Research Article

pISSN: 2345-5357
eISSN: 2423-4214

OPEN ACCESS

APPLIED FOOD BIOTECHNOLOGY, 2025, 12 (1): e10
Journal homepage: www.journals.sbmu.ac.ir/afb

O-carboxymethyl Chitosan-coated Bionanocomposite to Enhance Probiotic Viability under Gastrointestinal Digestion, Storage and Heat Treatment Conditions

Mohamadsadegh Mohamadzadeh [1], Ebrahim Vasheghani-Farahani [2], Ahmad Fazeli [1], Seyed Abbas Shojaosadati [1*]

1- Biotechnology Group, Faculty of Chemical Engineering, Tarbiat Modares University, Tehran, Iran
2- Biomedical Engineering Division, Faculty of Chemical Engineering, Tarbiat Modares University, Tehran, Iran

Abstract

Background and Objective: Improving probiotics viability in digestive and storage conditions is challenging for the food and pharmaceutical industries. The present study aimed to increase viability of the microencapsulated probiotic strain of *Lactobacillus reuteri* ATCC 23272 in O-carboxymethyl chitosan-coated bionanocomposite. The O-carboxymethyl chitosan was used to coat bionanocomposite containing prebiotics of pectin and inulin in presence of magnesium oxide nanoparticles.

Material and Methods: Pectin and inulin were used as prebiotics with magnesium oxide nanoparticles to improve the microgel structure and O-carboxymethyl chitosan for coating the microcapsules for increasing viability and stability of the probiotics. The extrusion efficiency, viability after microwave oven drying, survival in the simulated digestive fluids, viability after heat treatment and survival rate in long-term storage at 4 and 25 °C after 42 d were analyzed. Optimization of inulin, pectin and O-carboxymethyl chitosan in O-carboxymethyl chitosan-coated alginate-based bionanocomposite was achieved using Design-Expert software and simplex lattice mixture design.

Results and Conclusion: Optimal formulation was achieved using O-carboxymethyl chitosan coating polymer (68% w/v), inulin (29.4% w/v) and pectin (2.6% w/v) with magnesium oxide nanoparticles at a constant concentration. Results showed microencapsulation efficiency (96.43%), survival after microwave oven drying (99.45%) and survival in simulated gastrointestinal conditions (88.95%). Probiotic viability entrapped in O-carboxymethyl chitosan-coated microcapsules decreased by 1.46 log CFU.g^{-1} at 80 °C for 5 min. Moreover, O-carboxymethyl chitosan-coated bionanocomposite improved the stability of probiotics by 2.93 and 3.25 log CFU.g^{-1} at 4 and 25 °C after 42 d, compared to alginate beads. Additionally, it was observed that O-carboxymethyl chitosan coating enhanced the stability of probiotics entrapped in bionanocomposite beads. Results demonstrated that O-carboxymethyl chitosan-coated bionanocomposite, as a novel microencapsulation, could significantly increase the shelf life and viability of *Lactobacillus reuteri* in various harsh conditions, compared to alginate beads.

Conflict of interest: The authors declare no conflict of interest.

Article Information

Article history:

- Received 14 Feb 2025
- Revised 10 Apr 2025
- Accepted 18 Apr 2025

Keywords:

- Bionanocomposite
- *Lactobacillus reuteri*
- O-carboxymethyl chitosan
- Prebiotic
- Stability

[*] Corresponding author:

Seyed Abbas Shojaosadati

Tell: +982182883341
Fax: +982182883341
E-mail:
shoja_sa@modares.ac.ir

How to cite this article

Mohamadzadeh M, Vasheghani-Farahani E, Fazeli A, Shojaosadati SA. O-carboxymethyl Chitosan-coated Bionanocomposite to Enhance Probiotic Viability under Gastrointestinal Digestion, Storage and Heat Treatment Conditions. Appl Food Biotechnol. 2025; 12 (1): e10.
http://dx.doi.org/10.22037/afb.v12i1.47575

1. Introduction

Probiotics are beneficial live microorganisms that must be consumed sufficiently and survive the digestive tract for their effectiveness [1]. Due to the specific conditions of the mouth, stomach, small and large intestine, probiotics are susceptible to degradation. The multiple-covering technique enhances their stability and viability under these challenging conditions [2]. Therefore, coated microorganisms are expected to include higher viability than that non-coated microorganisms. One of the challenges is the efficacy of coating materials in improving viability and stability of

bacteria under harsh conditions. Therefore, it is critical to choose an appropriate composition for the coating and microencapsulation of probiotics [3]. Alginate and chitosan are the most widely used natural polymers for the microencapsulation of probiotics. Prebiotics have recently been addressed to improve the viability of probiotics. Prebiotics may decolonize pathogens by modulating gut diversity; thereby, improving the growth of probiotics and decreasing the number of pathogens [4]. In addition to microencapsulating probiotics, coating bacteria as a layer on microencapsulating polymers can increase bacterial resistance to digestive and storage conditions. Simultaneous microencapsulation and coating of probiotics can include a positive effect on improving cell viability.

Alginate, a carbohydrate polymer, is widely used in the food and pharmaceutical industries due to its non-toxicity, biodegradability, biocompatibility and ease of preparation. It can protect microorganisms from bile salts and stomach acid. However, alginate gels are susceptible to degradation in presence of monovalent ions, Ca^{2+} chelating agents, extreme pH levels and harsh chemical conditions, which can accelerate the release of encapsulated substances [5, 6]. Relatively, CMC is a water-soluble derivative of chitosan. In addition to cationic amine groups in chitosan, CMC contains further anionic carboxylic groups, which provide several potentials such as ampholytic characteristics. There are three categories of CMC based on the functional groups participating in the reaction such as O-CMC, N, O-CMC and N-CMC. The poor solubility of chitosan in water is one of the disadvantages of chitosan in drug delivery. The O-carboxymethyl chitosan (OCMC) effectively addresses the solubility issues associated with chitosan in aqueous solutions. Moreover, OCMC has attracted significant attentions due to its enhanced solubility, high viscosity, low toxicity and beneficial biocompatibility characteristics. Li et al. reported that ionic cross-linking through ionic interaction in alginate-CMC hydrogel could enhance the survival of *Lactobacillus casei* ATCC 393 against adverse conditions [7].

Inulin is a non-digestible fructan-type carbohydrate, soluble dietary fiber and includes short-chain fructooligo-saccharides (scFOS). It is widely addressed as a prebiotic because it can selectively be consumed by the gut microbiota, promoting growth of probiotic bacteria [8]. Zabihollahi et al. demonstrated that the survival of *L. plantarum* in carboxymethyl cellulose-based film increased significantly (36%) with the addition of inulin as a prebiotic during storage [9]. The pectin structure includes a linear chain of α-(1, 4)-linked D-galacturonic acid units, commonly identified as the homogalacturonan domain or the smooth region. As a prebiotic, pectin plays a critical role in modulating composition and metabolism of intestinal microbiota and decreasing possibility of intestinal colitis [10]. The pectin-inulin composite has enhanced the survival rate of *L. casei* and *L. rhamnosus*, compared to free cells in a simulated gastrointestinal tract (GIT) [11]. Magnesium oxide nanoparticles (MgONPs) have recently been highlighted as a potential candidate for controlled drug delivery systems due to their biocompatibility, non-toxicity, biodegradability, stability and various biomedical characteristics such as anticancer, antioxidant and antidiabetic characteristics. Using MgONPs in alginate-gelatin microgel showed significant advantages in enhancing viability of *Pediococcus pentosaceus* Li05 in heat treatment, simulated digestive fluid and long-term storage [12].

