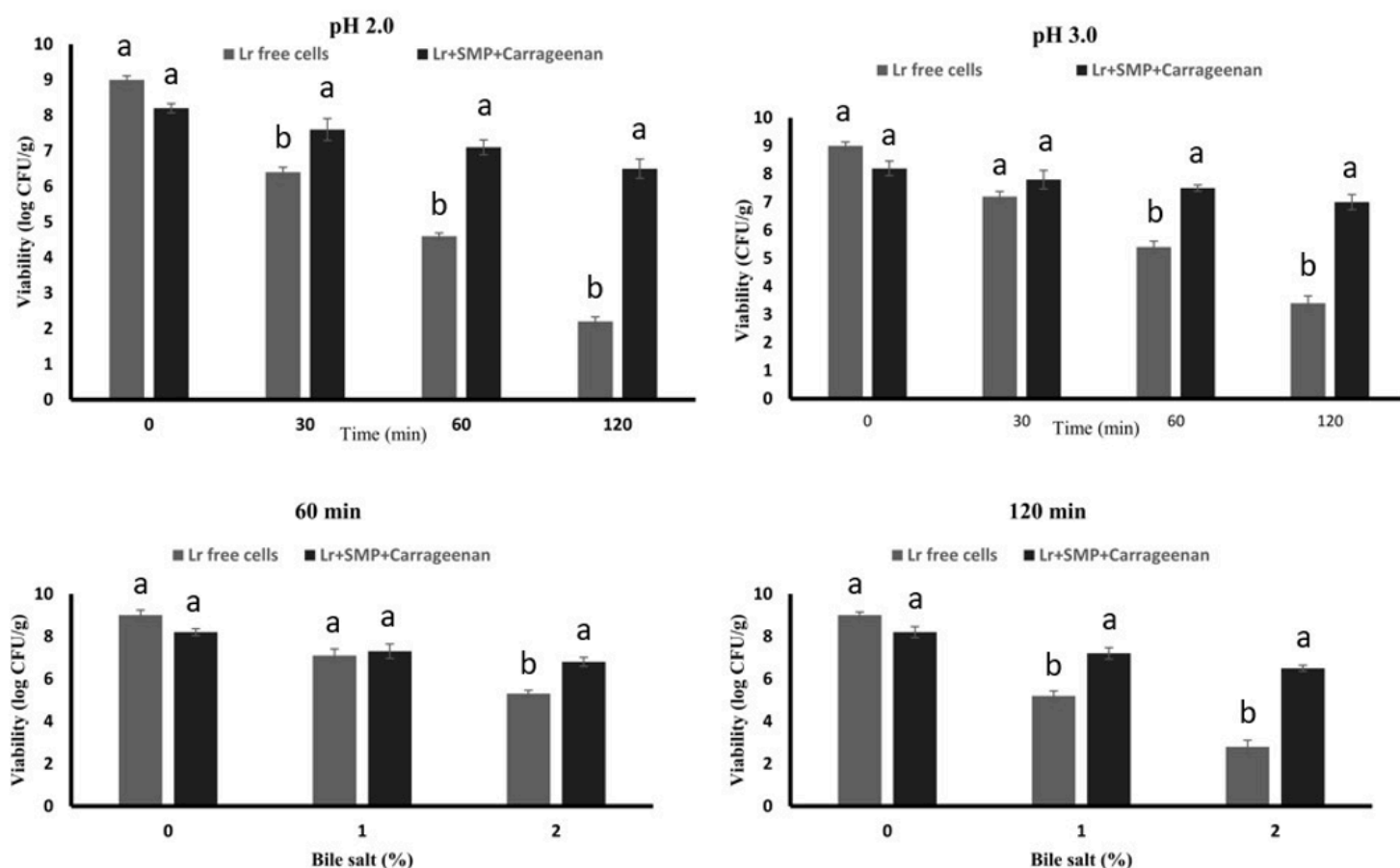


Figure 4

Effect of encapsulation on the viability of *Lactocaseibacillus rhamnosus* NCDC 347 under simulated gastrointestinal conditions. Viability of free cells and SMP-carrageenan encapsulated cells was evaluated in (a) simulated gastric fluid (SGF) at pH 2.0 for 0–120 min, (b) SGF at pH 3.0 for 0–120 min, (c) simulated intestinal fluid (SIF) containing 0–2% bile salts after 60 min, and (d) SIF containing 0–2% bile salts after 120 min. Viability is expressed as log CFU/g. Encapsulated cells generally retained higher viability than free cells under acidic and bile salt stresses, demonstrating the protective effect of the SMP–carrageenan matrix. Data are presented as mean $\pm$ standard deviation. Different lowercase letters indicate significant differences among treatments ($p < 0.05$).

