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Probiotics-encapsulated soy beverage spheres: Functional and technological characteristics

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Abstract

Soy beverage is a healthy and flavorful drink that can be a lactose-free dairy substitute. In this work, probiotic *Bifidobacterium animalis* BB-12 in soy-based beverages was encapsulated, and the viability of the microorganisms was evaluated under different conditions. Spheres were obtained by dissolving sodium alginate and xanthan gum in a grape-flavored soy beverage and cross-linked with calcium lactate by external ionic gelation. The condition for alginate 1.125% m v^{-1} and xanthan gum 0.50% m v^{-1} was selected for the optimal formation of the probiotic spheres by external ionic gelation. The encapsulated probiotics showed persistence at different pH values (2–5). The storage conditions of the spheres in soy beverage resulted in a microbial viability of 8 log CFU g^{-1} for 30 days. The findings reported in this work may be useful in the design and formulation of probiotic soy beverages.

Keywords: encapsulation; xanthan gum; alginate; *Bifidobacterium animalis* BB12; soy beverage.

Practical application: Probiotic spheres showed viability for 30 days.

1 INTRODUCTION

Previous studies have shown that a diet rich in probiotics can boost the immunity of the human body (immunomodulation) and inhibit the growth of pathogens capable of harming the host organisms (Marchesin et al., 2018). Moreover, the use of probiotics has been related to prophylactic benefits in different types of cancer and cancer-associated side effects (Juan et al., 2022; Liu et al., 2023; Vinceković et al., 2017). Other advances in the area of functional foods and nutraceuticals have also been reported (Campos et al., 2019; Morais et al., 2019; Nguyen et al., 2019). Probiotics are classified as live microorganisms that, when administered in adequate amounts per day, can bring benefits to the host. They are from the genera *Lactobacillus, Bifidobacterium, Lactococcus, Streptococcus,* and *Enterococcus* (Paludo et al., 2021; Vinceković et al., 2017). Benefits are achieved when probiotics survive the gastrointestinal transit, which is a challenge for the food industry since temperature and oxygen variations can influence cell survival.

Soy-based foods have a stimulating effect on the growth of some bacterial strains, such as those of the *Bifidobacterium* genus, because these foods contain oligosaccharides that are used as a nutritional source for probiotics (Rasika et al., 2021). The fermentation process with bacterial cells increases protein content, improves protein solubility, amino acid composition, and availability, and optimizes the probiotic viability over time (Bastida et al., 2023). Moreover, soy beverages can be a good source of plant proteins and are rich in isoflavones, which promote health benefits such as the prevention of osteoporosis and cardiovascular diseases (Xu et al., 2022).

Since probiotics must survive gastrointestinal transit and adhere to and colonize the intestinal mucosa, encapsulation technology may be an alternative to protect probiotics in food formulations (Vivek et al., 2023). External ionic gelling is an attractive encapsulation technique because it is low in cost and uses no organic solvent or high temperature (Alexandre et al., 2024; Kurozawa & Hubinger, 2017). Studies report that alginate is one of the most commonly used in the encapsulation of probiotics (Li et al., 2023; Tan et al., 2022; Yuan et al., 2022) due to its biocompatibility and non-toxicity. Alginate is a natural polymer capable of gelling when divalent cation ions (e.g., Ca^{2+}) bind to the polyuronate blocks of alginate chains. The polyuronate blocks of a polymer then form junctions with the polyuronate blocks adjacent to the polymer chains (called the egg-box crosslinking model), resulting in a stable gel structure. However, the stability of this structure can be improved by combining it with other polymers, such as xanthan gum (Ta et al., 2021). Xanthan gum is a polysaccharide used as a common stabilizing and emulsifying agent in food and pharmaceutical products (Cofelice et al., 2023).

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This polymer can be used to reinforce calcium-alginate granules (Pongjanyakul & Puttipipatkhachorn, 2007). The application of two polymers as wall materials in optimal proportions provides greater resistance to the medium and can delay the release of encapsulants.

