Food and Bioprocess Technology (2025) 18:10406–10418
https://doi.org/10.1007/s11947-025-04026-x

RESEARCH

# Design of Multi-Component Beads (Alginate/Xanthan/Glycerol): Influence of Polymer Concentration on *Lactobacillus acidophilus* Viability and Release in Complex Food Systems

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Received: 14 June 2025 / Accepted: 21 August 2025 / Published online: 12 September 2025


**Abstract**
The growing interest in probiotic food matrices has driven research toward the development of innovative products that ensure the stability and functionality of beneficial microorganisms. This study investigated the effects of varying alginate concentrations (2, 4, and 6%) on the encapsulation efficiency, viability, and release control of *L. acidophilus* (LA) loaded in multi-component hydrogel beads for applications in peach juice and animal feed. Higher alginate concentrations improved capsule stability, probiotic protection, and survival. Increasing alginate from 2 to 6% reduced moisture content, increased swelling capacity, bead size, and encapsulation efficiency. LA viability exceeded recommended levels after the encapsulation process in all bead types, with 6% alginate providing the highest protection ($\geq$ 80%) under gastrointestinal conditions. In peach juice, higher alginate concentrations (4% and 6%) significantly improved probiotic retention and decreased LA release. However, in feed, 2% of alginate capsules showed greater LA viability. The study highlights the importance of modulating alginate concentration to optimize the characteristics of the beads with LA, differentially influencing its viability and release pattern, depending also on the specific characteristics of the final food matrix. These findings are fundamental for the design of stable and functional foods in future.

**Keywords** Functional foods · Probiotics · Encapsulation · Viability · Feeds · Juices

## Introduction

Probiotics, which are live microorganisms with established health benefits, have gained considerable attention for their potential to enhance intestinal microbiota, boost immune function, and promote mental well-being (Misra et al., 2021). Nevertheless, the viability of probiotics can be compromised by several factors, including acidic environments, oxygen exposure, and adverse conditions during processing, storage, transportation, and gastrointestinal transit (Sultana et al., 2022). To address these challenges, encapsulation technologies have emerged as a promising strategy for protecting probiotic cells during technological processing and gastrointestinal digestion; however, their effectiveness can be influenced by the choice of encapsulation material (Somera et al., 2024; Xu et al., 2022).

Among the various encapsulation methods, extrusion is recognized as a cost-effective, and scalable technique. This process involves extruding a hydrocolloid solution combined with probiotic bacteria through a syringe needle into a calcium chloride solution, forming encapsulated beads (Fangmeier et al., 2019). However, the resulting bead size is influenced by several factors, including needle diameter, syringe pressure, alginate concentration, stirring speed, and calcium chloride concentration (Valero-Cases & Frutos, 2015). Sodium alginate is a frequently used biopolymer in extrusion encapsulation due to its biocompatibility, biodegradability, non-toxic nature, and its capacity to form a three-dimensional network with divalent cations. Despite these advantages, alginate beads can exhibit limitations such as permeability, degradability, and porosity, which may reduce the protective effect on probiotic cells

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(Peanparkdee et al., 2025). To overcome these challenges, researchers have explored incorporating other biopolymers, like starches, proteins, and additional hydrocolloids, into the alginate solution (Misra et al., 2021; Xie, Ni, Cao, & Gu, 2022). Additionally, the combination of sodium alginate with prebiotic agents has also been documented (Ismail et al., 2023; Sharifi et al., 2023). Notably, investigating the effects of varying alginate concentrations on bead strength offers valuable insights for enhancing the encapsulation system (Zazzali et al., 2019). Therefore, hypothesizes that the strategic design of multi-component beads and the optimization of their polymer concentrations will significantly improve the protection and viability *Lactobacillus acidophilus* (LA) during processing and simulated gastrointestinal digestion, ultimately ensuring adequate concentrations ($10^6$–$10^7$ CFU/g or mL of food) for optimal efficacy in food products. This research provides a new framework for developing robust probiotic delivery systems, going beyond the limitations of individual polymers to design more effective and stable encapsulated probiotics. The primary objective of this study was to investigate the impact of varying alginate concentrations on the key properties of the beads and the functionality of encapsulated LA across different food matrices. Specifically, the study sought to: 1. Characterize the structural properties of the capsules by evaluating swelling capacity, moisture content, and water activity based on the different alginate concentrations employed. 2. Assess the viability of encapsulated LA under osmotic stress and its survival throughout the various stages of simulated gastrointestinal digestion. 3. Explore the practical application of these capsules by monitoring the viability and release pattern of LA in two complex food environments: peach juice and feed. The successful development of this optimized multi-component encapsulation system will contribute significantly to the advancement of functional foods and nutraceuticals by protecting probiotics from adverse environmental conditions and ensuring their controlled release, thereby enhancing their efficacy and providing consumers with innovative products that promote health and well-being.

## Materials and Methods

### Activation of *L. acidophilus*

*L. acidophilus* CECT 903 (LA) was sourced from the Spanish Type Culture Collection (CECT, Valencia, Spain). The activation of the probiotic culture was carried out using frozen glycerol stocks following the protocol described by Valero-Cases and Frutos (2017) using Man Rogosa Sharpe (MRS) broth (Oxoid, Madrid, Spain).

### Production of Hydrocolloid Alginate Solutions

Three hydrocolloid solutions were prepared in accordance with Valero-Cases and Frutos (2015) using three concentrations of sodium alginate: 2%, 4% and 6% (w/v) (Sigma-Aldrich, Madrid, Spain). The appropriate amount of sodium alginate was dissolved in distilled water using a homogenizer (T18 digital ULTRA-TURRAX®, Spain). Subsequently, 0.15% xanthan gum, 5% glycerol (Guinama Valencia, Spain) and 2% sodium bicarbonate (PanReac AppliChem, Barcelona, Spain) were incorporated into the various hydrocolloid solutions. Each solution was homogenized for 15 min at 500 rpm. All solutions, prior to probiotic inoculation, were sterilized at 121 °C for 15 min and cooled at 37 °C. The LA culture was subsequently added to each hydrocolloid solution to achieve a concentration of approximately 9 log CFU/mL.

### Preparation of Probiotic Beads by Extrusion Method

The extrusion encapsulation was carried out using various hydrocolloid solutions (2%, 4%, 6%) containing probiotic LA. These solutions were drawn into 60 mL sterile syringes and injected using a perfusion pump (mod. KDS 200/200P Legacy syringe pump, Holliston, USA) at a flow rate of 100 µL/min through a 21G (0.80 mm × 25 mm) needle connected to a silicone tube with a 1 mm internal diameter. The injection was directed into a sterile 0.1 M calcium chloride ($CaCl_2$) solution, maintaining 5 (± 0,1) cm between the needle and the $CaCl_2$ solution. The resulting beads were immersed in the $CaCl_2$ solution for 30 min, after which they were washed with sterilized distilled water and stored at 4 °C until further use.

### Survival of Encapsulated *L. acidophilus* Under Gastrointestinal Conditions

The simulation of gastrointestinal digestion (SGD) was carried out according to the method of E. Valero-Cases et al. (2017), which adheres to the standardized digestion protocols provided by the Infogest network. Briefly, various beads were introduced into simulated gastric juices (SGJ) containing 3 g/L of pepsin at pH of 2 maintained at 37 °C under agitation at 100 rpm for 120 min. Subsequently, the SGJ was neutralized to a pH of 7 using a $NaHCO_3$ (0.1 M) solution, and 1 g/L of pancreatin along with 4.5 g/L of bile salts (Sigma- Aldrich, Madrid, Spain) were added to simulated intestinal juices (SIJ) for an additional 60 min. Following the exposure to SGJ and SIJ, the

concentration of viable LA encapsulated within each type of bead was quantified as described below.

