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Development of a Functional Yogurt Containing Probiotics and Phenolic Compounds of Coffee Encapsulated in Alginate Beads

Aurora Viridiana Toalá-Gómez [1], Claudia Mendoza-Avendaño [1], María Celina Luján-Hidalgo [1], Miguel Ángel Ruiz-Cabrera [2], Alicia Grajales-Lagunes [2], Enna Berenice Estudillo-Díaz [1], Lucia María Cristina Ventura-Canseco [1], Gabriela Palacios-Pola [3] and Miguel Abud-Archila [1,*]

[1] National Technological Institute of Mexico, Technological Institute of Tuxtla Gutiérrez, Carr. Panamericana km 1080, 29050, Tuxtla Gutiérrez, Mexico

[2] Faculty of Chemical Science, University of San Luis Potosi, 6 Dr Manuel Nava Avenue, University Area, 78210, San Luis Potosí, Mexico

[3] Faculty of Nutrition, Universidad de Ciencias y Artes de Chiapas. Ciudad Universitaria, Libramiento Norte Poniente 1150, Potinaspak, Tuxtla Gutiérrez, Chiapas, CP 29039, Mexico

* Correspondence: miguel.aa@tuxtla.tecnm.mx

Abstract: This study evaluated the encapsulation of *Lactiplantibacillus fabifermentans* BAL-27 ITTG and phenolic compounds from coffee husks using alginate beads. The research considered variables such as alginate concentration (1.5% and 3%), crosslinking time (8 and 20 minutes), and the inclusion of chitosan. A 2^3 factorial design was employed, and the effects were analyzed using ANOVA ($p < 0.05$). The encapsulation efficiency for the probiotic exceeded 80%, and its viability following gastrointestinal simulation ranged from 73.65% to 85.34%. Phenolic compounds achieved encapsulation efficiencies of up to 20%. In yogurt, the alginate beads maintained probiotic viability at approximately 9 Log_{10} CFU/g and preserved the stability of antioxidant compounds over 28 days. Moreover, the incorporation of beads did not adversely affect the physicochemical properties or sensory acceptance of the yogurt, supporting their potential application in functional foods.

Keywords: *Lactobacillus fabifermentans* BAL-27 ITTG; functional food; husk coffee extract

1. Introduction

Currently, interest in functional foods and beverages has increased significantly due to growing awareness of the importance of proper nutrition and health, as well as increasing scientific evidence supporting their efficacy [1]. Among these foods, the roles of probiotics and phenolic compounds have been widely studied, as both have demonstrated health benefits when consumed in adequate amounts. However, the incorporation of these bioactive compounds into food matrices may alter their physicochemical and organoleptic properties or reduce their bioactivity during processing. To address these challenges, researchers have implemented microencapsulation techniques to protect and stabilize these compounds in a way that preserves both the sensory characteristics of the food and the functional properties of the encapsulated ingredients during storage. Over the years, various technologies have been developed to extend the shelf life of these bioactives, with encapsulation emerging as one of the most employed strategies.

For the encapsulation of lactic acid bacteria, spray drying, lyophilization, and encapsulation in alginate beads are among the most prominent techniques. However, the latter presents significant challenges, as the viability of probiotics tends to decline rapidly during storage, as reported by Silva et al. [2], De Prisco et al. [3], and Kumar and Kumar [4]. This decrease in viability is often attributed to the physicochemical properties of the beads and their water activity (Aw). Another critical factor is the ability of probiotics to withstand the encapsulation conditions, prompting ongoing efforts to

identify more resilient strains. In our laboratories, *Lactiplantibacillus fabifermentans* BAL-27 ITTG has shown promising probiotic potential [5], suggesting its suitability for food applications.

Another group of important bioactive components includes phenolic compounds (antioxidants), which are highly susceptible to degradation by high temperatures, light, and oxygen during processing and storage. The antioxidant capacity of these compounds is associated with various health benefits, including anti-inflammatory, anticancer, antiatherosclerotic properties, and improved gut microbiota health [6]. Several studies have reported promising encapsulation efficiencies for phenolic compounds, distinct from those observed for probiotics. For example, Belščak et al. [7] and Li et al. [8] reported encapsulation efficiencies exceeding 57% for caffeine and phenolic compounds in green tea. These studies highlighted the crucial role of chitosan in achieving high encapsulation efficiency. Given these favorable outcomes, researchers are actively exploring new sources of phenolic compounds, particularly from agricultural by-products.

The coffee cherry has long been utilized; however, due to its nutritional potential, its by-products have attracted growing global interest. One such by-product is the pericarp (outer skin), which contains phenolic compounds at concentrations ranging from 9.2 to 13 mg GAE/g, as reported by Ballesteros et al. [9], Heeger et al. [10], and Ribeiro et al. [11]. These phenolic compounds are particularly valued for their antimicrobial and antioxidant properties, making their extraction and application especially appealing.

Despite numerous reports on the individual encapsulation of probiotics and phenolic compounds, there is limited information on their co-encapsulation. This is largely due to the antimicrobial nature of phenolic compounds, which may inhibit beneficial microorganisms. Therefore, in the present study, *L. fabifermentans* was co-encapsulated with phenolic compounds extracted from coffee pericarp using alginate beads. The effects of alginate concentration, crosslinking time, and the presence or absence of chitosan were evaluated. Additionally, the impact of incorporating these capsules into yogurt was assessed by monitoring probiotic survival, survival under simulated gastrointestinal conditions, and the physicochemical and sensory properties of the final product.

2. Materials and Methods

2.1. Materials and Microorganisms

Lactiplantibacillus fabifermentans BAL-27 ITTG was provided by the National Technological Institute of Mexico/Technological Institute of Tuxtla Gutiérrez, Chiapas, Mexico. The microorganism was reactivated in de Man, Rogosa, and Sharpe (MRS; Difco) broth and incubated at 37 °C for 8 h. Subsequently, the culture was centrifuged at 4,500 rpm and 4 °C for 30 min. The resulting cell pellet was washed with 0.9% (w/v) saline solution and centrifuged again under the same conditions. Finally, the cell pellet was stored at 4 °C until further use. All unspecified reagents were purchased from Sigma-Aldrich (USA).

2.2. Extraction of Phenolic Compounds

Ripe coffee cherries (Coffea arabica) were supplied by Rilly de Lievano Co. (Chiapas, Mexico). The cherries were washed with soap and water, disinfected by immersion in chlorinated water (100 ppm), rinsed thoroughly, and shade-dried to remove surface moisture. The pericarp was manually separated from undamaged, fully ripe cherries. For the extraction of phenolic compounds, the pericarp was immersed in distilled water at a 1:5 (w/v) ratio and stirred at 60 °C for 24 h. The resulting mixture was filtered through Whatman No. 4 paper, and the filtrate was lyophilized using a freezing dryer (Labconco, Cole-Parmer, USA) at −40 °C and 0.03 mbar of pressure [12]. The resulting coffee cherry pericarp extract (hereafter referred to as "coffee extract") was stored in the dark at −18 °C until further use.

2.3. Encapsulation of Bioactive Compounds

Alginate beads were obtained via ionic gelation method using two concentrations of sodium alginate (1.5 and 3% w/v), two crosslinking times (8 and 20 min), and with or without chitosan coating (molecular weight: 100,000–300,000) (Sigma–Aldrich, USA).