Researchers have implemented various strategies to increase bacterial viability, including use of prebiotics, nanoparticles and microcapsule coating separately. For example, use of MgONPs in the microencapsulation of bacteria has been studied to improve viability of probiotics in the digestive system. Research has shown that using prebiotics improves probiotics viability during the microencapsulation process and various polymers to improve the stability of probiotics. To improve probiotic viability under various harsh conditions, the present study simultaneously used prebiotics, MgONPs and OCMC as microcapsule coatings. The simultaneous use of prebiotics, MgONPs and OCMC in the microencapsulation of probiotics can improve the viability of probiotics under various harsh conditions by creating a synergistic effect. To the best of the authors' knowledge, no studies are available that investigate bacterial viability by coating microcapsules containing MgONPs and prebiotics. The experimental design used a simplex lattice mixture method to assess the optimal percentage of OCMC, inulin and pectin. The quantities of MgONPs and sodium alginate (SA) were constant in all experiments. Drying was carried out using microwave oven. Encapsulation efficiency, survival in simulated digestive fluids, heat treatment of microencapsulated probiotics and stability in storage conditions were calculated as well.

2. Materials and Methods

2.1 Materials

The probiotic strain of *L. reuteri ATCC 23272* was provided by the Iranian Scientific and Industrial Research Organization, Tehran, Iran. The OCMC (purity greater than 98%, deacetylation degree of 80%, carboxyl substitution degree of greater than 80% and amino content of nearly 1.45%) was purchased from Macklin, Shanghai, China. The MgONPs (purity greater than 99% and APS of 20 nm) were purchased from US Research Nanomaterials, Houston, TX, USA. Inulin (molecular weight of nearly 5000 Da with food-grade), low methoxyl pectin of citrus source

(molecular weight of 70–140 kDa, degree of esterification of 27%) and SA (viscosity of 2000 cp, molecular weight of 80–120 kDa and M/G ratio of 1.56) were purchased from BSK Pharmaceutical, Tehran, Iran. Pancreatin from porcine pancreas (6000 FIP-U.g^{-1} lipase, 350 FIP-U.g^{-1} protease and 7500 FIP-U.g^{-1} amylase), pepsin from porcine gastric mucosa (P700, 250 U.mg^{-1}), lactic acid, sodium citrate, glucose, calcium chloride, bile salts, trypticase soy broth (TSB) and trypticase soy agar (TSA), deMan Rogosa Sharpe (MRS) agar and broth (Cat no. 110660) were purchased from Sigma Aldrich, St. Louis, USA.

2.2 Probiotic Culture Preparation

The *L. reuteri* ATCC 23272 was incubated in MRS broth at 37 °C for 24 h. The probiotics were harvested by centrifuging at 1790 g for 10 min at 4 °C. Probiotic cells were washed twice with sterile PBS (PBS) (pH 7.4). To ensure the elimination of the supernatant from the culture broth, bacteria were recentrifuged under similar conditions and then resuspended in 2 ml of PBS [13, 14].

2.3 Preparation of Polysaccharides-based Bionanocomposite

Various compositions have been prepared for inulin and pectin prebiotics. The ratio of inulin:pectin of 10.9:1 was chosen as the optimal concentration, which was achieved in a previous study [15]. Based on the concentrations presented in Table 1, prebiotics were suspended in deionized water with a certain concentration (Table 1), MgONPs at a concentration of 5 µg.ml^{-1} were added to the mixture and stirred at 27.95 g for 60 min at 60 °C. Then, polysaccharides-based bionanocomposite was autoclaved at 121 °C for 20 min.

2.4 Microencapsulation Process

The composition of bionanocomposites (Table 1) was added to the cell suspension at a 1:1 (v/v) ratio and 37 °C and mixed using vortex to microencapsulate *L. reuteri*. The suspension of synbiotic was homogenized entirely to a 2% SA (w/v) solution. Cell suspensions were extruded using an insulin syringe in 0.1 M $CaCl_2$ solution. The interaction of uronic acid carboxylic groups with calcium ions formed a gel network, creating probiotic beads. To verify complete gelation of the beads, the $CaCl_2$ solution was stored at 4 °C for 30 min. Probiotic microgels were separated from the $CaCl_2$ solution using Whatman grade-1 filter papers (pore size of 11 µm) (Whatman, USA). These were washed twice with PBS. During the microencapsulation process, sterile conditions were addressed.

2.5 Coating Microcapsule with O-carboxymethyl Chitosan

The OCMC solution was prepared using method described by Mi et al. [16] with some modifications. Based on Table 1, OCMC solution was prepared by dissolving a certain quantity of OCMC (% w/v) in 95 ml of lactic acid solution (1% v/v). The pH was adjusted to 6 using 1 M NaOH. The solution was adjusted to 100 ml with distilled water (DW) and then filtered using Whatman grade-4 filter papers (pore size of 20 µm) (Whatman, USA). This was autoclaved at 121 °C for 15 min. Then, the alginate beads were immersed in the OCMC solution and agitated for 40 min at room temperature (RT) and 1.12 g for coating OCMC-coated microcapsules were separated using Whatman grade 1 filter papers (Whatman, USA). Then, beads were washed twice with PBS.

2.6 Encapsulation Efficiency

The number of cells released from microcapsules was calculated using method described by Halim et al. [17] with some modifications. Briefly, 1 g of beads was mixed well in 9 ml of sodium citrate (50 mM) for 10 min at RT. Cells coated with OCMC were added to the sodium citrate solution after grinding for 1 min using mortar and pestle. The released *L. reuteri* was diluted in PBS and then counted using pour plate method on plates containing MRS Agar. The efficiency of the extrusion was calculated using Eq. 1.

$$\text{Survival rate (\%)} = (N/N_0) \times 100 \qquad \text{(Eq. 1)}$$

Where, EE was efficiency of encapsulation, N was probiotics count released from beads (Log CFU.g^{-1}) and N_0 was the initial probiotics count added to the mixture (Log CFU.g^{-1}).

Table 1. Design of experiments for the optimization of bionanocomposite formulations with O-carboxymethyl chitosan coating

Run	Prebiotic % (w/v) A		Coating % (w/v) B	EE (%)	Y_1 (%)	Y_2 (%)
	Inulin	pectin	OCMC			
1	0.92	0.08	0	98.11	98.05	89.11
2	0.46	0.04	0.5	96.41	98.94	89.28
3	0.69	0.06	0.25	96.14	98.12	88.91
4	0.23	0.02	0.75	96.21	99.41	88.58
5	0	0	1	95.35	98.96	87.11
6	0.46	0.04	0.5	96.44	98.91	89.3
7	0.92	0.08	0	98.13	98.07	89.12
8	0	0	1	95.38	98.99	87.13
Alginate bead	-	-	-	95.23	95.9	71.49

EE: Extrusion efficiency; Y_1: Viability after drying; Y_2: Viability in GIT

2.7 Survival Rate of Microencapsulated Cells after Drying

The encapsulated probiotics were dried in a microwave oven (Mwl210, Kenwood CO, UK) for 7 min with a power of 400 W. The microencapsulated cells were hydrated in PBS for 2 hours. Bacteria were serially diluted in a phosphate buffer. Probiotics were placed on an MRS agar medium by the pour-plate method. After incubation for 48 h at 37 °C, the colonies were counted. The survival rate was calculated using above mentioned Eq. 1. While, Where N: The Probiotics count after drying (Log CFU.g^{-1}) and N_0: The Probiotics count before drying (Log CFU.g^{-1}).

