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Enhancing Probiotic Survival and Stability by Encapsulation of Enterococcus Strains Using Sodium Alginate and κ-Carrageenan Microbeads

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KEYWORDS

Probiotics, microencapsulation, Enterococcus, sodium alginate, κ carrageenan

ABSTRACT:

Introduction: Probiotic-rich functional foods are crucial for supporting gut health, boosting immunity, and enhancing digestion. They also assist in keeping gut bacteria in check, they lower the chance of digestive problems and improve overall health. Microencapsulation shields probiotics from tough environments in gut, improving their durability and effectiveness, guaranteeing they reach the gut efficiently to provide health advantages.

Objectives: To microencapsulate probiotic *Enterococcus* strains using sodium alginate and κ-carrageenan and check its survival under *in vitro* digestive environments and higher temperature.

Methods: In the present study, probiotic strains *Enterococcus faecium* LABYP9 (NCBI Accession No. PP228215), *Enterococcus faecalis* LABYP30 (NCBI Accession No. PP228373), and *Enterococcus faecium* LABYP34 (NCBI Accession No. PP228654), were encapsulated in microbeads composed of sodium alginate and κ-carrageenan. The survival of these encapsulated probiotics in simulated gastric (pH 2.0) and intestinal juices (pH 8.0) was examined, along with their stability at varying temperatures (4°C and 65°C).

Results: Microbeads were prepared, ranged in size from 548 µm to 600 µm and had either a drop-like or rod-shaped appearance. Compared to free cells, the encapsulation with sodium alginate and κ-carrageenan significantly enhanced the survival of the probiotics in both artificial gastric (pH 2.0) and intestinal (pH 8.0) environments. Additionally, the encapsulated bacteria demonstrated better storage stability at 4°C and 65°C for 30 min., than free cells.

Conclusions: These findings propose that microbeads made from sodium alginate and κ-carrageenan are potentially effective for encapsulating, protecting, and releasing *Enterococcus* strains as nutritional supplements.

1. Introduction

A proposed new definition of functional food is: "Functional foods are modern foods engineered to include ingredients or live microorganisms that can improve or prevent disease, provided the concentration is sufficient to be effective without causing harm." These foods often contain additional components such as nutrients, dietary fiber, phytochemicals, or probiotics [1]. Probiotics are defined as living microorganisms that offer health benefits to the host when consumed in adequate amounts [FAO/WHO 2001]. Probiotic functional food products must contain a viable dose of 10^6–10^7 CFU/mL of probiotic culture [2, 3]. Probiotics provide various health benefits, including relief from

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gastrointestinal disorders and lactose intolerance, treatment of allergies and obesity, bacterial vaginosis treatment, improvement of the gastrointestinal tract, inflammatory bowel disease, diarrhoea, hypercholesterolemia, and more [2, 4]. In functional foods, probiotic cultures are typically added in the form of spray-dried or freeze-dried powder. These can be consumed as dietary supplements (in powder, capsule, or tablet forms) or as fermented or non-fermented food products [5]. The most common method for consuming probiotics is through food-based supplements. In manufacturing probiotic foods, cultures are added to the food in highly concentrated forms for direct vat (DVS) applications, either as concentrated frozen culture or lyophilized powder. Freeze-dried cultures generally contain at least 10^{11} CFU/g, while frozen cultures contain over 10^{10} CFU/g [5]. Post-COVID-19, consumers have become more conscious of the health benefits associated with functional foods, leading to a rising demand for those containing probiotics. The market for probiotic-containing functional foods has grown significantly, with an annual growth rate of 8.5%. This market is projected to increase from $6.94 billion in 2023 to $7.53 billion in 2024[source:https://www.giiresearch.com/report/tbrc1429922-probiotics-dietary-supplements-global-market.html].