In this study, probiotic spheres of *Bifidobacterium* BB-12 made with alginate (AG) and xanthan gum (XG) were formed in soy beverages to provide better conditions for probiotic viability.

2 MATERIALS AND METHODS

2.1 Materials

Bifidobacterium animalis BB-12 strains were from Chr. Hansen Inc. (Windsor, WI, USA). Sodium alginate (MW: 405.21 g mol^{-1}) and calcium lactate were obtained from Exodus Scientific (West Palm Beach, FL, USA). XG (MW: 933.75 g mol^{-1}), sodium citrate, and hydrochloric acid were purchased from Chemical Dynamics, Inc. (Plant City, Fl, USA). Grape-flavored soy beverage was purchased in local supermarkets in Brazil.

2.2 Preparation of probiotic inoculum for encapsulation

The probiotic culture of *B. animalis* BB-12 was grown in three steps in MRS broth (from de Man, Rogosa, and Sharpe) with 0.1% (v v^{-1}) cysteine 10% (m v^{-1}) at 37°C for a total of 56 h. Each step was performed under anaerobic conditions. Then, the cells were centrifuged at 5.509 × *g* for 15 min at 5°C and washed with a phosphate buffer solution (1.0 M, pH 7.4). Cells were resuspended in 10% maltodextrin solution (m v^{-1}) and 10% sucrose (m v^{-1}) to obtain a 10 log CFU mL^{-1} solution. Finally, the suspension was aliquoted (1 mL) in Eppendorf tubes and frozen at -80 °C.

2.3 Formation of probiotic spheres by external gelation

The process of encapsulation was performed according to Tanganurat (2020), with adaptations. Alginate and XG were mixed in 100 mL of soy beverage until complete dissolution at approximately 60°C. After cooling to room temperature (25°C), 1 mL of the probiotic culture (10 log CFU mL^{-1}) was added to the polymeric solution and agitated using a magnetic stirrer. The solution was extruded dropwise with a 120 µm drip nozzle into a 1% calcium lactate solution (m v^{-1}) and stirred for 2 min. The spheres were washed with distilled water to remove excess salt and stored at 4°C. Probiotic culture encapsulated without soy beverage (i.e., probiotic dispersed in water) and free probiotic were used as controls.

2.4 Experimental design

A rotational central composite design (RCCD) was used (Table 1) for the formation of probiotic spheres; the independent variables were AG and XG (%, m v^{-1}), while the response variables were probiotic viability, encapsulation efficiency, particle size, sphericity, and dynamic viscosity. The design consisted of 11 treatments with four factorial points at two levels (2^2), four axial points, and three central points.

2.5 Characterization of probiotic spheres

2.5.1 Viscosity of polymeric solutions

The viscosity of the polymeric solutions to form spheres was analyzed using a rheometer (Haake Mars III, Thermo Scientific). The polymeric solution was loaded into the conical plate geometry (C60° Ti L) with a 0.052 mm gap at 25°C. The samples were conditioned for 1 min at 25°C to recover the structure, and the readings were performed in 2 min intervals at a frequency of 0.5 Hz.

2.5.2 Encapsulation efficiency

The inoculum standardization curve was established by relating the absorbance measurements using a spectrophotometer (model Anthos Zenith 200rt) with probiotic counts on MRS–cysteine agar plates. The standardization curve is represented in Equation 1:

$$CFU = 0.0099 \ln(x) - 0.0861; \quad R^2 = 0.90 \tag{1}$$

The encapsulation efficiency (EE) was known from the results obtained in the number of probiotic cells using Equation 2:

$$EE = \frac{N0}{N} \times 100 \tag{2}$$

Where:

N_0: the number of cells after encapsulation;

N: the number of cells before encapsulation.

