

Encapsulation of *Lactiplantibacillus plantarum* and Beetroot Extract with Alginate and Effect of Capsules on Rheological Properties and Stability of an Oil-in-Water Emulsion Model Food

Minerva Bautista-Villarreal^{ID}, Sandra L. Castillo Hernández*^{ID},
Salvador López Uriarte, María Porfiria Barrón González^{ID}

Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas. Departamento de Alimentos, Av. Universidad s/n Cd. Universitaria, San Nicolás de los Garza Nuevo León, CP. 66455, Mexico

Alginate encapsulation is a viable alternative for the preservation of probiotics along the gastric route or within a food product during its shelf life. Furthermore, co-encapsulation with a vegetal material could act as a prebiotic and enhance the viability of the encapsulated probiotic. The rheological properties of dressing-type foods could be altered by adding an ingredient that would affect the quality of the final product. In this investigation, alginate beads loaded with *Lactiplantibacillus plantarum* and beetroot extract were obtained by two methods (emulsification and extrusion). They were characterized by size and morphology, encapsulation efficiency, and bacteria viability under simulated gastrointestinal conditions. Finally, they were added in an oil-in-water emulsion model food for which rheological properties and probiotic survival were monitored. The encapsulation efficiency ranged from 86.4 to 88%. Morphology and size of capsules varied depending on the method of encapsulation applied. No significant changes were evidenced in the rheological properties of the model food; the viscosity, the particle size ($d_{3,2}$), and the coalescence rate remained stable after the addition of the capsules. Survival of *L. plantarum* was significantly higher in the capsules with beetroot extract. These results suggest a prebiotic effect conferred by the beetroot extract when co-encapsulated. It is worth mentioning that the incorporation of capsules with beetroot extract does not cause any destabilization of the model food.

Key words: alginate bead, probiotic, co-encapsulation, dressing, storage, bacteria viability

ABBREVIATIONS

O/W, oil-in-water; %EE, encapsulation efficiency; NB, natural beads; BB, beetroot beads; DWB, dressing without beads; DNB, dressing with natural beads; DBB, dressing with beetroot beads; COD, commercial dressing; V_{24h} , viability after 24 h; LAB, lactic acid bacteria; cfu, colony forming unit.

INTRODUCTION

Probiotics such as *Lactiplantibacillus plantarum* are part of human intestinal microbes. They play a very important role in food fermentation processes [Nordström *et al.*, 2021]. In addition, they are live microorganisms which can confer health benefits by improving the host's intestinal microbial balance when administered in adequate amounts [Baek & Lee, 2009]. Likewise,

*Corresponding Author:

tel.: + 52 8183 294 110 ext. 3670; e-mail: sandra.castilloh@uanl.mx (S. Castillo-Hernandez)

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probiotics are being commercialized in many different forms, including supplements or fermented foods from animal or vegetal origin (dairy and non-dairy products, respectively). *L. plantarum* 299v is the most documented and popular *L. plantarum* strain in the world [Nordström *et al.*, 2021]. The consumption of probiotic foods is an actual demand and these products account for up to 70% of the total functional food market [Rajam & Subramanian, 2022]. On the other hand, prebiotics are defined as ingredients that promote the growth or metabolic activity of the beneficial bacteria; when probiotics and prebiotics are in combination, they are known as “synbiotics” and are designed to improve the survival of the microorganisms and their colonization [Vijaya Kumar *et al.*, 2015]. The trend of incorporating natural sources of bioactive compounds to obtain healthier food products has led researchers to investigate their prebiotic potential [Lazar *et al.*, 2022].

The beetroot (*Beta vulgaris* L.) is rich in bioactive compounds and confers health benefits due to its antioxidant and anti-inflammatory effects, among other features [Chhikara *et al.*, 2019; Mirmiran *et al.*, 2020]. It is applied as a functional ingredient due to its content of betalains, flavonoids, phenolic acids, and saponins [Otalora *et al.*, 2020; Płatosz *et al.*, 2020; Spórna-Kucab *et al.*, 2022]. Moreover, its benefits on the gut microbiota, due to the content of oligo- and polysaccharides, have also been demonstrated and therefore it has received special attention as a potential prebiotic [Alexandrino de Oliveira *et al.*, 2021].

The encapsulation of probiotic cells serves to protect them from hostile environments; this is how they can reach the intestine without being damaged. On the other hand, the probiotic cell is immobilized and could be added to a product in order to extend the cell viability, along with its shelf life [Vijaya *et al.*, 2015]. Dressings are oil-in-water (O/W) emulsions which are widely consumed by the population; for instance, a variety of products such as mayonnaise or dressings are available in the market. The tendency to consume foods based on natural ingredients compels producers to incorporate ingredients from vegetal origin into dressings that could improve their functional properties [Castillo *et al.*, 2021]. The quality of the final product based on O/W emulsions is governed by diverse rheological characteristics that can be affected during the incorporation of new ingredients [Ma & Boye, 2013]. Due to the reasons stated above, the aim of the present research was to study the viability of *L. plantarum* co-encapsulated with beetroot extract loaded into alginate beads, and the effect of capsules on rheological properties when incorporated as an ingredient in a model food (O/W emulsion). This information may provide valuable data for the development of new, healthier products.

MATERIALS AND METHODS

■ Microbial strain and culture conditions

A bacterial strain of *Lactiplantibacillus plantarum* 299v (*L. plantarum* Orla-Jensen 1919 Bergery *et al.* 1923 strain 17–5) was acquired from the American type culture collection (ATCC; Manassas, VA, USA) and donated by the Laboratory of Cell Biology of the Autonomous University of Nuevo León (San Nicolás de los Garza,

Mexico). It was kept on De Man Rogosa Sharpe (MRS) broth (Difco, Becton & Dickinson, Sparks, MD, USA) in refrigeration until use. In order to obtain fresh cultures, an aliquot of 100 µL was taken from the refrigerated culture and placed in tubes containing 10 mL of MRS medium; they were incubated at 37°C for 24 h for further analyses. For the encapsulation, the cells were recovered by centrifugation (Hermle Labnet Z326, Labnet International, Inc., Wehingen, Germany) at 590×g for 20 min at 25°C and washed twice with 10 mL of 8.5 g/L of a saline solution. The cells were then suspended in 1 mL of the saline solution and used for the respective encapsulation method. A plate count method in MRS was used to determine the final count of cells in the suspension [Mahmoud *et al.*, 2020].

■ Preparation of beetroot material

The beetroots (*Beta vulgaris* L.) were purchased from retail markets in the metropolitan area of Monterrey, NL, Mexico. They were washed and disinfected with mycodin® (Tavistock Group, Mexico City, Mexico) for 15 min. After that, the peel was separated and discarded. Then, the beetroots were chopped into small pieces using a knife (squares of 0.5 cm) and placed in a tunnel drier (model LO2001, Procomm, Mexico City, Mexico) at 45°C for 24 h. The dried material was ground in a mill (model 4 Wiley mill, Thomas Scientific, Swedesboro, NJ, USA) with a mesh of 1 mm and stored in a dry, sterile container protected from light [Castillo *et al.*, 2021]. This material was used later for the beetroot extract preparation.

■ Preparation of the beetroot extract

An aqueous extract of dried beetroot powder was prepared according to the procedure described by Sánchez *et al.* [2016], with minor modifications. Briefly, the dried material was soaked in sterile distilled water (20 g/L) and left at room temperature (25°C) for 48 h in a sterile screw-capped glass flask. After that, the aqueous material was recovered by filtration (Whatman No.1) and placed in a sterile glass container at 4°C until use. Finally, the extract was incorporated into the capsules according to the method mentioned below.