The composition of food and its delivery form can directly impact the survival and multiplication of probiotics, as well as help preserve their viability during storage [6]. However, for probiotics to positively affect health, they must remain highly viable during colonic transit [7]. Probiotic viability can be diminished by factors such as high processing temperatures, low pH, water activity, titratable acidity, hydrogen peroxide, dissolved oxygen content, and prolonged storage [8] Probiotics are particularly sensitive to oxygen and freezing temperatures, which affects their viability mainly in dairy products [5, 9]. Probiotic viability can be maintained through technologies such as microencapsulation, which effectively protects probiotics by isolating them from external conditions [10, 11]. Microencapsulation involves enclosing probiotic cells in a suitable substance to ensure adequate cell release in the colonic environment [12]. This technique has long been used to enhance the viability of probiotic bacteria in fermented milk and the gastrointestinal tract [12]. Various materials, such as gelatine, alginate, carboxymethyl cellulose (CMC), chitosan, pectin, gum arabic, guar gum, and carrageenan, have been employed as wall materials in the microencapsulation process to protect the core material [13,14,15]. Alginate is particularly favoured for encapsulation due to its non-toxicity, lack of antigenicity, biocompatibility, and biodegradability, and it is classified as generally recognized as safe (GRAS) [14]. Alginate forms hydrogel matrices through ionic cross-links with divalent cations, such as calcium ions, under mild conditions without toxic solvents [16]. Derived from brown marine algae, alginate is a co-polymer of uronic acids, including mannuronic and glucuronic acids. It exhibits excellent rheological, gelling, and stabilizing properties, as well as good water retention. Alginate can be combined with various natural and synthetic polymers to create films, fibers, beads, capsules, and fiber forms [14, 17]. Marine algal polysaccharides like alginate, agarose oligosaccharides, and κ-carrageenan oligosaccharides have shown greater prebiotic activity in vitro than fructo-oligosaccharides (FOS) [18]. Carrageenan is a sulfated polygalactan containing 15–54% charged sulfate ester groups, composed of D-galactose and 3,6-anhydro-galactose linked by α-1,3 and β-1,4-glycosidic bonds. Alginate/carrageenan mixtures are widely used in the production of edible films for both food and non-food applications, providing excellent barrier and protection properties. Blending alginate with κ-carrageenan enhances gel elasticity and mechanical stability [14]. This combination also forms larger beads with higher gel water content and improved thermal stability compared to alginate beads alone [19]. Sodium alginate and κ-carrageenan are often used for microencapsulating probiotic microorganisms due to their non-toxic nature, cost-effectiveness, heat and acid tolerance, and biocompatibility [20]. In this study, a consortium of probiotic *Enterococcus* species was encapsulated using alginate and κ-carrageenan gel matrices. The viability of both free and encapsulated bacteria was tested under gastrointestinal conditions, heat, and low pH.

2. Objectives

The objective of present study is microencapsulation of probiotic *Enterococcus* strains using sodium alginate and κ-carrageenan. Checking the survival of encapsulated bacteria *in vitro* conditions.

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3. Methods

3.1 Materials: Encapsulating material Sodium alginate and κ-carrageenan, pepsin, and pancreatin were procured from Sigma Aldrich India.de Man Rogosa Sharpe medium (MRS), agar agar, sodium chloride and sodium taurocholate were obtained from Hi Media India.

3.2 Culture activation: Three probiotic cultures belonging to *Enterococcus faecium* LABYP9 NCBI Accession no. PP228215, *Enterococcus faecalis* LABYP30 NCBI Accession no. PP228373 and *Enterococcus faecium* LABYP34 NCBI Accession no. PP228654 were used in this study. All three cultures were activated in MRS broth (100ml) for 24-48 hrs. at 37ºC. MRS broth was then centrifuged (10 min. at 10000xg at 4ºC), and cell pellet was collected and washed three times with phosphate buffered saline. The collected cell mass concentration was adjusted to 10^{10}CFU/ml for encapsulation.