2.5.3 Particle size distribution and sphericity

Particle size was determined by measuring the transverse and longitudinal diameters of 20 spheres with a micrometer

Table 1. Coded values and uncoded values (observed experimental values) of the independent variables, alginate and Xanthan gum, according to the RCCD for preparation of polymeric solutions.

	Coded values		Decoded values	
Treatment	**Alginate**	**Xanthan gum**	**Alginate (%, m.v^{-1})**	**Xanthan gum (%, m.v^{-1})**
T1	-1	-1	0.86	0.15
T2	+1	-1	1.39	0.15
T3	-1	+1	0.86	0.43
T4	+1	+1	1.39	0.43
T5	-1.41	0	0.75	0.25
T6	+1.41	0	1.50	0.25
T7	0	-1.41	1.125	0
T8	0	+1.41	1.125	0.50
T9	0	0	1.125	0.25
T10	0	0	1.125	0.25
T11	0	0	1.125	0.25

(Mitutoyo). The average size was calculated using the Ferret diameter (Zanetti et al., 2002). The degree of sphericity of the particles was determined by using the Riley method (Riley, 1941).

2.6 Viability of free and encapsulated probiotics

The viable probiotic count was determined by plating on MRS–cysteine agar. In total, 1 g of the spheres was dispersed in 9 mL of 2% sodium citrate solution at 50 ± 1°C to completely dissolve them. A volume of 0.1 mL of the dissolved material was inoculated in plates and incubated at 37°C ± 1°C for 48 h. For the evaluation of the influence of the soy beverage, samples of probiotic spheres in water were tested as a control under the same conditions. Free probiotic was also used as a control.

2.7 Resistance of encapsulated probiotics at different pH

The effect of pH on probiotic survival was evaluated according to Silva et al. (2018), with adaptations. Solutions containing 2% sodium citrate (m v^{-1}) were adjusted to pH 2, 3, 4, and 5 using 2 M hydrochloric acid. Approximately 1 g of probiotic bubble was added to 9 mL of each solution. Free and water-encapsulated probiotics were used as controls. Probiotic resistance was evaluated by counting after 1 h of exposure to pH. Probiotics were counted according to Section 2.6.

2.8 Evaluation of probiotic viability during storage

The survival of *B. animalis* BB-12 in spheres was evaluated for 0, 2, 5, 10, 15, and 30 days at 8°C stored in (a) reverse osmosis water (pH 7.0), (b) in soy beverage (pH 3.9), and (c) without liquid. Probiotics were counted according to Section 2.6.

2.9 Statistical analysis

The response surface and analysis of variance were performed using the Statistica® software (StatSoft version). The regressions were evaluated for their determination coefficients (R^2) and statistical significance ($p < 0.05$).

3 RESULTS AND DISCUSSION

3.1 Probiotic spheres formation

During the formation of spheres by ionic gelation, the sodium alginate interacts with the divalent ions present in the crosslinking solution. These ions bind to the carboxyl groups of guluronate blocks present in alginate. Despite forming a strong gel, alginate spheres can have a porous structure, facilitating the diffusion of some molecules inside or outside them and affecting the probiotic viability (Li et al., 2023; Noor et al., 2022). This problem can be solved with the incorporation of another biopolymer into the polymeric solution, such as XG.

Alginate is made up of mannuronic and guluronic acid residues, while XG contains mannose, glucose, and glucuronic acid. The intermolecular interactions between these acids and the constituent hydroxyls of the monosaccharides make the structure of the spheres more compact (Cai et al., 2019; Wen et al., 2022) and can better retain the probiotics within the spheres. Thus, the role of XG as alginate reinforcement is essential to increase sphere persistence. Nsengiyumva and Alexandridis (2022) observed that the performance of XG is based on its macromolecular conformation. In aqueous media, XG undergoes conformational transitions from helix to random spiral in response to some stimuli such as pH, ionic strength, and temperature (Nsengiyumva & Alexandridis, 2022).