■ Preparation of capsules and their characterization

Two methods, including emulsification and extrusion, were used for the encapsulation of *L. plantarum* with alginate (natural beads, NB) and co-encapsulation of *L. plantarum* and beetroot extract with alginate (beetroot beads, BB). The particle size and morphology of the capsules obtained by both methods were determined. The encapsulation efficiency (%EE) was determined only for the method that gave the best results in terms of particle size and morphology of capsules.

■ Encapsulation by emulsification

The emulsification method was carried out according to the procedure described by Motalebi Moghanjoughi *et al.* [2021], with minor modifications. First, a sodium alginate solution (10 g/L) was prepared and autoclaved (121°C/15 min). After that, 1 mL of the harvested cells of *L. plantarum* in saline solution was mixed

with 99 mL of the alginate solution. The mixture (20 mL) was then added dropwise with a sterile syringe with needles of different calibers (G22×32 mm, G27×13 mm, G31×6 mm), to a previously sterilized solution containing 100 mL of vegetable oil (canola) with 0.1% (w/v) Tween 80 (Sigma-Aldrich, Saint Louis, MO, USA); then, it was magnetically stirred for 5 min. Subsequently, 100 mL of cooled 0.15 M CaCl₂ were added slowly to the mixture to break the emulsion. The mixture was left at room temperature for 30 min and the capsules were formed. The oil layer was removed, and the probiotic beads were recovered by filtration (Whatman No.1). The beads were washed twice with 0.15 M CaCl₂ and stored at 4°C for the analyses. For the incorporation of the beetroot extract to the bead, the same procedure mentioned above was used, but part of the sterile alginate solution (10 mL) was replaced by the same volume of the plant extract.

■ Encapsulation by extrusion

The encapsulation of *L. plantarum* through the extrusion technique was performed according to the method reported by Mahmoud *et al.* [2020] and Krasaekoopt & Watcharapoka [2014], with some modifications. Briefly, 1 mL of the harvested probiotic culture of *L. plantarum* was added to 99 mL of alginate solution (10 g/L) previously prepared and autoclaved. The suspension was extruded dropwise with a syringe through needles of different calibers (G22×32mm, G27×13mm, G31×6mm) in a sterile 0.15 M CaCl₂ with gentle magnetic stirring. The encapsulated cells were then washed with 0.15 M CaCl₂ and stored in a sealed bag at 4°C in sterile peptone water. For the incorporation of the beetroot extract to the beads, the plant extract (10 mL) was added to the alginate solution (89 mL). After adding 1 mL of the *L. plantarum* suspension, the same procedures of extrusion and capsule washing were used as described above.

■ Analysis of particle size and morphology of capsules

The average diameter of particles and the morphology of the capsules were evaluated by optical microscopy using a Leica EZ4HD digital stereo microscope with a high-definition CMOS camera (Leica, Wetzlar, Germany) according to the method reported by Krasaekoopt & Watcharapoka [2014] and Holkem *et al.* [2016]. Magnification of 10× was used. The diameters of 100 randomly selected capsules were measured using the Image-Pro Plus 2D analysis software (Media Cybernetics, Rockville, MD, USA) with the caliper tool.

■ Encapsulation efficiency determination

Once the capsules were obtained using the protocol that produced the best results in particle size and morphology, the encapsulation efficiency as a function of percentage of entrapped cells was determined according to Krasaekoopt & Watcharapoka [2014]. Briefly, 1 g of fresh beads was blended in a stomacher for 1 min with 99 mL of sterile peptone solution (1 g/L) and then allowed to stand for 10 min to dissolve. Appropriate serial dilutions were made and then a plate count method was deployed onto MRS broth (Difco, Becton & Dickinson). The same procedure was carried out after 24 h of the encapsulation for monitoring

the viability of *L. plantarum* in order to discard any antimicrobial activity.

The encapsulation efficiency (%EE) was calculated according to Equation (1), as suggested by Pupa *et al.* [2021].

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

where: N₀ is the initial population of *L. plantarum* before encapsulation, and N is the population of encapsulated *L. plantarum*.

The viability of bacteria after 24 h (%V₂₄) was calculated from Equation (2):

$$\%V_{24} = (\log N_{24} / \log N) \times 100 \quad (2)$$

where: N is the initial population of encapsulated *L. plantarum*, and N₂₄ is the remaining encapsulated population after 24 h.

■ Determination of *L. plantarum* viability under simulated gastrointestinal conditions

In order to determine the viability of *L. plantarum* under the gastrointestinal conditions, the method mentioned by Krasaekoopt & Watcharapoka [2014] and Chávarri *et al.* [2010] was used with minor modifications. One gram of freshly prepared beads was incubated in a tube containing 10 mL of simulated gastric juice (0.2% NaCl, 0.08 M of HCl, without pepsin and adjusted to pH 1.5 with HCl). This mixture was incubated at 37°C for 90 min; afterwards, the beads were recovered by filtration and resuspended in 9 mL of sterile simulated intestinal juice containing saline (0.5%) with pancreatin (0.1%) and bile salts (0.5%) at pH 8. This mixture was incubated at 37°C for 120 min. At the end of the process, the capsules with probiotics were recovered to determine their viability by means of a plate count (log cfu/g) as described above.

■ Determination of rheological properties and physical stability of model food with the capsules

A dressing (O/W emulsion) was used as model food. After addition of the capsules, the rheological properties, physical stability of model food, and the viability of encapsulated probiotics were determined throughout 28 days.

■ Preparation of the O/W emulsion food model

The ingredients used to formulate the O/W emulsion are presented in Table 1. The procedure performed according to Castillo *et al.* [2021] is described as follows: first, the water and egg yolk were placed in a bowl and mixed at 500 rpm for 5 min (T50 Digital Ultra Turrax, IKA, Wilmington, NC, USA). After that, vinegar, salt, sugar, and xanthan gum were added and mixed for 15 more min. Finally, the oil was added manually in a continuous manner until it was fully incorporated into the mix at 3,000 rpm for 2 min, and at 5,000 rpm for 3 more min. Once the emulsion was finished, the beads were added according to the formulations reported in Table 1. The first formulation was the dressing without the addition of beads (DWB), the second one was the dressing with the addition

Table 1. Composition (g/100 g) of oil-in-water emulsion dressings without beads (DWB), and with natural beads (DNB) obtained by encapsulation of *Lactiplantibacillus plantarum* with alginate and with beetroot beads (DBB) obtained by co-encapsulation of *L. plantarum* and beetroot extract with alginate.

Ingredient	DWB	DNB	DBB
Water	28.31	25.48	25.48
Egg yolk	3.0	2.7	2.7
Oil	50.0	45.0	45.0
Xanthan gum	0.29	0.26	0.26
White vinegar	11.50	10.35	10.35
Salt	1.15	1.04	1.04
Sugar	5.75	5.18	5.18
Natural beads	----	10.0	----
Beetroot beads	----	----	10.0

of natural beads (DNB), and the third one was the dressing with the addition of beetroot beads (DBB). The formulated dressings were packed in a sealed bag and stored at 4°C.

■ Analysis of flow properties of the O/W emulsion food model

The flow curves of the O/W emulsions (DWB, DNB and DBB) were obtained for the shear rate in range from 1 to 100 1/s using a rotational test with a RheolabQC rheometer (Anton Paar, Mexico City, Mexico) and a CC27 geometry at a temperature of 25°C [Castillo *et al.*, 2021]. Consistency indexes (k , Pa·s ^{n}) and flow behavior indexes (n) were obtained according to the power law model described in Equation (3) [Sakin-Yilmazer *et al.*, 2014].

$$\text{Shear stress (Pa)} = k \times \text{shear rate}^n \quad (3)$$

The commercial dressing was used as a point of comparison. Analyses were carried out for fresh dressings and dressings stored for 28 days.