### *L. acidophilus* Viability in the Alginate Beads

The viability of encapsulated LA was assessed following the formation of the beads and throughout the various stages of SGID. Each variant of probiotic beads (2%, 4% and 6%) underwent serial dilution in peptone water and was subsequently plated on MRS agar (Oxoid, Madrid, Spain). The plates were incubated for 72 h at 37 °C under anaerobic conditions prior to enumeration. The results were expressed as log CFU/g.

### Encapsulation Efficiency

The encapsulation efficiency (EE) was determined using Eq. (1) as proposed by Verruck et al. (2017) to assess the efficiency of cell retention in the different types of beads.

$$EE(\%) = \left(\log N / \log N_0\right) \times 100 \quad (1)$$

where N represents the number of viable cells released from the beads, and N0 denotes the number of free cells introduced into the different alginate solutions prior to the encapsulation process.

### Moisture and Water Activity

The moisture content of the probiotic hydrogel beads was determined using the three distinct types of beads. Each type of bead was weighed, placed on aluminum pans and subjected to drying at 105 °C until a constant weight was achieved. The results were expressed as a percentage of the initial weight. The water activity (aw) was measured using a Novasina water activity meter (Aw Sprint TH 500).

### Evaluation of the Mean Diameter and Structure

The dimensions of 50 beads were measured using an electronic digital micrometer (Mitutoyo JP67, Vitoria-Gasteiz, Spain), with the mean values reported in millimeters (minimum resolution = 0.01 mm). The morphology and surface characteristics of the beads were analyzed after gastrointestinal digestion using a Leica MZ95 stereomicroscope (Leica, Spain).

### Swelling Capacity

The swelling capacity (Sw) of the beads was assessed following exposure to deionized water, utilizing the methodology outlined by Foglio Bonda et al. (2020) with certain modifications. All three types of dry beads were weighed and then immersed in deionized water at a ratio of 1:10 (beads/deionized water) in sterile vials, which were sealed and stored for 24 h at 4 °C. Afterwards, the beads were retrieved, weighted and the swelling percentage was determined using Eq. (2).

$$Sw(\%) = (Wt - Wo/Wo) \times 100 \quad (2)$$

where Wt represents the weight of the alginate beads after 24 h in deionized water and Wo denotes the initial weight of the dried alginate beads.

### Viability Assessment of Encapsulated *L. acidophilus* in Osmotic Stress

To evaluate the viability of LA under osmotic stress conditions, four sodium chloride (NaCl) solutions were prepared at varying concentrations (Yang et al., 2024). Specific quantities of NaCl were dissolved in sterile distilled water to achieve final concentrations of 5%, 10%, 15%, and 20% (w/v). The beads were aseptically transferred to containers containing 100 mL of each NaCl solution in a 1:10 (w/v) ratio. The samples were maintained at room temperature for 4 h. Subsequently, the beads were separated from the saline solution through filtration, and the number of viable LA cells was determined by plating on MRS agar (as described in Sect. 2.5). The results were expressed as log CFU/g of beads.

### Viability and Preservation Assessment of *L. acidophilus* in Peach Juice

The viability and preservation of LA in peach juice was investigated over 15 days of refrigerated storage. The juice was prepared from a commercial concentrate obtained from Tomates del Guadiana S. Coop (Badajoz, Spain). To achieve the desired Brix levels (10.6°Brix), the concentrate was diluted in boiling water for proper dissolution. It was then cooled, and beads containing 2%, 4%, and 6% alginate, each with an initial LA concentration of ca. 9 log CFU/mL, were incorporated into 100 mL of peach juice at a 1:3 (w/v) ratio. This procedure resulted in three distinct formulations: juice with 2% capsules (2%), juice with 4% capsules (4%), and juice with 6% capsules (6%). These formulations were stored at 4 °C and sampled on days 1, 7, and 15. At each sampling interval, the beads were meticulously removed to quantify the number of viable LA cells present both in the peach juice and within the capsules. This methodology facilitated the assessment of probiotic release into the liquid medium and its retention within the encapsulation matrix throughout the duration of the storage.

### Evaluation of the Viability and Preservation of *L. acidophilus* in Feed

A standard commercial pellet diet from Fresh Mediterranean (Spain) consisting of 26% crude protein, 14% crude fat, 2.75% crude fiber, 8% crude ash, 1.80% calcium, 1.20% phosphorus, and 4500 mg/kg EPA + DHA was ground. Freshly prepared LA capsules (2%, 4%, and 6%) were then combined directly with the ground feed in a 1:1 (w/w) ratio to produce four different formulations. These resulting formulations, containing the LA capsules, were subsequently dried to a constant weight and stored at 4 °C. Samples were collected on days 1, 7, and 15 to evaluate the number of viable LA cells in feeds by plating on MRS agar (as detailed in Sect. 2.5). The results were expressed as log CFU/g of beads.

### Statistical Analysis

In each experiment, the measurements were conducted in triplicate, and the results were presented as the mean ± standard deviation. The experimental data were analyzed using SPSS v 21.0 software (SPSS Inc., Chicago-Illinois, USA) through analysis of variance (ANOVA), followed by the Tukey test, which was applied when significant differences ($p < 0.05$) were detected.

## Results and Discussion

### Effects of Water Activity and Moisture Content on Alginate-Xanthan Gum-Glycerol Probiotic Beads

The influence of water activity (aw) and moisture content on the different probiotic beads encapsulated with varying concentrations of alginate is shown on Table 1. These parameters are essential for ensuring the long-term stability and viability of encapsulated probiotics, particularly in dry food applications. Additionally, the polymeric matrix of the beads may experience water diffusion, affecting the hydrogel volume and potentially leading to bead contraction or expansion (Caccavo et al., 2018).

All bead formulations showed the same water activity (aw = 0.96) with no significant differences ($p > 0.05$) among them, indicating that alginate concentration did not significantly influence this parameter. Water activity denotes the amount of free water available in the system, which can affect microbial growth and chemical reactions. While high aw values can promote microbial growth, low aw values can result in protein denaturation and lipid oxidation, adversely affecting probiotic viability (Reyes et al., 2018; Silva et al., 2018). Previous research has demonstrated that factors such as extrusion tip size, storage conditions, and synthesis pH have minimal impact on the water activity of alginate beads (Zazzali et al., 2019).

In contrast, the moisture content exhibited significant differences ($p < 0.05$) among the different probiotic beads, with alginate concentration levels of 2%, 4%, and 6%. Beads with 2% alginate exhibited the highest moisture content (94,92%), whereas those with 6% alginate had a moisture content of 91.45%. Increased moisture content can compromise the stability of encapsulated materials, leading to water mobility and accelerated viability loss (Tonon et al., 2009; Ying et al., 2010). In addition, high moisture can weaken the physical structure of the encapsulating matrix, causing increased swelling and a more porous structure, which compromises the protective barrier for probiotics. Finally, high humidity can prematurely reactivate cellular metabolism, deplete cellular energy stores and make cells more susceptible to stress and death over time.