2.8 Survival Rate Of L. reuteri In Harsh Conditions

2.8.1 Simulated Gastrointestinal Digestion

Simulated intestinal and gastric fluids were prepared using method described by Mohamadzadeh et al. [15]. Pepsin at a concentration of 3 g.l^{-1} was added to a saline solution (0.5% v/v) to prepare simulated gastric fluid. The final pH of the solution was adjusted to 2 using 1 N HCl. Simulated intestinal fluid was prepared by adding 4.5% (w/v) bile salt and pancreatin USP at a concentration of 1 g.l^{-1} to a saline solution. The pH of the solution was adjusted to 8 by adding 1 N NaOH. The encapsulated-dried probiotics were added to 1 ml of simulated gastric fluid and incubated at 37 °C for 4 h. The mixture was centrifuged at 16100 g for 15 min. The supernatant was discarded and the cells were mixed with 1 ml of simulated intestinal fluid. As in the previous step, probiotics were incubated at 37 °C for 4 h and then centrifuged. After discarding the supernatant, probiotics were diluted using PBS. The released probiotics were counted. The viability under simulated gastrointestinal conditions was calculated using above mentioned Eq. 1. While, N was the probiotics count after exposure to simulated gastrointestinal digestion (SGD) (Log CFU.g^{-1}) and N_0 was the probiotics count before exposure to SGD (Log CFU.g^{-1}).

2.8.2 Heat Treatment of the Microencapsulated Probiotics

Alginate, bionanocomposite and OCMC-coated bionanocomposite beads were assessed for heat resistance at 60 °C for 60 min, 70 °C for 30 min and 80 °C for 5 min. One gram of the dried beads was added to 9 ml of PBS. After heat treatment, tubes were cooled down to 37 °C and serially diluted. Cells were counted using pour-plate method and results were reported as log CFU.g^{-1}.

2.8.3 Long-term Storage

Alginate, bionanocomposite and OCMC-coated bionanocomposite beads were dried using described methods. The probiotics stability was assessed at refrigerator temperature (4 °C) and ambient temperature (25 °C) for 6 w. Cell counts on MRS agar were carried out weekly and results were reported as log CFU.g^{-1}.

2.9 Characterization of the Microparticles

2.9.1 Scanning Electronic Microscopy

The microstructural characteristics and surface morphology of the dried samples, including inulin, pectin, bionanocomposites containing *L. reuteri* and OCMC-coated bionanocomposites containing *L. reuteri*, were analyzed using scanning electron microscopy (SEM). To enhance conductivity, samples were coated with a thin layer of gold and then analyzed at an accelerating voltage of up to 15 kV.

2.9.2 Fourier transform infrared spectroscopy (FTIR)

The structure of inulin, pectin, SA, OCMC, MgONPs and bionanocomposite microcapsules with and without *L. reuteri* and OCMC-coated bionanocomposite microcapsules were investigated using Fourier transform infrared spectroscopy (FTIR) analysis. First, KBr spectrum was recorded as a control. Then, samples were mixed with KBr and a thin pellet was formed by compressing them at a pressure of 60 kPa for 10 min. Findings were present with a resolution of 0.5 cm^{-1} within the wavelength of 400–4000 cm^{-1}.

2.9.3 X-Ray Diffraction

The X-ray diffraction (XRD) analysis was carried out using X-ray diffractometer (X'Pert MPD, Philips, the Netherlands) that used Cu Kα radiation at 40 kV, 30 mA and $\lambda = 0.1542$ nm. The diffraction patterns were recorded by monitoring diffractions with a scan speed at 0.02◦/s, within a 2θ angle range of 10–70◦ [18]. The XRD analysis was carried out on inulin, pectin, OCMC, bionanocomposite microcapsules with and without probiotics and OCMC-coated bionanocomposite microcapsules.

2.10 Experimental Design and Statistical analysis

This study used simplex lattice mixture design to optimize concentration of OCMC and various concentrations of prebiotics at a fixed ratio (inulin:pectin of 10.9:1). Table 1 presents the experimental design data. Results analysis was carried out using Design-Expert software. To assess the importance and effects of each element on the response, analysis of variance was carried out with a significance level of 95%. The coefficient of determination, R^2, verified validity of the regression model. All experiments were carried out in triplicate. A numerical optimization technique was used for the optimization process.

3. Results and Discussion

3.1 Encapsulation Efficiency of *L. reuteri*

The effects of various concentrations of inulin, pectin and OCMC on encapsulation efficiency were investigated. Table 1 shows various bionanocomposite formulations with and without OCMC coating. The encapsulation efficiency ranged from 95.35 (Run 5) to 98.13% (Run 7). The highest efficiency was achieved in absence of OCMC with a

previously optimized prebiotic formulation (inulin:pectin of 10.9:1). The lowest extrusion efficiency was observed at 1% (w/v) concentration of OCMC without prebiotics. No use of OCMC resulted in easier releases of probiotics from the beads. Decreases in the viability of probiotics coated with OCMC could be due to the use of mortar and pestle to release the probiotics. The microencapsulation efficiency of alginate beads coated with chitosan decreased, compared to that of alginate beads [19]. Parsana et al. demonstrated that the encapsulation efficiency of *L. reuteri* in alginate beads (92.06%) was higher than that in alginate beads coated with chitosan with prebiotic inulin (90.63%) [20]. The coating process (e.g. agitation, pH changes and exposure to chitosan solution) could affect mechanical or osmotic stress that decreased integrity of the alginate matrix, resulting in decreased microencapsulation efficiency. Using inulin and pectin prebiotics in the microencapsulation of probiotics could improve probiotic growth as well. Poletto et al. showed that efficiency of the extrusion in presence of inulin (96.75%) was 2.65% higher than that in microencapsulation using alginate (94.10%) [21]. Probiotics could ferment prebiotics, providing an immediate source of metabolic energy. Even before reaching the gut, inulin and pectin could serve as nutritional reserves within the microcapsule. Efficiency of the extrusion in alginate beads was 95.23%, which showed a decrease of 1.29%, compared to the average efficiency of various formulations in the experimental design. Zaeim et al. reported that the efficiency of probiotics microencapsulated with alginate (98.12%) was 1.21% higher than that of microencapsulated probiotics with alginate-chitosan (96.91%) [22].

In Table 2, a positive value indicates a synergistic effect and a negative value indicates an antagonistic effect on the response. Based on statistical analysis, total inulin-pectin concentration and OCMC alone positively affected the extrusion efficiency. The inulin-pectin concentration was more effective than the OCMC concentration. The interaction of two variables included negative effects on the microencapsulation efficiency. The lack of fit in all models was greater than 0.1 with insignificancy. The microencapsulation efficiency was predicted using cubic model with a value of $p < 0.001$ (Table 2).

3.2 Survival Rate of Microencapsulated Cells after Drying

The drying efficiency of probiotics using microwave oven is shown in column Y_1 of Table 1. The highest survival efficiency was 99.41 (Run 4) and the lowest survival efficiency was 98.05% (Run 1). Protein denaturation, fatty acid (FA) oxidation, DNA damage and free radical formation were factors that decreased viability of the probiotics due to thermal drying. The lowest drying survival of *L. reuteri* occurred in absence of OCMC. Survival of bacteria in encapsulation depended on the thickness and type of coating materials [23]. Without OCMC, the coating depended on lighter materials such as alginate and prebiotics, which did not form an equally robust barrier, resulting in thinner layers. The presence of OCMC significantly increased viscosity and density of the outer layer, creating a thicker, further cohesive protective coating around the microcapsules against thermal stress. Drying efficiency of the alginate beads was 95.98%. A 2.7% decrease in the viability of probiotics was observed, compared to an average of various formulations in the experimental design. The absence of inulin, pectin and OCMC as a coating layer decreased thickness of the bacterial coating layer and hence caused further heat damages to the probiotics. Jantarathin et al. showed that in addition to increasing the viability of cells during the drying process, chitosan improved the viability of bacteria after drying [24].

Based on the data fitting, the cubic model produced the lowest *p*-value regarding viability after drying (Table 2). The model significance was verified with a *p*-value < 0.0001. Two variables, total inulin-pectin concentration and OCMC, positively affected drying efficiency and the effect of OCMC concentration was greater than that of the inulin-pectin concentration. Decreasing the survival rate of probiotics during drying included a negative effect on the effectiveness of probiotics. The 99.41% viability of *L. reuteri* in the microwave process was a significant efficiency.

