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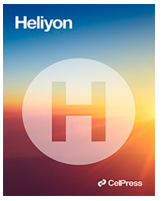

Research article

Microencapsulation and controlled release of *Bacillus clausii* through a novel non-digestible carbohydrate formulation as revolutionizing probiotic delivery

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ABSTRACT

Probiotics have gained significant attention in recent years due to the growing awareness of physical health and well-being. However, maintaining high concentrations of probiotics throughout the product's shelf life and during the gastrointestinal tract is crucial for ensuring their health-promoting effects. After determining an optimal formulation through a fractional factorial model, this study optimizes probiotic *Bacillus Clausii* delivery through spray-drying microencapsulation using a novel maltodextrin-alginate-inulin (MDX-ALG-IN) formulation (optimized ratio: 7:2:1). Notably, this formulation exclusively comprises non-digestible carbohydrates, marking a novel approach in probiotic encapsulation. Achieving a high Product Yield (51.06 %) and Encapsulation Efficiency (80.53 %), the study employed SEM for morphological analysis, revealing an irregular form and extensive surface in dentations characteristic of maltodextrin involvement. With a low moisture content of 3.02 % (±0.23 %) and 90.52 % solubility, the powder displayed exceptional properties. Probiotic viability remained robust, surviving up to 60 % even after 180 days at 4 °C, 25 °C, and 37 °C. Thermal characterization unveiled microcapsule resilience, exhibiting a glass transition temperature (Tg) at 138.61 °C and a melting point of 177.28 °C. The study systematically addresses crucial aspects of microencapsulation, including formulation optimization, morphological characteristics, and powder properties. Notably, the MDX-ALG-IN microcapsules demonstrated stability in simulated gastrointestinal conditions, indicating potential application for supplements and complex food matrices. In summary, this research contributes to microencapsulation understanding, emphasizing the MDX-ALG-IN formulation's efficacy in preserving probiotic viability across production stages and simulated digestive processes.

1. Introduction

Probiotics and prebiotics have gained significant attention in recent years due to the increasing awareness of physical health and well-being. Probiotics aim to assist in reestablish the normal balance of bacteria in the gut after an illness or treatment disrupted it. There is some evidence that probiotics may be beneficial in some situations, such as alleviating the symptoms of irritable bowel syndrome (IBS) [1]. They have become essential components in human health, leading to a surge in research focused on characterizing and confirming their potential health benefits. Alongside the study of prebiotics and probiotics, other strategies for modulating the

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Abbreviations

EE%	Encapsulation Efficiency
Y	Product yield
MDX	Maltodextrin
ALG	Sodium alginate
IN	Inulin
SSF	Simulated Salivary Fluid
SGF	Simulated Gastric Fluid
SIF	Simulated Intestinal Fluid

microbiome, such as symbiotics, postbiotics, microbial consortia, live biotherapeutic products, and genetically manipulated organisms, are also being developed [2].

One crucial area of research in the functional foods market is the development of probiotic-enhanced foods. These products may contain a single bacterial strain or a combination of multiple strains. However, it is essential to maintain high concentrations of probiotics throughout the shelf life of the product [3]. Guidelines and data from the Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) suggest that for viable probiotic strains to provide a "minimum therapeutic" level of functionality, the product should contain at least 10^6 colony-forming units (CFU) per gram of viable probiotic strains. Moreover, these probiotics must survive not only throughout the product's shelf life but also during transit through the upper intestinal tract to ensure their health-promoting effects upon reaching their site of action. Encapsulation techniques, such as microencapsulation, can help achieve this goal [4].

Microencapsulation involves enclosing minimal amounts of core materials containing nutrients or chemical compounds inside a protective wall material (S. & [5]. While the primary objective of encapsulation is to safeguard probiotics from adverse environmental conditions, microencapsulation processes are widely used in the food industry for various purposes, including protecting the core from degradation, reducing core material evaporation, improving material handling, modifying release characteristics, and masking undesired flavors, colors, or tastes [6]. Also, in a broader context of microencapsulation, the pharmaceutical and drug industries particularly emphasize the importance of microencapsulation for controlled release applications, enabling the gradual and sustained release of active compounds. Hydrogels, distinguished by their high-water content and biocompatibility, represent a notable group in this domain, for example, herbal hydrogels based on encapsulated *Enterococcus faecium* ABRIINW. N7 for potential probiotic anti-microbial activity against *Streptococcus iniae* [7]. Simultaneously, various microencapsulation techniques, including spray drying and coacervation, contribute to the diverse approaches in this field.

Spray drying is a commonly employed encapsulation method for probiotic cells in the food sector, primarily due to its cost-effectiveness and suitability for large-scale production [8]. The spray drying process involves atomizing a mixture containing probiotic cells and dissolved or suspended protective materials into droplets, which are then rapidly dried in a heated airflow, resulting in the formation of particles [9]. The primary advantage of spray drying technology is the ability to adjust particle size based on the desired form and morphology. However, the high temperatures involved in the process can be detrimental to heat-sensitive materials [10]. Therefore, it is advantageous to develop a drying method that efficiently removes flaws while preserving the biological activity of the probiotics [11]. Depending on the probiotic bacterium's nature and the desired product, several operational factors need to be carefully evaluated and improved, these factors include choosing the best protective matrix and adjusting the drying conditions [12].

Drying conditions might have a significant impact on the encapsulation process, based on Taguchi statistical analysis, a previous study of the *Bacillus thuringiensis* micro-encapsulation process found that the contribution of the wall material was only about 46 % leaving the remaining to inlet temperature (22.53 %), outlet temperature (23.56 %), and possible error (7.90), hence its importance [13]. Another study investigated the relationship between air inlet temperature and feed flow rate for two species of *Lactobacillus acidophilus* during spray drying. The study concluded that maintaining intermediate air inlet temperatures and feed flow rates is necessary to ensure probiotic survival, achieve high powder recovery rates at the cyclone separator, and minimize residual water activity [14].