Consequently, it is hypothesized that beads with higher alginate concentrations, such as 6%, may offer enhanced protection and preservation for LA. This hypothesis is supported by the water-binding capacity of alginate hydrogels (Bušić et al., 2018), where higher alginate concentrations create a denser, more cohesive network that immobilizes water more effectively The results indicate that while aw values remained constant across all bead formulations, the moisture content varied depending on the alginate concentration. These findings suggest that alginate concentration influences the moisture retention capacity of the beads. It is important to note that moisture content can directly impact the stability and viability of the encapsulated probiotics. Therefore, beads with higher alginate concentrations, such as 6%, may provide better protection and preservation for the

**Table 1** Physicochemical parameters of beads with different concentrations of alginate (2, 4, and 6%) obtained by extrusion method

| Alginate concentration | Parameters | | | |
|---|---|---|---|---|
| | Size (mm) | Moisture (%) | Aw | Swelling capacity (%) |
| **2%** | $2.10 \pm 0.21^{A}$ | $94.92 \pm 0.00^{C}$ | $0.96 \pm 0.01^{A}$ | $81.52 \pm 0.01^{A}$ |
| **4%** | $2.28 \pm 0.26^{AB}$ | $92.92 \pm 0.01^{B}$ | $0.96 \pm 0.01^{A}$ | $84.11 \pm 0.00^{B}$ |
| **6%** | $2.61 \pm 0.26^{B}$ | $91.42 \pm 0.01^{A}$ | $0.96 \pm 0.00^{A}$ | $97.55 \pm 0.01^{C}$ |

Within a column, means ± standard ($n = 3$) deviations with different superscript uppercase letters denote significant differences ($p < 0.05$) among samples

encapsulated microorganisms by reducing water mobility and improving the physical integrity of the barrier, resulting in greater long-term stability. To further explore the effect of alginate concentration on the viability of LA, subsequent sections will explore the survival rates of probiotics during simulated gastrointestinal digestion.

## Particle Size and Swelling Capacity

Particle size and swelling capacity are critical parameters in the development of alginate-based probiotic beads. As shown in Table 1, particle size analysis indicated a direct correlation between particle size and the concentration of alginate used. An increase in alginate concentration resulted in a significant enlargement ($p < 0.05$) of particle size. Beads formulated with 6% alginate showed a significantly larger size than the ones with 2% (2.66 vs 2.10 mm), a finding consistent with previous research that has established a positive correlation between increased polysaccharide concentration, and consequently the viscosity of the gel-forming solution, due to larger size of the resulting hydrogel particles (Qi et al., 2020a, 2020b). Essentially, higher viscosity solutions yield larger hydrogel particles. This phenomenon may be attributed to the greater water retention capacity of 6% alginate beads, which allows them to incorporate more water and swell, leading to increased size.

Furthermore, the higher viscosity of the polymer solution with increased alginate concentration contributed to the formation of larger and more robust beads, thereby enhancing encapsulation efficiency (Zhang et al., 2016). Extrusion encapsulation with alginate typically produces beads ranging from 2 to 5 mm in diameter (Frakolaki et al., 2021). Variations in size observed across studies can be attributed to differences in encapsulation materials, their concentrations, and specific methodologies employed. These factors include manual or mechanical extrusion, distance travelled, stirring speed, needle size, and flow rate. Additionally, particle diameter has been linked to the inclusion of prebiotics during encapsulation and the beads' ability to protect probiotics (Ismail et al., 2023; Valero-Cases & Frutos, 2015). However, beads formed at acidic pH exhibited alterations in both size and morphology, with larger dimensions and reduced roundness.

As the concentration of alginate increased from 2 to 6%, there was a corresponding increase in the swelling capacity (Sw) from 81.52% to 97.55% (Table 1). Surprisingly, a higher alginate concentration did not necessarily equate to greater resistance to volume change. Beads with the highest alginate concentration (6%) exhibited the greatest swelling capacity ($p < 0.05$), effectively regaining their original size. This observed relationship between capsule size and water retention capacity (WRC) implies a potential link between bead size and their water retention ability. WRC is influenced by various factors, including electrostatic and hydrophobic forces, as well as hydrogen bonding between different polysaccharides (e.g., alginate and xanthan gum) that form the capsule wall (Yao et al., 2018). Additional studies have indicated that greater water retention capacity results in larger beads due to their ability to incorporate more water internally (Qi, Simsek, Ohm, Chen, & Rao, 2020a, 2020b). The findings indicate that the concentration of alginate used in the encapsulation process directly affects both the particle size and swelling capacity of the probiotic beads. The higher swelling capacity associated with higher alginate concentrations suggests improved water retention within the polymer matrix. This controlled water retention could be beneficial for maintaining the structural integrity of the matrix by reducing the permeability of the beads to harmful external agents (such as oxygen, pH changes, or digestive enzymes) and, therefore, better protecting the probiotics.

## Encapsulation Efficiency and Probiotic Viability in the Alginate-Xanthan Gum-Glycerol Probiotic Beads

In this study, we utilized the extrusion encapsulation method, employing a peristaltic pump with different concentrations of alginate (2%, 4%, and 6%) as the wall material to protect LA. The encapsulation process yielded high EE values, exceeding 81% (Fig. 1). Notably, this parameter demonstrated a significant dependence on the alginate concentration employed. The least efficient system exhibited an EE of 81.52% with 2% alginate. However, previous studies have shown that EE was enhanced when the 2% alginate formulation included 3% of protein, 1% cocoa butter, and 0.1% (w/v) anthocyanin, achieving values of 94% (Morsy et al., 2022). Furthermore, the combination of alginate with proteins (pea, rice and protein isolate) improved the entrapment of probiotic microorganisms in the beads, resulting in encapsulation yields exceeding 95% (Camelo-Silva et al., 2023). Conversely, another study indicated that lyophilized beads containing a mixture of 1% (w/v) alginate, and 15% (w/v) protein isolate did not significantly ($p > 0.05$) enhance encapsulation performance (82.46%), compared to the values obtained in the present study with 2% alginate. This may be attributed to the sensitivity of bacterial cells to dehydration (Obradović et al., 2022). Nevertheless, our research demonstrated that the addition of alginate significantly increased the encapsulation efficiency (EE) of the probiotic. Specifically, the highest EE of 97.55% was achieved in the probiotic beads with the highest alginate concentration (6%). This improvement in LA entrapment can be attributed to the formation of alginate beads with a more rigid and compact membrane, effectively preventing

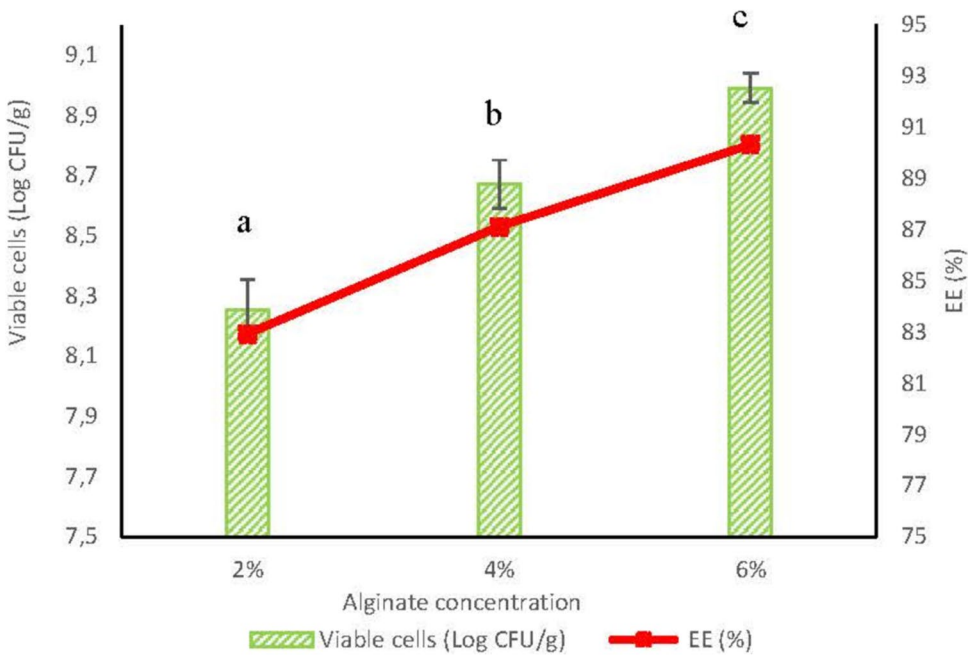


**Fig. 1** Number of live *Lactobacillus acidophilus* cells (green bar for FX) and their encapsulation efficiency (EE) (red bar for FX) after using the extrusion method with different alginate levels (2, 4, and 6%). The error bars show the variation in the data. Different lowercase letters indicate significant differences ($p < 0.05$) between samples

the diffusion of the probiotic during the cross-linking process. Consequently, higher amounts of probiotic microorganism were retained within the beads.