Table 2. Predicted models of the responses for the optimization of bionanocomposite formulations

Response	Model	Statistical value				
		R^2	Adj. R^2	Pred. R^2	Adeq. precision	p-Value
EE	98.09 A + 95.34 B - 1.79 AB – 7.72 AB(A-B)	0.9829	0.9702	0.8774	21.034	0.0005
Y_1	98.06 A + 98.97 B - 1.54 AB – 4.44 AB(A-B)	0.9972	0.9951	0.9821	53.842	< 0.0001
Y_2	89.10 A + 87.10 B + 4.29 AB – 3.56 AB(A-B)	0.9865	0.9765	0.9031	20.704	0.0003

EE: Extrusion efficiency; Y_1: Viability after drying; Y_2: Viability in GIT

In addition to choosing the appropriate materials for microencapsulation, drying method significantly improved the viability of probiotics. Using microwave oven is a biocompatible method that can maintain the viability of probiotics at high rates within short times. Drying in microwave ovens increases product purity, improves the quality of the pharmaceutical powder and decreases byproducts and energy consumption [25]. Microwave ovens serve as innovations for drying food and pharmaceutical products.

3.3 Viability of Probiotics in Simulated Gastrointestinal Conditions

The viability rate of *L. reuteri* (Table 1, column Y_2) varied between 87.11 (Run 5) and 89.3% (Run 6). The lowest survival rate of probiotics was observed at 1% (w/v) concentration of OCMC in absence of prebiotics within the bionanocomposite structure. The MgONPs could serve as a buffering agent, enhancing the survival rate of probiotics by decreasing the acidity in the stomach. The lack of MgONPs release due to the OCMC coating with a concentration of 1% (w/v) could be a reason for decreasing the viability of probiotics in SGI conditions. The highest survival rate of probiotics in SGI conditions was achieved at a concentration of 0.5% (w/v) of OCMC and 0.5% (w/v) of prebiotics (inulin:pectin of 10.9:1). Combining OCMC with prebiotics provided mechanical protection and metabolic support to the probiotics, which could include a synergistic effect. A decrease of 2.87 log $CFU.g^{-1}$ of the microencapsulated cells in alginate microcapsules was observed in the simulated digestive fluids. The survival rate of alginate beads in the SGI conditions was 71.49%, which was 17% less than the average efficiency of various formulations in the experimental design. Afzaal et al. demonstrated that the survival of *L. acidophilus* ATTC 4356 microencapsulated within alginate in the simulated gastric fluid was associated to a decrease of 3.57 log $CFU.ml^{-1}$ [26]. The resistance of *Lactobacillus* strains at low pH could be attributed to F_0F_1-ATPase activity in probiotics [27]. The survivability of alginate-encapsulated probiotics significantly decreased by 2.26 ±0.24 log $CFU.g^{-1}$ [28]. Based on the data fitting, the cubic model produced the lowest *p*-value (0.0003) regarding the SGI conditions (Table 2). The model significance was verified with a *p*-value < 0.001. Three variables, including the total inulin-pectin concentration, concentration of OCMC and interactions of variables (AB), positively affected cell viability in SGI conditions. Similar to extrusion efficiency, inulin-pectin concentration was more effective than OCMC concentration. The effect of AB (A-B) parameters negative affected the viability of probiotics.

3.4 O-Carboxymethyl Chitosan and Prebiotic Concentration Optimization

Optimizing the inulin and pectin as prebiotics, as well as the OCMC quantity for coating microcapsules, is critical. Three parameters of encapsulation efficiency, survival rate of microencapsulated cells after microwave drying and survival rate of *L. reuteri* in SGI conditions were selected for optimization. The efficiency of encapsulation is important due to the presence of probiotic microgels in food, pharmaceutical and cosmetic industries. Optimizing viability after drying greatly affected the viability of dried powder and improved the long-term stability of probiotics. Optimizing survival in simulated digestive conditions enhanced the delivery of probiotics to the clone by increasing its survival in these conditions. Quantities of inulin and pectin with a fixed ratio (inulin:pectin of 10.9:1) and the concentration of OCMC were assessed to coat the microgels in a 0–1 g range. Optimization was achieved when the encapsulation efficiency, viability after microwave drying and viability after exposure to SGI conditions were simultaneously at their highest levels. After defining the highlighted conditions in Design-Expert software, the ratio of inulin to pectin was 32% (inulin, 0.294, and pectin, 0.026) and the OCMC concentration was reported as 68% (Table 3). The quantities of MgONPs and SA were similar to them in all compositions. Optimization was valid when the desirability function was acceptable. The desirability was 90%. The optimization results were verified by carrying out experiments. The optimization results revealed that the OCMC-coated bionanocomposite could serve as a novel approach to protect cells and enhance survival of bacteria in harsh conditions in food, pharmaceutical and cosmetic industries.

Table 3. Optimum response values for the prebiotics and O-carboxymethyl chitosan

Response	Optimum response value (validated)	Optimum formulation % (w/v)		
		Inulin	pectin	OCMC
EE	96.4365			
Y_1	99.3587	0.294	0.026	0.68
Y_2	88.9543			

EE: Extrusion efficiency; Y_1: Viability after drying; Y_2: Viability in GIT

3.5 Heat Treatment of Microencapsulated Probiotics

To investigate the heat stability of probiotics, alginate, bionanocomposite and OCMC-coated bionanocomposite microcapsules were assessed at three various temperatures and times. Microcapsules were assessed at 60, 70 and 80 °C for 60, 30 and 5 min, respectively. It was observed that bionanocomposite and OCMC-coated bionanocomposite beads included significant resistance, compared to that alginate beads did. The viability of probiotics in OCMC-coated bionanocomposite microcapsules was higher than

that in bionanocomposite microcapsules. In microcapsules coated with OCMC, probiotic viability decreased by 1.46 log CFU.g^{-1} at 80 °C for 5 min. In comparison, decrease of *L. reuteri* viability in alginate beads in similar conditions was 5.91 log CFU.g^{-1}. Results demonstrated that OCMC was an appropriate coating polymer for probiotics against thermal treatments. The viability loss of the micro-encapsulated *L. plantarum* EMCC1039 with chitosan-coated alginate was 3.06 log CFU.g^{-1} after exposure to 65 °C for 30 min [29]. Cheow et al. demonstrated that the viability of *L. rhamnosus* GG coated with alginate-chitosan decreased by 5.9 log CFU.ml^{-1} after incubating at 60 °C for 30 min [30]. Alginate beads demonstrated higher sensitivity to temperature. The highest decrease in the viability of probiotics occurred in bacteria encapsulated with alginate beads. Halim et al. reported that *P. acidilactici* ATCC 8042 encapsulated in alginate was destroyed at 60 °C for 60 min [31]. Based on the thermal stability results in Table 4, OCMC-coated bionanocomposite beads demonstrated higher resistance and promise stability to severe harsh thermal conditions, compared to those other microcapsules did.

