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RESEARCH ARTICLE

Comparative Evaluation of Extrusion and Emulsification Techniques for Alginate-Based Microencapsulation of *Bifidobacterium longum*

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Comparative Evaluation of Extrusion and Emulsification Techniques for Alginate-Based Microencapsulation of Bifidobacterium longum

SUMMARY

Microencapsulation enhances probiotic viability by protecting cells during processing and storage. This study compared two alginate-based microencapsulation techniques, extrusion and emulsification, for the encapsulation of Bifidobacterium longum using 1% and 2% (w/v) sodium alginate. Probiotic cultures were encapsulated by extrusion into $CaCl_2$ solution or by emulsification in an oil phase followed by $CaCl_2$ addition and subsequently freeze-dried. The resulting microcapsules were evaluated for size, morphology, moisture content, hygroscopicity, encapsulation efficiency, and cell viability. Encapsulation efficiencies ranged from 94.4 ± 0.9% to 96.6 ± 2.2% for extrusion and from 87.7 ± 0.7% to 91.2 ± 0.8% for emulsification. The highest encapsulation efficiency (96.6 ± 2.2%) and viability (8.7 ± 2.2 log CFU/g) were achieved with the extrusion method using 1% sodium alginate. Compared with extrusion, emulsification produced slightly lower viability values of 7.9 ± 2.2 and 8.2 ± 2.0 log CFU/g at 1% and 2% sodium alginate, respectively, although all formulations maintained probiotic counts above the recommended minimum level of 10^7 CFU/g. Extrusion generated larger, more uniform spherical capsules, whereas emulsification produced smaller and less uniform particles. Increasing alginate concentration significantly increased capsule size while reducing hygroscopicity in both methods ($p < 0.05$). Moisture content ranged from 8.58–9.92 g/100 g for extrusion and 4.42–6.24 g/100 g for emulsification. Overall, both sodium alginate-based techniques effectively preserved B. longum viability. The physicochemical characteristics of the microcapsules indicate their suitability for incorporation into functional foods and pharmaceutical formulations, providing a promising approach for improving probiotic stability and delivery.

Keywords: *Bifidobacterium longum, extrusion, emulsification, microencapsulation*

Sodyum Aljinat Tabanlı Bifidobacterium longum Mikroenkapsülasyonunda Ekstrüzyon ve Emülsifikasyon Tekniklerinin Karşılaştırmalı Değerlendirilmesi

ÖZ

Mikroenkapsülasyon, probiyotik hücreleri üretim ve depolama sırasında koruyarak canlılıklarını artırmaktadır. Bu çalışmada, Bifidobacterium longum'un mikroenkapsülasyonu için %1 ve %2 (a/h) sodyum aljinat kullanılarak ekstrüzyon ve emülsifikasyon yöntemleri karşılaştırılmıştır. Probiyotik kültürler, $CaCl_2$ çözeltisine damlatılarak (ekstrüzyon) veya yağ fazında emülsifiye edilip ardından $CaCl_2$ ilavesiyle kapsüllenmiş ve liyofilize edilmiştir. Elde edilen mikrokapsüller; boyut, morfoloji, nem içeriği, higroskopiklik, enkapsülasyon verimi ve hücre canlılığı açısından değerlendirilmiştir. Enkapsülasyon verimi ekstrüzyon yöntemi için %94,4 ± 0,9 ile %96,6 ± 2,2, emülsifikasyon yöntemi için ise %87,7 ± 0,7 ile %91,2 ± 0,8 arasında değişmiştir. En yüksek enkapsülasyon verimi (%96,6 ± 2,2) ve canlılık (8,7 ± 2,2 log KOB/g), %1 sodyum aljinat kullanılan ekstrüzyon yöntemiyle elde edilmiştir. Emülsifikasyon yönteminde canlılık değerleri bir miktar daha düşük olmasına rağmen, tüm formülasyonlarda probiyotik sayıları önerilen minimum seviye olan 10^7 KOB/g değerinin üzerinde kalmıştır. Ekstrüzyon yöntemi daha büyük ve daha düzgün küresel kapsüller oluştururken, emülsifikasyon yöntemi daha küçük ve daha düzensiz partiküller üretmiştir. Aljinat konsantrasyonunun artırılması kapsül boyutunu artırırken higroskopikliği anlamlı olarak azaltmıştır ($p < 0,05$). Nem içeriği ekstrüzyon yöntemi için 8,58–9,92 g/100 g, emülsifikasyon yöntemi için ise 4,42–6,24 g/100 g arasında belirlenmiştir. Sonuç olarak, her iki yöntem de B. longum canlılığını etkili şekilde korumuş ve fonksiyonel gıda ile farmasötik uygulamalar için uygun fizikokimyasal özelliklere sahip mikrokapsüller sağlamıştır.