A higher concentration of alginate results in increased viscosity of the solution, leading to the formation of larger beads. This creates a greater interface between the alginate droplet and the $CaCl_2$ solution, facilitating a faster gelation upon exposure to Ca ions. Consequently, there is a shorter time for bacteria to be released into the $CaCl_2$ solution, resulting in higher encapsulation efficiency. As a result, beads containing 2% alginate exhibited the lowest LA concentration (8.25 log CFU/g). Hence, employing higher concentrations of alginate (4% or 6%) in the system can limit diffusion between the interior and exterior of the beads, enhancing probiotic retention (Blandino et al., 1999). On the other hand, previous studies have observed that the addition of inulin and chitosan significantly decreased ($p < 0.05$) EE (Frakolaki et al., 2020; Yonekura et al., 2014). It is important to highlight that the viability of encapsulated LA remained in all beads above the adequate levels (> 6–7 log CFU/g of beads) throughout the encapsulation and gastrointestinal digestion processes, ensuring the capacity to deliver beneficial effects to the host, as outlined by the FAO/WHO (2002).

The results indicate that the concentration of alginate employed during encapsulation plays a significant role in influencing both the EE and the viability of the encapsulated LA. Higher concentrations of alginate are associated with enhanced encapsulation efficiency and improved probiotic viability offering more effective protection for the probiotic.

## Effect of Alginate Concentration on *L. acidophilus* Survival in Beads Under Simulated Gastrointestinal Digestion Conditions

The survival of LA within alginate beads during simulated gastrointestinal digestion (SGD) was assessed (Fig. 2). After 2 h of exposure to simulated gastric juice (SGJ), the concentration of probiotics within the alginate beads was lower respect to the initial concentration across all bead types. Notably, the retention percentage was significantly influenced by the alginate concentration. Beads containing 6% alginate showed the highest probiotic retention (8.24 log CFU/g), whereas beads with lower alginate concentrations (4 and 2%) exhibited reduced LA retention respect to the initial concentration, approximately 7.5 and 6.0 log CFU/g for 4 and 2%, respectively. These findings suggest that increasing alginate concentration may lead to a decrease in pore size within the capsules, resulting in a denser structure. This structural change restricts the diffusion of H + ions through the bead pores, thereby enhancing the survival of LA during gastric digestion. Furthermore, the formulation with the highest encapsulation efficiency during bead preparation (6% alginate) also provided the greatest protection for LA against adverse gastric and intestinal conditions. Consequently, increasing the alginate concentration in the formulation significantly enhances bead stability, preventing premature probiotic release in the stomach and reducing exposure to the harsh gastric environment, thereby improving survival rates. The beads offer approximately 70% protection with 4% alginate and 80% with 6% alginate. Therefore, utilizing these


**Fig. 2** Effect of gastrointestinal digestion on the survival of *L. acidophilus* encapsulated with different alginate concentrations (2, 4, and 6%). Different uppercase letters above the bars denote significant differences ($p < 0.05$) on *L. acidophilus* survival between the different alginate concentration (2, 4, and 6%) at the same step of gastrointestinal digestion. Different lowercase letters above the bars denote significant differences ($p < 0.05$) in *L. acidophilus* survival for the same alginate beads along the different step of gastrointestinal digestion. GID, gastrointestinal digestion; SGD, simulated gastric juices; SIJ, simulated intestinal juices

concentrations effectively mitigates probiotic loss during gastric digestion and increases the number of viable cells reaching the colon.

In the simulated intestinal juice (SIJ), the viability of LA within beads produced using a lower alginate concentration (2%) decreased to approximately 46% after 1 h. Ensuring that probiotics reach the colon alive is crucial for them to exert their beneficial effects. Fortunately, all tested beads demonstrated satisfactory protection for LA following exposure to SIJ. However, significantly higher survival rates ($p < 0.05$) were observed when using 4% and 6% alginate concentrations compared to beads produced with 2% alginate (46% survival rate). This difference in survival can be attributed to the neutral pH conditions of the SIJ, which create an electrostatic repulsion between the strongly anionic polysaccharide chains, resulting in increased porosity in the bead wall and allowing rapid diffusion of the probiotic through the pores (Mokarram et al., 2009).

The alginate beads exhibit a spherical shape (Fig. 3). It is notable that, at the end of the simulated intestinal digestion process, the beads composed of 2% and 4% alginate show irregularities and fractures in the surface. In contrast, beads composed of 6% alginate show improved surface morphology while retaining their structural integrity. In future research, extending the duration of digestion until complete fragmentation of the beads occurs would be a valuable avenue to explore. This would allow a comprehensive assessment of LA viability, providing valuable information on its stability and controlled release potential. It should be noted that in our previous study, encapsulation of *L. plantarum* CECT 220 using 2% alginate resulted in a high survival rate after gastrointestinal digestion (Valero-Cases & Frutos,

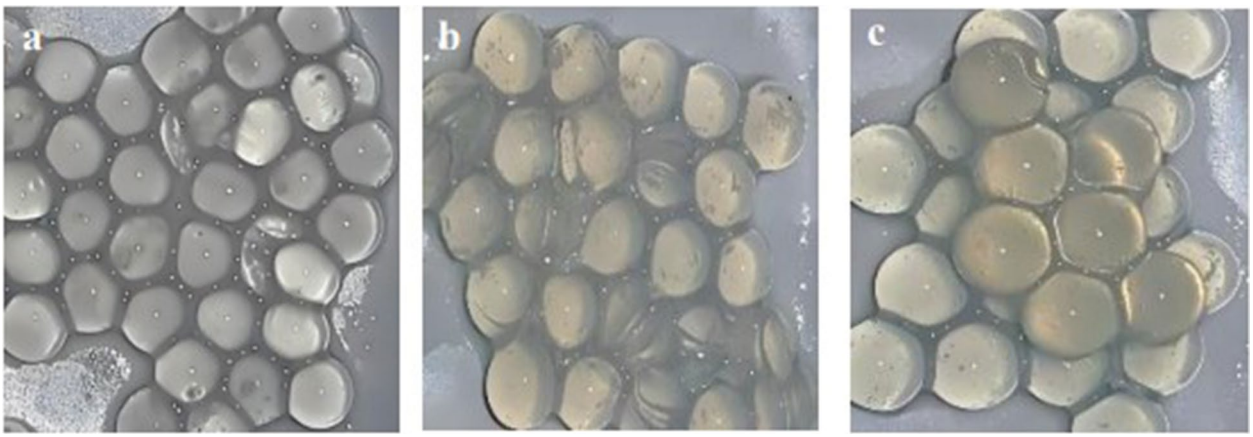


**Fig. 3** **a**, **b**, and **c** The Design of Multi-Component Beads obtained with different alginate concentrations (2%, 4%, and 6%, respectively) after the gastrointestinal simulation process

2015). These findings underline the possible strain specificity within the alginate matrix, emphasizing the need for individual investigations into the efficiency of encapsulation and protection of each probiotic strain. In future studies, exploring the viability of LA viability in 2% alginate beads enriched with prebiotic or bioactive compounds would be of particular interest. Babot et al. (2023), reported results comparable to those achieved with 4% alginate beads during 1 h of intestinal digestion, encapsulating *L. salivarius* CRL2217 in soy protein-alginate particles using the water-in-oil emulsion technique. However, in the beads made with 6% alginate, survival was higher, reaching a cell count higher than 7 log CFU/g. Other studies that incorporated double coating of the beads or a double emulsion before extrusion have demonstrated similar protection results during in vitro digestion ($\geq 80\%$) to those obtained in our present study by increasing the alginate concentration using the single extrusion method with a peristaltic pump (Frakolaki et al., 2020; Sultana et al., 2022). Thus, high encapsulation efficiency and protection during gastrointestinal digestion can be achieved without the use of additional agents or costly extra steps in the encapsulation process.