3.3 Preparation of probiotic beads: Probiotic beads were prepared following the method described by Pupa et al, [21] with some modifications. Initially, 2% and 4% (w/v) solutions of sodium alginate and κ-carrageenan were prepared for encapsulation and sterilized by autoclaving at 121°C (15 psi) for 15 minutes. Under aseptic conditions, the 2% sodium alginate, a mixture of sodium alginate (4%), and κ-carrageenan (4%) solutions were mixed in equal proportions with an activated cell mass of 10^{10} CFU/mL at a 1:5 (v/v) ratio. To form the beads, the sodium alginate and κ-carrageenan mixtures were added dropwise through a 3 mL syringe into 100 mL of 1 M CaCl2 with continuous stirring, and the beads were allowed to harden at 4°C for 24 hours. After hardening, the beads were washed with sterile distilled water and used for further experimentation. Using this method, probiotic beads with 2% sodium alginate, a mixture of sodium alginate (4%), and κ-carrageenan (4%) were prepared and subsequently used in further studies.

3.4 Characterization of beads

3.4.1 Morphological study: The bead size was measured using a stage micrometer according to the method described by Afzaal et al, [20]. A total of 100 beads were randomly selected to record their size and diameter.

3.4.2 Determination of Encapsulation Efficiency (EE): The encapsulation efficiency of the probiotic consortia was determined using the standard reported method [22]. One gram of probiotic beads was mixed with 9 mL of 0.1 M phosphate buffer saline (PBS) at pH 7.0 and fragmented in a stomacher for 10 minutes. One millilitre of the free cells and the mixture of disintegrated beads were serially diluted with PBS for viable cell enumeration. The viable cell counts of microencapsulated probiotic bacteria were determined using the pour plate technique. The encapsulation efficiency was calculated using the following formula.

$$\text{Encapsulation efficiency} = \frac{\text{Log no. of cell count after encapsulation}}{\text{Log no. of free cells added before encapsulation}} \times 100$$

3.5 FTIR analysis: Fourier Transform Infrared spectra of beads were recorded using PerkinElmer UATR Two [23]

4. Checking the survival of microencapsulated and free probiotic consortia in synthetic gastric and intestinal juices: Synthetic gastric juice and intestinal juice were prepared [10]. The synthetic gastric juice was formulated by dissolving pepsin (Sigma P 7000) at a concentration of 3g/L in a sterile sodium chloride solution (0.5%, w/v), with the final pH adjusted using 0.1N HCl. This mixture was sterilized through membrane filtration using a 0.22µm membrane filter. The synthetic intestinal juice was (pH 8.0) was created by dissolving pancreatin (Sigma P7545) at a concentration of 1g/L and sodium taurocholate (Hi media RM 011) at 4.5% in sterile sodium chloride solution (0.5%, w/v), with the final pH adjusted using sterile NaOH (0.1 M) [24]. This juice was sterilized through membrane filtration using a 0.22µm membrane filter. In brief, one gram of microencapsulated beads made from sodium alginate, κ-carrageenan, and 2% and 4% mixtures of sodium alginate and κ-carrageenan (2% each), respectively, as well as one milliliter of free cells of the probiotic consortia, were exposed to synthetic gastric and intestinal juices for 3 hours at 37°C. Samples were withdrawn at half-hour intervals (0, 30, 60, 90, and 120 minutes) and plated onto sterile MRS agar media to enumerate CFU/mL. The plates were then incubated at 37°C for 24 to 48 hours.

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5. Checking the thermal stability of microencapsulated and free probiotic consortia

The thermal stability of both microencapsulated beads and free cells was assessed by incubating one gram of microencapsulated beads and 1 mL of free cells in 10 mL of sterile saline in a water bath set to a temperature of 65°C for 30 minutes, followed by rapid cooling of the test tubes [10]. Samples were withdrawn at 10-minute intervals, and the number of viable cells was determined by plating 0.1 mL of each sample onto sterile MRS agar plates. The plates were then incubated at 37°C for 24 to 48 hours.

6.0 Checking survival of microencapsulated and free probiotic consortia at low temperature

The viability and stability of both free and microencapsulated probiotic consortia were evaluated at temperatures of 40°C and 25°C over a period of 15 days, following the procedure outlined by Lu-E et al. The number of viable cells was determined using the pour plate method [25]

4. Results

Characterization of Beads: Immediately after encapsulation, the bead's morphology was evaluated. It was observed that the concentration of the sodium alginate solution, κ-carrageenan, and the mixture of both solutions influenced the characteristics of the produced beads. Beads composed solely of sodium alginate exhibited a soft texture, whereas beads comprising a mixture of sodium alginate solution and κ-carrageenan were moderately firm.