Anahtar Kelimeler: *Bifidobacterium longum, ekstrüzyon, emülsifikasyon, mikroenkapsülasyon*

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INTRODUCTION

Probiotics are "a group of live microorganisms that when consumed in adequate amounts, confer certain health advantages on the host," according to international agencies such as the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). The most common species used in commercial products include members of the *Lactobacillus* and *Bifidobacterium* genera. However, the health advantages have also been demonstrated mainly for specific probiotic strains of the following genera: *Bacillus*, *Enterococcus*, *Lactobacillus*, *Saccharomyces*, *Leuconostoc*, *Streptococcus*, and *Pediococcus* (Fijan, 2014; Krasaekoopt, Bhandari, & Deeth, 2006). Probiotics have proven to be useful in treating conditions including obesity, lactose intolerance, insulin resistance syndrome, lower blood cholesterol, type 2 diabetes, and non-alcoholic fatty liver disease in several clinical investigations. Moreover, the beneficial impacts of probiotics on human health have been established through their ability to enhance immunity. Scientific studies also highlight the advantages of using probiotics as a preventative measure against certain cancer models, intestinal infections, and irritable bowel syndrome (Asgari, Pourjavadi, Licht, Boisen, & Ajalloueian, 2020; Markowiak & Ślizewska, 2017). In order to benefit from their therapeutic effects, the viability count of probiotics must be at least 10^7 CFU/g at the time of consumption, and they should be able to survive the digestive processes (Rodrigues et al., 2011). Probiotics must remain alive to exhibit their potentially beneficial effects when they pass through the different parts of the upper gastrointestinal tract (mouth, stomach, and small intestine) before finally being released in the colon (Liu et al., 2019). For this reason, they must remain viable in food and beverage items during manufacturing, storage, and consumption.

Probiotics are available in various forms of delivery, including capsules, sachets, gels, pastes, powders/liquids, granules, and others, and they are used in a wide range of foods, dietary supplements, and pharmaceutical products (Saxelin, Tynkkynen, Mattila-Sandholm, & De Vos, 2005). However, there are many challenges associated with maintaining the high viability of probiotics in these products. Probiotics are known to be extremely sensitive to stress factors that may be present in commercial products, such as high oxygen levels, high temperatures, and water activities. Additionally, the components of gastrointestinal fluids, particularly gastric acids, bile salts, and digestive enzymes, may cause harm to them, thereby reducing their potency as probiotics (Kailasapathy & Chin, 2000). The increasing importance of microbiota in health has led to improved development of new types of oral probiotic delivery systems (Yao et al., 2020).

Microencapsulation technology is a process of entrapping an active compound into a wall material to produce capsules with a thin but strong, semipermeable membrane having diameters ranging from a few nanometers to millimeters. Probiotics are often encapsulated using various methods, including emulsification, extrusion, spray drying, freeze drying, supercritical-fluid-based techniques, electrospinning, coacervation, and liposomes. However, most of the research in this field has focused on using extrusion and emulsification methods. In this research, extrusion and emulsification methods were preferred because of their simplicity and adaptable steps. The extrusion process has several advantages, including being simple to perform, cost-effective, and operating under moderate conditions that ensure high cell viability.

In the extrusion method, a polymeric solution is first mixed with microbial cells and then extruded through an orifice as droplets into a solution using a syringe needle or nozzle of a cross-linking agent. Gelation occurs when the cross-linking agent meets the polymer solution, cools, or does both simultaneously (Brun-Graeppi, Richard, Bessodes, Scherman, &

Merten, 2011). Capsule size and sphericity can be adjusted based on operational parameters, such as needle diameter, distance from output to gel bath, concentration, temperature, viscosity, and flow rate of the biopolymer solution (Frakolaki, Giannou, Kekos, & Tzia, 2021). The emulsification technique involves dispersing the polymer suspension into a continuous phase, such as soybean, sunflower, or corn oil, and encapsulating the probiotics in the oil/water system. The capsule sizes used in emulsification processes have a diameter range of 25 μ to 2 mm. The capsule size can be controlled by adjusting the mixing speed and depends on the water/oil ratio (Chen & Chen, 2007).