3.6 Stability of Encapsulated Probiotics in Long-term Storage

Assessment of the probiotic stability of micro-encapsulated *L. reuteri* in various time and temperature conditions is shown in Table 5. Due to a better cell protection of bionanocomposites during the extrusion and drying processes, the initial number of cells coated with bionanocomposite was higher than that with alginate microcapsules. Over time, the death slope of probiotics decreased; thus, the highest decrease in *L. reuteri* viability was recorded within the first week. The highest rate of viability loss of probiotics, with a value of 3.02 log CFU.g^{-1} at 25 °C, was linked to alginate beads. The lowest decrease in survival within the first week was observed with OCMC-coated bionanocomposite microcapsules at 4 °C with a value of 0.47 log CFU.g^{-1}. Using OCMC to coat bionano-composites improved the stability of probiotics by 0.06 and 0.42 log CFU.g^{-1} at 4 and 25 °C after 42 d. Microencapsulated probiotics in OCMC-coated bionano-composite recorded significantly higher viability, compared to that those in alginate microcapsules did. Qi et al. concluded that the survival rate of *L. rhamnosus* GG microencapsulated in alginate/chitosan at 25 °C after 42 d was nearly 1 log CFU.g^{-1} higher than that of probiotics microencapsulated in alginate was [32]. Adding chitosan significantly increased the 35-d storage stability of *L. acidophilus* NCIMB 701748 dried powders [33]. The survival of probiotics encapsulated with OCMC-coated bionanocomposite was 3.25 and 2.93 log CFU.g^{-1} higher than that of alginate microcapsules at 25 and 4 °C after 42 d. A decrease of 2 log CFU.g^{-1} was reported for microencapsulated probiotics in alginate/bentonite nanocomposite at 25 °C after 14 d [34]. In general, the viability of probiotics decreased with increasing temperature. In all microcapsules, the viability of probiotics at 4 °C was higher than that at 25 °C. Shu et al. demonstrated that probiotic viability decreased at high temperatures due to increased intracellular water activity and membrane oxidation stress.

Table 4. Viability of various microcapsulated probiotics after various heat treatments

Microcapsule type	Initial count (log CFU.g^{-1})	Viable cells (log CFU.g^{-1})		
		60 °C, 60 min	70 °C, 30 min	80 °C, 5 min
Alginate beads	10.09	5.72±0.42	4.36±0.33	4.18±0.36
Bionanocomposite beads	10,32	7.53±0.19	7.74±0.09	7.79±0.15
OCMC-coated bionanocomposite beads	10.39	8.52±0.13	8.81±0.07	8.93±0.29

All experiments were carried out in triplicates. Data represented as mean ± standard deviation ($p<0.05$)

Table 5. Viability of the microencapsulated *Lactobacillus reuteri* during storage for 42 d at 4 and 25 °C

Parameter	Time (day)	Alginate microcapsules		Bionanocomposite microcapsules		OCMC-coated bionanocomposite microcapsules	
		4°C	25°C	4°C	25°C	4°C	25°C
Viability (log CFU.g^{-1})	0	10.09	10.09	10.32	10.32	10.39	10.39
	7	7.29±0.12	7.07±0.24	9.62±0.09	9.32±0.19	9.92±0.19	9.75±0.21
	14	6.91±0.31	6.19±0.11	9.24±0.13	9.01±0.29	9.59±0.23	9.24±0.18
	21	6.23±0.21	5.53±0.25	9.15±0.24	8.88±0.25	9.27±0.25	9.10±0.15
	28	6.20±0.25	5.41±0.29	8.98±0.31	8.47±0.29	9.09±0.14	8.93±0.19
	35	6.09±0.12	5.40±0.13	8.97±0.21	8.26±0.27	8.99±0.16	8.63±0.16
	42	5.98±0.08	5.36±0.23	8.85±0.14	8.19±0.18	8.91±0.22	8.61±0.17

All experiments were carried out in triplicate. Data represented as mean ± standard deviation ($p<0.01$)

Analysis of the stability results for *L. reuteri* was carried out using SPSS statistics software, which indicated statistically significant results (p-value < 0.01). Results indicated that OCMC polymer was appropriate for coating microencapsulated probiotics; thereby, enhancing their stability. Microencapsulated probiotics with OCMC-coated bionanocomposite were promising to increase the microorganism stability.

3.7 Characterization of the Microparticles

3.7.1 Surface Morphology

The SEM images of inulin, pectin, beads containing *L. reuteri* and OCMC-coated microcapsules containing *L. reuteri* are shown in Figure 1. The surface morphology indicated that inulin possessed a spherical structure. The physicochemical characteristics of the mixture could be affected by various sizes of inulin. Strength of the gel structure was directly linked to the size of inulin particles. Inulin with a higher molecular weight was further resistant to hydrolysis and was further stable [35]. The SEM images showed that pectin included a non-spherical structure. The quantity of moisture in the particles and various extraction methods could affect the size of particles such as inulin and pectin. Drying the microgels using microwave oven resulted in the dehydration of the beads within a short time. Deng et al. showed that drying carbohydrate compounds using microwave ovens could improve gelling characteristics of the compounds in addition to maintaining the structure [36]. No probiotics were observed on the surfaces of microcapsules containing bacteria. The high efficiency of the entrapment of *L. reuteri* (96.43%) could be a reason for the absence of *L. reuteri* on the surface of the beads. moreover, OCMC coating on microcapsules could be another reason for the absence of bacteria on the surface of beads. The surface structure of uncoated microcapsules containing *L. reuteri* was non-porous and cohesive. The cohesive structure of the microcapsules was attributed to the egg-box structure formed by specific and strong interactions between Ca^{2+} and the G-blocks of alginate. Optimal drying resulted in no cracks or pores in the microcapsule structure. The absence of pores prevented the penetration of acids, hydrogen ions, bile salts and enzymes and improved the stability of probiotics in harsh conditions. Presence of MgONPs could also create a non-porous structure by filling the nanometer pores. The OCMC-coated microcapsules shrank and wrinkled during the drying process. The peaks and valleys in dried OCMC-coated microcapsules could be attributed to the ionic interaction between alginate and OCMC. Alginate cross-linking with OCMC in a hydrogel could increase stability and improve microgel structures. The ionic interaction between carboxyl residues in alginate and amino residues in OCMC formed a polyelectrolyte complex.