Morphological study: The study findings indicated that the cells exhibited a drop-like or rod-shaped morphology. The size of the microbeads ranged from 548 µm to 600 µm in diameter. Microbeads produced with a combination of sodium alginate solution and κ-carrageenan were larger than those made with sodium alginate and κ-carrageenan alone. This study suggests that the size and diameter of the microbeads were influenced by the encapsulation material. These results are consistent with previous studies [26], where the size of microbeads fabricated from calcium alginate (IMs samples) and a mixture of calcium alginate and skim milk differed significantly, measuring 311 µm and 325 µm, respectively.

Determination of Encapsulation Efficiency (EE): In the current study, utilizing two different coating materials resulted in varying levels of encapsulation efficiency. Microbeads crafted with 2% sodium alginate exhibited a microencapsulation efficiency of 88.51±1.74%, whereas microbeads formed with a combination of sodium alginate and κ-carrageenan demonstrated a higher efficiency of 92.29±0.69%.

The microencapsulation efficiency was significantly greater in the mixture of sodium alginate and κ-carrageenan as compared to the use of 2% sodium alginate alone ($P< 0.05$). In a study by Pupa et al., *Lactobacillus plantarum* was microencapsulated using the extrusion method with sodium alginate (1.5%) and chitosan (0.5%), yielding a microencapsulation efficiency of 93.52 ± 0.11% [21].

Praepanitchai et al., (2019) prepared hydrogel beads of *L. plantarum* using alginate (1%) and soy protein (12%), achieving a microencapsulation efficiency of 91.50 ± 1.30% [27].

Previous research has shown that increasing the concentration of polymer leads to higher microencapsulation efficiency [25].

Golnari et al., microencapsulated *Enterococcus faecium* in chitosan-alginate nanoparticles, achieving an encapsulation efficiency of 92.97±0.1% [28].

FTIR analysis: Microbeads created using 2% sodium alginate showed significant peaks at 3306.91 cm^{-1}, which correspond to O-H vibrational stretching. The peak at 1629.96 cm^{-1} is associated with carboxylic [COO^-] stretching. When Ca+ ions are present, this peak shifts to a higher wavenumber, and its intensity decreases to 1425.2 cm^{-1}, indicating symmetric stretching of the carboxylic [COO^-] groups.

FTIR spectrum of microencapsulated beads is presented in fig.1a and b. Microbeads prepared using a blend of sodium alginate and κ-carrageenan exhibited a prominent peak at 3307.86 cm^{-1}, and smaller peaks at 1627.02 cm^{-1} and 1333.07 cm^{-1}. Additional minor peaks at 884.05 cm-1 and 922.36 cm^{-1} indicated the presence of 3, 6-anhydro-galactose, and secondary sulfates within the galactose structure. These peaks suggest that the structural organization is enhanced in the presence of calcium ions [20].

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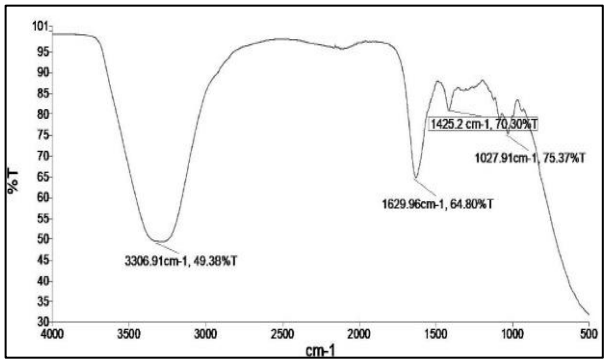
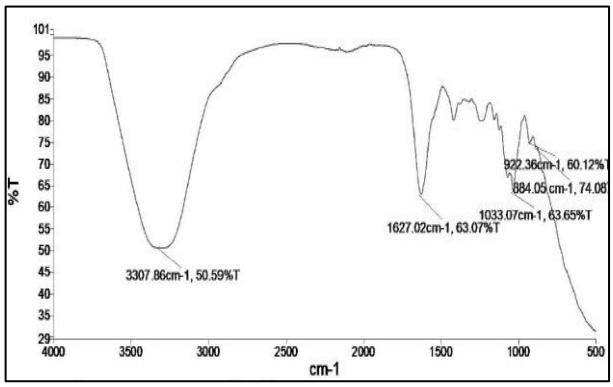


a.



b.