Wall materials used for microencapsulation must possess certain favorable qualities. They should be safe for consumption, protect against environmental conditions, and also control the release of the encapsulated probiotics. Some common materials used for encapsulating probiotics include alginate, whey proteins, starch, chitosan, xanthan gum, gellan gum, and gelatin. Alginate is the most commonly used biopolymer for microencapsulation of probiotics using extrusion and emulsification techniques. Alginate is a naturally occurring polysaccharide consisting of a-L-guluronic acid and b-D-mannuronic acids and is present in many different types of algae. It has several advantages, including being nontoxic to cells, increasing viscosity, forming soft matrices with calcium chloride to protect cells, being low-cost, and enabling the release of confined cells at neutral pH, such as intestinal pH (Krasaekoopt, Bhandari, & Deeth, 2003).

In this study, extrusion and emulsification techniques were used to produce microcapsules using natural polymers of alginate, which were used to encapsulate *Bifidobacterium*. The structure, capsule size, encapsulation efficiency, cell viability, and release of encapsulated cells were evaluated for potential applications in the food, pharmaceutical, or biotechnological industries. Although both extrusion and emulsification methods have been widely investigated, limited comparative studies have systematically evaluated their physicochemical and stability-related properties under identical formulation conditions. Therefore, this study aims to provide a direct comparison of these two techniques using the same probiotic strain and alginate concentrations.

MATERIALS AND METHODS

Preparation of cells for microencapsulation

The lyophilized probiotic culture of *Bifidobacterium longum* was incubated in MRS broth at 37 °C for 48 hours under anaerobic conditions, which were provided by an anaerobic jar and gas-pack system. After the incubation period, the probiotic cells were separated by centrifugation at 2000 x g for 10 minutes at 4 °C, followed by washing with 10 mL of peptone water and another centrifugation step at 2000 x g at 4 °C for 10 minutes. The initial concentration of cells used in all experiments was approximately 10^9 CFU/g. The cells were subsequently subjected to microencapsulation or used as free cells.

Encapsulation of *Bifidobacterium longum* using extrusion technique

The microcapsules were prepared using an extrusion technique with different concentrations of sodium alginate (1% and 2% w/v) as the wall material, following the method described by Krasaekoopt, Bhandari and Deeth (2004). First, the sodium alginate solutions were prepared in distilled water and sterilized in an autoclave at 121 °C for 15 minutes. After cooling, 20 mL of *B. longum* cell cultures were mixed with 180 mL of the sterilized sodium alginate solutions at a 9:1 (v/v) ratio. The mixture was homogenized at 5,000 rpm for 2 minutes using a homogenizer, after which the resulting suspension was carefully introduced dropwise into a sterile 0.2 M $CaCl_2$ solution through a 0.11 mm needle under gentle stirring. The formed capsules were allowed to harden in the $CaCl_2$ solution for 30 minutes while shaking at 300 rpm. Subsequently, they were filtered using Whatman No. 4 filter paper, rinsed with sterile distilled water (Figure 1.), and stored at 4 °C until further use.