To prepare the beads, hydrated alginate (12 h under refrigeration) was mixed with the cell pellet of L. fabifermentans and the resuspended lyophilized coffee extract to final concentration of 10 Log_{10} CFU/mL and 2% (w/v), respectively. The mixture was homogenized at 1,000 rpm for 10 minutes and then added dropwise into a sterile 5% (w/v) calcium chloride solution. After, the beads were immersed in the $CaCl_2$ solution for 8 or 20 minutes, according to the experimental design. A batch of beads was subsequently coated by immersion in a 0.4% (w/v) chitosan solution according to the methodology described by Krasaekoopt et al. [13]. Previously, low-molecular-weight chitosan was dissolved in 1% (v/w) acetic acid. The pH of the solution was subsequently adjusted to 5.7 ± 0.3 with 1 M NaOH. The mixture was filtered with filter paper (Whatman # 4) and sterilized at 121 °C for 15 minutes prior to immersion of the beads. Fifteen g of beads were immersed in 100 mL of the chitosan solution and stirred at 100 rpm for 40 min. Finally, the beads were removed from the chitosan solution, washed twice with sterile distilled water and stored under refrigeration until use. Importantly, the beads were used for no more than 5 hours for future determinations.

2.4. Determination of the Viability of Lactobacillus Fabifermentans

To evaluate the viability of microorganisms, 1 g of beads was mixed with 5 mL of 2% sodium citrate (p/v). The mixture was homogenized at 3,200 rpm for 5 minutes using an Ultra-Turrax homogenizer (IKA T25, USA) following the protocol described by De Prisco et al. [3]. The viable cell concentration was determined by plating on MRS agar according to Ramírez-Pérez et al. [5]. The encapsulation efficiency (EE) was determined via Equation (1):

$$EE(\%) = (Log_{10}\ Ni/Log\ No) \times 100 \qquad (1)$$

where Ni is the concentration (CFU/g) of cells encapsulated in the beads and No is the concentration (CFU/g) of cells in the encapsulation mixture.

2.5. Gastrointestinal Simulation

Gastrointestinal simulation was performed according to Ramírez-Pérez et al. [5]. Briefly, the tolerance of the microorganisms to acidic conditions was evaluated by exposing them to a pH of 1.9 for 30 minutes. The passage through the small intestine was subsequently simulated via intestinal juices (pH 7.5), and the beads were incubated for 6 hours. The resistance gastrointestinal simulation (RGS) was calculated by Equation 2.

$$RGS\ (\%) = (Log_{10}\ Ni/Log_{10}\ No) \times 100 \qquad (2)$$

where Ni represents the cell concentration present at the end of the intestinal stage and No represents the initial cell concentration.

2.6. Quantification of Phenolic Compounds in the Beads

The phenolic compound content in the alginate beads was quantified by dissolving 1 g of the beads, as described in Section 2.3. The concentration of phenolic compounds was determined using the Folin–Ciocalteu spectrophotometric method [14]. Briefly, 1 mL of the sample was mixed with 4.2 mL of distilled water, followed by the addition of 0.5 mL of 2 N Folin–Ciocalteu reagent and 1 mL of 20% (w/v) sodium carbonate. The mixture was incubated at room temperature for 2 hours. The absorbance was measured at 765 nm using a spectrophotometer (Cole-Parmer UV-2100, USA). A standard calibration curve was prepared using gallic acid (GA). The results are expressed in mg gallic acid equivalents per gram of bead (mg GAE/g).

The encapsulation efficiency of phenolic compounds (PCEE) was determined by Equation (3):

$$PCEE\ (\%) = (CF_i/CF_o) \times 100 \tag{3}$$

where CF_i represents the concentration of phenolic compounds encapsulated in the beads and CF_o corresponds to the initial concentration of phenolic compounds in the mixture before encapsulation.

2.7. Preparation of Yogurt

A commercial starter culture from the BIOPROX brand (Mexico) was used, activated in sterile distilled water, and incubated at 37 °C for 1 h according to the manufacturer's instructions. For inoculum preparation, whole milk powder was reconstituted to a concentration of 12% (w/v) and sterilized at 121 °C for 15 minutes. The starter culture was subsequently inoculated at 1% (v/v) and incubated at 35 °C for 8 h [15].

Yogurt preparation was conducted following the methodology described by Morales-Ruiz [15], with slight modifications. Milk was standardized by dissolving 6 g of powdered whole milk (Nido, Mexico) and 7 g of sugar in 100 mL of liquid whole milk (Pradel, Mexico). The mixture was subjected to low-temperature pasteurization (73 °C for 20 s), followed by inoculation with the activated culture at 10% (v/v). The mixture was stirred for 20 minutes, and 30 mL aliquots were transferred into 40 mL polyethylene containers containing alginate beads at a 1:10 (w/v) ratio. Fermentation was carried out at 40 °C for 2 h, after which the samples were refrigerated (approximately 5 °C) to allow for gelation.

The yogurt was stored at 5 °C for 28 days, and samples were collected every 7 days to assess the viability of the microorganisms, as well as the physicochemical and sensory properties of the product. In addition, the phenolic compound content, and the release of *L. fabifermentans* under simulated gastrointestinal conditions were quantified at the end of the storage period.

2.7.1. Viability of Lactobacillus Fabifermentans in Yogurt

To determine the viability of the probiotic on the beads within the yogurt, the beads were removed and carefully washed with sterile water to remove excess yogurt. The determination of the viability of microorganisms was then carried out as described in Section 2.4.

2.7.2. Physicochemical Analysis of Yogurt

Syneresis

The syneresis of the yogurt was determined according to Zainoldin and Hi Baba [16]. For this purpose, 20 g of yogurt was weighed in 50 mL tubes and centrifuged at 4,500 rpm for 20 min at 4 °C. The supernatant was subsequently weighed, and the percentage of syneresis was calculated using Equation (4).

$$Syneresis\ (\%) = ((weight\ of\ the\ supernatant)/(weight\ of\ the\ sample)) * 100 \tag{4}$$

pH

The pH of the yogurt was measured during storage using a digital pH meter (Deluxe pH meter, India).

Titratable acidity

The titratable acidity, expressed as a percentage of lactic acid, was determined according to NOM-243-SSA1-2010 [17]. Eighteen grams of yogurt were mixed with 36 g of distilled water. Ten drops of 1% phenolphthalein (w/v) were added and titrated with 0.1 N NaOH. The acidity was calculated using Equation (5).

$$Acidity\ (\%) = (V * N * 9)/M \tag{5}$$

where V is the volume (mL) of NaOH used in the titration, N is the normality of NaOH (0.1 N), and M is the weight of the sample.

Sensory analysis

The degree of global acceptance, as well as the properties of odor, color, flavor and texture, was evaluated using a structured seven points hedonic test (1: dislike extremely; 7: like extremely). The evaluation was performed by a panel of 60 untrained judges (aged 18-24 years). Assessment was conducted at the beginning and end (28 days) of yogurt shelf-life.