In summary, the findings of this study are based on an analysis of how alginate concentration influences the retention and protection of probiotics within alginate beads during simulated gastrointestinal digestion. These results offer valuable insights into the development of effective encapsulation formulations aimed at enhancing probiotic survival in food and supplement applications.

## Viability of *L. acidophilus* in Alginate Beads Under Osmotic Stress

The impact of various sodium chloride (NaCl) concentrations on the viability of LA encapsulated in alginate beads was evaluated across four salinity levels: 0%, 5%, 10%, 15%, and 20% (w/v) (Fig. 4). The findings indicated a significant interaction between the alginate concentration in the bead wall and the salt concentration of the medium, collectively influencing the viability of LA within the beads.

Across all salinity conditions, the NaCl concentration adversely affected the viability of LA compared to time zero, with a progressive decline in survival observed as the NaCl concentration increased. This phenomenon may be attributed to the osmotic stress imposed by high salt concentrations in the environment, leading to cell dehydration (plasmolysis) and alterations in the microorganism's metabolic functions. However, the protective capacity of the beads varied significantly with alginate concentration. Beads prepared with 2% alginate demonstrated significantly lower protection for LA viability (Fig. 4) compared to concentrations of 4% and 6%. This can be explained by the lower density of the 2% alginate matrix, which provides a less robust physical barrier to external osmotic stress, allowing greater diffusion of sodium ions and a more pronounced loss of water from the microbial cell. In contrast, beads formulated with 4% and 6% alginate exhibited enhanced stability and protection for probiotic viability. This higher alginate concentration forms a more compact and less permeable polymeric network, which confers greater resistance to capsule disintegration and a "buffering" effect against osmotic shock. However, in these 4% and 6% alginate microbeads, probiotic activity continued to progressively decline as NaCl concentration increased. This may be attributed to the denser matrix of

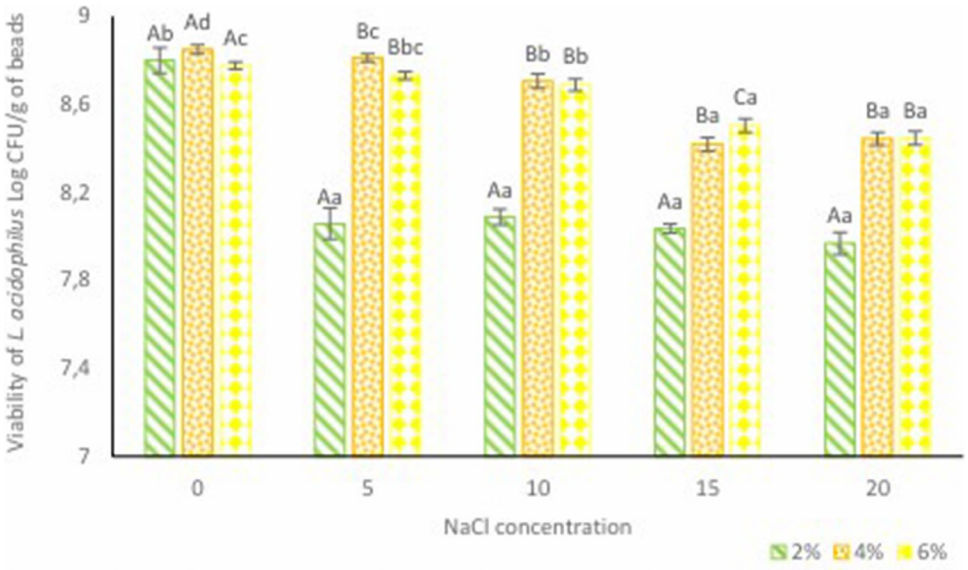


**Fig. 4** Viability of *Lactobacillus acidophilus* encapsulated in alginate beads with different alginate concentrations (2, 4, and 6%) under different salt stress conditions (NaCl). Different uppercase letters above the bars indicate significant differences ($p < 0.05$) between alginate concentrations for the same NaCl concentration. Different lowercase letters above the bars indicate significant differences ($p < 0.05$) between different NaCl concentrations for the same alginate formulation at 4 °C

these higher alginate concentrations, which provides more gradual but sustained protection. Although initially more effective, the continuous increase in external NaCl concentration (from 5 to 15% and 20%) allowed for prolonged and cumulative osmotic stress to penetrate the beads. This led to a more continuous decline in viability, as the cells within these better-protected beads were subjected to a progressively more hostile environment over time, rather than an immediate shock that would quickly eliminate the most susceptible cells. Despite the improved protection of the 4% and 6% alginate beads, the greatest loss of viability in these formulations was specifically observed at the highest NaCl concentrations (15% and 20%), decreasing to survival levels of ca. 8.4 log CFU/g with no significant differences between concentration. Importantly, even after this decrease, viability remained above the minimum concentrations recommended by the World Health Organization (WHO) for a beneficial probiotic effect. This suggests that, while higher alginate concentrations enhance the capsule integrity and protection capacity, there are limits to this protection when the osmotic stress of the external environment becomes more severe. Therefore, the interaction between the alginate concentration in the capsule wall and the NaCl concentration in the solution is a critical determinant of the viability of the encapsulated probiotic. The composition of the wall material directly influences the ability of the alginate beads to mitigate salt stress, which is essential for the survival of LA under adverse conditions. A denser alginate matrix (4% and 6%) provides superior protection by mitigating the impact of osmotic stress and potential ionic toxicity, thereby maintaining the integrity of the probiotic cells for an extended period.

## Viability of Encapsulated *L. acidophilus* in Peach Juice

Figure 5-A illustrates the release of LA from the capsules into peach juice over a 15-day period of refrigerated storage at 4 °C. Concurrently, Fig. B depicts the retention of the probiotic within the capsules in the same juice and over the same duration.

At 24 h of storage (day 1), significantly greater release of LA into the juice was observed when the capsules contained 2% alginate (5.5 log CFU/mL of juice). This finding suggests that a lower concentration of alginate in the capsule matrix facilitates a more rapid release of LA into the surrounding medium. In contrast, for capsules with 4% and 6% alginate, no significant differences in LA release were observed at this early stage. ca. 4.7 log CFU/mL of juice). At the same time, an inverse trend was observed in the retention of probiotics within the capsules: the formulation with the highest alginate concentration (6%) showed a greater capacity to encapsulate and retain the probiotic (8.9 log CFU/g) compared to those with 4% and 2% (8.5 and 8.4 log CFU/g, respectively) with significant differences between all concentrations.

After 7 and 15 days of storage, the trend of LA release continued to increase in the lower concentration capsules. During these last periods, significant differences were observed among all the alginate concentrations studied: the higher the alginate concentration, the greater the probiotic retention, and the lower the LA release into the juice. This reduced LA release is crucial, as it helps to preserve the original physicochemical properties of the juice, minimizing alterations in its flavor and stability that could result from excessive acidification due to the metabolic activity of the microorganism when released outside the capsule.

In summary, our results demonstrate that increasing the alginate concentration substantially enhances the probiotic retention capacity of the capsules in peach juice, establishing a direct and significant relationship between the integrity of the alginate matrix and the stability of the probiotic load over storage time, while contributing to maintaining the inherent characteristics of the juice.