■ Droplet size and coalescence rate determination

The particle size of fresh emulsions and those stored for 14 and 28 days were monitored with the Malvern Mastersizer 3000 equipment (Malvern Instruments, Ltd, Worcestershire, UK) using the Hydro LV unit with water as a dispersant. Angular scattering intensity data from the analyzer were used to calculate the size of the particles that create the scattering pattern using Mie's theory of light scattering [Bautista Villarreal *et al.*, 2018]. The software Malvern Mastersizer v3.63 (Worcestershire, UK) was used to calculate the Sauter mean diameter ($d_{3,2}$). The optical properties of the emulsions were defined as follows: the refractive index was 1.460, while the absorption index was 0.100. The coalescence rate (K_c , 1/s) was obtained based on the first order kinetics and the Equation (4).

$$N_t/N_0 = e^{-K_c t} \quad (4)$$

where: N_t is the concentration in the number of drops at a time = (t), N_0 is the concentration in the number of the newly formed drops (time = 0), and K_c is the rate constant, which is related to the probability that the interfacial film will break at a certain time (t). The volume of the emulsion droplets remains constant when there is no oil release in the emulsion [Castillo *et al.*, 2021].

■ Determination of *L. plantarum* viability in the food model under storage conditions

In order to monitor the viability of encapsulated *L. plantarum* over time in the food model, a plate count technique was used. Briefly, after preparation of the dressing, a portion of 10 g containing 1 g of respective beads was packed in a sealed bag and stored at 4°C. Each sample was processed in time intervals (on day 0, 2, 4, 8, 12, 18, 22 and 28), by serial dilutions and plating. Results were expressed as log cfu/g.

■ Statistical analysis

All measurements were performed three times in triplicate. To determine statistically significant differences between the values, a one-way analysis of variance (ANOVA) and a Tukey's test were used ($p < 0.05$).

RESULTS AND DISCUSSION

Beetroot was selected as vegetal material to be included in the encapsulation of *L. plantarum* since it has been implicated in different health benefits and recognized as an important source of antioxidants [Chhikara *et al.*, 2019]. It has also been reported as functional food [Chhikara *et al.*, 2019; Platosz *et al.*, 2020]. Beetroot has become a very popular food and actually is one of the top ten planted vegetables associated with superior health benefits [Borjan *et al.*, 2022]. On the other hand, some investigations have suggested the possibility of beetroot juice encapsulation to preserve its functional characteristics [Tumbas Šaponjac *et al.*, 2020]. For this reason, in this research, beetroot aqueous extract was obtained and co-encapsulated with *L. plantarum*.

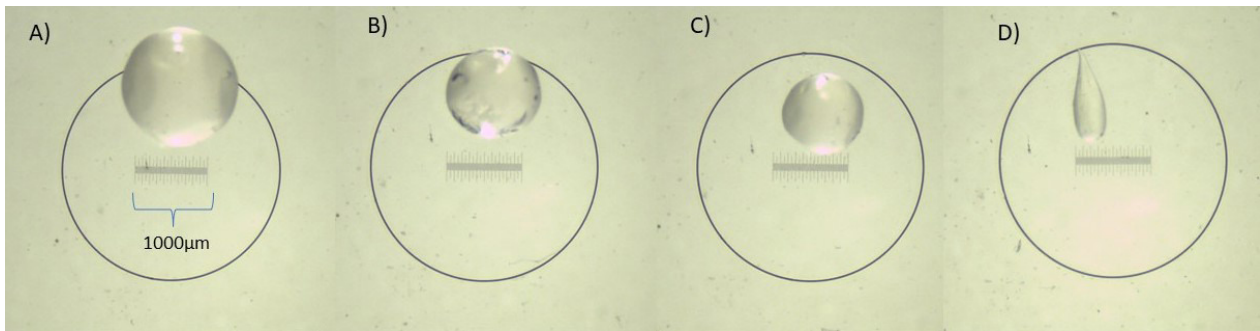


Figure 1. Morphology of the alginate beads loaded with *Lactiplantibacillus plantarum* co-encapsulated with beetroot extract obtained by extrusion (A–C) and emulsion (D) techniques using different needle calibers: G22×32 mm (A), G27×13 mm (B), G31×6 mm (C), G31×6 mm (D). Magnification was 10×. The measurements of the capsules were obtained with the Image-Pro Plus 2D analysis software with the caliper tool.

■ Characterization of capsules

■ Size and morphology

Considering that particle size can influence the aroma, texture, and appearance of the food, it may be deemed a very important physical parameter [Holkem *et al.*, 2016]. In our study, the alginate beads loaded with *L. plantarum* and with *L. plantarum* and beetroot aqueous extract varied in size and morphology depending on the protocol of capsule preparation used. However, the particle size was independent of the addition of beetroot extract (data not shown). The extrusion technique derived in spherical alginate beads (Figure 1) with an average particle diameter between 2,260 to 978 µm (for capsules with *L. plantarum* and beetroot extract), depending on the needle caliber used (Table 2). In general, the beads obtained by this protocol presented a homogeneous size for each needle caliber. On the other hand, beads obtained by the emulsion technique had an irregular morphology (Figure 1) and differed greatly in diameters. The average particle diameter of these beads ranged from 600 to 2,850 µm (Table 2). The smaller beads in size were obtained by the emulsification and needle calibers of G31×6 mm, G27×13 mm compared to analogous beads obtained using the extrusion technique. These results are in agreement with Capela *et al.* [2007], who reported a wide size distribution of alginate capsules loaded with *Lactobacillus acidophilus*, *L. casei* and *L. rhamnosus* when using the emulsion technique with bead sizes ranging from 200 to 1,000 µm. Also, Li *et al.* [2009] and Lopes *et al.* [2017], reported more uniform alginate beads loaded with probiotics obtained by the extrusion technique with diameters ranging from ~900 to 1,400 µm, which is consistent with our study results. Pupa *et al.* [2021] reported varied alginate-chitosan bead shapes and sizes depending on the preparation technique used,

with mean diameters of ~1,500 µm for those obtained by extrusion, and ~500 µm for those obtained by emulsion technique, while Mahmoud *et al.* [2020] obtained alginate microcapsules loaded with *L. plantarum* and biopolymers by the extrusion technique with sizes ranging from ~501 to 1,200 µm. According to Holkem *et al.* [2016], particle sizes below 100 µm are desirable to avoid the negative impact on the sensory characteristics; however, some marketing strategies regarding flavor may help in the acceptance of the product. Nevertheless, the optimum size of the microcapsules could be debatable as it varies according to their applications. Actually, encapsulating lactic acid bacteria (LAB) and applying them in a food product is a challenge due to the large sizes of bacterial cells that lead to the production of large beads [Mahmoud *et al.*, 2020].

The variability in the beads' morphology could affect the encapsulation efficiency and the results could hardly be replicated. Due to the variability in size and morphology of the beads obtained by the emulsification, we selected the extrusion technique for further analyses. The smallest bead sizes produced by this last method were for the needle with the caliber of G31×6mm, thus it was chosen for bead production.

■ Encapsulation efficiency and viability of *L. plantarum* after 24 h

The counts of encapsulated *L. plantarum* (natural beads, NB) and *L. plantarum* co-encapsulated with beetroot extract (beetroot beads, BB), the count of bacteria in both types of beads after 24 h, as well as encapsulation efficiency (%EE) and percentage of bacteria viability after 24 h (%V_{24h}) are presented in Table 3. The counts of encapsulated cells were 7.59 and 7.82 log cfu/g for NB and BB, respectively, with an encapsulation efficiency

Table 2. Average particle diameter ($d_{3,2}$, µm) of capsules of *Lactiplantibacillus plantarum* and beetroot extract with alginate obtained by different encapsulation techniques.