2.8. Experimental Design and Statistical Analysis

A completely randomized 2^3 factorial experimental design was implemented with three replicates. The following factors were evaluated: alginate concentration (1.5% and 3%), immersion time (8 and 20 minutes), and the presence or absence of chitosan. All data were analyzed using analysis of variance (ANOVA) at a significance level of $p < 0.05$. Mean comparisons were performed using Tukey's test, with the aid of the Statgraphics Centurion XVI software. For the sensory analysis, yogurt without alginate beads was used as the control.

3. Results

3.1. Encapsulation of Lactobacillus Fabifermentans BAL-27-ITTG and Phenolic Compounds

3.1.1. Viability of Encapsulated Lactobacillus Fabifermentans BAL-27-ITTG

The viability of *L. fabifermentans* microencapsulated with coffee extract had a range of 8.83 to 9.47 Log_{10} CFU/g of beads (Table 1). The highest alginate concentration (3%) combined with the longest crosslinking time (20 minutes) resulted in the greatest probiotic viability (9.45 and 9.47 Log_{10} CFU/g), regardless of the presence or absence of a chitosan coating. In contrast, treatments T1 and T5 presented the lowest content of viable microorganisms in the beads (8.83 and 8.91 Log_{10} CFU/g, respectively). This is related to the low alginate concentration (1.5% w/v) and short crosslinking time (8 min), regardless of the presence or absence of chitosan.

Table 1. Viability and MEE of *L. fabifermentans* and phenolic compounds. PCEE and RGS of *L. fabifermentans* encapsulated in beads.

Treatment	Viability (Log_{10} CFU/g bead)	MEE (%)	PC (mg GAE/g bead)	PCEE	RGS (%)
T1	8.83[c]	82.97[b]	0.48[a]	14.22[c]	84.38 [ab]
T2	9.09[abc]	82.86[b]	0.49[a]	17.02[bc]	83.82 [b]
T3	9.45[a]	84.70[a]	0.47[a]	19.75[ab]	85.34 [a]
T4	9.47[a]	84.84[a]	0.51[a]	20.99[a]	73.65 [e]
T5	8.91[bc]	81.00[c]	0.48[a]	20.86[a]	75.51 [d]
T6	9.00[abc]	81.79[c]	0.52[a]	19.12[ab]	84.08 [ab]
T7	9.29[abc]	83.47[b]	0.52[a]	19.06[ab]	77.94 [c]
T8	9.38[ab]	83.31[b]	0.52[a]	20.89[a]	76.11 [d]
Tukey	0.532	1.01	0.07	3.03	1.50

MEE = microbial encapsulation efficiency, PC= phenolic compound, PCEE = phenolic compound encapsulation efficiency, RGS = resistance gastrointestinal simulation, T1 = 1.5% (w/v) alginate, 8 min, without chitosan. T2 = 3.0% (w/v) alginate, 8 min, without chitosan. T3 = 3.0% (w/v) alginate, 20 min, without chitosan. T4 = 3.0% (w/v) alginate, 20 min, with chitosan. T5 = 1.5% (w/v) alginate, 8 min, with chitosan. T6 = 1.5% (w/v) alginate, 20 min, without chitosan. T7 = 3.0% (w/v) alginate, 8 min, with chitosan. T8 = 1.5% (w/v) alginate, 20 min, with chitosan. Similar lowercase letters in the same column indicate no significant difference between treatments ($p > 0.05$).

Although achieving high cell viability within the beads is one of the main goals of encapsulation, it is also essential to ensure a high microbial encapsulation efficiency (MEE). Treatments T3 and T4 exhibited the highest MEE values, at 84.70% and 84.84%, respectively (Table 1). This outcome may be influenced by the alginate concentration and the crosslinking time, as illustrated in the Pareto

diagram (Figure 1a). The diagram indicates that both factors had a positive effect on MEE, whereas the addition of chitosan showed no significant influence. MEE increased with higher alginate concentrations and longer crosslinking times. In contrast, treatments T5 and T6 exhibited the lowest MEE values, at 81.00% and 81.79%, respectively (Table 1). Although the mean comparison test revealed statistically significant differences among treatments, the actual differences in MEE values were small. The higher MEE observed with increased alginate concentration may be attributed to a reduction in osmotic stress, ionic imbalance, and protein denaturation, thereby protecting the integrity of the probiotic cell membrane [18].
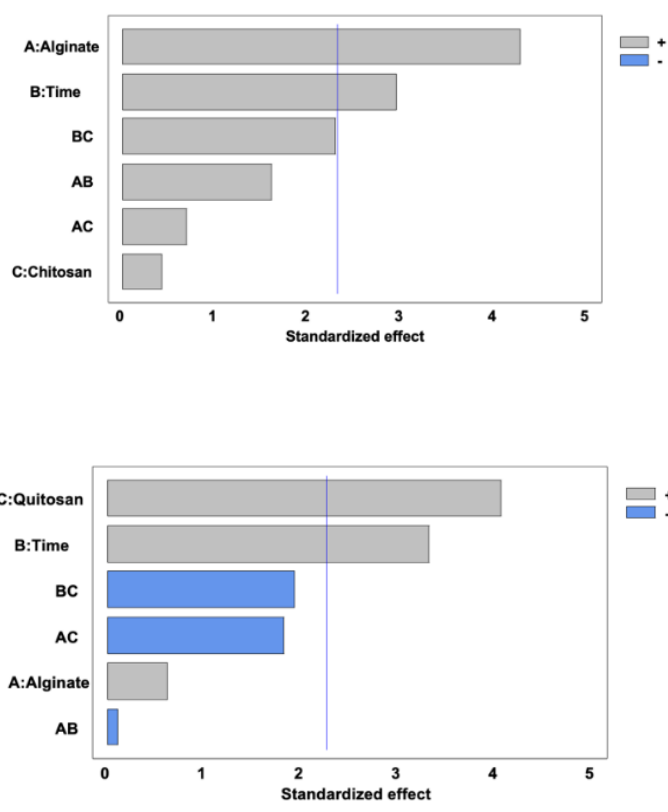

Figure 1. Pareto diagram showing the effects of the sodium alginate concentration, time and application of chitosan: MEE of *L. fabifermentans* (A) and PCEE (B).

3.1.2. Encapsulated Phenolic Compounds

The encapsulation of phenolic compounds is another important criterion, as it directly affects their stability, bioavailability, and biological activity in the consumer. These compounds play a crucial role in preventing oxidative stress, as their consumption is associated with reduced cellular damage, enhanced immune defense, and a lower risk of developing diseases related to free radicals [19]. The concentration of phenolic compounds in the beads ranged from 0.47 to 0.51 mg GAE/g across all treatments (Table 1), corresponding to phenolic compound encapsulation efficiencies (PCEE) between 14% and 20%.

Analysis of variance revealed that while the factors studied did not significantly affect the concentration of encapsulated phenolic compounds, they did have a significant influence on PCEE (Figure 1b). The lowest PCEE values were observed in treatments T1 and T2 (14.22% and 17.02%, respectively), both of which used an 8-minute crosslinking time. Conversely, the addition of chitosan contributed to higher PCEE values, even under short crosslinking conditions, as seen in treatments T1 and T2 (Table 1).