## Viability of Encapsulated *L. acidophilus* in Feed

The viability during 15 days of refrigerated storage of encapsulated LA with different alginate composition when used in feed formulation is depicted in Fig. 6. During the 15 days of storage, capsules formulated with 2% alginate showed higher LA viability in feed compared to those formulated with 4% and 6%. This finding suggests that, in this context, a lower alginate concentration could be compromising the integrity of the encapsulation and the retention capacity of the probiotic, rather than effectively protecting it within the bead. Beads with 2% alginate, due to their less dense and more porous polymeric matrix, facilitate a more substantial release of LA cells into the feed matrix. Once released, microorganisms have direct access to the nutrients present in the feed. This substrate availability allows LA to actively initiate fermentation processes and proliferate, resulting in the high overall viability observed in the feed (greater than 8 log CFU/g throughout storage). Therefore, the higher viability observed in the feed with the 2% alginate beads is not attributed to better protection within the capsule, but rather to an uncontrolled release of the probiotic into the feed matrix. However, this proliferation outside the capsule could lead to undesirable alterations in the physicochemical and sensory properties of the feed due to the metabolic activity of the released microorganisms, such as lactic acid production among other compounds. The evaluation of these alterations in the feed matrix will be addressed in future trials specifically designed for this purpose. Therefore, this finding, rather than suggesting successful encapsulation, indicates that a lower alginate concentration is compromising the integrity of the encapsulation and the retention capacity

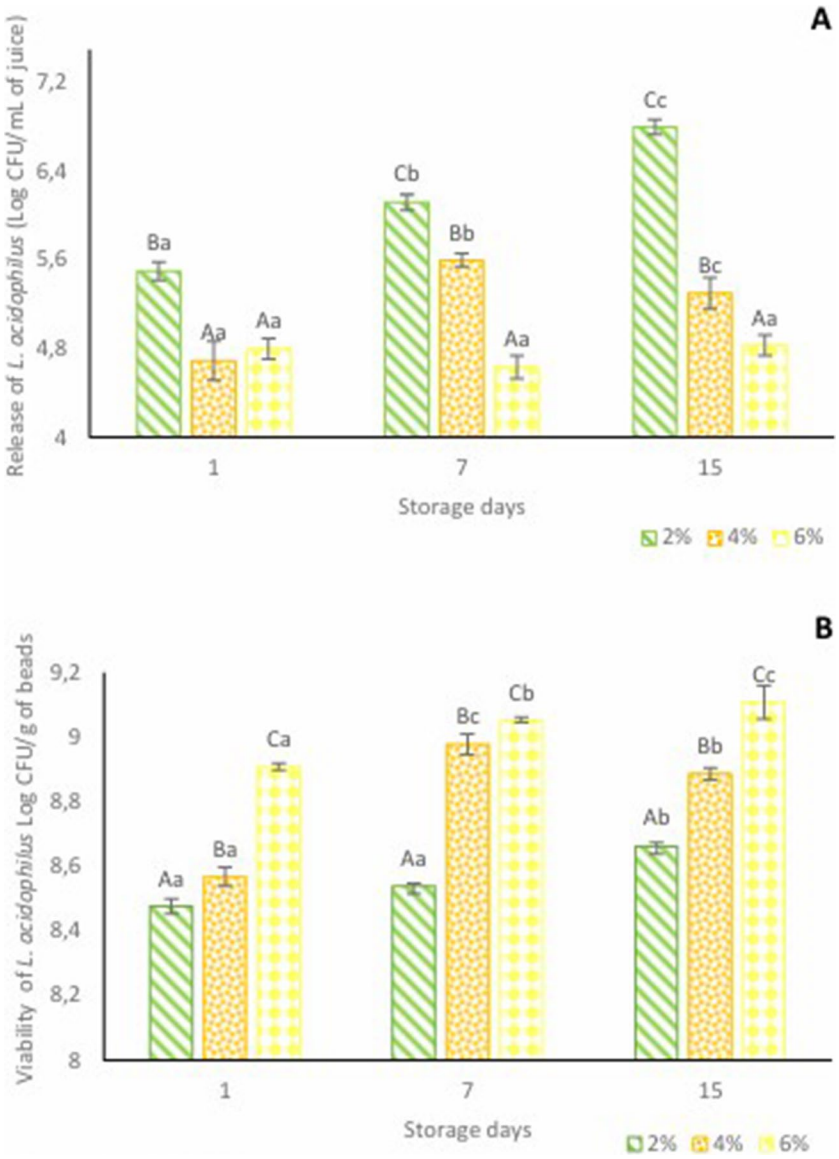


**Fig. 5** Viability and release of *Lactobacillus acidophilus* (LA) encapsulated from alginate beads with different alginate concentrations (2, 4, and 6%) in peach juice over 15 days of refrigerated storage at 4 °C. **A** The concentration of LA released into the peach juice and **B** the concentration of LA remaining in the beads. Different uppercase letters above the bars indicate significant differences between samples ($p < 0.05$) for the same day of refrigerated storage at 4 °C. Different lowercase letters above the bars indicate significant differences ($p < 0.05$) for the same alginate beads throughout the storage

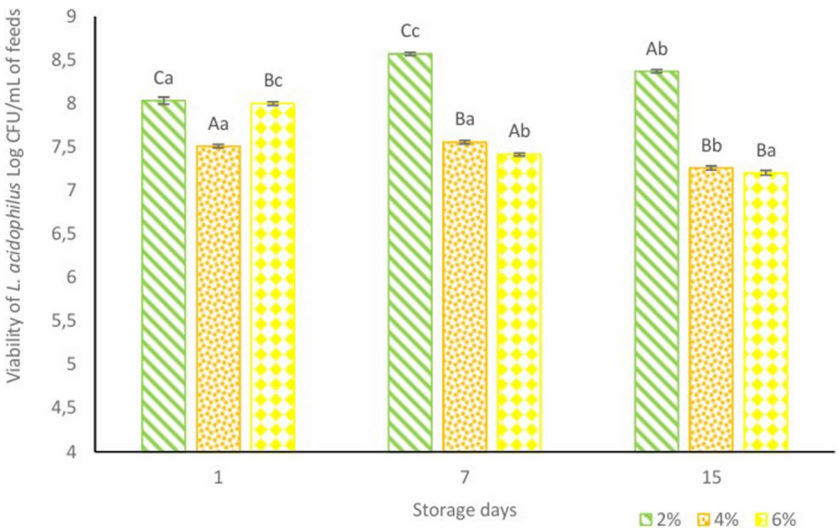


**Fig. 6** Viability of *Lactobacillus acidophilus* from alginate beads with different alginate concentrations (2, 4, and 6%) in feeds over 15 days of refrigerated storage. Different uppercase letters above the bars indicate significant differences between samples ($p < 0.05$) for the same day of refrigerated storage at 4 °C. Different lowercase letters above the bars indicate significant differences ($p < 0.05$) for the same alginate beads throughout refrigerated storage at 4 °C

of the probiotic, not effectively protecting it within the bead, which is the main objective of encapsulation. In contrast, the 4% and 6% alginate capsules, being denser and more robust, likely achieved better containment of the probiotic, reaching concentrations of approximately 7.5 log CFU/g with no significant differences between samples. Although this could result in a lower overall viability observed in the feed matrix, it indicates that the beads integrity was better maintained, which is crucial to prevent migration of the encapsulated LA and thus the feed spoilage. The challenge with these denser beads could be confinement microbial stress or limited nutrient diffusion within the capsule, affecting the viability of the microorganisms that remain encapsulated. In conclusion, the apparent the apparent increased viability of LA with 2% alginate in the feed is interpreted because of the uncontrolled release of the probiotic into the feed. However, the goal is to minimize feed spoilage during storage by ensuring probiotic retention in the capsules. Therefore, capsules with higher alginate concentrations (4% and 6%) would be the most suitable for this purpose, as they demonstrate better probiotic retentions within the capsule matrix, limiting their interaction with feed components. It is important to highlight that in all samples analyzed over 15 days of storage, LA viability remained above the recommended concentrations.