Needle caliber	Extrusion	Emulsion
G31×6mm	978±42 ^e	600±150 ^f
G27×13mm	1,250±37 ^c	1,050±120 ^d
G22×32mm	2,260±120 ^b	2,850±260 ^a

Results are expressed as mean of three repetitions in triplicate ± standard deviation. Different letters (a–f) represent significant differences of values ($p < 0.05$).

Table 3. Encapsulation efficiency (%EE) of *Lactiplantibacillus plantarum* with alginate (natural beads, NB) and *L. plantarum* and beetroot extract with alginate (beetroot beads, BB), percentage of bacteria viability after 24 h (%V_{24h}) and percentage of bacteria survival after incubation under simulated gastrointestinal conditions.

	NB	BB
Count of <i>L. plantarum</i> (log cfu/g)	7.59±0.21 ^a	7.82±0.10 ^a
%EE	86.4±2.1 ^a	88.0±2.8 ^a
Count of <i>L. plantarum</i> after 24 h (log cfu/g)	6.07±0.11 ^b	7.28±0.41 ^a
%V _{24h}	80.2±2.2 ^b	92.4±2.1 ^a
Survival (%) under gastrointestinal conditions	57.4±3.1 ^b	73.3±1.0 ^a

Results are expressed as mean of three repetitions ± standard deviation. Initial count of *L. plantarum* before encapsulation was ≈8.89 log cfu/mL. Values with different letters (a,b) in the same row are significantly different ($p < 0.05$).

of 86.4–88.0%. No significant differences ($p \geq 0.05$) in %EE were evidenced between the different formulations when cells were incorporated. These results are in agreement with the study by Pupa *et al.* [2021], who reported an efficacy of alginate – *L. plantarum* encapsulation in the range from 73.64 to 94.10%. They found a higher efficiency in the extrusion method compared to the spray drying method. Mahmoud *et al.* [2020] reported an efficiency of up to 94% with no significant differences between different encapsulating agents (skim milk, dextrin and chitosan) in combination with the alginate loaded with *L. plantarum*. In addition, our results showing that the content of beetroot extract did not affect the %EE are in line with the literature data; according to Krasaekoopt & Watcharapoka [2014], the effectiveness of encapsulation is independent of the concentration of prebiotics incorporated. Otherwise, the encapsulated bacteria viability after 24 h (%V_{24h}) varied among formulations with better survival noted in beetroot beads (Table 3). A significantly ($p < 0.05$) lower bacterial count by 1.52 log cfu/g was evidenced after 24 h in NB. The much smaller loss of 0.54 log cfu/g was noted for BB. This last assay is important to determine any negative effect of the beetroot extract on *L. plantarum* cells. A significant decrease within 24 h in the number of bacteria co-encapsulated with beetroot extract could indicate the occurrence of antimicrobial activity; if that is the case, co-encapsulation would not be feasible. On the contrary, if the population is maintained or increased, we can infer that the presence of the beetroot extract does not exert a negative effect or that it may actually be beneficial for the bacteria. Our results indicate that the incorporation of the beetroot extract in the alginate beads does not exert negative effects on *L. plantarum*. Since *L. plantarum* showed higher viability after 24 h in BB than in NB (Table 3), the effect of beetroot extract incorporation was found positive. According to Shafizadeh *et al.* [2020], some compounds co-encapsulated with bacteria could confer certain protective effect. Other investigations reported that the viability of encapsulated probiotics was improved when adding prebiotics, *i.e.*, raftilose [Capela *et al.*, 2006], inulin [Akhari, 2010], galacto-oligosaccharides and fructo-oligosaccharides [Amine *et al.*, 2014], as well as dextrin and whey [Mahmoud *et al.*, 2020] among others. These previous reports are consistent with our results, in which the V_{24h} was higher in BB than NB. Based

on this literature data and our results, it can be concluded that the beetroot extract acted as a prebiotic.

The results presented in Table 3, show that the encapsulation efficiency was less than 95% for the beads produced. Even though other investigations have reported %EE above 95%, this could be improved by increasing the number of initial cell counts [Pupa *et al.*, 2021].

■ Viability of *L. plantarum* under simulated gastrointestinal conditions

The survival percentages of *L. plantarum* encapsulated in NB and BB after the incubation under simulated gastrointestinal conditions are shown in Table 3. For this assay, the free *L. plantarum* was used as a respective control. Encapsulated *L. plantarum* had better viability compared with the free cells, which were not detectable at the end of the process. These results are in agreement with those reported by Khosravi *et al.* [2014], who found no survival of free *Bifidobacterium bifidum* after exposure to simulated gastrointestinal conditions. Several other studies have reported better survival of bacteria in the capsules than in the non-encapsulated form, during exposure to unfavorable conditions [Chávarri *et al.*, 2010; Khosravi *et al.*, 2014; Krasaekoopt & Watcharapoka, 2014; Mahmoud *et al.*, 2020; Pupa *et al.*, 2021]. Besides this, significant difference ($p < 0.05$) in the survival percentage was evidenced between the NB and BB, proving that BB ensured a better cell survival rate (73.3%) when compared with NB (57.4%) (Table 3). The losses of bacteria were 3.1 and 2.0 log cfu/g for NB and BB, respectively. This could suggest that beetroot had a protective effect on *L. plantarum*, which allows major survival of the bacteria in the gastric environment, compared with the bead that does not contain beetroot. Other studies reported a protective effect generated by the incorporation of skim milk [Mahmoud *et al.* 2020] or amylo maize [Khosravi *et al.*, 2014], with losses ranging from 2.5 to 4.6 log cfu/g after exposure to gastrointestinal conditions, depending on the bacteria and the co-encapsulated material. It has been reported that beetroot represents a rich source of bioactive compounds and contains certain amounts of nutrients (*i.e.*, carbohydrates and proteins) that could protect the bacterial cells and thus maintain their viability [Barbu *et al.*, 2020]. Although it has been

reported that buffering capacity of some ingredients can protect probiotic bacteria in the product or during intestinal transit [Shori, 2017; Tripathy & Giri, 2014], with the data presented here we cannot ensure which compound of beetroot was specifically responsible for this protection. According to Mahmoud *et al.* [2020], this protection may be influenced by the strong structure of the bead, or the nutritional factor conferred by the incorporation of beetroot extract.

■ Effect of the addition of capsules on the rheological properties of model food

To obtain functional food with capsules, different formulations of dressings based on O/W emulsion were prepared (Table 1). For monitoring the stability of the products, flow properties, the droplet size and coalescence rate were monitored during storage. The dressing without the addition of beads (DWB) was used as the control.