Table 1 lists the polymer proportions used for the 11 treatments of the RCCD. The response variables of EE, viscosity, particle size, and sphericity were studied in this work, but according to ANOVA only viscosity and EE were significant ($p < 0.05$) (supplementary material). Despite the significant variances found, it was not possible to establish a mathematical model for these variables, as the model fit was lower than 90% (supplementary material).

3.2 Characterization of the probiotic sphere

3.2.1 Encapsulation efficiency

The EE represents the efficiency of the wall materials to hold or encapsulate the molecules of interest within the particle. In this study, the spheres presented EE ranging between 50. and 87.5% (Figure 1A). Higher concentrations of XG did not contribute to higher EE (Figure 1B). However, higher concentrations of alginate led to a higher EE. This happens because there is a greater availability of active sites in the chains of alginate to bind to Ca^{2+}, resulting in a greater degree of crosslinking. Similar results were found by Farahmand et al. (2022). The authors encapsulated probiotics with alginate by ionic gelation and complexed the spheres with chitosan.

Furthermore, high concentrations of alginate result in greater viscosity of the polymeric solution. In contrast, under the conditions of lower viscosity of the polymeric solution, the extrusion dripping movement is facilitated, and a high leakage occurs in the gelation step that plays a role in reducing the EE.

3.2.2 Particle size distribution and sphericity

Physical characterization of the spheres is very important for an understanding of the probiotic release and viability. Particle size can affect the solubility, storage stability, and release of the core (Jang & Koh, 2023). The particle size varied between 4.25 ± 0.15 and 5.57 ± 0.25 mm, and the sphericity varied between 0.94 ± 0.04 and 0.98 ± 0.01 (Table 2). In this work, a tendency of smaller size and greater dispersion was observed with lower levels of XG and alginate, although a significant difference ($p > 0.05$) was not found.

These results of particle size are directly related to the viscosity of the treatments. In the case where the polymeric solution had a higher viscosity, there was a tendency for greater uniformity between the particles obtained. Sphericity close to 1.0 (Table 2) is considered a perfect sphere and can help to prevent bacterial overgrowth in encapsulated granules (Tanganurat, 2020).

3.2.3 Viscosity of polymeric solutions

The combination of polysaccharides is a strategy to create functional materials with synergistic properties and low cost (Kondaveeti et al., 2022), providing the formation of a complex matrix that could help in the release and viability of probiotics. However, a study of the proportion of each polymer needs to be realized to achieve the maximum potential of the combination.

There was a significant difference between the viscosity parameter ($p \leq 0.05$) of the polymeric solutions of alginate and XG. The dynamic viscosity found varied from 212.4 Pa.s (T3) to 1670.8 Pa.s (T8) (Figure 2). These values were higher with the increase in the percentage of XG, which can be explained by the presence of hydrogen bonds formed between XG and alginate, as reported in other reports, too (Cai et al., 2019; Wen et al., 2022).

During encapsulation, the treatments that presented lower viscosities, and therefore more fluidity, were easily dripped into the reticulant. However, this resulted in greater heterogeneity of the spheres, while those with higher viscosities were dripped more slowly, generating greater homogeneity of the spheres. Khoshdouni Farahani et al. (2023) encapsulated jujube extract with alginate and gellan gum and noticed that these biopolymers increase the binding capacity with water and reduce the flow rate, forming a stronger network of gels.

In view of the results of physical and physicochemical characteristics found in this work, the T8 treatment (alginate 1.125% m v^{-1} and XG 0.50% m v^{-1}) was chosen as the best condition for the spheres formation. This treatment showed good EE, viscosity, and homogeneity in particle size and sphericity, and exhibited attractive sensory characteristics for its application in new probiotic food products.

3.3 Persistence of encapsulated probiotics at different pH values

With the chosen treatment, the encapsulated probiotics were studied in soy beverage and water at different pHs (Figure 3).