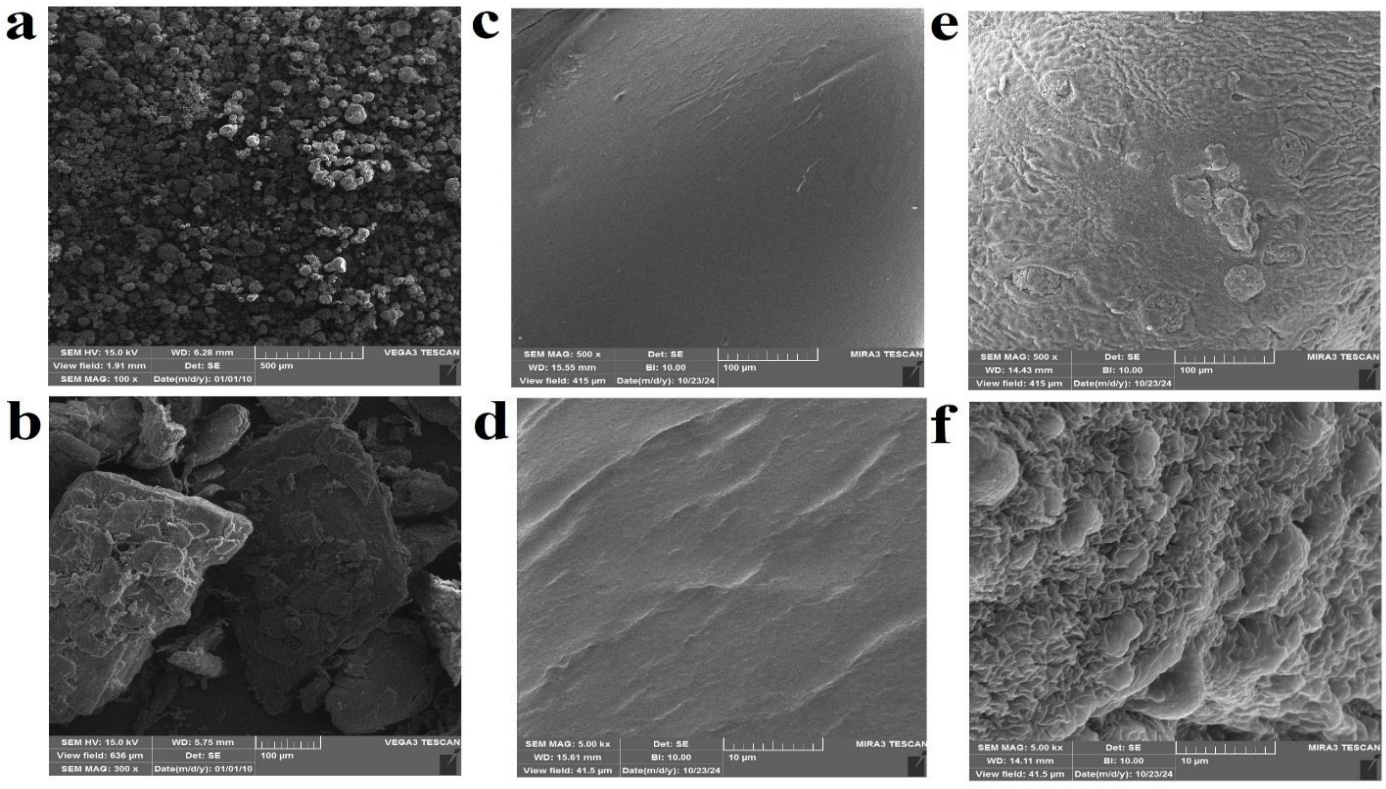

Figure 1. The surface morphology of (a) inulin, (b) pectin, (c, d) bionanocomposites containing *Lactobacillus reuteri* and (e, f) O-carboxymethyl chitosan-coated bionanocomposites containing *Lactobacillus reuteri*.

3.7.2 Fourier-transform Infrared Spectroscopy Analysis

Inulin, pectin, SA, OCMC, MgONPs, bionanocomposite microcapsules with and without *L. reuteri* and OCMC-coated bionanocomposite microcapsules were assessed via FTIR analysis (Figure 2). The presence of an absorption band at 3640 cm^{-1} in SA and at 3700 cm^{-1} in MgONPs showed O–H stretching vibrations that did not involve hydrogen bonding [37]. The absorption band of 3400–3450 cm^{-1} in all three microcapsules was linked to hydrogen bonding in hydroxyl groups. These bands indicated presence of moisture in the microcapsules. The peak in 3000–3600 cm^{-1} wavenumbers range in inulin, pectin and OCMC indicated the stretching vibrations of O-H groups. The peaks in the wavenumber range of 2800–3000 cm^{-1} observed in all analyses, except for MgONPs, associating to C–H stretching vibrations. Peaks at 1650, 1140 and 1000 cm^{-1}, respectively, represented vibrations of C=O, C–C and C–O–C groups in the inulin structure [38]. The peak at a wavenumber of 1745 cm^{-1} in pectin was linked to a carbonyl group. The peak at 1610 cm^{-1} was due to the asymmetric stretching vibrations of COO^- ions in the C=O group. In SA FTIR, 1410 and 1030 cm^{-1} peaks corresponded to symmetric stretching vibrations of carboxylate ions and C–O–C, respectively. In OCMC FTIR, the peak at 1610 cm^{-1} corresponded to the N–H bending of primary amines and the peak at 1430 cm^{-1} corresponded to the C–N group. Peak 1320 cm^{-1} was linked to C–O group vibrations. Peak 1060 cm^{-1} represented the C–O stretch of –CH2–OH in primary alcohols. The peak at a wavenumber of 1640 cm^{-1} represented carbonyl vibrations in MgONPs. The 1435 cm^{-1} peak corresponded to the vibrational activity of water molecules on the surface of MgONPs. The peak at 555 cm^{-1} in nanoparticles was associated to Mg–O bending vibrations [39].

The absorption range of stretching vibrations associated with hydroxyl bonds in microcapsules was denser than that of SA. A reason for this difference was the participation of carboxylate and alginate hydroxyl groups with calcium ions to create the egg-box structure. The egg-box model could improve the stability of microcapsules by creating a compact structure. All three types of microcapsules showed high similarities in their FTIR analysis. The difference between microcapsules included presence or absence of *L. reuteri* and OCMC. Presence of bacteria in the bionanocomposite caused a slight difference in FTIR analysis. The peak at 1235 cm^{-1} in microcapsules containing *L. reuteri* could be attributed to the amide III band of cytoplasmic and membrane proteins in *L. reuteri*. Adding OCMC led to the formation of new hydrogen bonds between the $–NH_2$ and –COOH groups of OCMC and the C=O and –OH groups of alginate. Transfer of peaks from 1437 and 1625 cm^{-1} to 1428 and 1620 cm^{-1} could be a reason for the formation of bonds between OCMC and calcium alginate. The slight shift of the carboxylate bands to lower wavenumbers was due to the sharing of bonds with amine groups in OCMC on the surface of the microcapsules. The increase in intensity of the peak at 1620 cm^{-1} verified formation of strong polyelectrolyte complexes [40].

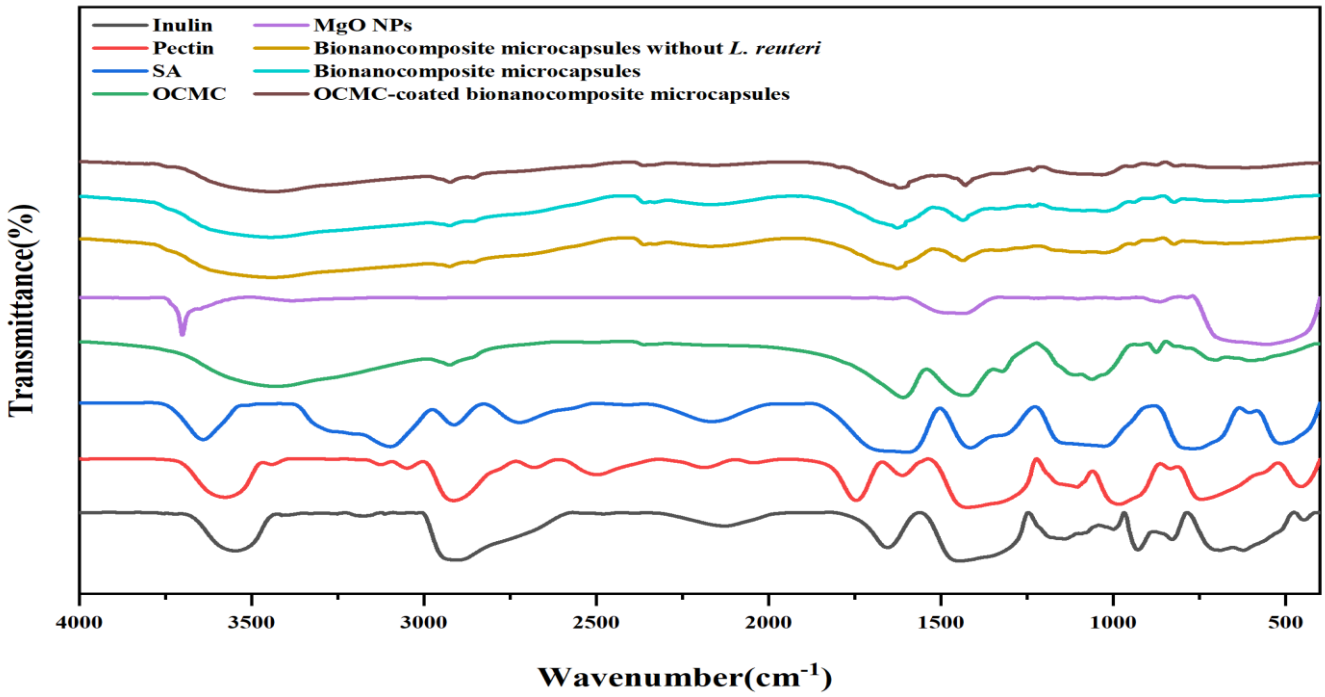

Figure 2. Fourier-transform infrared spectroscopy spectra of inulin, pectin, sodium alginate, O-carboxymethyl chitosan, MgONPs and bionanocomposite microcapsules with and without *Lactobacillus reuteri* and O-carboxymethyl chitosan-coated bionanocomposite microcapsules.