## Conclusions

This study elucidates that the concentration of alginate is a critical determinant in the encapsulation efficacy of *L. acidophilus*. An increase in alginate concentration markedly enhances probiotic viability and stability, both during the encapsulation process and under the stress of simulated gastrointestinal digestion. Beads formulated with higher alginate concentrations provided substantial protection against gastric conditions, with remarkable survival rates: approximately 70% protection with 4% alginate and 80% with 6% alginate. It is pertinent to note that a 4% alginate concentration already yields significant improvements in encapsulation efficiency, retention, and protection ($p < 0.05$).

However, the highest efficiency and protection were achieved with beads produced with 6% alginate, reaching an encapsulation efficiency greater than 90%. This suggests that selecting a 4% or 6% alginate concentration for encapsulating *L. acidophilus* is not only feasible and cost-effective but also provides high levels of microbial protection for food and feed formulations, supporting their practicality and efficacy in those matrices. The applicability of these beads was evaluated in two different food matrices during storage, highlighting the significance of the interaction between the capsule and the environment. In peach juice, higher alginate concentrations (4% and 6%) were crucial for enhanced probiotic retention and reduced lactic acid release, which is essential for preserving the intrinsic physicochemical properties of the juice and ensuring the stability of the final product without unwanted modifications. Conversely, in feed, the formulations with the 2% alginate capsules exhibited higher viability due to a faster release of the probiotic to the feed matrix, with also a higher nutrient availability in the medium. However, the beads with higher alginate concentrations (4% and 6%) offered superior retention, a critical aspect when the objective is to minimize feed spoilage due to microbial activity.

In summary, our alginate-xanthan gum-glycerol encapsulation methodology not only ensures the efficiency and reproducibility of *L. acidophilus* encapsulation but also presents an economically viable and technologically feasible solution for various food and feed applications, adjusting the alginate concentration to optimize probiotics viability during storage and gastrointestinal digestion and controlling their interactions with specific matrices such as peach juice or feed.

**Acknowledgements** This work is part of the ThinkInAzul program supported by MCIN with funding from European Union Next Generation EU (PRTR-C17.I1) and by the Comunidad Autónoma de la Región de Murcia-Fundación Séneca (Spain).

**Author Contribution** EVC: conceptualization, investigation, methodology, supervision, formal analysis, writing-original draft, writing-review and editing. ARG: investigation. MAE: writing-review and editing funding acquisition and resources. MJF: writing-review and editing.

**Funding** Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature. This work has been financed by MCIN/AEI/https://doi.org/10.13039/501100011033(grant number PID2020-113637RB-C21).

**Data Availability** No datasets were generated or analysed during the current study.

## Declarations

**Consent for Publication** All authors agree to publish.

**Conflicts of interest** The authors declare no competing interests.

## References


Babot, J. D., Argañaraz-Martínez, E., Apella, M. C., & Perez Chaia, A. (2023). Microencapsulation of probiotics with soy protein isolate and alginate for the poultry industry. *Food and Bioprocess Technology, 16*(7), 1478–1487. https://doi.org/10.1007/s11947-023-03007-2

Blandino, A., Macías, M., & Cantero, D. (1999). Formation of calcium alginate gel capsules: Influence of sodium alginate and CaCl2 concentration on gelation kinetics. *Journal of Bioscience and Bioengineering, 88*(6), 686–689. https://doi.org/10.1016/S1389-1723(00)87103-0

Bušić, A., Belščak-Cvitanović, A., Vojvodić Cebin, A., Karlović, S., Kovač, V., Špoljarić, I., Mršić, G., & Komes, D. (2018). Structuring new alginate network aimed for delivery of dandelion (*Taraxacum officinale* L.) polyphenols using ionic gelation and new filler materials. *Food Research International, 111*, 244–255. https://doi.org/10.1016/j.foodres.2018.05.034

Caccavo, D., Cascone, S., Lamberti, G., & Barba, A. A. (2018). Hydrogels: Experimental characterization and mathematical modelling of their mechanical and diffusive behaviour. *Chemical Society Reviews, 47*(7), 2357–2373. https://doi.org/10.1039/C7CS00638A

Camelo-Silva, C., Figueredo, L. L., Cesca, K., Verruck, S., Ambrosi, A., & Di Luccio, M. (2023). Membrane emulsification as an emerging method for *Lacticaseibacillus rhamnosus* GG® encapsulation. *Food and Bioprocess Technology*. https://doi.org/10.1007/s11947-023-03099-w

de Araújo Etchepare, M., Nunes, G. L., Nicoloso, B. R., Barin, J. S., Moraes Flores, E. M., de Oliveira Mello, R., & Ragagnin de Menezes, C. (2020). Improvement of the viability of encapsulated probiotics using whey proteins. *LWT, 117*, Article 108601. https://doi.org/10.1016/j.lwt.2019.108601

Fangmeier, M., Lehn, D. N., Maciel, M. J., & Volken de Souza, C. F. (2019). Encapsulation of bioactive ingredients by extrusion with vibrating technology: Advantages and challenges. *Food and Bioprocess Technology, 12*(9), 1472–1486. https://doi.org/10.1007/s11947-019-02326-7

Foglio Bonda, A., Regis, L., Giovannelli, L., & Segale, L. (2020). Alginate/maltodextrin and alginate/shellac gum core-shell capsules for the encapsulation of peppermint essential oil. *International Journal of Biological Macromolecules, 162*, 1293–1302. https://doi.org/10.1016/j.ijbiomac.2020.06.194

Frakolaki, G., Giannou, V., Kekos, D., & Tzia, C. (2021). A review of the microencapsulation techniques for the incorporation of probiotic bacteria in functional foods. *Critical Reviews in Food Science and Nutrition, 61*(9), 1515–1536. https://doi.org/10.1080/10408398.2020.1761773

Frakolaki, G., Katsouli, M., Giannou, V., & Tzia, C. (2020). Novel encapsulation approach for *Bifidobacterium* subsp. *lactis* (BB-12) viability enhancement through its incorporation into a double emulsion prior to the extrusion process. *LWT - Food Science and Technology, 130*, Article 109671. https://doi.org/10.1016/j.lwt.2020.109671

Gomathi, T., Susi, S., Abirami, D., & Sudha, P. (2017). Size optimization and thermal studies on calcium alginate nanoparticles. *IOSR J. Pharm., 2017*(48), 1–7.

Ismail, S. A., Hassan, A. A., Nour, S. A., & El-Sayed, H. S. (2023). The production of stirred yogurt fortified with prebiotic xylooligosaccharide, probiotic and synbiotic microcapsules. *Biocatalysis and Agricultural Biotechnology, 50*, Article 102729. https://doi.org/10.1016/j.bcab.2023.102729

Misra, S., Pandey, P., & Mishra, H. N. (2021). Novel approaches for co-encapsulation of probiotic bacteria with bioactive compounds, their health benefits and functional food product development: A review. *Trends in Food Science & Technology, 109*, 340–351. https://doi.org/10.1016/j.tifs.2021.01.039

Mokarram, R. R., Mortazavi, S. A., Najafi, M. B. H., & Shahidi, F. (2009). The influence of multi stage alginate coating on survivability of potential probiotic bacteria in simulated gastric and intestinal juice. *Food Research International, 42*(8), 1040–1045. https://doi.org/10.1016/j.foodres.2009.04.023

Morsy, M. K., Morsy, O. M., Abdelmonem, M. A., & Elsabagh, R. (2022). Anthocyanin-colored microencapsulation effects on survival rate of *Lactobacillus rhamnosus* GG, color stability, and sensory parameters in strawberry nectar model. *Food and Bioprocess Technology, 15*(2), 352–367. https://doi.org/10.1007/s11947-022-02758-8