After 5 min, it was possible to verify that the spheres with probiotics formed in soy beverage, water, and free cells exhibited similar behavior, with a viability of 8 log CFU g^{-1} (Figure 3). After 60 min of immersion in sodium citrate solution under different pH levels, the spheres formed in soy beverage were preserved and maintained their viability at 8 log CFU g^{-1}. However, the free cells underwent a loss of 2 log CFU g^{-1} at pH 2, while the spheres formed in water lost 1 log CFU g^{-1}. Thus, even under stressful conditions, there was greater persistence of probiotics encapsulated in soy beverage. This result is especially interesting for the soy-based beverage market. These soy beverages contain oligosaccharides that are used as a nutritional source for probiotics (Bastida et al., 2023; Rasika et al., 2021).

On the basis of the present results, the encapsulation promoted greater probiotic protection in acidic conditions, even for the treatments in which spheres were formed in water. Chotiko and Sathivel (2016) reported the positive influence of encapsulation on probiotic survival in acidic conditions. The authors

Table 2. Experimental responses for particle size and sphericity of probiotic spheres.

Treatment (AG+XG)	Particle size (mm)	Sphericity
T1 (0.86+0.15)	4.25 ± 0.15	0.97 ± 0.02
T2 (1.39+0.15)	4.63 ± 0.09	0.96 ± 0.02
T3 (0.86+0.43)	4.65 ± 0.27	0.94 ± 0.04
T4 (1.39+0.43)	4.89 ± 0.25	0.97 ± 0.02
T5 (0.75+0.25)	5.12 ± 0.26	0.96 ± 0.04
T6 (1.50+0.25)	4.97 ± 0.11	0.98 ± 0.01
T7 (1.125+0)	5.57 ± 0.25	0.95 ± 0.03
T8 (1.125+0.50)	5.46 ± 0.15	0.97 ± 0.02
T9 (1.125+0.25)	5.39 ± 0.20	0.97 ± 0.02
T10 (1.125+0.25)	5.30 ± 0.16	0.97 ± 0.02
T11(1.125+0.25)	5.30 ± 0.19	0.98 ± 0.02

AG: alginate; XG: xanthan gum.

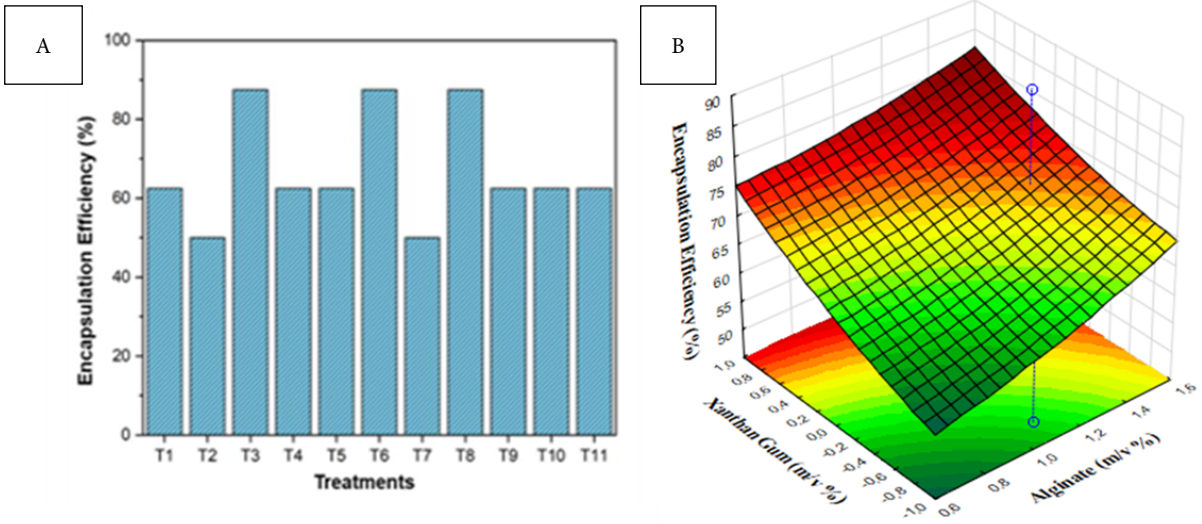

Figure 1. (A) Response surface with decoded values of alginate and xanthan gum for encapsulation efficiency of probiotic spheres. (B) Experimental response for encapsulation efficiency of probiotic spheres.