Various polymeric materials, including lipids, proteins, and polysaccharide hydrocolloids (such as alginate, xanthan, chitosan, carrageenan, and cellulose), have been used as microencapsulation materials [15]. As consumer awareness grows, there is a heightened demand for encapsulated probiotic products that ensure viability throughout shelf life, reflecting a broader trend toward dietary choices that promote overall well-being. For example, a previous study used an alginate–basil seed mucilage formulation with various concentrations of prebiotics and selected for microencapsulation for improving probiotic survival, stability, and release in digestive settings during yogurt storage [16].

Meeting these consumer requirements, which include the necessity for formulations free from allergens and suitable for vegetarians and vegans, underscores the significance of innovative approaches like using non-digestible ingredients as wall materials for the microencapsulation. This emphasis becomes particularly pronounced when comparing formulations that may not align with these characteristics, emphasizing the crucial role of a novel formulation meeting diverse dietary preferences and requirements [17].

The specific choice of materials depends on the product being encapsulated and its specific conditions. Maltodextrin is a commonly used encapsulating ingredient in spray drying processes due to its high solubility in water and high glass transition temperature [18]. Alginate is another widely used substance known for its ability to withstand the gastric pH of 2–2.5 and expand under neutral to

Table 1
Mixture of microencapsulation matrices, product yield and encapsulation efficiency of *B. Clausii.*

Encapsulation Efficiency (EE%)	77.48	75.78	71.57	82.11	71.85	69.69	70.17	83.58	83.40	73.18	82.35	77.49	84.59	83.40	80.53	78.78	–
Product Yield (Y%)	38.46	27.38	47.87	19.38	41.88	40.46	42.71	43.41	32.38	41.71	17.85	48.11	28.53	21.68	51.96	49.38	–
Viable Probiotic (log CFU g-1 matrix)	9.76 ± 0.01	9.55 ± 0.02	9.02 ± 0.04	10.35 ± 0.03	9.05 ± 0.01	8.78 ± 0.01	8.84 ± 0.03	10.53 ± 0.01	10.51 ± 0.02	9.22 ± 0.03	10.38 ± 0.01	9.77 ± 0.01	10.66 ± 0.01	10.51 ± 0.02	10.15 ± 0.02	9.93 ± 0.03	12.60
FOS (g g-1 matrix)	0.10	0.10	0.00	0.00	0.00	0.14	0.00	0.00	0.10	0.08	0.14	0.20	0.00	0.14	0.00	0.00	–
XOS (g g-1 matrix)	0.00	0.10	0.00	0.00	0.09	0.14	0.20	0.00	0.00	0.08	0.14	0.00	0.14	0.00	0.00	0.10	–
Inulin (g g-1 matrix)	0.00	0.00	0.20	0.00	0.09	0.14	0.00	0.00	0.10	0.08	0.00	0.00	0.14	0.14	0.10	0.00	–
Alginate (g g-1 matrix)	0.20	0.10	0.20	0.40	0.18	0.14	0.20	0.12	0.10	0.17	0.29	0.20	0.29	0.29	0.20	0.20	–
Maltodextrin (g g-1 matrix)	0.70	0.70	0.60	0.60	0.64	0.43	0.60	0.88	0.70	0.58	0.43	0.60	0.43	0.43	0.70	0.70	–
Mixture	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Initial Count

alkaline pH conditions [19]. Sodium alginate is extracted from brown seaweeds, its low cost, low toxicity, biocompatibility, biodegradability, and gelatinous die-forming ability have made it one of the most researched polymers that can be used in controlled-release pharmaceutical systems with other polymers [20]. Previous studies have explored the combination of maltodextrin, sodium alginate, and inulin as encapsulation matrices for various applications [18,21,22].

In the context of probiotics, the ability of certain substances to adhere to mucosal membranes can enhance probiotic retention in the gastrointestinal (GI) tract, directly affecting their bioavailability, delivery frequency, and targeted effects [23]. Extensive research has been conducted on different probiotic strains, encapsulation methods, natural materials, and nutraceutical substances to assess their viability and successful release and survival under simulated gastric, intestinal, and bile acid conditions. These studies have also explored the application of encapsulated probiotics in the food industry for the development of innovative functional foods [24].

When considering probiotics, it is important to consider the microorganism itself, including its strain and sporulation characteristics, along with the encapsulating materials and drying conditions [25]. For example, a study on twelve different *Bacillus cereus* strains demonstrated significant variability in spore survival during spray drying, highlighting the variation in spore resistance among strains of the same species [26]. The spore resistance of *Bacillus* species can vary not only between species but also among different strains within the same species, although this variability has been poorly documented to date [27,28]. However, considering well-documented probiotic properties, established safety profiles, and demonstrated resilience in various environmental conditions; *B. Clausii* makes a suitable candidate for microencapsulation studies aimed at enhancing probiotic viability [29].

This study is motivated by the growing significance of probiotics in promoting health and well-being, especially in probiotic-enhanced products. Robust encapsulation techniques are essential to ensure the viability and functionality of probiotics throughout their shelf life and transit through the gastrointestinal tract. Recognizing the pivotal role of encapsulation materials in achieving these objectives, our research focuses on exploring the functional properties of non-digestible wall materials, including maltodextrin, sodium alginate, and other prebiotics, for targeted probiotic delivery. The importance of this investigation lies in its potential to offer novel formulations, addressing current challenges associated with maintaining probiotic viability in commercial products that align with modern consumer needs. By analyzing the shelf life and bioaccessibility of encapsulated probiotics in the gastrointestinal tract, our aim is to provide valuable insights that can enhance the feasibility of commercializing probiotic products. Ultimately, this research seeks to advance our understanding of probiotic delivery systems, offering innovative solutions to meet the evolving demands of consumers for functional foods that support overall well-being.

2. Materials and methods

2.1. Cells & materials

The probiotic cells of *B. Clausii* were obtained from a commercial suspension Sinuberase® containing 4×10^{12} UFC/5 ml and was in liquid presentations. Commercial maltodextrin was obtained by NOW Foods Fibersol®-2 Digestion-Resistant. Sodium alginate was purchased at Sigma-Aldrich Prebiotics and all other chemicals and reagents were of analytical grade and used without further purification.