3.7.3 The X-ray Diffraction Analysis

The XRDs of inulin, pectin, OCMC, bionanocomposite microcapsules with and without *L. reuteri* and OCMC-coated bionanocomposite microcapsules are shown in Figure 3. The X-ray analysis was used to assess the amorphous or crystalline structure of polymers. The XRD analysis showed that the structures of inulin and pectin were amorphous and crystalline, respectively. Non-sharp peaks in the inulin structure and sharp peaks in the pectin structure showed a significant structural difference between these prebiotics. The six sharp peaks at 2θ = 27.623°, 31.976°, 38.178°, 45.671°, 56.679° and 66.474° were important regions of the OCMC, representing that this polymer structure is crystalline. The XRD analysis demonstrated that structures of bionanocomposite microcapsules with and without *L. reuteri* were amorphous. One of the reasons for the amorphous structure of beads was the cross-linking of SA and pectin with Ca^{2+} ions. Presence or absence of *L. reuteri* include no significant effect on the amorphous structure of microcapsules. Addition of crystalline OCMC included a significant effect on the structure of amorphous microcapsules. The OCMC coating caused the formation of semi-crystalline structures in the microcapsules. The ionic interaction between amino groups in OCMC and carboxyl groups of alginate could be one of the reasons for changing the structure of microcapsules.

Rao et al. demonstrated that calcium alginate-pectin microcapsules containing *L. paraplantarum* LR-1 included an amorphous structure. Presence of polymers with various concentrations in the microcapsule, as well as the method of microencapsulating probiotics, could affect intensity of the peaks and thus structure of the microcapsules [41]. Generally, polymers with semi-crystalline structures included higher temperature resistance than that amorphous structures did. Menegazzi et al. showed that use of polymers with a semi-crystalline structure decreased heat and oxygen transfer into the microcapsule and improved the viability of probiotics [42]. Based on Section 3.5, it was observed that the semi-crystalline structure of microcapsules included higher temperature resistance in all three temperatures of 60, 70 and 80 °C and hence higher *L. reuteri* survival was recorded. Additionally, semi-crystalline morphologies showed higher chemical resistance and biocompatibility, compared to that amorphous polymers did.

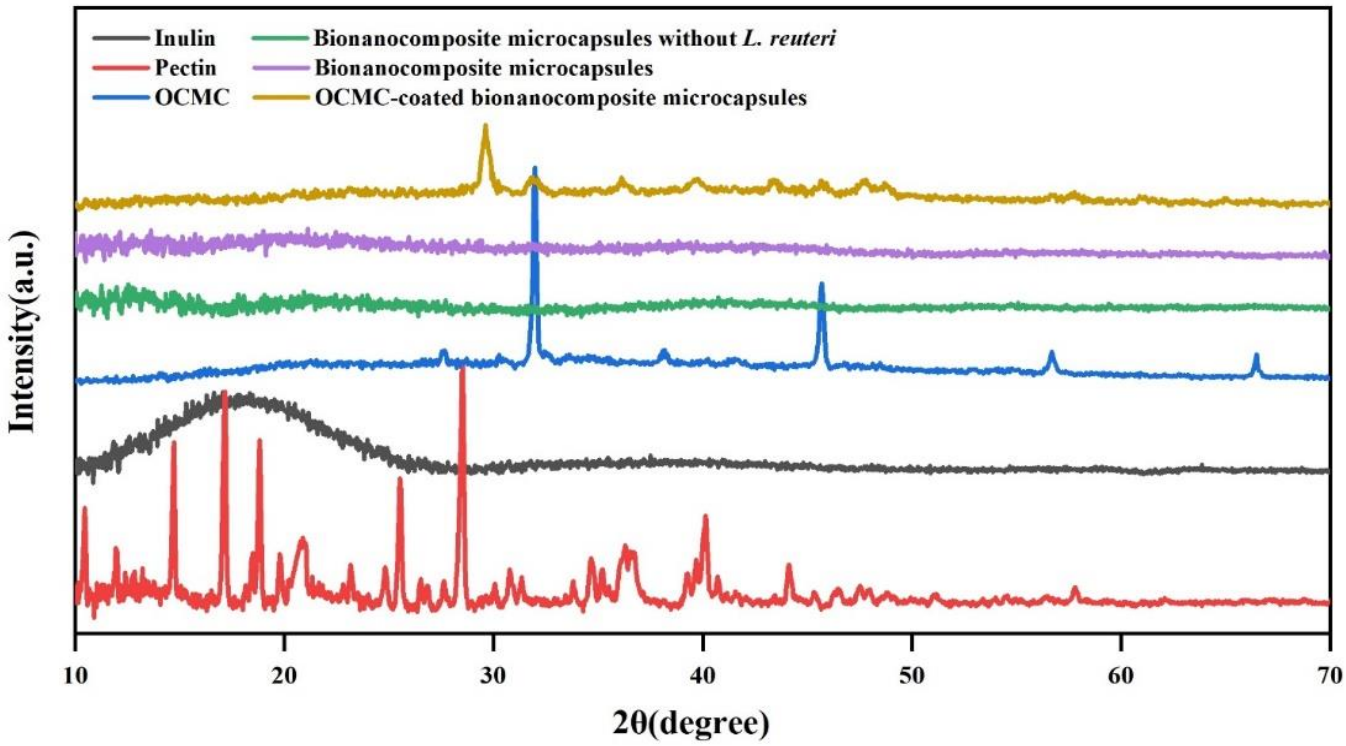

Figure 3. The X-ray diffraction of inulin, pectin, OCMC, bionanocomposite microcapsules with and without *L. reuteri* and OCMC-coated bionanocomposite microcapsules.

4. Conclusion

The present study focused on the optimization and assessment of an OCMC-coated bionanocomposite with inulin and pectin as prebiotics with MgONPs at constant concentrations. Based on the optimization result, concentration of 68% of OCMC with 29.4% inulin and 2.6% pectin could include the highest protection of *L. reuteri* in various conditions. Results showed that OCMC included high-heat protections from probiotics. High thermal stability of probiotics revealed that the use of OCMC-coated bionanocomposites could be effective in preparing functional foods, where bacteria were usually exposed to high temperatures. The stability of alginate beads decreased respectively at 4 and 25 °C, 3.25 and 2.93 log $CFU.g^{-1}$ after 42 d, compared to the coated probiotics. Physicochemical analysis of the optimal microcapsule revealed a coherent compact structure, which enhanced the stability and survival of bacteria in harsh conditions. Microencapsulation using the investigated bionanocomposite could increase the viability of probiotics during the production, formulation, storage and packaging processes. Further studies on the binding of OCMC to intestinal epithelial cells, *in-vivo* assessment of the release rate of microencapsulated probiotics and investigation of the viability of microencapsulated probiotics in OCMC-coated bionanocomposites in food products such as juices or fillets validate these findings.

5. Acknowledgements

The authors would like to express their thanks to the Department of Drug and Food Control, Pharmaceutical Quality Assurance Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, for the support it provided for this study.

6. Conflict of Interest

The authors declare no competing interest.

7. Authors' Contributions

Mohamadsadegh Mohamadzadeh, conceptualization, validation, investigation, visualization and writing original draft, formal analysis, software; Ebrahim Vasheghani Farahani, supervision, review and editing; Ahmad Fazeli, conceptualization, supervision, review and editing; Seyed Abbas Shojaosadati, conceptualization, validation, supervision, review and editing.