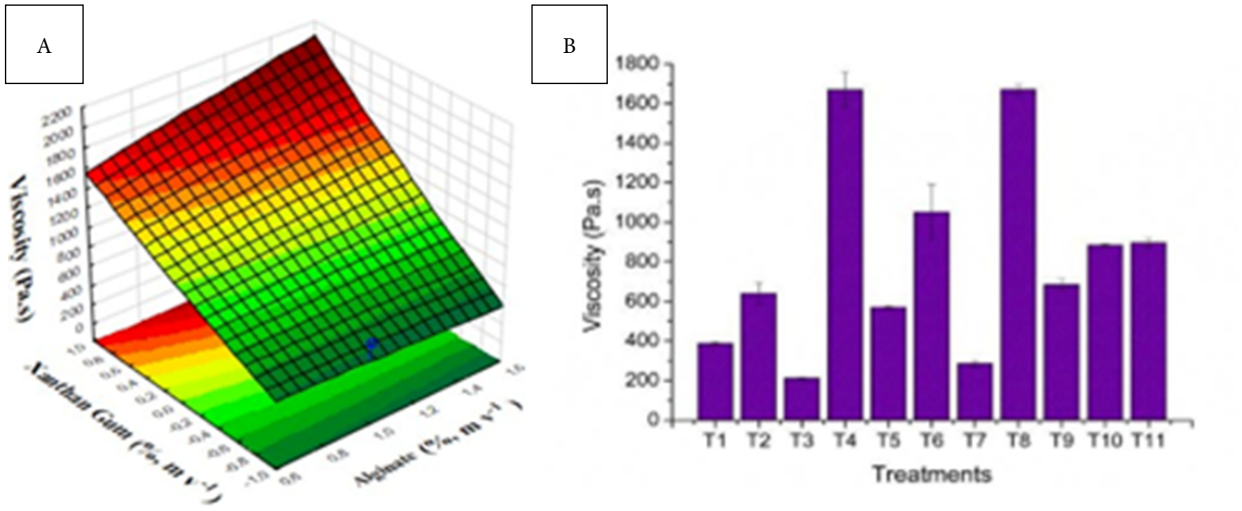

Figure 2. (A) Response surface with decoded values of alginate and xanthan gum for the viscosity of polymeric solutions of alginate and xanthan gum. (B) Experimental response for the viscosity of alginate and xanthan gum solutions.