2.2. Optimum determination of maltodextrin-alginate-prebiotic concentration in microcapsules

Maltodextrin (Bulk Density: 0.48 g per ml. Now Foods, IL, USA) sodium alginate (Sigma-Aldrich) and prebiotics (inulin, fructooligosaccharides and/or xylooligosaccharides) were used as wall materials at different combinations. Table 1 shows the concentrations proposed on a fractional factorial design model. A 50 % w/v aqueous solution of maltodextrin was prepared, left to fully hydrate for 10 min at room temperature and stirred until it was completely dissolved. Prebiotic or mixture of them (when applied) were added together with probiotic *B. Clausii* and were mixed with sodium alginate. Mixture was left stirring for 10–20 min with a magnetic stirrer at 50 °C, once the mixture was fully dissolved temperature was set at 35 °C and spray dried. The mixture was dried using a Yamato Spray Dryer ADL311S (Monterrey, Monterrey, México). Spray-drying conditions were settled based on past *Bacillus* spp. Studies to enable the encapsulation matrix's concentrations entirely up to the wall-material components and were as follows: air-inlet temperature of 125 °C, outlet temperature 70 °C, feed flow rate of 200 mL h^{-1}, and spray pressure of 0.15 MPa [13,30–32]. Each concentration was repeated at least three times.

2.3. Product yield (Y)

Product yield was determined as the ratio between the weight of the samples that were recovered and the weight of the suspended feed (both in grams of dry matter) [33].

2.4. Cell enumeration and encapsulation efficiency (EE%)

1 g of *B. Clausii* microcapsule was suspended and diluted in 9 ml of sterile peptone saline solution (peptone bacteriological 1 g L^{-1} and sodium chloride 8.5 g L^{-1}). Serial dilutions were done and then pour plated onto tryptic soy agar (TSA). The plates were incubated at 37 °C for 24 h. In this study, the total counts of the viable bacteria were expressed as colony forming units per gram (CFU g^{-1}) using a plate count for bacterial colonies.

The following formula was used to determine Encapsulation Efficiency after spray drying [8]:

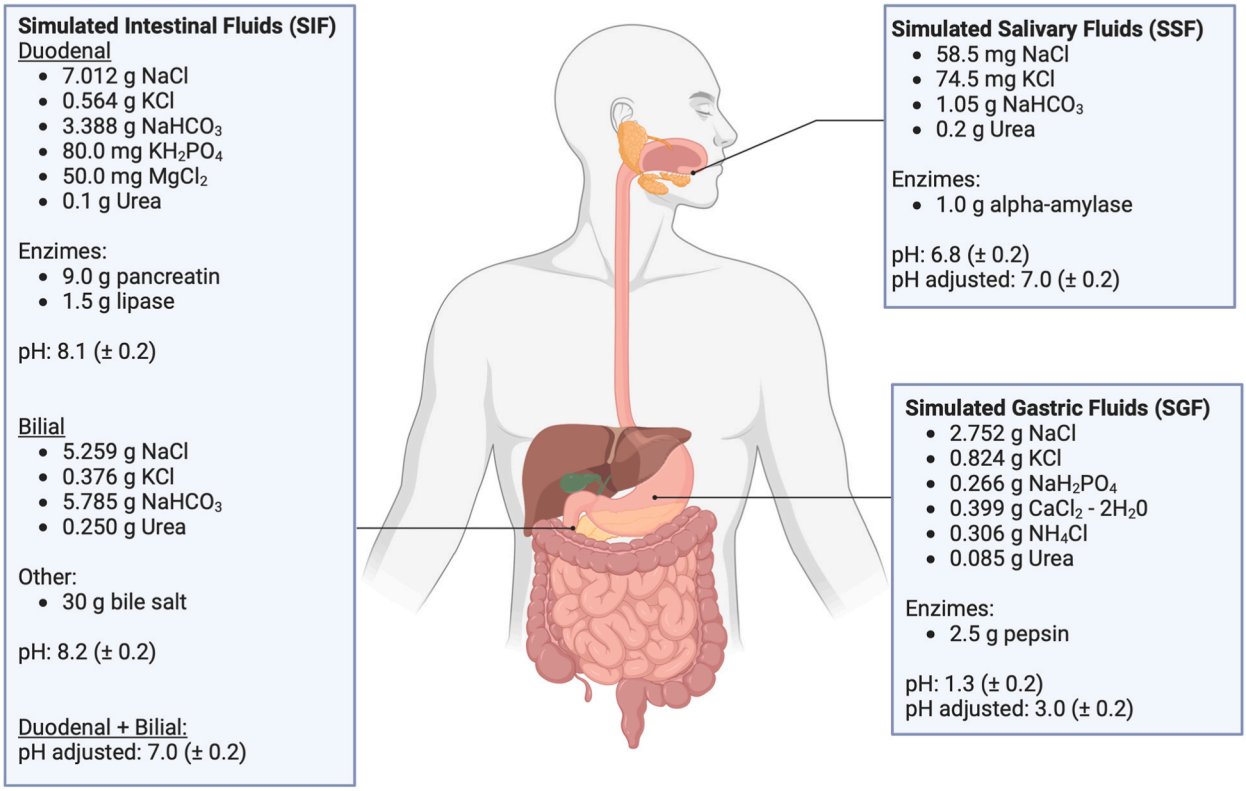

Fig. 1. In vitro summary digestion model. Conditions settled for simulated salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) including reactants, salts, pH adjustment, and enzymes.

$$\text{Encapsulation Efficiency } \% = \frac{\text{Log}_{10}\ (\text{CFUg}^{-1}\ (\text{powder after spray drying}))}{\text{Log}_{10}\ (\text{CFUg}^{-1}\ (\text{culture before spray drying}))} * 100$$

2.5. Scanning Electron Microscopy (SEM)

Scanning electron microscopy was used to obtain information about the microstructure, surface morphology and particle size of the carriers. The powders were sputter-coated with gold and after metallization, the samples were visualized using the EVO MA25 Zeiss (Monterrey, Monterrey, Mex.) field-emission scanning electron microscope (FE-SEM), at an accelerating voltage of 20 kV.

2.6. Moisture content

The moisture content of the optimum encapsulation matrix was determined using a modified version published before [34]. Powder samples (0.5 g) were placed in Petri dishes in the laboratory oven for 24 h at 105 ± 2 °C. Samples were weighed and compared to the mass of the initial samples after the period mentioned before. The tests were carried out in triplicate.