Fig. 1 a. FTIR spectra of sodium alginate beads, b. FTIR spectra of beads prepared using a mixture of sodium alginate and κ-carrageenan

Checking the survival of microencapsulated and free probiotic consortia in synthetic gastric and intestinal juices: In the present study, the survival and stability of free and microencapsulated probiotic consortia were assessed in synthetic gastric juice (pH 2.0) and intestinal juice (pH 8.0). The findings indicated a decrease in the logarithmic number of free cells following exposure to synthetic gastric and intestinal juices (Fig. 2 and 3). Specifically, when free cells were subjected to synthetic gastric juice, cell viability declined from 2.510 log cfu/ml to 0.957 log cfu/ml after 120 minutes of exposure. Conversely, when probiotic consortia encapsulated with 2% sodium alginate were exposed to synthetic gastric juice, cell count decreased from 2.497 log cfu/gm to 1.893 log cfu/gm. Similarly, the use of a mixture of sodium alginate and κ-carrageenan as encapsulating material resulted in a decrease in cell count from 2.503 log cfu/gm to 1.983 log cfu/gm after 120 minutes of exposure to synthetic gastric juice. These results highlight the sensitivity of free cells to the low pH (2.0) of gastric juice [12, 38], with microencapsulation serving as a barrier against direct exposure to acidic pH [25, 39]. The combination of sodium alginate and κ-carrageenan demonstrated enhanced protection for probiotic consortia in the present study.

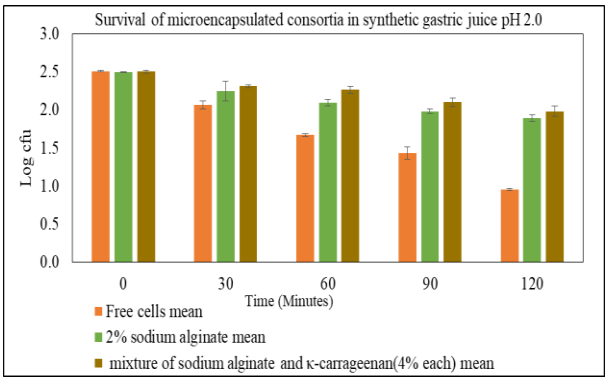


Fig.2. Survival of probiotic bacterial free cells and microencapsulated bacteria with sodium alginate (2%) and a mixture of sodium alginate and κ-carrageenan(4% each) in synthetic gastric juice pH2.0 from 0 min to 120 min.

In this study, both free and microencapsulated probiotic consortia underwent exposure to synthetic intestinal juice (pH 8.0) for a designated duration of 2 hours. A notable decrease in the logarithmic count of free probiotic bacterial cells was observed upon exposure to synthetic intestinal juice (see Fig. 3). Specifically, when free cells were subjected to exposure of synthetic intestinal juice at pH 8.0, the cell count decreased from 8.91±0.12 to 3.26±0.42 cfu/ml. However, the utilization of 2% sodium alginate protected the probiotic consortia against the conditions of synthetic intestinal juice, resulting in a reduction in cell count from 8.15±0.16 to 6.47±0.41 cfu/gm. Similarly, when microencapsulated beads composed of a mixture of sodium alginate and κ-carrageenan were exposed to synthetic intestinal juice, the cell count decreased from 9.04±0.08 to 6.56±0.57 log cfu/gm. These findings underscored the enhanced effectiveness of a combination of sodium alginate and κ-carrageenan in safeguarding probiotic consortia compared to sodium alginate alone. Alginate and κ-carrageenan synergistically acted as coadjuvants, imparting effective gelling properties to the microencapsulated beads.