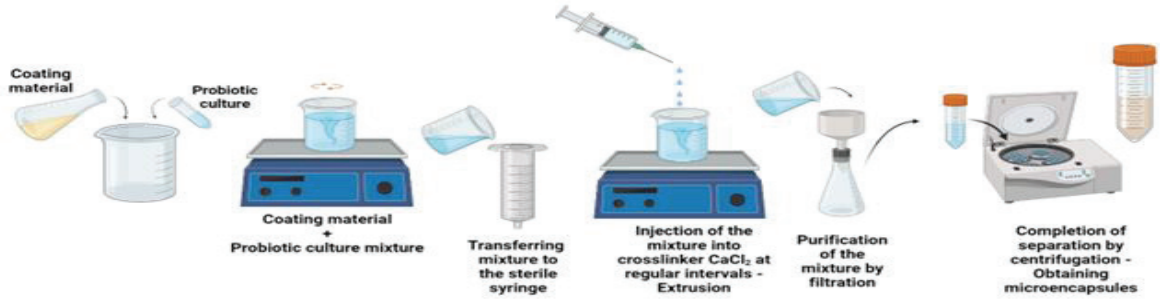


Figure 1. Extrusion method scheme

Encapsulation of *Bifidobacterium longum* using emulsification technique

The microcapsules were produced using the emulsification technique developed by Zanjani, Ehsani, Ghiassi Tarzi and Sharifan (2018). First, sodium alginate solutions were mixed with 20 mL of *B. longum* cell cultures for 5 minutes. The resulting mixture was then dropped into a sterile continuous phase containing 500 mL of sunflower oil with 2% Tween 80 emulsifier, using a 0.11-mm needle. The mixture was magnetically stirred at 350 rpm for 20 minutes to obtain a uniform emulsion. Subsequently, 0.1 M $CaCl_2$ was added to initiate microcapsule formation, and the reaction was maintained under continuous stirring at 400 rpm for 30 minutes until encapsulation was complete. Afterward, the oil phase was carefully removed, and the capsules were separated from the $CaCl_2$ solution by centrifugation at 324 × g for 10 minutes. Finally, the microcapsules were rinsed twice with sterile distilled water (Figure 2.) and stored at 4 °C until further analysis.

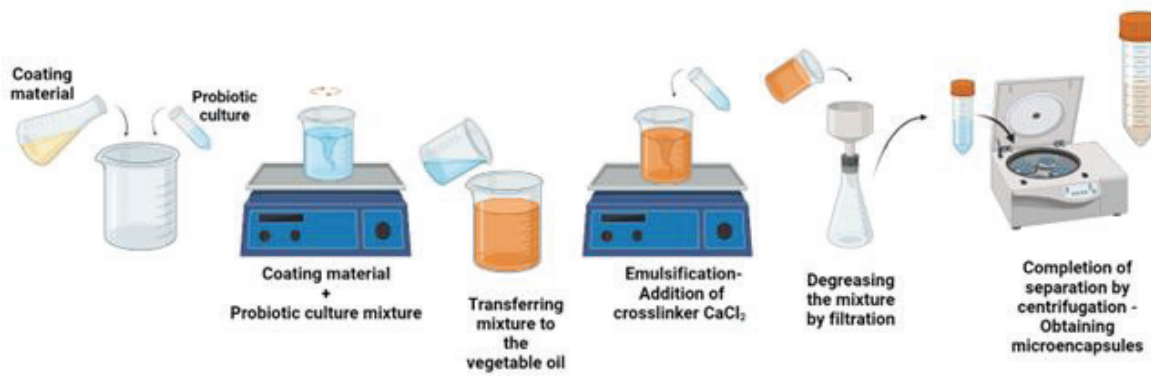


Figure 2. Emulsification method scheme

Freeze drying

The microcapsules were frozen at −20 °C for 24 h before freeze-drying. Lyophilization was carried out for 28 h using a programmed drying cycle, with a condenser temperature of −85 °C, a shelf temperature of −60 °C, and a chamber pressure maintained at 100 mTorr. During the process, the condenser pressure was monitored and maintained within the range of 50–60 mTorr.

***Bifidobacterium longum* count in the microcapsules**

The determination of the entrapped microcapsules was performed according to the method proposed by Chandramouli, Kailasapathy, Peiris and Jones (2004) with modifications. To count the bacteria entrapped in microcapsules, 0.1 g of prepared capsules were re-suspended to 9 ml of a sterile solution of phosphate buffer (0.1 M and pH 7) and homogenized in a

stomacher for 7 min at room temperature until the bacteria were released into the sterile phosphate buffer solution. The survival of viable *B. longum* was determined by plate-counting method on MRS agar. Serial dilutions were prepared in 9 mL of 0.1% peptone water and inoculated in MRS agar to count the *B. longum*. Cultures were subsequently incubated for 48 h at a temperature of 37 °C under anaerobic conditions using anaerobic jar. After the incubation period the viable *B. longum* were counted, and results were given as colony forming units per gram (log CFU/g).