2.7. Solubility analysis

A previous method was used to determine the solubility [34]. 2 g of powder sample was dissolved in 50 mL of distilled water, vortexed rapidly, and then centrifuged at 3000 rpm for 5 min and 4 °C. The supernatant was poured into freshly weighted Petri dishes and dried in an oven at 105 °C until complete drying. The solubility (%) was determined using the weight difference. The experiment was done in triplicate.

2.8. Shelf life & storage condition stability

Powder samples were kept in a hermetically sealed jar on the shelf at 4 °C, 25 °C and 37 °C. For 180 days, storage studies were carried out in three replicate trials. Storage information was shown as logarithmic viable cell counts.

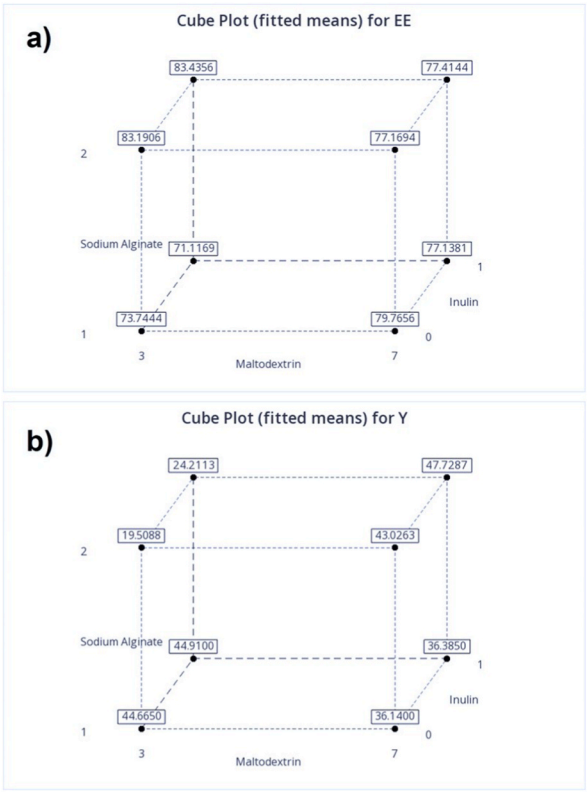

Fig. 2. Cube plot means for Encapsulation efficiency (a) and Product Yield (b) respectively. The cubes display all combinations of factor settings for the three factors (maltodextrin, sodium alginate and inulin) and the fitted mean for each combination. The cube on the left shows the response means for the encapsulation efficiency while the cube on the right shows the response means for the product yield. The model predictions are evaluated at every stage of the design by the fitted means on the cube plot.

2.9. Differential scanning calorimetry (DSC)

For the thermal characterization of the samples, a Pyris Diamond DSC (PerkinElmer) under a nitrogen gas flow was used. The melting point of indium (PerkinElmer #LA94121V) was measured for equipment calibration. Samples (4–6 mg) of mixtures MDX-ALG (mixture 8) and MDX-ALG-IN (mixture 15) were sealed in 50 μL aluminum pans (PerkinElmer). Samples were equilibrated at 30 °C and then heated from 30 °C to 210 °C at a heating rate of 10 °C/min with the purpose of determining melting temperature of crystalline samples.

2.10. Survival in simulated gastrointestinal conditions

Simulated digestive solutions were prepared according to method described previously with slightly modifications [35]. Fig. 1 shows the compositions of the simulated salivary, gastric, and intestinal (duodenal and bile) juices, pH was adjusted with 1 M HCl or 1 M NaOH respectively. The sample was digested separately on each fluid and sequentially in the following order: mouth, stomach, and intestine. For the isolated digestions, mouth digestion involved adding 6 ml of salivary juice and mixing for 30 s and after that 1 ml of sample was taken and serial diluted as section 2.4. For the stomach digestion, it involved adding 12 ml of gastric juice to the sample and then it was incubated at 37 °C in a water bath at 200 rpm, 1 ml of the sample was taken at 1 h, 2 h and 4 h. For the intestine 12 ml of duodenal and 6 ml of bile juices were added and incubated as in gastric juice, sample was collected at 4 h and 8 h. Finally, for the sequential digestion the samples were in the same concentration and samples were taken at salivary (30 s), gastric (1 h) and intestinal (4 h and 8 h) respectively. All digestions were repeated three times.

2.11. Statistical analysis

Each experiment was performed at least in triplicate. The one-way ANOVA method was used to analyze the results. The significance of the difference between the samples was determined using the Tukey test ($p < 0.05$). Microsoft Excel and Minitab software (version 19.2020.2.0) were used for all analyses.

Table 2
Multiple response prediction for fractional model. Optimized response prediction with variable setting 7:2:1 for maltodextrin, sodium alginate, inulin respectively.

Response	Fit	SE Fit	95 % CI	95 % PI
Encapsulation Efficiency (EE%)	80.332	0.765	(78.366, 82.298)	(77.251, 83.412)
Product Yield (Y)	51.98	1.73	(47.90, 56.06)	(45.18, 58.78)