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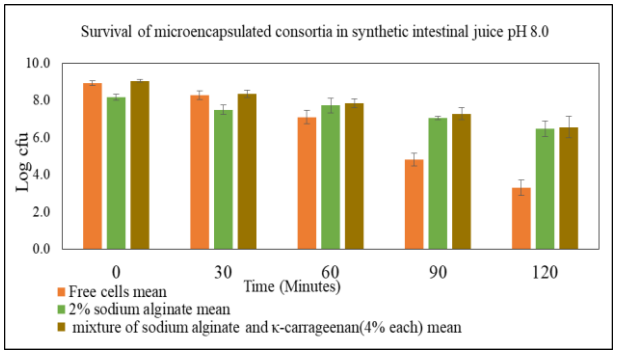

Fig. 3. Survival of probiotic bacterial free cells and microencapsulated bacteria with sodium alginate (2%) and a mixture of sodium alginate and κ-carrageenan(4% each) in synthetic intestinal juice pH8.0 from 0 min to 120 min.

Checking the thermal stability of microencapsulated and free probiotic consortia

The heat tolerance of probiotic bacteria is crucial for maintaining the viability of probiotic cultures, especially considering their exposure to pasteurization and heat treatments during the manufacturing of various products. In this study, both free cells and microencapsulated probiotic consortia were subjected to a temperature of 65°C for 30 minutes. The current findings demonstrate that microencapsulation provides thermal stability to probiotic consortia (see Fig. 4.0). When free cells were exposed to 65°C for 30 minutes, a decrease in cell count was observed from 8.90±0.12 to 2.52 log cfu/ml. However, microencapsulated beads made from sodium alginate alone and a mixture of sodium alginate and κ-carrageenan effectively protected the probiotic consortia, resulting in minimal reduction in cell count.

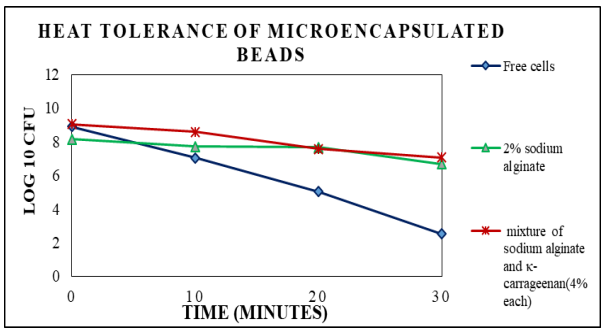

Fig. 4. Survival of probiotic bacterial free cells and microencapsulated bacteria with sodium alginate (2%) and a mixture of sodium alginate and κ-carrageenan (4% each) at 65ºC for 30 minutes.

Checking survival of microencapsulated and free probiotic consortia at low temperature: The current study aimed to investigate whether microencapsulation affects the viability of probiotic consortia during storage at temperatures of 4°C and 25°C over 15 days. The results (see Fig. 5 a & b demonstrated that microencapsulation effectively preserves cell viability, with good survival observed for up to 15 days. Both microencapsulated beads containing sodium alginate and those containing a mixture of sodium alginate and κ-carrageenan exhibited similar viability after the 15-day storage period, indicating that encapsulation protects the internal environment with minimal damage to the probiotic consortia. Conversely, the viability of free cells decreased over time, particularly after 5 days of storage. At 4°C, the count of free cells decreased from 8.90±0.12 to 4.54±0.29, suggesting an adverse effect of low temperature on survival (see Fig. 5a). Similarly, at 25°C, the cell count decreased to 3.18±0.19, indicating that higher temperatures resulted in a loss of viability for free cells. Therefore, higher temperatures are unsuitable for probiotic storage, a finding supported by previous studies [12, 20, and 41].