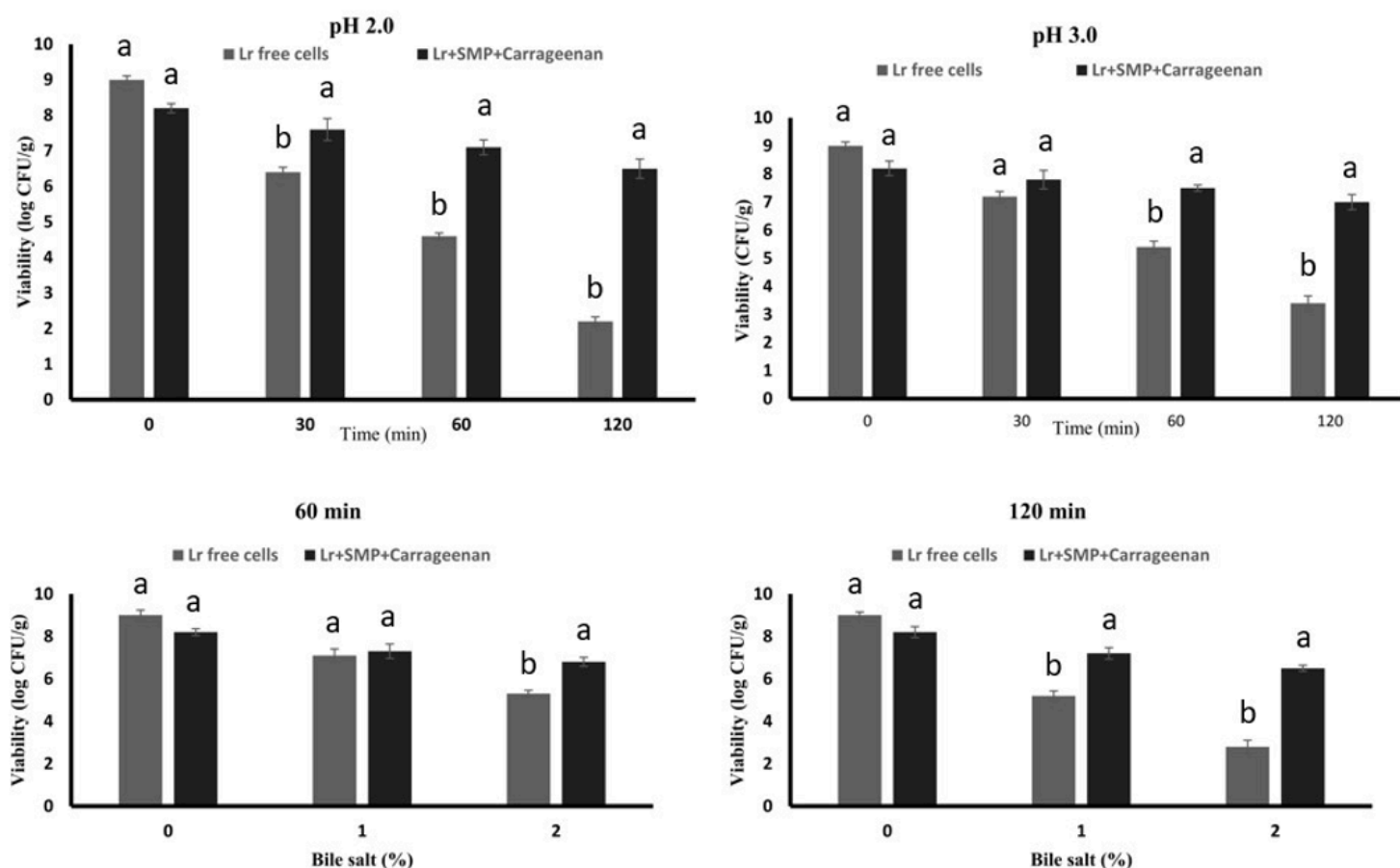


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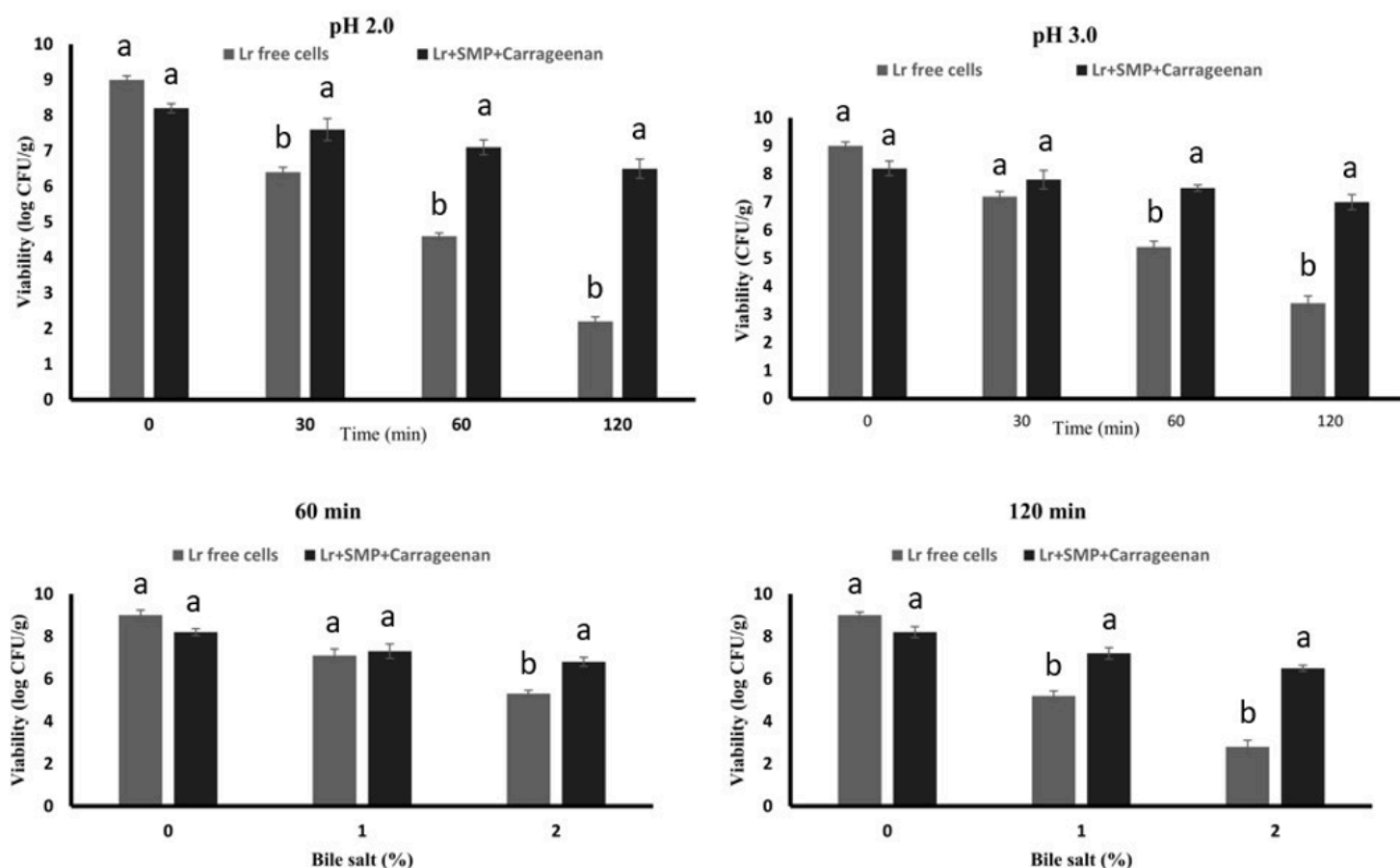


Figure 4

Effect of encapsulation on the viability of *Lacticaseibacillus rhamnosus* NCDC 347 under simulated gastrointestinal conditions. Viability of free cells and SMP-carrageenan encapsulated cells was evaluated in **(a)** simulated gastric fluid (SGF) at pH 2.0 for 0–120 min, **(b)** SGF at pH 3.0 for 0–120 min, **(c)** simulated intestinal fluid (SIF) containing 0–2% bile salts after 60 min, and **(d)** SIF containing 0–2% bile salts after 120 min. Viability is expressed as log CFU/g. Encapsulated cells generally retained higher viability than free cells under acidic and bile salt stresses, demonstrating the protective effect of the SMP–carrageenan matrix. Data are presented as mean $\pm$ standard deviation. Different lowercase letters indicate significant differences among treatments ($p < 0.05$).