■ Flow properties of dressings

The flow behavior of O/W emulsions is important because the behavior of different formulations can be compared and appropriate process conditions, as well as optimal quality control strategies may be chosen accordingly [Mun *et al.*, 2009]. In our study, to evaluate changes in viscosity of dressings over time, emulsion flow curves (the changes in apparent viscosity as a function of shear rate) were obtained at the initial time of storage (zero time) and at the end of storage (28 days). Flow curves are presented in Figure 2. The behavior of the different dressings was consistent with the power law. Overall, dressings with NB and BB added exhibited non-Newtonian stress thinning behavior and a creep point related to the systems' initial resistance to flow. The control sample and formulated dressings showed a similar trend. To identify the flow characteristics of the dressings, the values of consistency coefficient (k) and flow behavior index (n) were obtained as a function of the shear rate. The k is often used as an indicator of fluid viscosity, which is a measure of the fluid's ability to resist deformation; high values indicate high viscosity and a stronger and more stable network structure

[Castillo *et al.*, 2021]. When evaluating the flow behavior index, a value less than 1 represents pseudoplasticity; this characteristic is desirable for obtaining a dressing with a good mouth feel [Ma & Boye, 2013; Primacella *et al.*, 2019]. On the other hand, a high k value is desirable for the stability of the system. The k and n values for the formulated dressings (DWB, DNB, and DBB) at zero time and after 28 days of storage are shown in Table 4. Additionally, a commercial dressing (COD) was analyzed as a point of comparison. The initial k values varied from 12.52 to 14.52 $\text{Pa}\cdot\text{s}^n$, with no significant differences ($p \geq 0.05$) among the formulations prepared, including the COD, indicating a similar viscosity of all the formulations tested. When the initial flow behavior index was considered, low values (< 1) were evidenced for all the formulations. The initial n values varied from 0.239 to 0.299. Significant difference ($p < 0.05$) was noted only between the DWB and COD formulations. Anyway, there was $n < 1$ for all the formulations, so we could expect a good texture of the product. After 28 days of storage, the k values ranged from 13.93 to 17.23 $\text{Pa}\cdot\text{s}^n$. On the other hand, the n values ranged from 0.249 to 0.300. The results showed no significant changes ($p \geq 0.05$) in k and n values over the storage period (after 28 days) regardless of the formulation, which indicates that the addition of the capsules did not affect the rheological properties of the system.

■ Droplet size and coalescence rate of dressings

An incorporation of a new ingredient can destabilize the system and may affect shelf life. Stability of the system can be monitored through changes in the average particle diameter over time and also implies prevention of coalescence [Castillo *et al.*, 2021]. Averages of droplet size ($d_{3,2}$) of the formulations were measured at zero time and after 14 and 28 days of storage. Changes in droplet size varied along time in the three formulations (Table 5). A significant difference ($p < 0.05$) was found on day fourteen of storage only for DNB ($d_{3,2}=4.380$) compared with DWB ($d_{3,2}=3.942$) and DBB ($d_{3,2}=3.836$). On the other hand, on day 28 of storage, significant differences ($p < 0.05$) were found for DNB ($d_{3,2}=4.326$) and DBB ($d_{3,2}=3.925$) compared with DWB ($d_{3,2}=3.488$). Despite the differences in those points, no significant

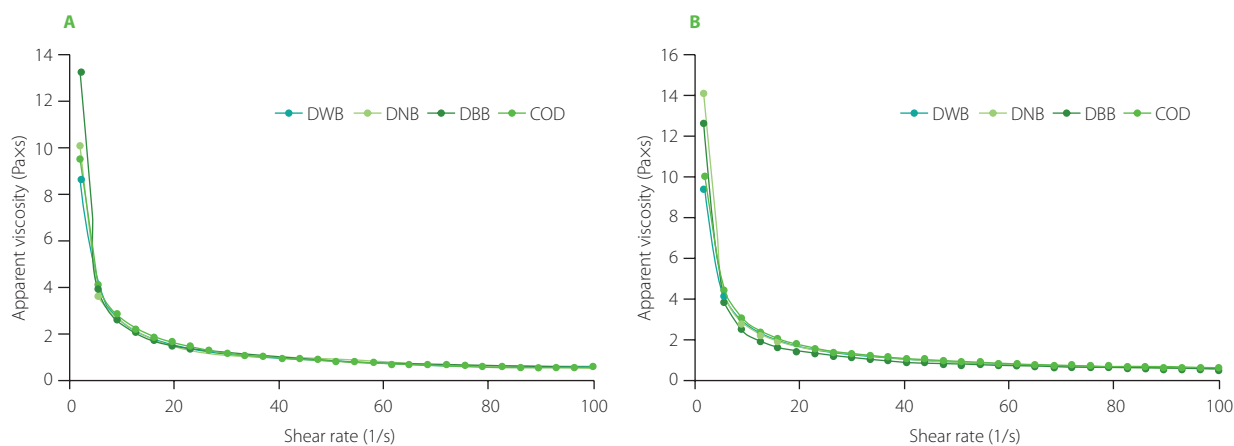


Figure 2. Apparent viscosity as a function of shear rate of the dressings at the initial (A) and final time of storage (28 days) (B). DWB, dressing without beads; DNB, dressing with encapsulated *Lactiplantibacillus plantarum* with alginate (natural beads); DBB, dressing with co-encapsulated *L. plantarum* and beetroot extract with alginate (beetroot beads); COD, commercial dressing.

Table 4. Consistency coefficient (k) and flow behavior index (n) of dressings at the initial (day 0) and final time (28 days) of storage, and coalescence rate (K_c) of model food.

Dressing	k (Paxs ⁿ)		n		K_c (1/s)
	0 day	28 days	0 day	28 days	
COD	14.29±0.89 ^{aA}	15.47±1.04 ^{aA}	0.239±0.020 ^{bA}	0.279±0.028 ^{aA}	-2×10 ^{-7a}
DWB	12.52±1.23 ^{aA}	13.93±1.11 ^{aA}	0.299±0.018 ^{aA}	0.300±0.015 ^{aA}	-4×10 ^{-8b}
DNB	13.20±3.61 ^{aA}	17.23±2.66 ^{aA}	0.279±0.044 ^{abA}	0.249±0.044 ^{aA}	-3×10 ^{-7a}
DBB	14.52±3.26 ^{aA}	15.85±2.77 ^{aA}	0.251±0.050 ^{abA}	0.251±0.039 ^{aA}	-1×10 ^{-7a}

Results are expressed as mean of three repetitions ± standard deviation. Values with different minor letters (a–c) in the same column are significantly different ($p < 0.05$). Values with different capital letters (A, B) in the same row for each parameter separately (k or n) are significantly different ($p < 0.05$). COD, commercial dressing; DWB, dressings without beads; DNB, dressing with encapsulated *Lactiplantibacillus plantarum* with alginate (natural beads); DBB, dressing with co-encapsulated *L. plantarum* and beetroot extract with alginate (beetroot beads).

Table 5. Average particle diameter ($d_{3,2}$, μm) of the dressings at 0, 14 and 28 days of storage.

Dressing	0 day	14 days	28 days	Mean
DWB	3.514±0.011 ^a	3.942±0.115 ^b	3.488±0.015 ^b	3.624±0.215 ^a
DNB	3.518±0.013 ^a	4.380±0.027 ^a	4.326±0.053 ^a	4.070±0.408 ^a
DBB	3.512±0.007 ^a	3.836±0.025 ^b	3.925±0.041 ^a	3.786±0.217 ^a

Results are expressed as mean of three repetitions in triplicate ± standard deviation. Different letters (a,b) in the same column represent significant differences ($p < 0.05$). DWB, dressings without beads; DNB, dressing with encapsulated *Lactiplantibacillus plantarum* with alginate (natural beads); DBB, dressing with co-encapsulated *L. plantarum* and beetroot extract with alginate (beetroot beads).

differences ($p \geq 0.05$) were found in the global behavior throughout the 28 days.

Another parameter that provides important information about stability of the emulsion is the coalescence rate (K_c), which can be measured through changes in the average diameter of the drops over time. This parameter allows the collection of quantitative data on system stability. The coalescence rate of the different formulations is reported in Table 4. Despite a significant difference ($p < 0.05$) was noted for DWB (-4×10^{-8}) compared with the other formulations, the coalescence rate for all the dressings tested had very low values with exponents -7 and -8 . The formulations with encapsulated probiotic showed similar behavior compared with the COD with values ranging from -1×10^{-7} to -3×10^{-7} . According to Bautista *et al.* [2018], values at this level indicate a stable system. When the droplet coalescence rate is high, a decrease in the emulsion's viscosity may occur because the coalescence of emulsions is inversely related to the viscosity of the aqueous phase [Krstonošić *et al.*, 2009]. We can observe in our results that k of the formulations did not decrease during storage (Table 4).