3.1.3. Viability of Lactobacillus Fabifermentans BAL-27-ITTG During Gastrointestinal Simulation

The resistance of a probiotic to gastrointestinal simulation is key to ensuring its arrival in the large intestine and ability to colonize it. The highest resistance to gastrointestinal simulation was 85.34% for Treatment T3, compared with treatments T5 and T8, with values of 75.51 and 76.11%, respectively (Table 1). This may be because treatment T3 contained a higher concentration of alginate (3%) without the addition of chitosan than did treatments T5 and T8, which presented the lowest concentration of alginate (1.5%) supplemented with chitosan in both treatments.

3.2. Evaluation of the Quality of Alginate Beads During Yogurt Storage

To demonstrate the functionality of yogurt as a functional food, it was essential to ensure the viability of the encapsulated probiotic during storage. Therefore, the viability of the probiotic and the stability of the phenolic compounds encapsulated within the beads incorporated into the yogurt were evaluated. The beads were carefully extracted from the yogurt, washed, and analyzed weekly over a 28-day storage period.

3.2.1. Viability of Lactobacillus Fabifermentans BAL-27-ITTG

Beads prepared with 1.5% (w/v) sodium alginate and crosslinked for 8 or 20 minutes, both with and without chitosan, maintained a probiotic concentration of approximately 9 Log_{10} CFU/g throughout the 28-day storage period. The viability kinetics (Figure 2a) showed that from week 0 to week 3, the concentration of viable microorganisms remained stable. However, a significant increase in viability was observed between weeks 3 and 4. A similar trend was observed with higher alginate concentrations (3%), as shown in Figure 2b.
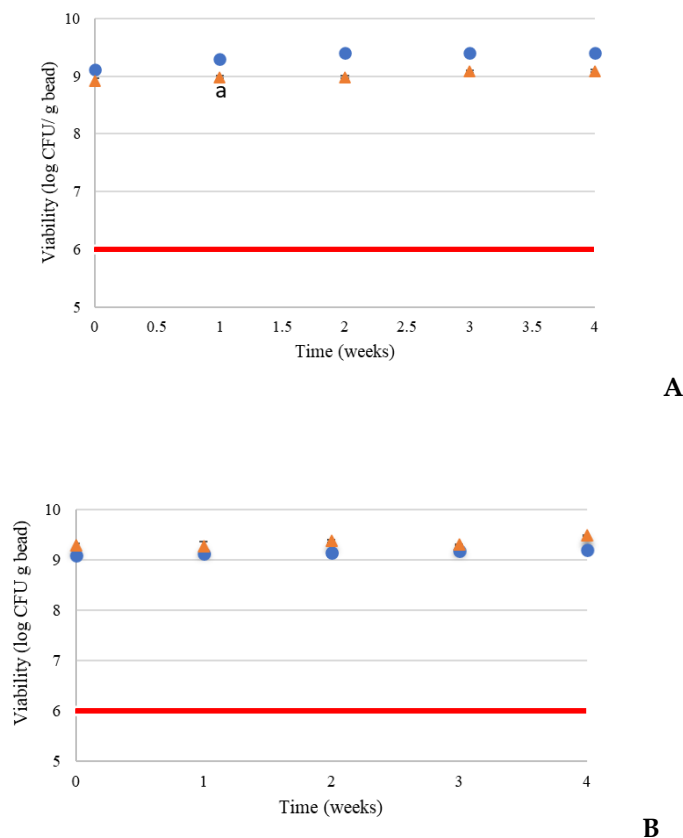

A

B

Figure 2. Chitosan effect of alginate beads concentration 1.5% (A), 3% (B), during immersion time of 8 min on viability of probiotic for 4 weeks. Similar lowercase showed no significant statistical difference between

treatments (p > 0.05). Without chitosan (●) with chitosan (■). Red line is the value (6 Log_{10} CFU/g) reported by the FAO/WHO [30] for food to have a beneficial effect.

3.2.2. Phenolic Compounds in Beads During Storage

At the beginning of the storage period, no significant differences were observed in the phenolic compound content among the treatments (p > 0.05). However, by the end of the storage period, treatments T1, T2, T3, and T6 showed significant differences (p < 0.05), indicating that storage time had a measurable effect on these treatments (Table 2).

Table 2. Concentration of phenolic compounds (mg EAG/g bead) in alginate beads during yogurt storage.

Treatment	Storage time (weeks)		Tukey
	0	4	
T1	0.48[Aa]	0.43[Bb]	0.04
T2	0.49[Aa]	0.45[Bb]	0.04
T3	0.47[Aa]	0.41[Babc]	0.08
T4	0.51[Aa]	0.52[Aa]	0.07
T5	0.48[Aa]	0.47[Aabc]	0.13
T6	0.52[Aa]	0.48[Babc]	0.02
T7	0.52[Aa]	0.52[Aa]	0.04
T8	0.52[Aa]	0.50[Aab]	0.02
Tukey	0.07	0.04	

T1 = 1.5% (w/v) alginate, 8 min, without chitosan. T2 = 3.0% (w/v) alginate, 8 min, without chitosan. T3 = 3.0% (w/v) alginate, 20 min, without chitosan. T4 = 3.0% (w/v) alginate, 20 min, with chitosan. T5 = 1.5% (w/v) alginate, 8 min, with chitosan. T6 = 1.5% (w/v) alginate, 20 min, without chitosan. T7 = 3.0% (w/v) alginate, 8 min, with chitosan. T8 = 1.5% (w/v) alginate, 20 min, with chitosan. Similar lowercase letters in the same column indicate no significant difference between treatments (p > 0.05). A similar capital letter in the same row indicates no significant difference between treatments (p > 0.05).

The treatments with chitosan (T4, T5, T7 and T8) did not significantly differ (p < 0.05) during storage, which suggests that the chitosan layer also functioned as a protective barrier, limiting the release and diffusion of phenolic compounds.

3.2.3. Viability of Lactobacillus Fabifermentans BAL-27-ITTG from Alginate Beads During Gastrointestinal Simulation After Four Weeks of Yogurt Storage

After four weeks of yogurt storage, the viability of *Lactobacillus fabifermentans* under simulated gastrointestinal conditions ranged from 71% to 89% (Table 3). The highest survival rate was observed in treatment T1, which used 1.5% alginate, an 8-minute immersion time, and no chitosan coating. In contrast, the lowest survival was recorded in treatment T8, which involved 1.5% alginate, a 20-minute immersion time, and chitosan coating.

Table 3. Survival of *L. fabifermentans* on alginate beads during gastrointestinal simulation after 4 weeks of storage

Treatment	RGS (%)
T1	89.82[a]
T2	83.82[c]
T3	84.22[c]

T4	79.86[d]
T5	73.25[f]
T6	88.09[b]
T7	77.23[e]
T8	71.88[g]
Tukey	2.8104

RGS = Resistance Gastrointestinal Simulation, T1 = 1.5% (w/v) alginate, 8 min, without chitosan. T2 = 3.0% (w/v) alginate, 8 min, without chitosan. T3 = 3.0% (w/v) alginate, 20 min, without chitosan. T4 = 3.0% (w/v) alginate, 20 min, with chitosan. T5 = 1.5% (w/v) alginate, 8 min, with chitosan. T6 = 1.5% (w/v) alginate, 20 min, without chitosan. T7 = 3.0% (w/v) alginate, 8 min, with chitosan. T8 = 1.5% (w/v) alginate, 20 min, with chitosan. Similar lowercase letters in the same column indicate no significant difference between treatments (p > 0.05).