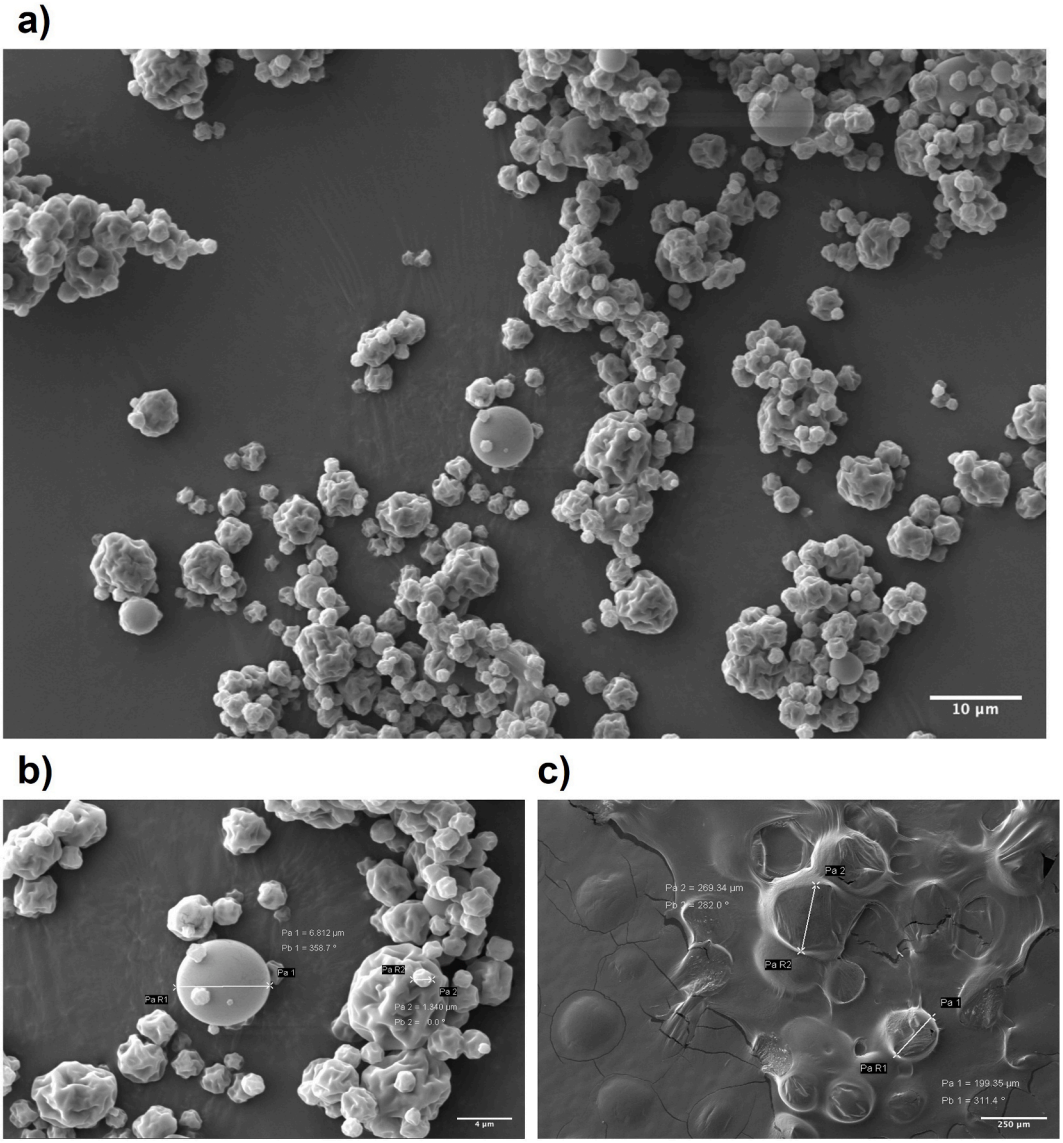

Fig. 3. Scanning Electron Microscopy (SEM) images of MDX-ALG-IN microencapsulated (optimal mixture) by spray-drying technique. Powder encapsulation at a) 1000 ×, b) 3000× magnification and c) after soaking with distilled water at 50× magnification.

3. Results and discussion

3.1. Microencapsulation matrix design, product yield and encapsulation efficiency

Sixteen different compositions were created for the encapsulating matrix during the factorial design (Table 1). Among the mixtures, the combination of Maltodextrin-Alginate-Inulin-XOS (13th) resulted in the highest encapsulation efficiency (84.59 %), but its product yield was one of the lowest (28.53 %). This decrease in product yield with decreasing maltodextrin content has been reported

previously [36]. The high amount of sodium alginate in this combination increased viscosity rates and reduced the flow rate, which can be a limiting factor in the spray drying technique. Excessive viscosity can lead to clogging of the atomizer nozzle, insufficient solvent evaporation, and inadequate process yields, which negatively affect particle morphology [37].

Fig. 2 illustrates the behavior of the model to understand the relationship between the proposed encapsulation matrixes. For Encapsulation Efficiency (EE%), the highest amount was estimated as 3:2:1 of Maltodextrin-Alginate-Inulin (Fig. 2a). For product yield, the highest amount was observed in the combination of Maltodextrin-Alginate-Inulin (15th) as observed in Fig. 2b. The high concentration of maltodextrin in this combination may be the reason for the increased yield. Previous studies in *Bacillus subtilis* have shown a relationship between increased maltodextrin concentration and higher average survival rates. Concentrations of 80 % and higher resulted in significantly higher mean survival rates compared to 20 %, 40 %, and 60 % [30]. Therefore, this combination was chosen as the best formulation for probiotic protection among the compositions for creating the microencapsulation matrix, which coincided with the recommended combination according to the response optimizer in Minitab's Statistical Analysis (Table 2).

3.2. Microstructure and morphology characteristics

Scanning electron microscopy (SEM) was employed to analyze the structure of the probiotic microcapsules, as shown in Fig. 3. The particles exhibited an irregular shape with extensive surface indentations, which are characteristic of spray-dried materials containing maltodextrin (Fig. 3a). These indentations are a result of rapid water loss and shrinkage during the initial drying cycle [38]. Previous studies have reported similar surface topologies with surface depressions in spray-dried particles incorporating maltodextrin as a component of the wall materials. It has been observed that maltodextrin-based wall systems exhibit an asymmetrical structure, with pronounced surface depressions becoming more prominent as the maltodextrin ratio in the wall system increases [39]. Additionally, it is widely known that alginate capsules undergo shrinkage during the drying phase, which often leads to shape distortion, pore formation, and interference with the evaporation of residual water [18].

Furthermore, the inulin-containing matrix used for the microcapsules has been reported to have irregular surfaces (both rough and smooth) with small pores, regardless of the addition of maltodextrin. The introduction of sodium alginate enlarges these pores [22]. Conversely, another investigation found that microcapsules produced solely with maltodextrin exhibited some holes compared to those made with prebiotic inulin, which provides stronger protection for the probiotics [40]. Nevertheless, the microcapsules in our study displayed walls with no visible pores or cracks (Fig. 3b), thereby preserving the encapsulated probiotics by preventing the entry of external factors. This smooth surface could be attributed to the sodium alginate concentration used. Previous research has reported that the concentration of sodium alginate affects the structure of microcapsules, with higher concentrations resulting in smoother surfaces. In our study, microcapsules created with 3 % sodium alginate exhibited a smoother surface with relatively smaller pores compared to those created with 1 % sodium alginate, which displayed a rougher surface with significantly broader and wider pores [41].