The coalescence of emulsions in storage conditions are influenced by different factors, as collision frequency and drainage time [Ye *et al.*, 2004]. This phenomenon could take place in different stages of emulsion formulation, *i.e.*, whipping process or storage [Petruć *et al.*, 2016]. The addition of certain ingredients including those with different functional characteristics could cause changes in stability. Preserving the original viscoelastic properties is very important to the quality of the product that is strongly influenced by its rheological properties and appearance attributes [Ma & Boye, 2013]. Losses in stability provoked by

the increase in droplet mean diameter of the emulsion shorten the shelf life. Therefore, obtaining a product with an extended shelf life is a challenge during food product formulation [Traynor *et al.*, 2013].

■ Viability of encapsulated *L. plantarum* in a model food in storage conditions

The NB and BB loaded with *L. plantarum* were added to a dressing (DNB and DBB, respectively) and the viability of the probiotic was determined over time. Viable counts of *L. plantarum* during 28 days of storage at 4°C are presented in Figure 3A. Also, the percentage of viability was calculated and results are shown in Figure 3B. Even though the number of viable cells of *L. plantarum* decreased as time progresses in both models, the DBB maintained a greater viability of the probiotic over time, ranging from 7.09 to 4.3 log cfu/g, and a percentage of viability ranging from 94.4% to 60.6% along the storage time, respectively. On the other hand, the levels of cells recovered for the DNB were significantly lower ($p < 0.05$) from the 8th day on. The bacterial population decreased from 6.90 to 2.1 log cfu/g at the end of storage for this last model, while the percentage of viability ranged from 79.2% to 30.6%, respectively. At the end of storage, a total diminution of 2.8 log cfu/g was detected for DBB and 4.8 log cfu/g for DNB, suggesting that encapsulated beetroot could act as a prebiotic ingredient. Our results are in agreement with Chavarri *et al.* [2010], who reported losses of encapsulated probiotic bacteria ranging from 2.1 to 3.4 log cfu/g along the time of storage (28 days), depending on the bead matrix. Additionally, Rodríguez Huevo *et al.* [2011] reported losses of 4.0 log cfu/g for encapsulated probiotics without prebiotic ingredient in a period of 25 days.

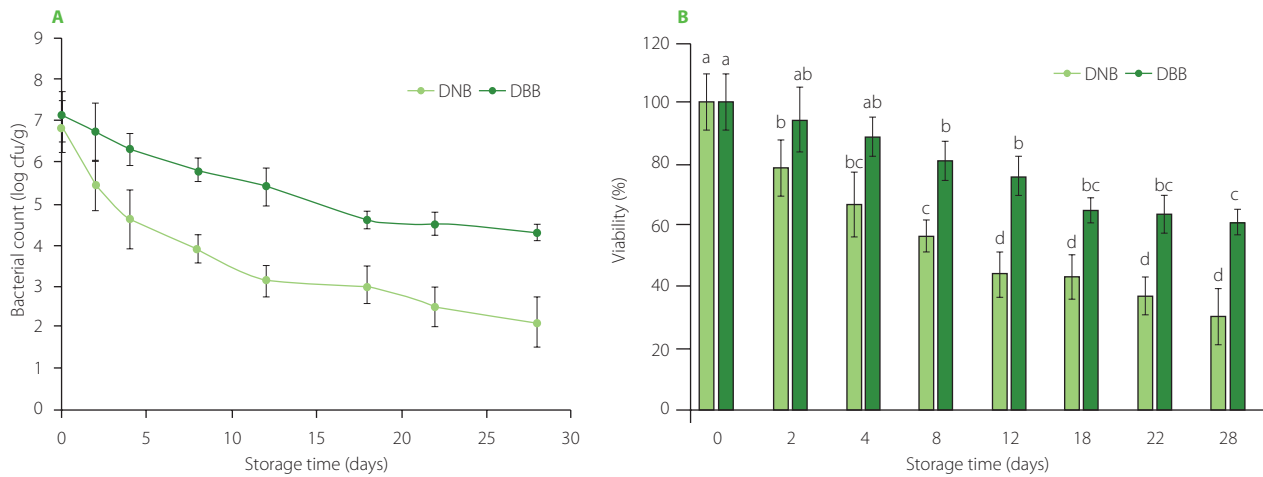


Figure 3. Viable counts of encapsulated *Lactiplantibacillus plantarum* along time of storage at 4°C in the model food (A). Percentage of viability of *L. plantarum* along storage time in the model food (B). Error bars represent standard deviation. Different letters in the top of the bars (a–d) represent significant differences ($p < 0.05$). DNB, dressing with encapsulated *L. plantarum* with alginate (natural beads); DBB, dressing with co-encapsulated *L. plantarum* and beetroot extract with alginate (beetroot beads).

Although the population of *L. plantarum* continued to decline by the 28th day, the population remained over 60% in DBB, while the DNB model had less than 35% of viability at the end of storage. These results are consistent with other reports, in which greater viability of probiotics was observed when encapsulated with prebiotics rather than with natural beads [Capela *et al.*, 2006; Krasaekoopt & Watcharapoka, 2014; Mahomud *et al.*, 2020]. Krasaekoopt & Watcharapoka, [2014] reported greater viability of *L. casei* and *L. acidophilus* encapsulated with galacto-oligosaccharides compared with those without this prebiotic. The authors also reported minimal losses of population (0.5 to 2.5 log cfu/g) along four weeks of storage. Additionally, Mahomud *et al.* [2020] reported better survival in storage conditions for *L. plantarum* when co-encapsulated with skim milk, and in some cases, an increase in population was evidenced. According to Barbu *et al.* [2020], diverse factors may influence the growth of the probiotics; in these cases, it could be attributed to biocomposites and nutrients from the bead matrix that somehow protect the bacterial cells, and thus maintain their viability. In a dressing, a probiotic bacterium must be kept immobilized to prevent proliferation, and in this way prevent food decomposition; when encapsulating the bacteria, it remains immobilized, protecting the integrity of the food while maintaining its attributes, such as pH and texture. Likewise, it has been demonstrated that beetroot consumption has a beneficial effect on the gut microbiota in humans, induced by a prebiotic effect [Alexandrino de Oliveira *et al.*, 2021]. Our results have shown a higher microbial recovery during storage time in the DBB model, so this model could have been influenced by the presence of the beetroot extracts inside the capsules.

Industrially, encapsulated probiotic bacteria can be used in many products (*i.e.*, yogurt, cheese or frozen dairy desserts). This strategy provides higher stability of bacteria also protecting the components incorporated against nutritional loss [Anal & Singh, 2007; Khosravi *et al.*, 2014].

CONCLUSIONS

The extrusion technique demonstrated greater feasibility compared to emulsification for obtaining capsules of *L. plantarum* with alginate and *L. plantarum* and beetroot extract with alginate with homogeneous morphology and size. The co-encapsulation of *L. plantarum* and beetroot extract with alginate conferred better protection for bacteria under gastrointestinal conditions than the encapsulation of *L. plantarum* along with alginate. Although the protection exerted by beetroot on the bacteria was evidenced, due to the number of components that beetroot contains, it is difficult to determine the driving mechanisms of this protection. Further studies are ongoing on this aspect. When beads were incorporated to the O/W emulsion model food, they did not cause changes in the flow properties or droplet coalescence, indicating that the addition of the capsules does not cause system destabilization. Additionally, beetroot beads preserved the viability of *L. plantarum* during 28 days of storage in the product, indicating that the incorporation of beetroot in the capsule could have a prebiotic effect on *L. plantarum*. Future studies should be carried out to evaluate the acceptability of the developed product.