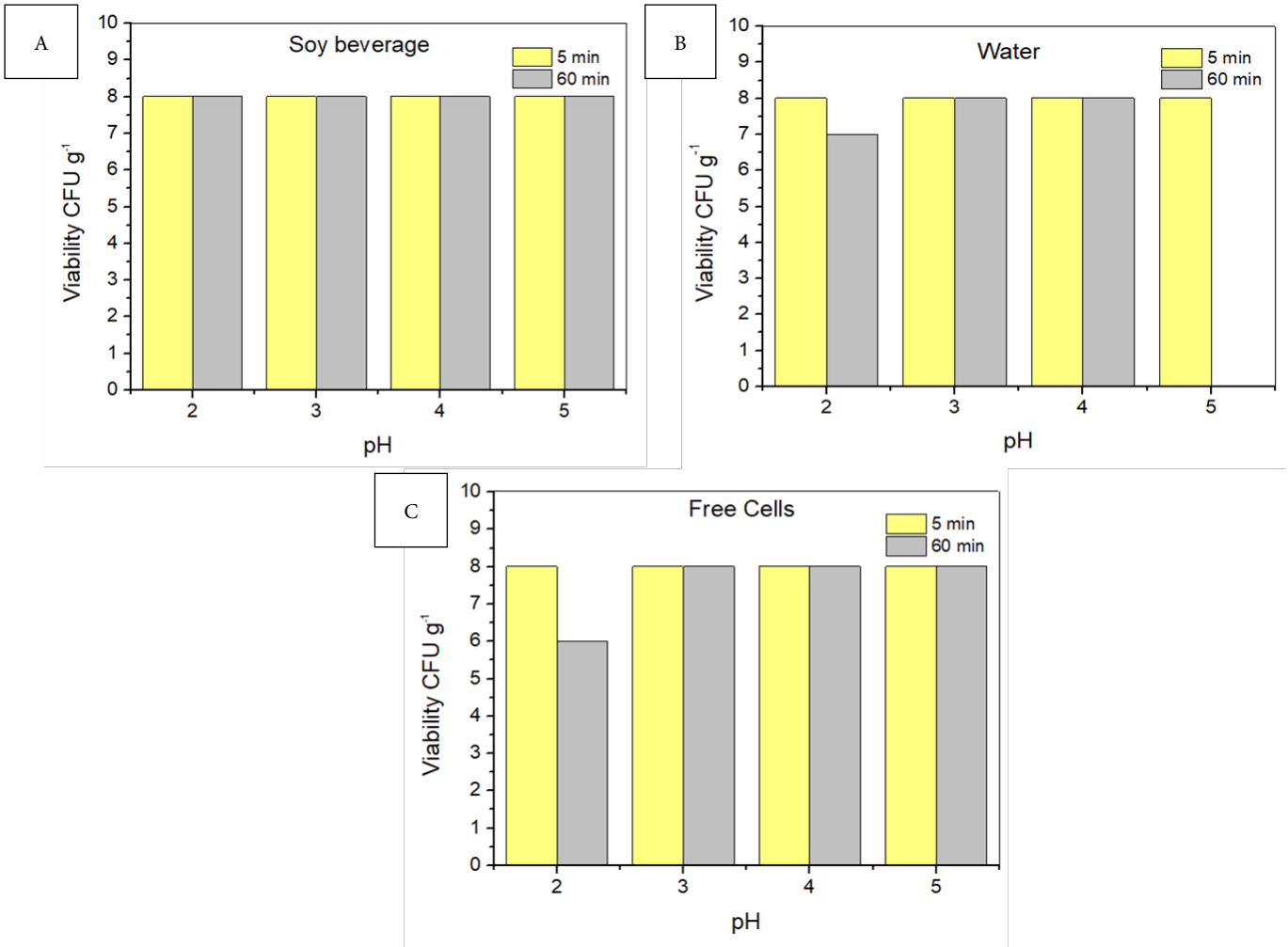

Figure 3. Viability of probiotics from spheres formed in (A) soy beverage, (B) water, and (C) free probiotics at 5 and 60 min after preparation in 2% sodium citrate solution (m v^{-1}), pH 2, 3, 4, and 5.

encapsulated *Lactobacillus plantarum* with rice bran and pectin by ionic gelation and observed a reduction of 1.0 log CFU g^{-1} in free cells in pH 3.0, while encapsulated cells showed a smaller reduction (0.5 log CFU g^{-1}).

After 1 h in pH 5, the spheres formed in water swelled and released the probiotic cells. This occurs because when the polymers are above the pKa values of mannuronic acid (*pKa* = 3.38) and guluronic acid (*pKa* = 3.65), which are the building blocks of alginate, there is a disorder in the calcium-alginate structure (Alexandre et al., 2024; Dalponte Dallabona et al., 2020). At higher pH, the presence of the negative charge (COO^-) causes repulsion of the alginate chain, causing polymer swelling and consequently facilitating the release of the encapsulated material (Camacho et al., 2019). The opposite happens when the pH is below the pKa, where the alginate carboxyl groups are in its protonated state and there is a disorder in the calcium-alginate structure.