3.3. Effects of the Addition of *Lactobacillus Fabifermentans* and Encapsulated Phenolic Compounds on the Physicochemical and Sensory Properties of Yogurt

Once the yogurt was confirmed to function as a functional food, it was necessary to evaluate whether its physicochemical and sensory properties were affected. For the analysis of syneresis, pH, and titratable acidity, the encapsulated beads were removed from the yogurt prior to measurement. However, for the sensory evaluation, the beads were left intact.

3.3.1. Determination of the Physicochemical and Microbiological Properties of Yogurt

Yogurt Syneresis

Syneresis is a key physical parameter used to assess yogurt quality, as it reflects the instability of the gel network and its inability to retain the serum phase within the matrix [20]. Elevated levels of syneresis are generally undesirable from a consumer perspective. The incorporation of alginate beads significantly increased (p < 0.05) the syneresis of the yogurt compared to the control sample (yogurt without beads) (Table 4). The highest syneresis percentages (22–29%) were observed in treatments T4, T5, T7, and T8, all of which included chitosan.

Table 4. Syneresis (%) of yogurt supplemented with alginate beads during storage

Treatment	Time (weeks)					
	0	1	2	3	4	Tukey
T1	20.65[Ac]	23.40[Bc]	25.52[Cc]	25.29[Cc]	28.48[Dc]	1.42
T2	21.03[Acd]	24.74[Bc]	24.74[Bc]	24.91[Bc]	25.09[Bb]	0.97
T3	21.84[Ade]	24.1[Bc]	24.21[Bc]	25.41[Cc]	25.91[Cb]	1.01
T4	29.70[Ag]	31.37[Ad]	34.57[Bf]	39.26[Cf]	39.93[Cf]	1.88
T5	22.42[Ae]	24.45[Bc]	27.37[Cd]	29.91[Dd]	35.45[Ed]	1.50
T6	17.60[Ab]	18.84[Ab]	21.33[Bb]	23.36[Cb]	25.56[Db]	1.88
T7	23.73[Af]	27.69[Bd]	29.65[Ce]	30.46[Cd]	38.33[De]	1.90
T8	23.06[Af]	25.65[Bcd]	28.52[Cde]	32.86[De]	37.54[Ee]	0.86
Control	13.63[Aa]	15.29[Ba]	17.71[Ca]	20.14[Da]	20.58[Da]	1.33
Tukey	1.00	2.77	1.95	1.04	1.02	

T1 = 1.5% (w/v) alginate, 8 min, without chitosan. T2 = 3.0% (w/v) alginate, 8 min, without chitosan. T3 = 3.0% (w/v) alginate, 20 min, without chitosan. T4 = 3.0% (w/v) alginate, 20 min, with chitosan. T5 = 1.5% (w/v) alginate, 8 min, with chitosan. T6 = 1.5% (w/v) alginate, 20 min, without chitosan. T7 = 3.0% (w/v) alginate, 8 min, with chitosan. T8 = 1.5% (w/v) alginate, 20 min, with chitosan. Similar lowercase letters in the same column indicate no significant difference between treatments (p > 0.05). A similar capital letter in the same row indicates no significant difference between treatments (p > 0.05).

On the other hand, treatments T1, T2, T3, and T6, which did not contain chitosan, also exhibited significantly higher syneresis (p < 0.05) compared to the control yogurt. The presence of beads in yogurt from the beginning of fermentation disrupted the homogeneity of the product, as illustrated in Figure 3. This disruption interfered probably with the formation of a uniform casein network, thereby contributing to the increased syneresis observed. The syneresis of the yogurt during storage (Table 4) increased even for the yogurt without beads. Table 4 shows that the chitosan treatments presented the highest percentage of syneresis, which was above 35%.
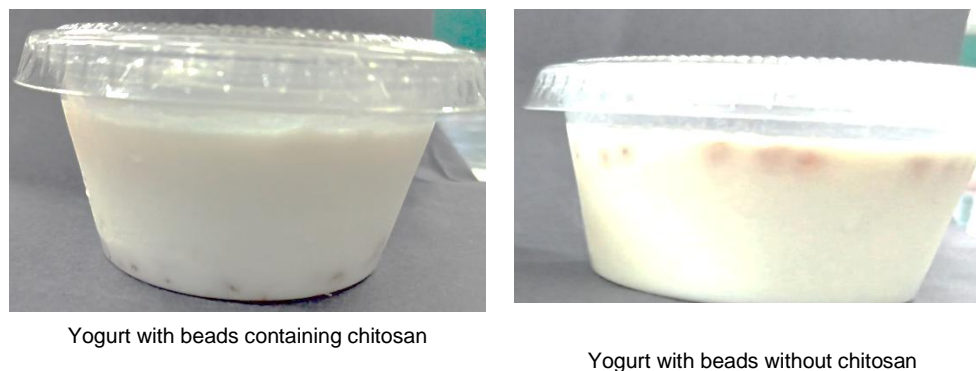

Yogurt with beads containing chitosan

Yogurt with beads without chitosan

Figure 3. Yogurt with added alginate beads.

Titratable pH and acidity

The initial pH of the yogurt ranged from 4.26 to 4.34 (Table 5), values that are close to those of the control yogurt, which did not contain beads, and are within the maximum allowable limit (pH ~ 4.50) according to NOM-243-SSA1-2010 [17]. During yogurt production, the initial milk pH of 6.2 decreased due to the activity of the starter culture, as approximately 20–40% of the lactose is converted into lactic acid, as reported by Cheng [21]. Throughout storage, the pH of the yogurt decreased, with statistically significant differences (p < 0.05) observed between treatments. At the end of storage, the pH of the yogurt ranged from 3.93 to 4.0, with a significant difference (p < 0.05) between the control yogurt and the yogurt stored without chitosan (treatments T1, T2, T3, and T6). This variation could be attributed to the potential release of L. fabifermentans into the yogurt. The titratable acidity of yogurt supplemented with alginate beads increased slightly during storage, which accounts for the observed decrease in pH (Table 6). A similar decrease in pH was also observed in the control yogurt without beads. According to NOM-243-SSA1-2010 [17], the minimum allowable titratable acidity is 0.50%, and all treatments met this standard.

Table 5. pH of yogurt fermented with alginate beads for four weeks.