Obradović, N., Volić, M., Nedović, V., Rakin, M., & Bugarski, B. (2022). Microencapsulation of probiotic starter culture in protein–carbohydrate carriers using spray and freeze-drying processes: Implementation in whey-based beverages. *Journal of Food Engineering, 321*, Article 110948. https://doi.org/10.1016/j.jfoodeng.2022.110948

Peanparkdee, M., Janchai, P., Aksornsri, T., & Vaithanomsat, P. (2025). Design and evaluation of bromelain-encapsulated alginate beads reinforced with gum Arabic: Formulation, characterization, and stability in simulated gastrointestinal conditions. *Journal of Agriculture and Food Research, 19*, Article 101698. https://doi.org/10.1016/j.jafr.2025.101698

Qi, X., Simsek, S., Chen, B., & Rao, J. (2020). Alginate-based double-network hydrogel improves the viability of encapsulated probiotics during simulated sequential gastrointestinal digestion: Effect of biopolymer type and concentrations. *International Journal of Biological Macromolecules*, *165*(Part B), 1675–1685. https://doi.org/10.1016/j.ijbiomac.2020.10.028

Qi, X., Simsek, S., Ohm, J.-B., Chen, B., & Rao, J. (2020b). Viability of *Lactobacillus rhamnosus* GG microencapsulated in alginate/chitosan hydrogel particles during storage and simulated gastrointestinal digestion: Role of chitosan molecular weight. *Soft Matter, 16*(7), 1877–1887.

Reyes, V., Chotiko, A., Chouljenko, A., & Sathivel, S. (2018). Viability of *Lactobacillus acidophilus* NRRL B-4495 encapsulated with high maize starch, maltodextrin, and gum arabic. *LWT - Food Science and Technology, 96*, 642–647. https://doi.org/10.1016/j.lwt.2018.06.017

Sharifi, Z., Jebelli Javan, A., Hesarinejad, M. A., & Parsaeimehr, M. (2023). Application of carrot waste extract and *Lactobacillus plantarum* in *Alyssum homalocarpum* seed gum-alginate beads to create a functional synbiotic yogurt. *Chemical and Biological Technologies in Agriculture*. https://doi.org/10.1186/s40538-022-00377-1

Silva, M. P., Tulini, F. L., Martins, E., Penning, M., Fávaro-Trindade, C. S., & Poncelet, D. (2018). Comparison of extrusion and co-extrusion encapsulation techniques to protect *Lactobacillus acidophilus* LA3 in simulated gastrointestinal fluids. *LWT - Food Science and Technology, 89*, 392–399. https://doi.org/10.1016/j.lwt.2017.11.008

Somera, T., Mafaldo, I. M., Bezerril, F. F., Rosset, M., Magnani, M., & Pimentel, T. C. (2024). Unconventional foods and by-products as sustainable encapsulating materials carrying probiotic cultures: An overview of the encapsulation methods, and incorporation on food products. *Food Bioscience, 61*, Article 104892. https://doi.org/10.1016/j.fbio.2024.104892

Sultana, M., Chan, E.-S., Pushpamalar, J., & Choo, W. S. (2022). Advances in extrusion-dripping encapsulation of probiotics and omega-3 rich oils. *Trends in Food Science & Technology, 123*, 69–86. https://doi.org/10.1016/j.tifs.2022.03.006

Tonon, R. V., Brabet, C., Pallet, D., Brat, P., & Hubinger, M. D. (2009). Physicochemical and morphological characterisation of açai (*Euterpe oleraceae* Mart.) powder produced with different

carrier agents. *International Journal of Food Science and Technology, 44*(10), 1950–1958. https://doi.org/10.1111/j.1365-2621.2009.02012.x

Valero-Cases, E., & Frutos, M. J. (2015). Effect of different types of encapsulation on the survival of *Lactobacillus plantarum* during storage with inulin and in vitro digestion. *LWT - Food Science and Technology, 64*(2), 824–828. https://doi.org/10.1016/j.lwt.2015.06.049

Valero-Cases, E., & Frutos, M. J. (2017). Effect of inulin on the viability of *L. plantarum* during storage and in vitro digestion and on composition parameters of vegetable fermented juices. *Plant Foods for Human Nutrition, 72*(2), 161–167. https://doi.org/10.1007/s11130-017-0601-x

Valero-Cases, E., Nuncio-Jauregui, N., & Frutos, M. J. (2017). Influence of fermentation with different lactic acid bacteria and in vitro digestion on the biotransformation of phenolic compounds in fermented pomegranate juices. *Journal of Agricultural and Food Chemistry, 65*(31), 6488–6496. https://doi.org/10.1021/acs.jafc.6b04854

Verruck, S., de Carvalho, M. W., de Liz, G. R., Amante, E. R., Vieira, C. R. W., Amboni, RDd. M. C., & Prudencio, E. S. (2017). Survival of *Bifidobacterium* BB-12 microencapsulated with full-fat goat's milk and prebiotics when exposed to simulated gastrointestinal conditions and thermal treatments. *Small Ruminant Research, 153*, 48–56. https://doi.org/10.1016/j.smallrumres.2017.05.008

Xie, H., Ni, F., Cao, M., & Gu, Q. (2022b). The encapsulation of probiotics by polysaccharides. In J. Venkatesan, S.-K. Kim, S. Anil, & R. P. D (Eds.), *Polysaccharide nanoparticles* (pp. 31–64): Elsevier.

Xu, C., Ban, Q., Wang, W., Hou, J., & Jiang, Z. (2022). Novel nano-encapsulated probiotic agents: Encapsulate materials, delivery, and encapsulation systems. *Journal of Controlled Release, 349*, 184–205. https://doi.org/10.1016/j.jconrel.2022.06.061

Yang, Zhen-Quan and Mgomi, Fedrick C. and Yuan, Lei and Farooq, Rimsha and Lu, Chun-lei, Survivability and characterization of the biofilm-like probiotic Pediococcus Pentosaceus encapsulated in calcium alginate gel beads. Available at SSRN: https://doi.org/10.2139/ssrn.4702759

Yao, J., Zhou, Y., Chen, X., Ma, F., Li, P., & Chen, C. (2018). Effect of sodium alginate with three molecular weight forms on the water holding capacity of chicken breast myosin gel. *Food Chemistry, 239*, 1134–1142. https://doi.org/10.1016/j.foodchem.2017.07.027

Ying, D. Y., Phoon, M. C., Sanguansri, L., Weerakkody, R., Burgar, I., & Augustin, M. A. (2010). Microencapsulated *Lactobacillus rhamnosus* GG powders: Relationship of powder physical properties to probiotic survival during storage. *Journal of Food Science, 75*(9), E588–E595. https://doi.org/10.1111/j.1750-3841.2010.01838.x

Yonekura, L., Sun, H., Soukoulis, C., & Fisk, I. (2014). Microencapsulation of *Lactobacillus acidophilus* NCIMB 701748 in matrices containing soluble fibre by spray drying: Technological characterization, storage stability and survival after in vitro digestion. *Journal of Functional Foods, 6*, 205–214. https://doi.org/10.1016/j.jff.2013.10.008

Zazzali, I., Aguirre Calvo, T. R., Pizones Ruíz-Henestrosa, V. M., Santagapita, P. R., & Perullini, M. (2019). Effects of pH, extrusion tip size and storage protocol on the structural properties of Ca(II)-alginate beads. *Carbohydrate Polymers, 206*, 749–756. https://doi.org/10.1016/j.carbpol.2018.11.051

Zhang, Z., Zhang, R., & McClements, D. J. (2016). Encapsulation of β-carotene in alginate-based hydrogel beads: Impact on physicochemical stability and bioaccessibility. *Food Hydrocolloids, 61*, 1–10. https://doi.org/10.1016/j.foodhyd.2016.04.036