3.4 Evaluation of probiotic survival during storage

The viability of the probiotics encapsulated in soy beverage or water (control) stored in the same media (beverage or water) and dry conditions (no media) was carried out in this work to evaluate the best conditions for the storage of probiotic spheres (Figure 4).

Our studies found that for 10 days, all storage conditions tested had viable probiotics (7 and 8 log CFU g^{-1}). After 15 days, probiotics encapsulated in soy and under dry storage conditions lost their viability. After 30 days of storage, spheres formed in soy beverage and stored in the same media maintained bacterial cells at 8 log CFU g^{-1}, which was the best condition for probiotic survival. At the same time, after 30 days, the spheres formed and stored in water reduced the bacterial concentration, reaching 6 log CFU g^{-1}. Considering the recommended daily portion of probiotic intake, the concentration of 6 log CFU g^{-1} would still be acceptable, making clear the advantage of the probiotic encapsulation process. Similar results were reported by other authors.

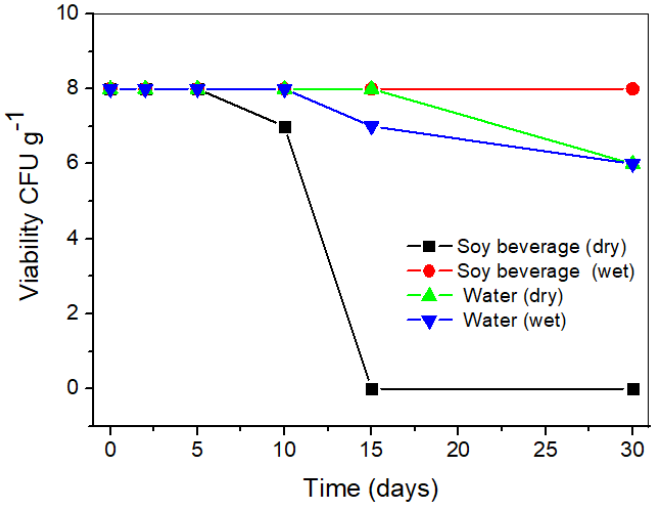

Figure 4. Probiotic viability over time for the spheres formed and stored in soy beverage (wet), water (wet), and no liquid immersion (dry).

D'Alessandro et al. (2023) developed fermented soy beverages containing encapsulated and nonencapsulated probiotics. The viability of the strains remained at approximately 7 log CFU mL^{-1} from the beginning to final time. This finding complies with the literature, as lactic acid bacteria can reach 8–9 log CFU g^{-1} of viable cells in soy beverages without the need to add other carbohydrates. Cui et al. (2021) reported the application of probiotic BB-12 in soy yogurt and milk yogurt and verified that the probiotic counting in soy yogurt was significantly higher, confirming our findings. This occurs because most *Bifidobacteria* have the enzyme α-galactosidase, capable of fermenting raffinose (Cui et al., 2021), present in high levels in soy beverages. Thus, raffinose can be a source of carbohydrates, preserving the probiotic viability over time. According to Kumari et al. (2022), soy beverage is an affordable and appropriate medium for growth and recommended as a suitable vehicle for probiotics.

4 CONCLUSION

The combination of XG and alginate proved to be efficient in the encapsulation and viability of probiotic *B. animalis* BB-12. The chosen treatment for the formation of the spheres was 1.125 g mL^{-1} alginate and 0.5 g mL^{-1} XG. For this treatment, the morphological characteristics of particle size and sphericity were homogeneous, with potential future food applications. In addition, the spheres were efficient in protecting the probiotics in acidic conditions close to those of the stomach. The probiotics encapsulated and stored in soy beverage were viable for 30 days, showing that the soy-based beverage was important in the viability of the probiotics for a longer period.

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