Encapsulation efficiency

The encapsulation efficiency (EE%), which is the survival ratio of the *B. longum* during the microencapsulation procedure, was calculated according to Eq. (1) (Holkem et al., 2016; Martin, Lara-Villoslada, Ruiz, & Morales, 2013).

$$EE\ \% = \text{Log } N / \text{Log } N_0 \times 100 \quad (1)$$

N is the cell number of viable cells released from the microcapsules (log CFU/g) and N_0 is the number of viable cells (log CFU/g) in the cell concentrate used for microencapsulation.

Characterization of the microcapsules

The morphology and sizes of the microcapsules obtained by extrusion and emulsification processes were evaluated using an optical microscope equipped with a digital microscope. For the determination of diameter, each measurement received a random selection of microcapsules containing 20 capsules. The diameter of the microcapsules was determined in micrometers using a digital micrometer and ocular micrometer.

Moisture content

One gram of the sample was placed in a container and dried in a hot air oven at 105 ± 2 °C until reaching a constant weight. The experiments were carried out in triplicate, and the average values were taken.

Hygroscopicity

The hygroscopicity was performed according to the method proposed by Fritzen-Freire et al. (2012). One gram of capsules was placed in a desiccator containing a saturated sodium chloride solution (relative humidity 75%) at 25 °C. After one week, the samples were weighed, and the hygroscopicity was measured as the amount of moisture absorbed by 100 g of capsules on a dry weight basis.

$$\text{Hygroscopicity } \% = mf - mi \,/\, mi \times 100\% \quad (2)$$

m_i and m_f express the moisture of the samples before and after storage at 75% relative humidity.

Statistical analysis

All experiments were carried out in triplicate, and data were presented as mean ± standard deviation (SD). Statistical evaluation was conducted using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test, implemented in GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA). A p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Encapsulation efficiency and viability

Probiotic microorganisms are sensitive to various environmental and processing conditions, including oxygen exposure, pH, relative humidity, and shear stress. In this study, both the encapsulation method and sodium alginate concentration significantly affected encapsulation efficiency and cell viability ($p < 0.05$) (Table 1, Figure 3.). Following microencapsulation, the viable cell counts of B. longum remained above 10^6 CFU/g, satisfying the minimum recommended level for probiotic products. Specifically, for the extrusion method, viability values were 8.7 ± 2.2 log CFU/g and 8.5 ± 2.0 log CFU/g at 1% and 2% sodium alginate concentrations, respectively. In contrast, for the emulsification method, viability values were 7.9 ± 2.2 log CFU/g and 8.2 ± 2.0 log CFU/g at 1% and 2% sodium alginate concentrations, respectively (Figure 3a.). Encapsulation efficiency (EE) val-

ues also varied depending on the method and alginate concentration. The extrusion method resulted in higher EE values of 96.6 ± 2.2% and 94.4 ± 0.9% at 1% and 2% sodium alginate concentrations, respectively. In comparison, the emulsification method yielded EE values of 87.7 ± 0.7% and 91.2 ± 0.8% under the same conditions (Table 1, Figure 3b.). A slight decrease in both viability and encapsulation efficiency was observed with increasing alginate concentration in the extrusion method. Conversely, in the emulsification method, an increase in both viability and encapsulation efficiency was noted with higher alginate concentration. These results suggest that the response of microcapsules to alginate concentration is method-dependent. The higher encapsulation efficiency observed in extrusion may be attributed to the milder processing conditions and reduced mechanical stress, which help preserve cell integrity. In contrast, emulsification involves exposure to shear forces and oil phases, which may adversely affect cell viability and encapsulation performance. These findings are in agreement with previous studies reporting high encapsulation efficiency for extrusion-based systems (Vodnar et al., 2010) and comparable results for emulsification techniques (Holkem et al., 2016; Motalebi Moghanjougi, Rezazadeh Bari, Alizadeh Khaledabad, Amiri, & Almasi, 2021). However, variations in encapsulation efficiency reported in the literature, such as the lower values observed by Chávarri et al. (2010), indicate that encapsulation performance is strongly influenced by formulation and process parameters, including polymer concentration, wall material properties, calcium chloride concentration, microorganism type, and particle size (Chávarri et al., 2010). The results demonstrate that B. longum cells were successfully encapsulated with high viability and efficiency, confirming that both extrusion and emulsification methods are suitable for probiotic microencapsulation, with extrusion showing superior performance under the tested conditions.