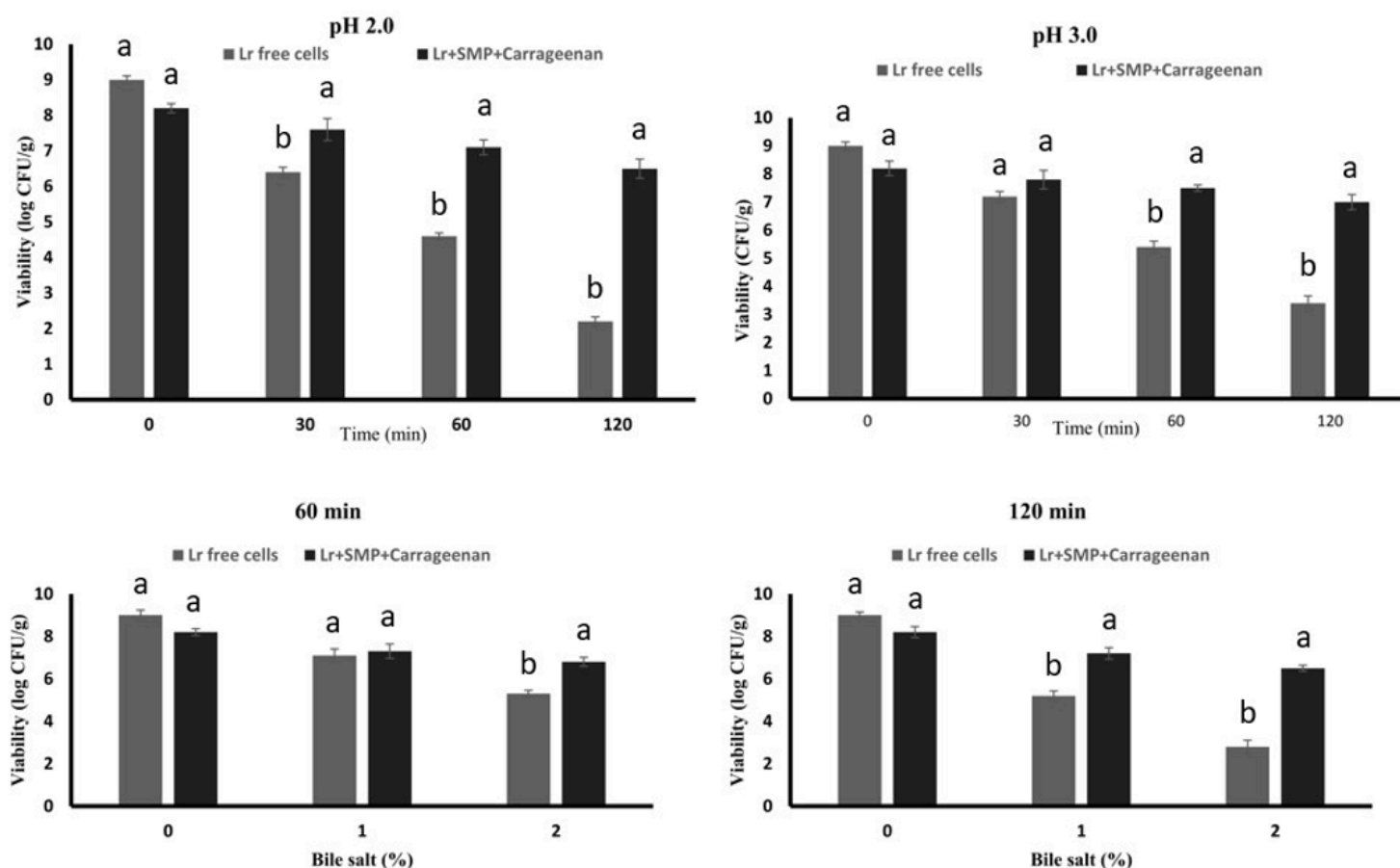


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