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CONFLICTS OF INTEREST

The authors declare no conflict of interests.

ORCID IDs

M.P. Barrón González
M. Bautista-Villarreal
S.L. Castillo Hernández

<https://orcid.org/0000-0002-8386-444X>
<https://orcid.org/0000-0001-7975-1488>
<https://orcid.org/0000-0001-8354-3200>

REFERENCES

- Akhiar, N.S.M. (2010). Enhancement of probiotics survival by microencapsulation with alginate and prebiotics. *MMG 445 Basic Biotechnology*, 6, 13–18.
- Alexandrino de Oliveira, S.P., do Nascimento, H.M.A., Sampaio, K.B., de Souza, E.L. (2021). A review on bioactive compounds of beet (*Beta vulgaris L. subsp. vulgaris*) with special emphasis on their beneficial effects on gut microbiota and gastrointestinal health. *Critical Reviews in Food Science and Nutrition*, 61(12), 2022–2033.
<https://doi.org/10.1080/10408398.2020.1768510>
- Amine, K.M., Champagne, C.P., Salmieri, S., Britten, M., St-Gelais, D., Fustier, P., Lacroix, M. (2014). Effect of palmitoylated alginate microencapsulation on viability of *Bifidobacterium longum* during freeze-drying. *LWT – Food Science and Technology*, 56(1), 111–117.
<https://doi.org/10.1016/j.lwt.2013.11.003>
- Anal, A.K., Singh, H. (2007). Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. *Trends in Food Science & Technology*, 18(5), 240–251.
<https://doi.org/10.1016/j.tifs.2007.01.004>
- Baek, Y.J., Lee, B.H. (2009). Chapter 12 – Probiotics and prebiotics as bioactive components in dairy products. In Park, Y.W. (Ed.). *Bioactive Components of Milk and Dairy Products*, Wiley- Blackwell, pp. 287–310.
<https://doi.org/10.1002/9780813821504.ch12>
- Barbu, V., Cotarlet, M., Bolea, C.A., Cantaragiu, A., Andronoiu, D.G., Bahrim, G.E., Enachi, E. (2020). Three types of beetroot products enriched with lactic acid bacteria. *Foods*, 9(6), art. no. 786.
<https://doi.org/10.3390/foods9060786>
- Bautista Villarreal, M., Gallardo Rivera, C.T., García Márquez, E., Rodríguez Rodríguez, J., Núñez González, M.A., Chávez Montes, A., Báez González, J.G. (2018). Comparative reduction of egg yolk cholesterol using anionic chelating agents. *Molecules*, 23(12), art. no. 3204.
<https://doi.org/10.3390/molecules23123204>
- Borjan, D., Šeregelj, V., Andrejč, D.C., Pezo, L., Tumbas Šaponjac, V., Knez, Ž., Vulić, J., Marevci, M.K. (2022). Green techniques for preparation of red beetroot extracts with enhanced biological potential. *Antioxidants*, 11(5), art. no. 805.
<https://doi.org/10.3390/antiox11050805>
- Capela, P., Hay, T.K.C., Shah, N.P. (2006). Effect of cryoprotectants, prebiotics and microencapsulation on survival of probiotic organisms in yoghurt and freeze-dried yoghurt. *Food Research International*, 39(2), 203–211.
<https://doi.org/10.1016/j.foodres.2005.07.007>
- Capela, P., Hay, T.K.C., Shah, N.P. (2007). Effect of homogenisation on bead size and survival of encapsulated probiotic bacteria. *Food Research International*, 40(10), 1261–1269.
<https://doi.org/10.1016/j.foodres.2007.08.006>
- Castillo, S., Rodríguez, A., Bautista-Villarreal, M., García-Solano, N., Gallardo-Rivera, C., Báez-González, J.G., Sánchez-García, E., García-Alanis, K.G. (2021). Rheological and nutritional characterization of sweet corn by-product (cob) to develop a functional ingredient applied in dressings. *Frontiers in Nutrition*, 8, art. No. 666654.
<https://doi.org/10.3389/fnut.2021.666654>
- Chávarri, M., Marañón, I., Ares, R., Ibañez, F.C., Marzo, F., del Carmen Villarán, M. (2010). Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions. *International Journal of Food Microbiology*, 142(1–2), 185–189.
<https://doi.org/10.1016/j.ijfoodmicro.2010.06.022>
- Chhikara, N., Kushwaha, K., Jaglan, S., Sharma, P., Panghal, A. (2019). Nutritional, physicochemical, and functional quality of beetroot (*Beta vulgaris L.*) incorporated Asian noodles. *Cereal Chemistry*, 96(1), 154–161.
<https://doi.org/10.1002/ccche.10126>
- Holkem, A.T., Raddatz, G.C., Nunes, G.L., Cichoski, A.J., Jacob-Lopes, E., Grosso, C.R.F., de Menezes, C.R. (2016). Development and characterization of alginate microcapsules containing *Bifidobacterium BB-12* produced by emulsification/internal gelation followed by freeze drying. *LWT – Food Science and Technology*, 71, 302–308.
<https://doi.org/10.1016/j.lwt.2016.04.012>
- Khosravi Zanjani, M.A., Tarzi, B.G., Sharifan, A., Mohammadi, N. (2014). Microencapsulation of probiotics by calcium alginate-gelatinized starch with chitosan coating and evaluation of survival in simulated human gastro-intestinal condition. *Iranian Journal of Pharmaceutical Research: IJPR*, 13(3), art. no. 843.
- Krasaekoopt, W., Watcharapoka, S. (2014). Effect of addition of inulin and galactooligosaccharide on the survival of microencapsulated probiotics in alginate beads coated with chitosan in simulated digestive system, yogurt and fruit juice. *LWT – Food Science and Technology*, 57(2), 761–766.
<https://doi.org/10.1016/j.lwt.2014.01.037>
- Krstonošić, V., Dokić, L., Dokić, P., Dapčević, T. (2009). Effects of xanthan gum on physicochemical properties and stability of corn oil-in-water emulsions stabilized by polyoxyethylene (20) sorbitan monooleate. *Food Hydrocolloids*, 23(8), 2212–2218.
<https://doi.org/10.1016/j.foodhyd.2009.05.003>
- Lazar, S., Constantin, O., Horincar, G., Georgeta, D., Stanciu, N., Muresan, C., Rapeanu, G. (2022). Beetroot by-product as a functional ingredient for obtaining value added mayonnaise. *Processes*, 10(2), art. no. 227.
<https://doi.org/10.3390/pr10020227>
- Li, X.Y., Chen, X.G., Cha, D.S., Park, H.J., Liu, C.S. (2009). Microencapsulation of a probiotic bacteria with alginate gelatin and its properties. *Journal of Microencapsulation*, 26(4), 315–324.
<https://doi.org/10.1080/02652040802328685>
- Lopes, S., Bueno, L., De Aguiar Júnior, F., Finkler, C.L.L. (2017). Preparation and characterization of alginate and gelatin micro-capsules containing *Lactobacillus rhamnosus*. *Anais Da Academia Brasileira de Ciências*, 89(3), 1601–1613.
<https://doi.org/10.1590/0001-3765201720170071>
- Ma, Z., Boye, J.I. (2013). Advances in the design and production of reduced-fat and reduced-cholesterol salad dressing and mayonnaise: A review. *Food and Bioprocess Technology*, 6(3), 648–670.
<https://doi.org/10.1007/s11947-012-1000-9>
- Mahmoud, M., Abdallah, N.A., El-Shafei, K., Tawfik, N.F., El-Sayed, H.S. (2020). Survivability of alginate-microencapsulated *Lactobacillus plantarum* during storage, simulated food processing and gastrointestinal conditions. *Heliyon*, 6(3), art. no. e03541.
<https://doi.org/10.1016/j.heliyon.2020.e03541>
- Mirmiran, P., Houshialsadat, Z., Gaeini, Z., Bahadoran, Z., Azizi, F. (2020). Functional properties of beetroot (*Beta vulgaris*) in management of cardio-metabolic diseases. *Nutrition & Metabolism*, 17, art. no. 3.
<https://doi.org/10.1186/s12986-019-0421-0>
- Motalebi Moghanjoug, Z., Rezazadeh Bari, M., Alizadeh Khaledabad, M., Amiri, S., Almasi, H. (2021). Microencapsulation of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium animalis* BB-12 in pectin and sodium alginate: A comparative study on viability, stability, and structure. *Food Science & Nutrition*, 9(9), 5103–5111.
<https://doi.org/10.1002/fsn3.2470>
- Mun, S., Kim, Y.L., Kang, C.G., Park, K.H., Shim, J.Y., Kim, Y.R. (2009). Development of reduced-fat mayonnaise using 4αGase-modified rice starch and xanthan gum. *International Journal of Biological Macromolecules*, 44(5), 400–407.
<https://doi.org/10.1016/j.ijbiomac.2009.02.008>
- Nordström, E.A., Teixeira, C., Montelius, C., Jeppsson, B., Larsson, N. (2021). *Lactiplantibacillus plantarum* 299v (LP299v®): Three decades of research. *Beneficial Microbes*, 12(5), 441–465.
<https://doi.org/10.3920/BM2020.0191>
- Otalora, C.M., Bonifazi, E., Fissore, E.N., Basanta, F., Gerschenson, L.N. (2020). Thermal stability of betalains in by-products of the blanching and cutting of *Beta vulgaris L. var conditiva*. *Polish Journal of Food and Nutrition Sciences*, 70(1), 15–24.
<https://doi.org/10.31883/pjfn/116415>
- Petruț, R.F., Danthine, S., Blecker, C. (2016). Assessment of partial coalescence in whippable oil-in-water food emulsions. *Advances in Colloid and Interface Science*, 229, 25–33.
<https://doi.org/10.1016/j.cis.2015.12.004>
- Platosz, N., Sawicki, T., Wiczowski, W. (2020). Profile of phenolic acids and flavonoids of red beet and its fermentation products. does long-term consumption of fermented beetroot juice affect phenolics profile in human blood plasma and urine? *Polish Journal of Food and Nutrition Sciences*, 70(1), 55–65.
<https://doi.org/10.31883/pjfn/116613>
- Primacella, M., Wang, T., Acevedo, N.C. (2019). Characterization of mayonnaise properties prepared using frozen-thawed egg yolk treated with hydrolyzed egg yolk proteins as anti-gelator. *Food Hydrocolloids*, 96(06), 529–536.
<https://doi.org/10.1016/j.foodhyd.2019.06.008>
- Pupa, P., Apiwatsiri, P., Sirichokchatchawan, W., Pirarat, N., Muangsin, N., Shah, A.A., Prapasarakul, N. (2021). The efficacy of three double-microencapsulation methods for preservation of probiotic bacteria. *Scientific Reports*, 11(1), art. no. 13753.
<https://doi.org/10.1038/s41598-021-93263-z>
- Rajam, R., Subramanian, P. (2022). Encapsulation of probiotics: Past, present and future. *Beni-Suef University Journal of Basic and Applied Sciences*, 11(1), art. no. 26.
<https://doi.org/10.1186/s43088-022-00228-w>
- Rodríguez-Huezo, M.E., Lobato-Calleros, C., Reyes-Ocampo, J.G., Sandoval-Castilla, O., Perez-Alonso, C., Pimentel-Gonzalez, D.J. (2011). Survivability of entrapped *Lactobacillus rhamnosus* in liquid-and gel-core alginate beads during storage and simulated gastrointestinal conditions. *Revista Mexicana de Ingeniería Química*, 10(3), 353–361.
- Sakin-Yilmazer, M., Dirim, S.N., Di Pinto, D., Kaymak-Ertekin, F. (2014). Yoghurt with candied chestnut: freeze drying, physical, and rheological behaviour. *Journal of Food Science and Technology*, 51, 3949–3955.
<https://doi.org/10.1007/s13197-012-0890-x>