Table 1. Probiotic viability and encapsulation efficiency results of extrusion and emulsification methods

	Extrusion		**Emulsification**	
Concentrations	**Encapsulation Efficiency (EE%)**	**Viability (log CFU/g)**	**Encapsulation Efficiency (EE%)**	**Viability (log CFU/g)**
1% SA	96.6±2.2	8.7±2.2	87.7±0.7	7.9±2.2
2% SA	94.4±0.9	8.5±2.0	91.2±0.8	8.2±2.0

* SA (sodium alginate)

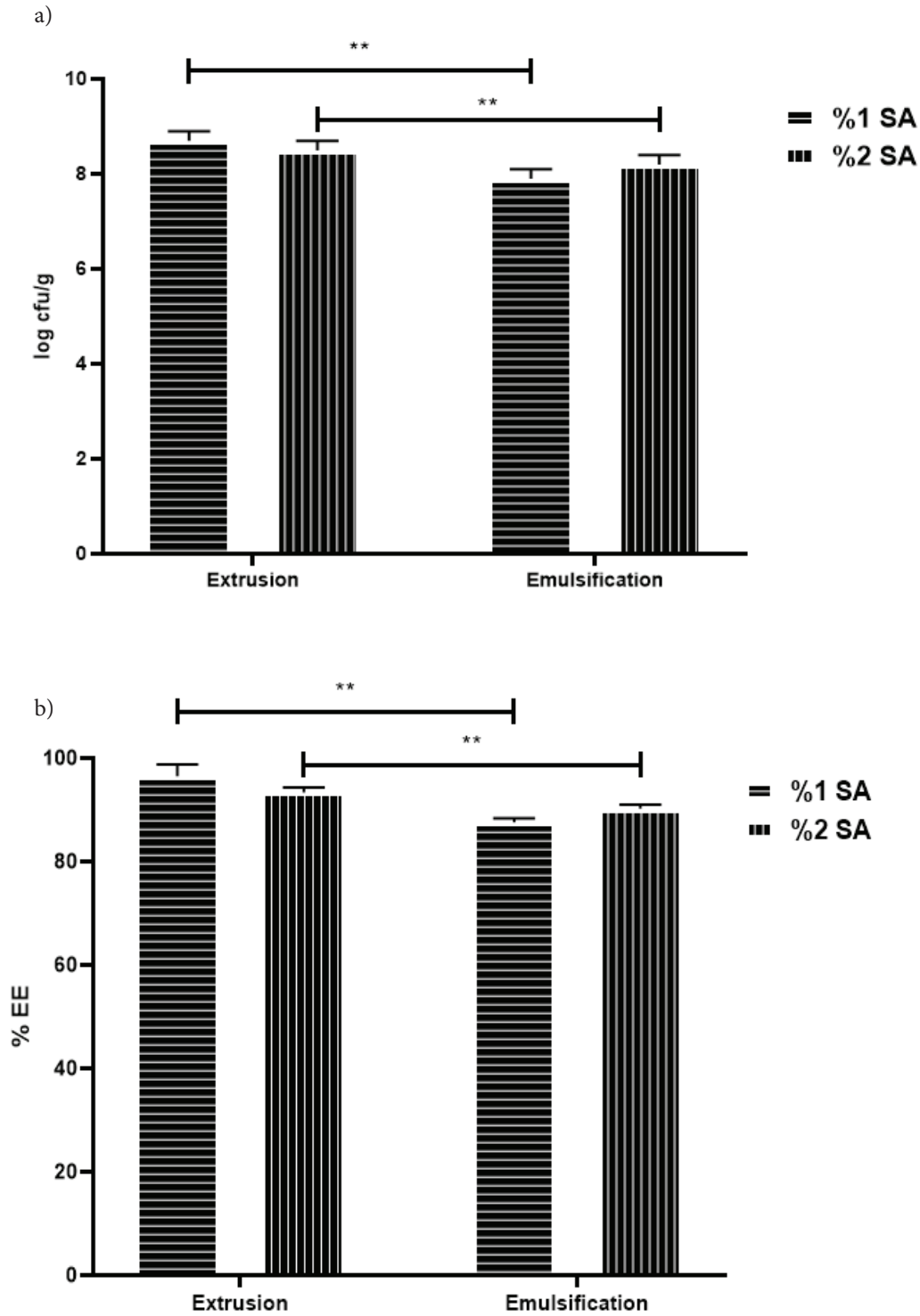


Figure 3. Microencapsulated a) probiotic viability (log CFU/g) and b) encapsulation efficiency (%EE) after extrusion and emulsification methods