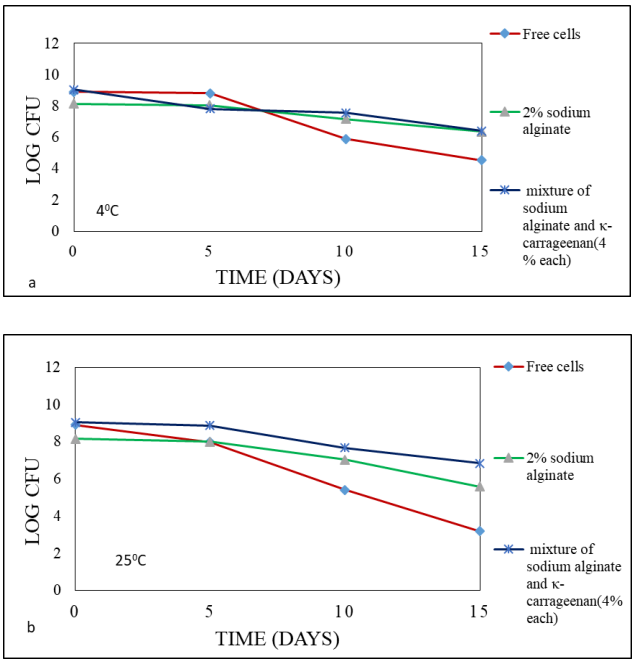

Fig.5. Survival of free and microencapsulated probiotic consortia during storage. a) Stability of free and microencapsulated probiotic consortia at 4ºC for 15 days of storage. b) Stability of free and microencapsulated probiotic consortia at 25ºC for 15 days of storage.

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5. Discussion

The initial parameter assessed in this study was the microencapsulation yield, which quantifies the percentage of cells that are successfully encapsulated. Qi et al. (2021) found that encapsulating *Saccharomyces boulardii* and *Enterococcus faecium* with sodium alginate increased cell survival rates by 25% and 40% at elevated temperatures and humidity [31]. When *S. cerevisiae* var. *boulardii* was encapsulated with 2% sodium alginate, the minimum microencapsulation yield observed was 54.07%, with cell survival lasting 30 days at 4°C [42]. Pupa et al. (2021) utilized the extrusion method to microencapsulate *Lactobacillus plantarum* with 1.5% sodium alginate and 0.5% chitosan, attaining a microencapsulation efficiency of 93.52 ± 0.11% [21]. In a similar study, Praepanitchai et al. (2019) achieved a microencapsulation efficiency of 91.50 ± 1.30% for *L. plantarum* by preparing hydrogel beads using 1% alginate and 12% soy protein [27]. Previous research has indicated that augmenting polymer concentration leads to elevated microencapsulation efficiency [25].

Golnari et al. (2024) employed chitosan-alginate nanoparticles to encapsulate *Enterococcus faecium*, attaining an encapsulation efficiency of 92.97 ± 0.1% [28]. In the present investigation, microencapsulation efficiency varied from 88.51 ± 1.74% for sodium alginate-coated beads to 92.29 ± 0.69% for a blend of sodium alginate and κ-carrageenan encapsulated bacteria exhibited superior viability under simulated gastric conditions when contrasted with non-encapsulated free bacterial cells [29, 30]. In particular, the viability percentages of *Saccharomyces boulardii* and *Enterococcus faecium*, when encapsulated using sodium alginate, rose by 60% and 25% correspondingly in simulated gastric fluid. Upon exposure to synthetic intestinal fluid, these survival rates experienced additional increments of 15% and 20% sequentially [31].

When subjected to simulated gastric conditions (pH 2.0), encapsulated *L. acidophilus* CSCC 2400 and *L. acidophilus* CSCC 2409 exhibited a notable increase ($p < 0.05$) in initial cell count from $3.5 \pm 0.9 \times 10^9$ to $6.1 \pm 1.1 \times 10^6$, with bacteria surviving for 3 hours [32]. Microencapsulated lactic acid cultures, after incubation in simulated gastric juice for 5 minutes, maintained total viable counts of 8.22 ± 0.83 log cfu/g, 8.21 ± 0.81 log cfu/g, and 8.42 ± 1.17 log cfu/g for *L. casei*, *L. brevis*, and *L. plantarum*, respectively, with no significant decline ($p > 0.05$) in viable counts even after 120 minutes of incubation [23]. The highest survival rate under simulated gastric conditions was observed in microencapsulated cells utilizing 1% alginate and 1% carrageenan gum, with a survival rate of 10.9 CFU/g in contrast to 4.4 CFU/g for free cells at 80 minutes [33]. Liu-E et al. (2015) demonstrated that probiotic *E. faecalis* HZNU P2, encapsulated in milk alginate microspheres, maintained viability after 120 minutes of exposure to simulated gastric fluid (SGF) at pH levels of 2.5 and 2.0. [25].