One of the main drawbacks of powdered products is the difficulty in rehydration. A functional encapsulated powder should quickly and completely moisten, sink into the liquid (water) rather than float on top, and dissolve within a short period without forming lumps [42]. To gain a better understanding of the microencapsulation after rehydration, the MDX-ALG-IN encapsulate was also examined by SEM after being wetted with distilled water (Fig. 3c). Since the water and powder were manually combined for a brief period, the examined solution contained fully dissolved, agglomerated, and undissolved components. The solution appeared as a pasty mixture that solidified upon drying under vacuum.

Moreover, Fig. 3c displays some cracks resulting from water loss, along with an elastic substance that appears to connect the agglomerated pieces with the dissolved solution, possibly through hydrogen bonds or due to the viscoelastic properties and rapid solidification of the wall materials before the expansion of the microcapsules. A previous study involving sodium alginate films has reported a similar behavior [43]. The shape of the undissolved portions observed in Fig. 3c, resembling the spheres seen in Fig. 3b, suggests the need for further investigations into the encapsulation behavior during rehydration states to gain a comprehensive understanding of microcapsule performance.

3.3. Powders characteristics and storage stability

Food products and encapsulation matrixes with lower moisture contents are said to promote improved stability, and it is also recommended that the acceptable value of must be less than 6 % [33,44]. In this case, the moisture content in the sample was of 3.02 ± 0.23 %, maltodextrins alone have been reported to range from 2.09 % to 6.47 % depending on the dextrose equivalents; while sodium alginate from 5 % to 11 % [45,46]. These two materials, in combination with the inulin were expected to have a low value of moisture content, since large polysaccharide molecules are known to produce a barrier that prevents water from diffusing to the surface particle [47]. However, the moisture content is reported to be more influenced by the packing protection and drying circumstances (primarily outlet air temperature) than it is by the molecular mass and specific content of microencapsulation materials [46]. Therefore, the variation of the drying conditions needs to be studied alongside the moisture variation of the content, as air temperature or humidity rises, the drying rate either increases or reduces depending on the amount of moisture content [48].

The solubility of the encapsulation after the spray-drying was of 90.52 %. Since sodium alginate and maltodextrin are both hydrophilic substances, this result may reflect the powder's excellent rehydration properties. Although inulin is not, its composition is not high enough to make a significant difference. It has been reported many factors, including pH, molecular weight, ionic strength, nature of ions present in the structure, and concentration, to affect a substance's solubility, specifically in sodium alginate [49]. As stablished before, the ratio of polysaccharide-water interactions is heavily influenced by maltodextrin's high concentration, because of this, the

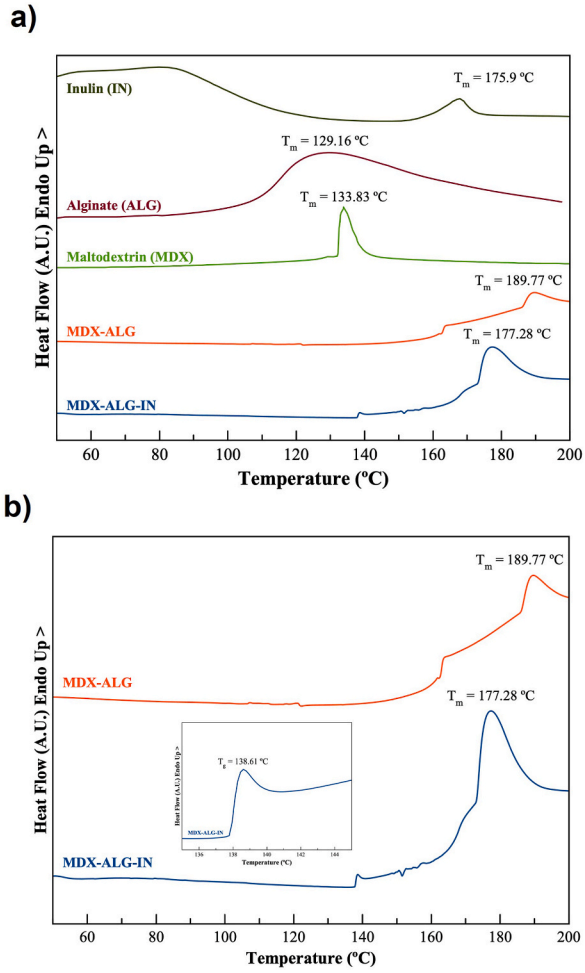

Fig. 4. a) DSC thermograms of inulin (IN), alginate (ALG), maltodextrin (MDX), mixture 8 (MDX-ALG) and optimal mixture (mixture 15, MDX-ALG-IN) and b) DSC thermograms of MDX-ALG and MDX-ALG-IN mixtures. The heating rate of all measurements was 10 °C/min.

number of structural components or aggregates grows along with the structuring action of maltodextrin on water, but their micro-structure remained the same [50]. This is related to what was discussed on the SEM characterization.

For 180 days, the cell viability of the encapsulation was studied, its behavior can be observed in Fig. 5a. For the first 15 days, the cell counts had a constant increase in the three temperatures (4 °C, 25 °C and 37 °C), followed by a continuous decrease until the last day of storage. For the first 70 days, the cell counts were almost equal between the conditions settled, however, after day 100, the temperatures started to separate from each other, but without being a significant difference between them according to Tukey test ($p > 0.05$). The similar behavior between temperatures is consistent with other investigations on spray-drying storage tests done with another probiotic bacteria [51].

Fig. 5b illustrates the survival rate of probiotic bacteria from day 0 (post-spray drying) throughout the entire storage test. The presented results are intended to elucidate the probiotic's ability to tolerate various storage conditions and highlight the efficacy of the carrier in safeguarding it. Specifically, the figure demonstrates the probiotic's resilience not only to elevated temperatures but also over extended periods of time. As can be observed, there are some small periods where the Log count increases between time periods, as a result for the different stages of adaptation during the probiotic growth phases. The inulin on the encapsulation matrix played an important role during the storage conditions since there is a high level of survival because of the partial integration of commercial prebiotics [52]. Therefore, it is important to consider adding prebiotics to an encapsulation matrix, although there may not have any statistical difference during the spray drying process (as can be observed in Table 1), it may during the storage time. On the other hand, a different study discovered when adding prebiotics to feed media, it did not increase probiotic viability after spray-drying or powder storage as much as the spray-dried culture's development phase; as it had an impact on storage survival with the stationary phase being the best, followed by lag and log phase [53].