35. Shori, A.B. (2017). Microencapsulation improved probiotics survival during gastric transit. *HAYATI Journal of Biosciences*, 24(1), 1–5. <https://doi.org/10.1016/j.hjb.2016.12.008>
36. Sánchez, E., Morales, C.R., Castillo, S., Leos-Rivas, C., Misael, D., Martínez, O. (2016). Antibacterial and antibiofilm activity of methanolic plant extracts against nosocomial microorganisms. *Evidence Based Complementary and Alternative Medicine*, 2016, art. no. 1572697. <https://doi.org/10.1155/2016/1572697>
37. Shafizadeh, A., Golestan, L., Ahmadi, M., Darjani, P., Ghorbani-HasanSaraei, A. (2020). Encapsulation of *Lactobacillus casei* in alginate microcapsules: improvement of the bacterial viability under simulated gastrointestinal conditions using flaxseed mucilage. *Journal of Food Measurement and Characterization*, 14, 1901–1908. <https://doi.org/10.1007/s11694-020-00437-w>
38. Spórna-Kucab, A., Tekieli, A., Skalicka-Woźniak, K., Grzegorzczak, A., Świergosz, T., Wybraniec, S. (2022). Characterization of triterpene saponin composition of white, yellow and red beetroot (*Beta vulgaris* L.). *Polish Journal of Food and Nutrition Sciences*, 72(2), 159–170. <https://doi.org/10.31883/pjfn/149515>
39. Traynor, M., Burke, R., Frias, J.M., Gaston, E., Barry-Ryan, C. (2013). Formation and stability of an oil in water emulsion containing lecithin, xanthan gum and sunflower oil. *International Food Research Journal*, 20(5), 2173–2181. <https://doi.org/10.21427/d7d189>
40. Tripathi, M.K., Giri, S.K. (2014). Probiotic functional foods: Survival of probiotics during processing and storage. *Journal of Functional Foods*, 9, 225–241. <https://doi.org/10.1016/j.jff.2014.04.030>
41. Tumbas Šaponjac, V., Čanadanović-Brunet, J., Četković, G., Jakišić, M., Vučić, J.J., Stajčić, S., Šeregelj, V. (2020). Optimisation of beetroot juice encapsulation by freeze-drying. *Polish Journal of Food and Nutrition Sciences*, 70(1), 25–34. <https://doi.org/10.31883/pjfn/115153>
42. Vijaya Kumar, B., Vijayendra, S.V., Reddy, O.V.S. (2015). Trends in dairy and non-dairy probiotic products-a review. *Journal of Food Science and Technology*, 52, 6112–6124. <https://doi.org/10.1007/s13197-015-1795-2>
43. Ye, A., Hemar, Y., Singh, H. (2004). Enhancement of coalescence by xanthan addition to oil-in-water emulsions formed with extensively hydrolysed whey proteins. *Food Hydrocolloids*, 18(5), 737–746. <https://doi.org/10.1016/j.foodhyd.2003.11.010>