Treatment	Time (weeks)					
	0	1	2	3	4	Tukey
T1	4.34^{Aa}	4.09^{Bd}	4.00^{BCc}	3.97^{Cb}	3.97^{Ccd}	0.08
T2	4.32^{Aab}	4.09^{Bd}	4.03^{Bc}	3.96^{Cb}	3.93^{Cd}	0.06
T3	4.26^{Aabc}	4.13^{Bcd}	4.02^{Cc}	3.97^{CDb}	3.96^{Dcd}	0.05
T4	4.29^{Aab}	4.15^{Bbc}	4.09^{Bb}	4.00^{Cab}	4.00^{Cabc}	0.08
T5	4.26^{Aabc}	4.17^{Bbc}	4.09^{Cb}	4.01^{Dab}	4.00^{Dabc}	0.04
T6	4.26^{Aabc}	4.18^{ABb}	4.09^{BCb}	4.03^{Cab}	3.98^{Cbcd}	0.13
T7	4.27^{Aabc}	4.17^{Bbc}	4.10^{Cb}	4.04^{Dab}	4.04^{Dab}	0.05
T8	4.27^{Aabc}	4.15^{Bbc}	4.10^{Cb}	4.04^{Dab}	4.00^{Eabc}	0.03
Control	4.34^{Aa}	4.27^{Ba}	4.20^{Ca}	4.11^{Da}	4.05^{Ea}	0.03

Tukey	0.08	0.04	0.05	0.12	0.06

T1 = 1.5% (w/v) alginate, 8 min, without chitosan. T2 = 3.0% (w/v) alginate, 8 min, without chitosan. T3 = 3.0% (w/v) alginate, 20 min, without chitosan. T4 = 3.0% (w/v) alginate, 20 min, with chitosan. T5 = 1.5% (w/v) alginate, 8 min, with chitosan. T6 = 1.5% (w/v) alginate, 20 min, without chitosan. T7 = 3.0% (w/v) alginate, 8 min, with chitosan. T8 = 1.5% (w/v) alginate, 20 min, with chitosan. Similar lowercase letters in the same column indicate no significant difference between treatments (p > 0.05). A similar capital letter in the same row indicates no significant difference between treatments (p > 0.05).

To demonstrate the potential release of *L. fabifermentans* from the beads, the concentration of *L. fabifermentans* in the yogurt was determined (Table 7). The concentration of *L. fabifermentans* was found to be extremely low (between ~ 10^3 and ~ 10^4 CFU/g) in treatments T1, T2, T3, and T6, which did not contain chitosan. This value was negligible compared to the concentration of the starter culture (9 Log_{10} CFU/g yogurt).

Sensory analysis of yogurt

A hedonic test was conducted to assess the palatability of the yogurt containing beads. The sensory evaluation was performed on freshly produced yogurt as well as after 28 days of storage, with 60 untrained judges aged between 18 and 24 years. In general (Table 8), the sensory attributes were slightly affected by the addition of beads compared to the control yogurt (without beads). However, the observed decrease was minimal, corresponding to only one point on the hedonic scale for each attribute. Although this comparison was made, it does not imply that the product is undesirable, as the products are inherently different.

Table 6. Titratable acidity (% lactic acid) of yogurt supplemented with alginate beads during four weeks of storage.

Treatment	Time (weeks)					
	0	1	2	3	4	Tukey
1	0.96^{Abc}	0.98^{Aab}	0.99^{Aab}	1.04^{Bab}	1.11^{Cabc}	0.03
2	0.94^{Aab}	0.96^{Aa}	1.04^{Bab}	1.09^{BCab}	1.12^{Cbc}	0.06
3	0.94^{Aab}	1.06^{Bc}	1.06^{Bb}	1.14^{Cb}	1.14^{Cc}	0.02
4	0.94^{Aab}	0.99^{ABab}	1.01^{ABab}	1.03^{ABa}	1.08^{Ba}	0.10
5	0.92^{Aa}	0.95^{Ba}	0.98^{Ca}	1.05^{Dab}	1.09^{Eab}	0.01
6	0.96^{Abc}	1.03^{ABbc}	1.05^{BCb}	1.12^{Cab}	1.14^{Cc}	0.08
7	0.95^{Abc}	1.02^{Bbc}	1.06^{Cb}	1.10^{Dab}	1.12^{Eab}	0.02
8	0.98^{Ac}	1.03^{ABbc}	1.06^{BCb}	1.11^{Cab}	1.09^{BCab}	0.07
Control	0.92^{Aa}	0.96^{ABa}	1.00^{Bab}	1.05^{Cab}	1.09^{Cab}	0.04
Tukey	0.02	0.05	0.02	0.05	0.04	

T1 = 1.5% (w/v) alginate, 8 min, without chitosan. T2 = 3.0% (w/v) alginate, 8 min, without chitosan. T3 = 3.0% (w/v) alginate, 20 min, without chitosan. T4 = 3.0% (w/v) alginate, 20 min, with chitosan. T5 = 1.5% (w/v) alginate, 8 min, with chitosan. T6 = 1.5% (w/v) alginate, 20 min, without chitosan. T7 = 3.0% (w/v) alginate, 8 min, with chitosan. T8 = 1.5% (w/v) alginate, 20 min, with chitosan. Similar lowercase letters in the same column indicate no significant difference between treatments (p > 0.05). A similar capital letter in the same row indicates no significant difference between treatments (p > 0.05).

Table 7. Concentration of *L. fabifermentans* and starter culture in yogurt at the end of storage (week 4)

Treatment	*L. fabifermentans* (CFU/g yogurt)	Starter culture (CFU/g yogurt)
T1	~10^4	~10^9
T2	~10^3	~10^9
T3	~10^4	~10^9
T4	ND	~10^9

T5	ND	$\sim 10^9$
T6	$\sim 10^3$	$\sim 10^9$
T7	ND	$\sim 10^9$
T8	ND	$\sim 10^9$

ND = NOT DETECTED.

Table 8. Results obtained in the seven-point hedonic test performed on days 0 and 28 of storage

	Time (days)	Treatment									Tukey
		Control	T1	T2	T3	T4	T5	T6	T7	T8	
Color	0	6.26a	5.25cd	5.08d	5.56bcd	6.05ab	5.38cd	5.66bcd	6.25ab	5.80abc	0.56
	28	6.50a	5.46c	5.57c	5.89abc	6.39ab	6.16abc	5.7bc	6.36ab	6.40ab	0.77
Smell	0	6.05a	5.36b	5.41b	5.71ab	5.48ab	5.18b	5.78ab	5.80ab	5.50ab	0.63
	28	6.14a	5.46a	5.67a	5.82a	5.82a	5.56a	5.50a	5.70a	5.86a	0.90
Flavor	0	6.29a	4.73d	5.21cd	5.3bcd	5.38bcd	5.70abc	5.55bc	5.91ab	5.51bc	0.69
	28	6.28a	5.35ab	5.42ab	5.67ab	6.0ab	5.60ab	5.16ab	5.66ab	6.16a	0.98
Texture	.0	5.85a	4.6c	4.91bc	5.15abc	5.16abc	4.56c	4.98bc	5.43ab	5.11bc	0.84
	28	6.17a	5.07bc	4.28c	5.10bc	5.75abc	5.33ab	5.33abc	5.76ab	5.86ab	0.95
Global Appearance	0	6.25a	5.01b	5.2c	5.40bc	5.41bc	5.50bc	5.45bc	5.95ab	5.46bc	0.11
	28	6.42a	5.42abc	5.17cd	5.5bcd	5.96abc	5.73abcd	5.10d	5.76abcd	6.06ab	0.84

T1 = 1.5% (w/v) alginate, 8 min, without chitosan. T2 = 3.0% (w/v) alginate, 8 min, without chitosan. T3 = 3.0% (w/v) alginate, 20 min, without chitosan. T4 = 3.0% (w/v) alginate, 20 min, with chitosan. T5 = 1.5% (w/v) alginate, 8 min, with chitosan. T6 = 1.5% (w/v) alginate, 20 min, without chitosan. T7 = 3.0% (w/v) alginate, 8 min, with chitosan. T8 = 1.5% (w/v) alginate, 20 min, with chitosan. A similar lowercase letter in the same row indicates no significant difference between treatments for each descriptor and time ($p > 0.05$).