Physical properties of the microcapsules

An optical microscope equipped with a digital camera was used to evaluate the morphology and particle size of Bifidobacterium longum microcapsules. The capsule size distributions obtained by extrusion and emulsification methods at different sodium alginate concentrations are presented in Table 2. For the extrusion method, the average capsule diameter was 1.48 ± 0.30 mm at 1% sodium alginate and increased to 1.62 ± 0.26 mm at 2% sodium alginate. Similarly, for the emulsification method, the average diameter increased from 0.49 ± 0.14 mm at 1% sodium alginate to 0.60 ± 0.21 mm at 2% sodium alginate. The size ranges were 1.5 ± 0.7 mm and 2.0 ± 0.8 mm for extrusion, and 0.60 ± 0.50 mm and 1.0 ± 0.96 mm for emulsification at 1% and 2% sodium alginate concentrations, respectively (Table 2.). The capsules produced by the emulsification method were considerably smaller than those obtained by extrusion. In addition, optical microscopy revealed that extrusion produced more regular and spherical capsules, whereas emulsification resulted in irregular and less uniform structures. The increase in capsule size with higher sodium alginate concentration was statistically significant for both methods ($p < 0.05$), which can be attributed to the increased viscosity of the alginate solution, leading to the formation of larger droplets during encapsulation. The relatively small capsule sizes observed in this study may be related to the use of a 22G injection needle with an inner diameter of 0.11 mm during the extrusion process. Previous studies have reported much smaller capsule sizes in emulsification systems, such as 19 μm and 67 μm (Hansen, Allan-Wojtas, Jin, & Paulson, 2002; Holkem et al., 2016), whereas extrusion-based methods typically yield larger capsules, ranging from 2 to 4 mm (Muthukumarasamy, Allan-Wojtas, & Holley, 2006). Capsule size is an important physical parameter that can influence the sensory properties of food products, including texture and mouthfeel. In food applications, particle sizes below 100 μm are generally preferred to avoid negative sensory perception. However, in the present study, the obtained capsule sizes are within the expected range for extrusion-based systems and remain suitable for controlled incorporation into functional formulations.

Table 2. The capsule size (mm) formed with different alginate concentrations by extrusion and emulsification methods

Extrusion		Emulsification		
Concentrations	Size Range	Average Diameter	Size Range	Average Diameter
1% SA	1.5±0.7	1.48 ± 0.30	0.60±0.50	0.49±0.14
2% SA	2.0±0.8	1.62 ± 0.26	1.0±0.96	0.60 ± 0.21

* SA (sodium alginate)

The moisture content of microcapsules ranged from 4.42% to 9.92%, increasing with higher alginate concentrations. This result can be attributed to the hydrophilic nature of alginate, which contains abundant hydroxyl and carboxyl groups capable of forming hydrogen bonds with water molecules. As the alginate concentration increases, the number of available binding sites within the polymer matrix also increases, leading to greater retention of bound water. This finding is consistent with previous studies reporting that higher polymer concentrations in alginate-based systems result in increased moisture content. From a stability perspective, this increase in moisture content highlights the importance of maintaining a balanced formulation. While moderate moisture levels contribute to maintaining matrix integrity and protecting encapsulated cells, excessive moisture may enhance molecular mobility and accelerate degradation reactions, ultimately reducing probiotic viability during storage. Conversely, very

low moisture levels may induce cellular stress and negatively impact survival (Tripathi & Giri, 2014). Therefore, the observed moisture content range in this study suggests that alginate concentration plays a critical role in determining water retention properties and, consequently, the stability of microcapsules. These findings emphasize the need to optimize alginate levels in order to achieve an appropriate balance between structural protection and long-term stability of encapsulated probiotics.