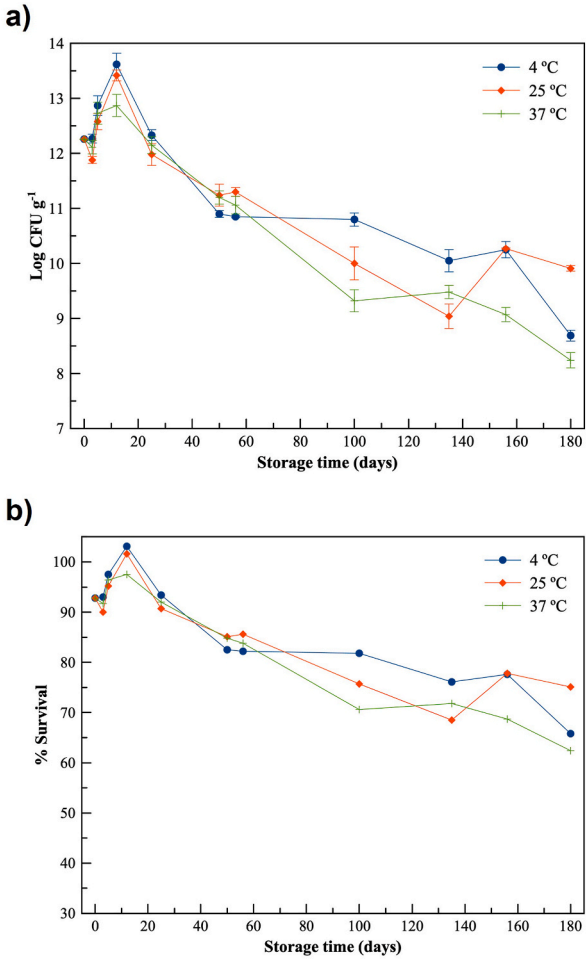

Fig. 5. a) The viability of probiotic encapsulation (Log10 $CFUg^{-1}$) and b) % of Survival during storage time (days) at the conditions studied (4 °C, 25 °C, 37 °C). The results are presented by the mean ± the standard error of 3 replicates.

3.4. Thermal properties

Differential scanning calorimetry (DSC) analysis was used to characterize the microcapsules, which provided details about the structure's transition temperature and the instability of solid structures because of a molecular disorder increase [54]. Fig. 4a shows the comparison between melting points of pure matrices reported in literature and the results obtained for mixtures 15 (optimal) and 8 (to study the matrix's thermal behavior without inulin). For pure compounds, melting points (endothermic peaks) for inulin, sodium alginate, and maltodextrin are reported as 175.9, 129.16, and 133.83 °C, respectively [55–57]. In the case of pure inulin, the DSC curve has two peaks, the first one at 98.8 °C and the second one corresponds to the anhydrous inulin sample, which was reported as the crystalline transition in solid state (form α → form β) of anhydrous inulin [56].

For the case of the mixtures analyzed, they revealed a melting temperature of 177.28 °C, which is higher than the isolated pure compounds. This phenomenon has already been documented in prior investigations, whereby mixing two or more matrices enhances the melting temperature of the encapsulation in an ideal combination [58]. The increase of melting point by using the optimum MDX-ALG-IN matrix indicates a rigid structure of microcapsules, which provides higher stability and protection to the probiotic. When comparing the two mixtures analyzed, the one without inulin has a higher melting point; however, its addition to the matrix improved the shelf life as it was discussed previously.

Due to the quick drying rate, spray drying is commonly employed in the production of amorphous solid dispersion systems, which enables kinetic trapping of the product in amorphous form where solvent content of the spray-drying environment can have a significant impact on the characteristics of the spray-dried dispersions [59]. In our case, the optimal mixture of MDX-ALG-IN showed an endothermic peak at 138.61 °C (Fig. 4b), this thermal transition corresponds to the glass transition temperature (T_g), where the microencapsulation passed from a glassy to a rubbery state by heating it [60]. For better understanding of the encapsulation matrix's properties, further investigations of the encapsulation compounds need to be addressed in the amorphous and crystal form.