The color of a product is a crucial indicator, as it is the first visual cue for consumers when selecting a product [22]. At the beginning of storage, the color (Table 8) was significantly different ($p < 0.05$) between the treated yogurt and the control yogurt. However, yogurt containing chitosan beads did not show a significant difference ($p > 0.05$), indicating that this attribute was favorable. The chitosan beads gave the yogurt a shinier appearance, which was considered an improvement.

Smell is another key property that distinguishes yogurt. On day zero, the acceptance scores ranged from 5.18 to 5.80 across treatments (Table 8). By week four, no significant difference ($p > 0.05$) was observed between the yogurt from the treatment groups and the control group. These results are favorable, suggesting that the addition of beads did not negatively impact on the product's aroma, and the yogurt remained acceptable at the end of storage.

Similarly, taste did not significantly differ ($p > 0.05$) between the treated yogurt and the control yogurt at the end of storage (Table 8). These findings align with those of Kailasapathy [23], who reported that the use of encapsulated beads in yogurt did not alter color, acidity, or flavor properties. During fermentation, milk protein proteolysis generates peptides and amino acids, which contribute to the formation of flavor compounds [24], while short-chain fatty acids are also produced [5]. Furthermore, although the addition of beads to the yogurt matrix can affect its texture, the yogurt with added beads showed no significant change in texture at either the beginning or the end of storage (Table 8).

Finally, in terms of general acceptance at the end of storage, treatments T1, T4, T5, T7, and T8 did not show significant differences (p > 0.05) compared to the control yogurt (Table 8). Therefore, the addition of alginate beads to yogurt did not affect its overall acceptance. The sensory attributes of taste, odor, texture, and general acceptance were not adversely affected by the incorporation of alginate beads. These results suggest that the decrease in pH during storage and the increase in acidity did not influence the sensory outcomes, due to the low concentration of lactic acid.

4. Discussion

A key aspect of this study was the evaluation of the survival of *Lactiplantibacillus fabifermentans* and the encapsulation efficiency of phenolic compounds under different formulation conditions. The survival of *L. fabifermentans* ranged from 8.83 to 9.47 Log_{10} CFU/g, while the encapsulation efficiency of phenolic compounds (PCEE) was approximately 20%. An increase in alginate concentration (3%) and crosslinking time (20 minutes) led to improved encapsulation efficiency. These findings are consistent with those reported by Abbaszadeh et al. [25], who demonstrated that higher alginate concentrations enhanced the encapsulation efficiency of *Lactobacillus rhamnosus*. This behavior is attributed to the increased rigidity provided by the alginate matrix, which limits the release of microorganisms during the hardening and coating stages [25,26].

Regarding crosslinking time, longer durations favor enhanced intermolecular interactions between the guluronic acid residues in sodium alginate, leading to a more cohesive gel network [26,27]. In contrast, shorter crosslinking times may result in incomplete gelation and reduced encapsulation efficiency [26]. Additionally, during the gelation process, alginate forms a more stable crosslinked matrix in the presence of calcium ions, thereby supporting probiotic survival [18].

Nevertheless, prior to gelation, alginate may interact with both the phenolic compounds from the coffee extract and the probiotic cell wall, creating potential competition for calcium ion (Ca^{2+}) binding. Caffeine and chlorogenic acid—two of the principal phenolic compounds identified in coffee husks [28]—may engage in hydrogen bonding with alginate. Specifically, interactions may occur between the hydroxyl groups of chlorogenic acid, the carbonyl groups of caffeine, and the carboxyl groups of alginate. Such interactions could potentially influence the structure and functionality of the encapsulation matrix.

These mechanisms may significantly influence the stability and encapsulation efficiency of phenolic compounds within the alginate matrix, as reported by Machado et al. [29]. Additionally, the low PCEE observed in this study could be attributed to the diffusion of phenolic compounds into the calcium chloride solution during the crosslinking process, thereby reducing their retention within the encapsulating matrix. The physical entrapment of these compounds in the "egg-box" structure formed by calcium–alginate interactions may also be insufficient to ensure complete encapsulation, ultimately compromising both stability and efficiency.

These findings are particularly noteworthy considering the report by Khochapong et al. [12], which indicated that aqueous coffee husk extract inhibits the growth of microorganisms belonging to the genus *Lactobacillus*. Despite this, the viability of *L. fabifermentans* remained above 6 Log_{10} CFU/g, the minimum threshold recommended by the FAO/WHO [30] for probiotics to confer health benefits through food consumption. Therefore, the successful co-encapsulation of *L. fabifermentans* with phenolic compounds from coffee husk supports the potential development of a novel functional food product.

The PCEE values obtained in this study are comparable to those reported by other authors. For instance, Arriola et al. [31] reported a PCEE of 20% in alginate beads containing dandelion extract, while Flamminii et al. [32] observed a PCEE of 21% when encapsulating olive leaf extract using a combination of alginate and pectin. However, our encapsulation efficiencies were lower than those reported by Belščak et al. [7], who achieved 68.94% encapsulation efficiency for caffeine using an alginate–chitosan matrix, and those reported by Li et al. [8], who obtained 57.76% efficiency for phenolic compounds from green tea using the same encapsulation system.

These discrepancies may be primarily attributed to methodological differences among the studies. In the present work, beads containing the coffee extract were immersed in the chitosan solution after gelation, whereas in previous studies, chitosan was incorporated directly into the mixture of phenolic compounds and $CaCl_2$ prior to the crosslinking process. This alternative strategy may have enhanced encapsulation efficiency by promoting earlier interaction and stabilization of the phenolic compounds within the polymer matrix.

Although the Pareto diagram (Figure 1b) indicates that both the addition of chitosan and extended crosslinking time had a statistically significant and positive effect on PCEE, the magnitude of this increase appears to be constrained by the nature of intermolecular interactions among phenolic compounds, sodium alginate, microbial cell wall components, and calcium chloride during gelation. Given the hydrophilic nature of phenolic compounds, they are more likely to remain in the aqueous phase, thereby reducing their entrapment within the polymer matrix and subsequently limiting the PCEE [32].

The observed increase in PCEE may be attributed to the cationic nature of chitosan, which can form an external membrane around alginate beads by establishing electrostatic interactions with the carboxyl groups of alginate. These interactions help to stabilize the matrix and reduce the diffusion and loss of phenolic compounds during the encapsulation process [33].

During the gastrointestinal simulation, the encapsulated probiotic demonstrated a certain degree of protection. It is plausible that under simulated gastric conditions (pH 1.9), the chitosan layer was partially solubilized, contributing to the degradation of the polymeric network. As a result, the probiotic was exposed to the gastric solution containing chitosan, which may have exerted a synergistic antimicrobial effect and reduced probiotic viability. Indeed, Barbosa et al. [34] reported antimicrobial activity of chitosan under acidic conditions.

In contrast, Ramírez-Pérez et al. [5] reported a viability of 90% for L. fabifermentans in gastrointestinal simulations when no encapsulation system was applied. Although higher viability may be observed in the absence of encapsulation under short-term conditions, in the long term, encapsulation remains essential to protect the microorganism from environmental stressors and to ensure its stability during prolonged storage.