Hygroscopicity reflects the ability of microcapsules to absorb moisture from the surrounding environment and is a critical parameter influencing the stability of encapsulated microorganisms during storage. This property is mainly governed by the presence of hydrophilic functional groups within the polymer matrix and the structural characteristics of the microcapsules (Anal & Singh, 2007; Desmond, Ross, O'Callaghan, Fitzgerald, & Stanton, 2002). Lower hygroscopicity is generally desirable, as excessive moisture uptake may negatively affect the stability of probiotic formulations (Tripathi & Giri, 2014). As shown in Table 3, both the production method and sodium alginate concentration significantly affected hygroscopicity ($p < 0.05$). Microcapsules produced by the extrusion method exhibited higher hygroscopicity values of 16.32 ± 0.62 g/100 g and 14.11 ± 0.55 g/100 g at 1% and 2% sodium alginate concentrations, respectively. In contrast, microcapsules obtained by emulsification showed lower hygroscopicity values of 10.48 ± 0.49 g/100 g and 8.14 ± 0.89 g/100 g under the same conditions. A decrease in hygroscopicity was observed with increasing sodium alginate concentration for both methods ($p < 0.05$). This reduction can be attributed to the formation of a denser and more compact polymeric network at higher alginate concentrations, which limits the diffusion of water molecules into the microcapsule structure (Chen & Chen, 2007; Lee & Mooney, 2012). Additionally, the lower hygroscopicity observed in emulsification-derived microcapsules may be associated with the presence of a residual oil layer on the capsule surface, which acts as a barrier to moisture absorption (Krasaekoopt et al., 2003). These results indicate that both formulation and production method play a key role in determining hygroscopic behavior, with emulsification providing improved resistance to moisture uptake compared to extrusion.

Table 3. The hygroscopicity and moisture content of capsules formed with different concentrations of alginate by extrusion and emulsification methods

	Extrusion		Emulsification	
Concentrations	**Hygroscopicity (g/100g)**	**Moisture content (g/100g)**	**Hygroscopicity (g/100g)**	**Moisture content (g/100g)**
1% SA	16.32 ±0.62	8.58	10.48 ±0.49	4.42
2% SA	14.11 ± 0.55	9.92	8.14 ±0.89	6.24

*SA (sodium alginate)

CONCLUSION

This research shows that B. longum can be successfully microencapsulated using sodium alginate via two different techniques, extrusion and emulsification, each providing distinct advantages. Encapsulation using 1% sodium alginate via extrusion resulted in the highest probiotic viability (8.7 ± 2.2 log CFU/g) and encapsulation efficiency (96.6 ± 2.2%), along with the formation of uniform and spherical capsules. In comparison, the emulsification method produced smaller capsules (0.49 ± 0.14–0.60 ± 0.21 mm) with slightly lower, yet still high, encapsulation efficiency (87.7 ± 0.7–91.2 ± 0.8%) and viability (7.9 ± 2.2–8.2 ± 2.0 log CFU/g). These microcapsules also exhibited lower hygroscopicity (8.14 ± 0.89–10.48 ± 0.49 g/100 g), likely due to the presence of a residual oil layer on the capsule surface. Increasing sodium alginate concentration from 1% to 2% resulted in an increase in capsule size (from 1.48 ± 0.30 to 1.62 ± 0.26 mm for extrusion and from 0.49 ± 0.14 to 0.60 ± 0.21 mm for emulsification), as well as a mod-

erate increase in moisture content (from 8.58 to 9.92 g/100 g for extrusion and from 4.42 to 6.24 g/100 g for emulsification), while significantly decreasing hygroscopicity in both methods ($p < 0.05$). All microcapsule formulations maintained probiotic counts above the recommended threshold of 10^6 CFU/g, confirming the effectiveness of these mild encapsulation techniques in preserving cell viability. From a practical perspective, alginate-based microcapsules offer a cost-effective and scalable strategy for probiotic delivery. The obtained microcapsules can protect B. longum during processing and storage, making them suitable for incorporation into functional foods such as dairy products and beverages, as well as for use in pharmaceutical formulations. The findings provide a strong basis for improving probiotic stability and enhancing their practical applicability. These results highlight the potential of the developed systems as oral probiotic delivery platforms within pharmaceutical dosage forms. Future studies are required to validate these outcomes, particularly by optimizing capsule size and incorporating additional coating layers to improve resistance to environmental stress conditions. Furthermore, the integration of these microencapsulated probiotics into functional foods and pharmaceutical formulations is expected to be confirmed in future studies for their contribution to gut health and overall well-being.

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AUTHOR CONTRIBUTION STATEMENT

Developing hypothesis (PS, AAÖ), experimenting and statistics (PS), study text preparation, literature research (PS), data interpretation, and reviewing (PS, AAÖ).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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