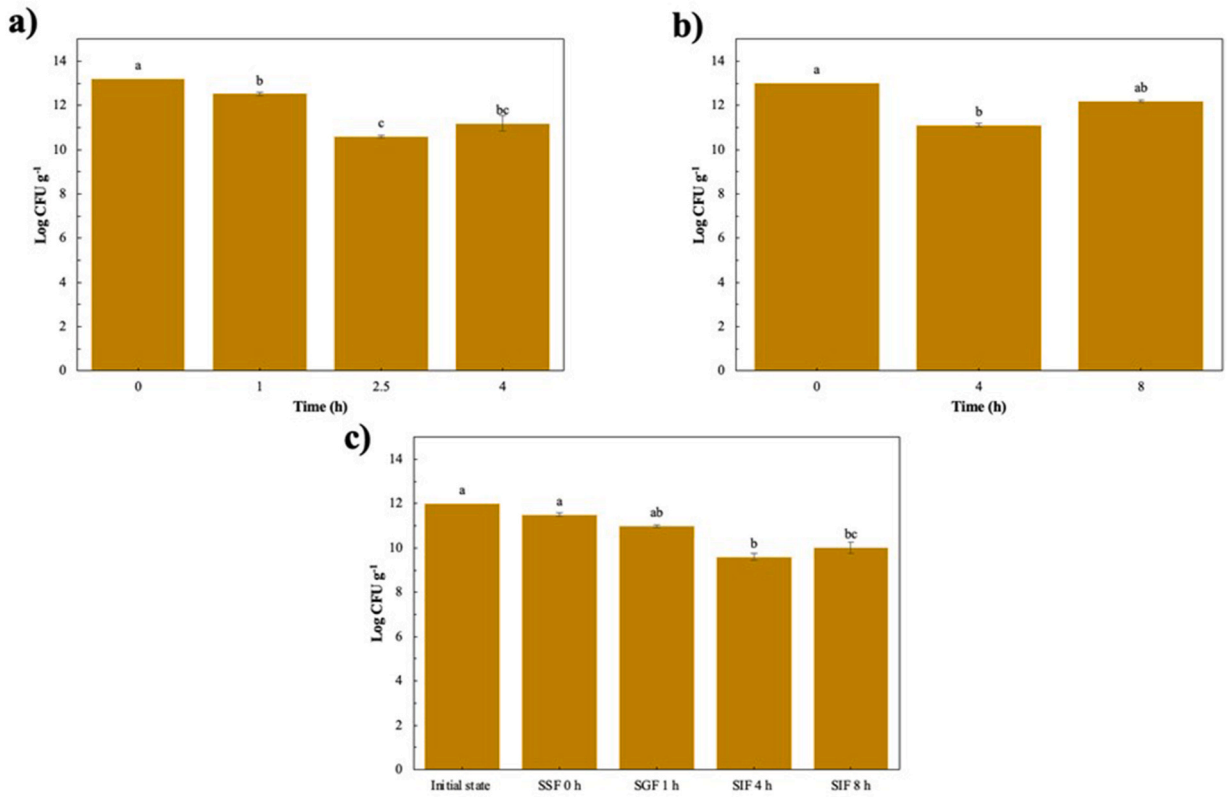

Fig. 6. *In vitro* digestion study of cell viability of encapsulated *B. Clausii* in a) simulated gastric fluid, b) simulated intestinal fluid and c) complete digestion. The bars with same letters are not significantly different ($p > 0.05$).

3.5. Digestive tolerance

Results of *in vitro* survival under simulated gastrointestinal conditions highlighted the importance of the encapsulation process for bacteria protection, particularly probiotic cells. The effect of viability loss of microencapsulated probiotic *B. Clausii* is shown on simulated gastric fluid (Fig. 6a), simulated intestinal fluid (Fig. 6b) and sequential digestion (6c), respectively, where viable cells generally showed a decreasing trend with increasing exposure time and a final increase in the simulated fluids.

For the case of gastrointestinal condition, a significant reduction happens from the initial state to 2.5 h, followed by a slightly increase in the 4 h. It is not common to observe an increase on the SGF isolated test (Fig. 6a) but considering the acid resistant bacteria used and the matrix, it can be explained [8,31,60,61]. In this situation, the probiotic used the matrix as a prebiotic to proliferate; the probiotic was the only one that could take advantage of it thus making its proliferation. Since carbohydrates are chemically broken down into simple sugars and cannot be digested by the stomach, maltodextrin used was a resistant starch and can only be fermented until it reaches the colon [62].

The behavior for intestinal conditions (Fig. 6b) demonstrated similar effects in cell survival decreases as those for simulated gastric conditions; however, SIF viable cells declined more slowly than SGF ones. This indicates the viability of the probiotic cells was not significantly affected by the concentration of bile salts, which has been previously reported [31].

For sequential digestion (Fig. 6c), there wasn't significant reduction between the initial state and the SSF 0 h according to Tukey test ($p > 0.05$). However, the activity of alpha-amylase in the mouth, which begins to break down starch and after it passes through the digestive tract, could continue to function for up to 30 min inside the food bolus according to literature [63]. Hence the importance to include it on the sequential digestion if the final application of the encapsulation is intended for a food matrix application [64].

Then, SGF 1 h had a similar reduction as the one done in the individual simulated gastric conditions (Fig. 6a); the behavior can also be interpreted with what has been previously discussed. Afterwards, the SIF 4 h had a higher decrease compared with the one done on the isolated intestinal conditions (Fig. 6b); this is explained by the SGF remnants that were present between the fluid change; however, proper carriers are required to ensure that viable probiotic cells move through the upper gastrointestinal system of the stomach through the intestinal, because the stress acid conditions have some impact on their ability to survive.

Finally, the increase in probiotic population at the end of the digestion (SIF 8 h) is attributed to cell growth at pH 7.5 (optimal pH conditions) and the recovery of viable but nonculturable probiotics. The increase in viable cell counts after 3 h incubation at the pH 7.5 in the presence of pancreatin and bile salts indicate that low pH stress had only caused temporary damage to the probiotic cells; according to previous studies, which is consistent with the whole digestive simulations performed [65].

4. Conclusion

In this study, the spray-drying technique was employed to prepare novel concentration mixtures of complex carbohydrates for the microencapsulation delivery of probiotic *B. Clausii*. Through the statistical response optimizer, it was determined that the mixture 15 (MDX-ALG-IN 7:2:1) was the optimal choice, demonstrating the highest production yield and encapsulation efficiency, both exceeding 80 %. The morphological analysis revealed an irregular form with extensive surface indentations and a lack of visible pores or cracks, which are characteristic features of maltodextrin-containing materials. Furthermore, upon rehydration, the solution exhibited complete dissolution, agglomeration, and undissolved components, forming a pasty mixture that solidified upon vacuum drying.

Regarding storage stability, the presence of inulin in the encapsulation matrix proved to be crucial under various storage conditions, as it enabled a survival rate of up to 60 % after 180 days of study at 4 °C, 25 °C, and 37 °C. The increased melting point observed when employing the optimal MDX-ALG-IN matrix suggests that the microcapsules possess a rigid structure, thereby enhancing the stability and protection of the probiotics. Additionally, the carrier exhibited good stability in simulated gastrointestinal conditions, and the microencapsulation process effectively protected the probiotics during gastric, intestinal, and sequential digestion.

Further investigations should focus on rehydration states and microcapsule behavior, as well as the encapsulation of compounds in amorphous and crystalline forms. The results presented in this study indicate that carbohydrate carriers prepared using the mentioned techniques have the potential to be utilized for probiotic stabilization, facilitating their incorporation into supplements and complex food matrices.

CRediT authorship contribution statement

Hugo Ramirez-Olea: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Conceptualization. **Sebastian Herrera-Cruz:** Methodology, Formal analysis. **Rocio Alejandra Chavez-Santoscoy:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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