Afzal et al. (2019) noted that sodium alginate-coated capsules experienced less reduction in cell count compared to carrageenan-coated capsules when subjected to simulated gastric juice, indicating the superior efficacy of sodium alginate as an encapsulating material[20]. The minimal survival rate of free cells can be attributed to the unrestricted penetration of synthetic intestinal fluid (SIF) into the bacterial cells. Sin Jin et al., (2020) observed the lowest survivability of probiotics in free cells when exposed to SIF conditions, due to lower permeability and lack of bacterial cell coating [34].

While the viable cell counts for free cells decreased marginally from 3.57 to 3.32 log cfu/g, the cell count in coated beads experienced a slight rise from 4.10 to 4.71 log cfu/g after 24 hours of incubation in synthetic intestinal fluid [34]. Shi et al. (2016) found that *E. faecalis* HZNU P2 encapsulated in milk alginate was released rapidly in the simulated intestinal fluid within 1 hour, with cell numbers increasing up to 2 hours, signifying its viability[35]. The higher pH of 8.0 in intestinal juice triggers the dissolution of the encapsulating polymer, leading to the liberation of encapsulated bacteria [13].

Microbeads' thermal resistance can be enhanced by sodium alginate and κ-carrageenan, according to prior studies [19, 20]. Lactobacilli's thermostability at 65°C is better protected by modest concentrations of sodium alginate (3% w/v), according to Ding and Shah's (2007) findings [36]. Mandal et al. (2006) observed that the reduced water diffusion rate within a 4% alginate matrix during heat exposure led to increased survival rates of *L. casei* NCDC-298 at 60°C for 20 minutes [37]. Probiotic viability was greatly increased by microencapsulation, at 72, 85, and 90°C, the average increase in lactobacilli

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viability microencapsulated in chitosan and poly-L-lysine was 61.5% [10]. Viable free cells of *L. acidophilus* were undetectable during storage at 4°C, whereas encapsulated cells demonstrated significantly higher viability, maintaining a level of 5.5 log CFU [12].

For example, Sultana et al. (2000) prepared probiotic yogurt with microencapsulated *L. acidophilus* and *Bifidobacterium infantis*, which retained good culture viability throughout 8 weeks of storage at 5°C [38]. Sun and colleagues (2023) achieved an initial viable cell count of 10.01 log CFU/g by encasing *Enterococcus faecalis* HZNU S1 using sodium alginate (SA) and flaxseed milk (FM) using the extrusion process [43]. After 8 days, this count dropped to 9.6 log CFU/g at 4°C and 9.2 log CFU/g at 25°C. After a month of storage at 4°C, the encapsulated *L. bulgaricus* retained its full viability [25]. *E. faecalis* HZNU P2's storage stability at 4 °C and 25 °C may be enhanced by soy protein isolate-alginate microspheres [44].

6. Conclusion:

The current study elucidates the role of microencapsulation in safeguarding probiotic communities within *in vitro* digestive environments, a novel approach not previously explored. The effectiveness of microencapsulation in protecting probiotic consortia is demonstrated, highlighting the synergy between sodium alginate and κ-carrageenan as carrier polymers for encapsulation. This combination proves superior to their individual use. Using a blend of sodium alginate and κ-carrageenan, probiotic consortia are shielded from harsh digestive environments. Moreover, microencapsulation ensures the preservation of probiotic consortia viability when subjected to various temperatures. Thus, microencapsulation technology serves as a remarkable outstanding method for maintaining the vitality of these cultures.

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