On the other hand, during the initial stages of gastric simulation, the volume of the alginate beads gradually increased, allowing for the controlled release of *L. fabifermentans*. At the end of the gastric phase and throughout the intestinal simulation, the beads continued to swell without undergoing structural disintegration. This volumetric expansion is likely related to the increase in internal pH caused by exposure to intestinal fluid, which promotes bead swelling. Under acidic conditions, the amino groups of chitosan are ionized, while the hydroxyl groups of phenolic compounds become deprotonated, leading to matrix expansion and facilitating the release of encapsulated microorganisms [33,35].

The survival rates observed in this study are consistent with those reported by Azam et al. [36], who documented approximately 80% viability of *L. rhamnosus* encapsulated in sodium alginate beads. According to these authors, the internal structure of alginate beads contains intramolecular voids that enable bile salt diffusion, which in turn contributes to the increased bead volume during intestinal simulation.

Throughout the storage period, *L. fabifermentans* maintained its viability within the encapsulation system. The "egg-box" structure of the calcium-alginate matrix likely played a crucial role in retaining both the microorganisms and phenolic compounds, while simultaneously reducing water availability within the beads. This decrease in water activity (Aw) likely limited microbial metabolism, rendering the probiotic less susceptible to environmental stress.

Moreover, as noted by Murthy and Naidu [37], coffee husk contains approximately 12% sugars, which may have served as a carbon source for *L. fabifermentans*, supporting minimal growth and survival over the 28-day storage period despite limited nutrient and water mobility inside the matrix. Similarly, Machado et al. [38] demonstrated that sugars extracted from coffee husks promoted the

growth of *L. paracasei*, suggesting that these carbohydrates exert a beneficial effect on probiotic viability.

These findings are particularly encouraging, as they confirm the sustained viability of the encapsulated probiotic in yogurt. In contrast, previous studies by Silva et al. [2], De Prisco et al. [3], and Chaikha [39] reported a decline of approximately three logarithmic cycles in the viability of encapsulated probiotics during storage. Likewise, Kumar and Kumar [4] observed a decrease in *Lactobacillus rhamnosus* viability from 8.8 to 4.35 Log_{10} CFU/g over a 15-day period using beads composed of 2% alginate and 2% $CaCl_2$.

The retention of gastrointestinal survival (RGS) of probiotics after four weeks of storage is particularly relevant; however, these values decreased over time. This reduction in probiotic viability may be attributed to prolonged storage of the beads in yogurt. As storage progresses, the acidity of the yogurt increases, potentially leading to the partial dissolution of the chitosan-coated beads under acidic conditions. This process facilitates the release of the encapsulated probiotic. Once released, chitosan may interact with the positively charged free amino groups present in the probiotic cell wall, causing structural damage and, consequently, cell death [40]. In contrast, beads composed solely of alginate as the encapsulating agent did not exhibit this detrimental effect, thereby allowing for higher survival rates of the probiotic throughout the four-week storage period.

Regarding phenolic compounds, a decline in their concentration was observed at the end of the storage period. This reduction may be attributed to the diffusion of phenolic compounds into the yogurt matrix. As previously described by Chan et al. [41] and Gombotz and Wee [42], water-soluble bioactive compounds can readily diffuse from the encapsulation matrix into the surrounding medium, driven by a concentration gradient.

Nevertheless, the presence of chitosan appeared to mitigate this diffusion, functioning as a protective barrier. The addition of chitosan likely reduced the release of phenolic compounds due to the formation of electrostatic interactions between the carboxyl groups of alginate and the amino groups of chitosan. These interactions lead to the development of a semi-permeable membrane around the beads, which restricts the outward diffusion of the encapsulated compounds [43].

At this stage, we confirmed both the high survival rate of *L. fabifermentans* and the retention of phenolic compounds within the encapsulated beads. However, it was also necessary to assess the impact of these beads on the yogurt matrix. Syneresis was evaluated, and higher levels were observed in formulations containing chitosan-coated beads. This phenomenon may be attributed to the behavior of the beads during milk fermentation, wherein the chitosan-coated beads tended to sink to the bottom of the container, as illustrated in Figure 3.

During fermentation, milk acidification leads to the formation of a gel, altering the density and allowing denser chitosan-coated beads to settle. As fermentation proceeds, this downward movement may disrupt the structural integrity of the gel, contributing to water release and consequently increasing syneresis. The sinking behavior of the beads may also be due to the formation of a polyelectrolyte complex between the amino groups of chitosan and the carboxylate groups of alginate, which results in a denser polymeric network [44] compared to uncoated beads. Moreover, increased syneresis may be partially explained by the acid-induced contraction of the protein network. Higher acidity promotes protein hydration and gel shrinkage, ultimately leading to enhanced syneresis [45]. Among the physicochemical parameters, pH and acidity are essential indicators of yogurt quality. The initial drop in pH is primarily due to lactic acid production by the starter culture. However, the continued decrease in pH during storage can be attributed to the gradual release of *L. fabifermentans* from the beads, which ferment lactose into organic acids, mainly lactic and acetic acids [5,46].

Several studies have examined the incorporation of alginate beads into yogurt and their influence on the food matrix. For example, Pourjafar et al. [47] and Budianto et al. [48] reported that the addition of alginate beads did not significantly alter the pH of yogurt compared to control formulations, and the pH remained stable throughout storage. These findings contrast with our

results, where a noticeable decrease in pH was observed during storage, presumably due to probiotic release and metabolic activity.

Considering the slight acidification observed, a sensory evaluation was conducted using a hedonic test to assess consumer acceptability. The results indicated that none of the treatments were negatively affected by the addition of beads. Even at the end of the storage period, the yogurt was rated as acceptable by the panelists. Notably, despite the release of *L. fabifermentans* and phenolic compounds, the sensory attributes of the yogurt—such as taste, texture, and appearance—remained unchanged. This finding contrasts with previous observations by Rahmani et al. [49], who reported that the direct addition of free phenolic compounds could negatively impact sensory properties. Nonetheless, the overall acceptability in their study remained above the threshold of consumer acceptance.

5. Conclusions

An increase in the concentration of sodium alginate and chitosan, as well as the gelation time during ionic gelation, positively influenced the encapsulation efficiency of coffee extract and the viability of *Lactobacillus fabifermentans* in alginate beads. This ionic gelation process enabled the encapsulation of phenolic compounds and *L. fabifermentans* in stable gel beads, which were successfully maintained in yogurt for 28 days of storage. The concentration of phenolic compounds and the viability of L. fabifermentans in chitosan-coated beads remained stable throughout the four-week storage period in yogurt. Although the physicochemical properties of yogurt supplemented with alginate beads were significantly different from those of the control yogurt at the end of storage, these changes did not negatively impact consumer acceptance, as assessed through sensory evaluation. Yogurt containing *L. fabifermentans* and coffee extract encapsulated in sodium alginate beads exhibited acceptable physicochemical and organoleptic properties, making it a promising functional food product. Given its beneficial characteristics, this formulated yogurt offers potential applications within the food industry.

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Abbreviations

The following abbreviations are used in this manuscript:.

EE	Encapsulation Efficiency
GAE	Gallic Acid Equivalents
MEE	Microbial Encapsulation Efficiency
PCEE	Phenolic Compounds Encapsulation Efficiency
RGS	Resistance of Gastrointestinal Simulation

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