8. Using Artificial Intelligent Chatbots

No artificial intelligence chatbots were used in this study.

9. Ethical Consideration

This study did not receive specific grants from funding agencies in the public, commercial and not-for-profit sectors.

Abbreviations: L, *Lactobacillus*; CMC, carboxymethyl chitosan; NPs, nanoparticles; SGI, simulated gastrointestinal conditions; CFU, colony forming units; SEM, scanning electron microscopy; FTIR, Fourier transform infrared spectroscopy; XRD, X-ray diffraction; scFOS, short-chain fructooligosaccharides; BNC, bacterial nanocellulose; SA, sodium alginate; MRS, De Man, Rogosa and Sharpe; TSB, tryptic soy broth; TSA, tryptic soy agar.

References

1. Swanson KS, Gibson GR, Hutkins R, Reimer RA, Reid G, Verbeke K, Scott KP, Holscher HD, Azad MB, Delzenne NM. The international scientific association for probiotics and prebiotics (isapp) consensus statement on the definition and scope of synbiotics. Nat Rev Gastroenterol Hepatol. 2020; 17(11): 687-701. https://doi.org/10.1038/s41575-020-0344-2

2. Zhang K, Wang W, Zhao K, Ma Y, Wang Y, Li Y. Recent development in foodborne nanocellulose: Preparation, properties and applications in food industry. Food Biosci. 2021; 44: 101410. https://doi.org/10.1016/j.fbio.2021.101410

3. Razafindralambo H. Bacillus subtilis - Functionalities and Emerging Applications [Working Title]. In, Mohamadzadeh M, Abbaspour S. Probiotic Applications of *Bacillus subtilis*. 2024. https://doi.org/10.5772/intechopen.1007134

4. Rahman MN, Barua N, Tin MC, Dharmaratne P, Wong SH, Ip M. The use of probiotics and prebiotics in decolonizing pathogenic bacteria from the gut; A systematic review and meta-analysis of clinical outcomes. Gut Microbes. 2024; 16(1): 2356279. https://doi.org/10.1080/19490976.2024.2356279

5. Mohamadzadeh M, Fazeli A, Shojaosadati SA. Polysaccharides and proteins-based bionanocomposites for microencapsulation of probiotics to improve stability and viability in the gastrointestinal tract: A review. Int J Biol Macromol. 2024: 129287. https://doi.org/10.1016/j.ijbiomac.2024.129287

6. Koushki M, Amiri-Dashatan N, Pourghadamyari H, Khodabandehloo H, Bagheri F, Farahani M, Tayebi L. An overview of the relationships between the food industry and nanotechnology. Appl Food Biotechnol. 2024; 11(2): e7. https://doi.org/10.22037/afb.v11i2.46737

7. Li XY, Chen XG, Sun ZW, Park HJ, Cha D-S. Preparation of alginate/chitosan/carboxymethyl chitosan complex microcapsules and application in *Lactobacillus casei* ATCC 393. Carbohydr Polym. 2011; 83(4): 1479-1485. https://doi.org/10.1016/j.carbpol.2010.09.053

8. Puspita AE, Pangastuti A, Listyawati S, Sari SLA. Investigating untapped potentials: Velvet beans as novel prebiotic sources and their effects on gut microbiota and

short-chain fatty acid level. Appl Food Biotechnol. 2024: 11(1): e17. https://doi.org/10.22037/afb.v11i1.44643

9. Zabihollahi N, Alizadeh A, Almasi H, Hanifian S, Hamishekar H. Development and characterization of carboxymethyl cellulose based probiotic nanocomposite film containing cellulose nanofiber and inulin for chicken fillet shelf life extension. Int J Biol Macromol. 2020; 160: 409-417. https://doi.org/10.1016/j.ijbiomac.2020.05.066

10. Sarmadi B, Nikmaram P, Mortazavian-Farsani A-M, Kiani H, Mousavi M, Khanniri E, Mohammadi R, da Cruz AG. High-methoxyl apple pectin improves rheological properties and storage stability of the flavored probiotic yogurt drinks, compared to pomegranate pectin. Appl Food Biotechnol. 2022; 9(2): 91-102. https://doi.org/10.22037/afb.v9i2.37565

11. Tarifa MC, Piqueras CM, Genovese DB, Brugnoni LI. Microencapsulation of *Lactobacillus casei* and *Lactobacillus rhamnosus* in pectin and pectin-inulin microgel particles: Effect on bacterial survival under storage conditions. Int J Biol Macromol. 2021; 179: 457-465. https://doi.org/10.1016/j.ijbiomac.2021.03.038

12. Yao M, Li B, Ye H, Huang W, Luo Q, Xiao H, McClements DJ, Li L. Enhanced viability of probiotics (*Pediococcus pentosaceus* Li05) by encapsulation in microgels doped with inorganic nanoparticles. Food Hydrocoll. 2018; 83: 246-252. https://doi.org/10.1016/j.foodhyd.2018.05.024

13. Mohamadzadeh M, Ghiasi M, Aghamollaei H. Optimization of plasmid electrotransformation into *Bacillus subtilis* using an antibacterial peptide. Arch Microbiol. 2024; 206(3): 116. https://doi.org/10.1007/s00203-024-03847-2

14. Maleki O, Khaledabad MA, Amiri S, Asl AK, Makouie S. Microencapsulation of *Lactobacillus rhamnosus* ATCC 7469 in whey protein isolate-crystalline nanocellulose-inulin composite enhanced gastrointestinal survivability. LWT. 2020; 126: 109224. https://doi.org/10.1016/j.lwt.2020.109224

15. Mohamadzadeh M, Fazeli A, Vasheghani-Farahani E, Shojaosadati SA. Viability and stability evaluation of microencapsulated *Lactobacillus reuteri* in polysaccharide-based bionanocomposite. Carbohydr Polym. 2024: 122693. https://doi.org/10.1016/j.carbpol.2024.122693

16. Mi Y, Su R, Fan D-D, Zhu X-L, Zhang W-N. Preparation of N, O-carboxymethyl chitosan coated alginate microcapsules and their application to *Bifidobacterium longum* BIOMA 5920. Mater Sci Eng C Mater Biol Appl. 2013; 33(5): 3047-3053. https://doi.org/10.1016/j.msec.2013.03.035

17. Halim M, Mustafa NAM, Othman M, Wasoh H, Kapri MR, Ariff AB. Effect of encapsulant and cryoprotectant on the viability of probiotic *Pediococcus acidilactici* ATCC 8042 during freeze-drying and exposure to high acidity, bile salts and heat. LWT. 2017; 81: 210-216. https://doi.org/10.1016/j.lwt.2017.04.009

18. Shitu IG, Katibi KK, Taura LS, Muhammad A, Chiromawa IM, Adamu SB, Iya SGD. X-ray diffraction (XRD) profile analysis and optical properties of Klockmannite copper selenide nanoparticles synthesized via microwave assisted technique. Ceram Int. 2023; 49(8): 12309-12326. https://doi.org/10.1016/j.ceramint.2022.12.086

19. Jantarathin S, Borompichaichartkul C, Sanguandeekul R. Microencapsulation of probiotic and prebiotic in alginate-chitosan capsules and its effect on viability under heat process in shrimp feeding. Mater Today Proc. 2017; 4(5): 6166-6172. https://doi.org/10.1016/j.matpr.2017.06.111

20. Parsana Y, Yadav M, Kumar S. Microencapsulation in the chitosan-coated alginate-inulin matrix of *Limosilactobacillus reuteri* SW23 and *Lactobacillus salivarius* RBL50 and their characterization. Carbohydr Polym Technol Appl. 2023; 5: 100285. https://doi.org/10.1016/j.carpta.2023.100285

21. Poletto G, Raddatz GC, Cichoski AJ, Zepka LQ, Lopes EJ, Barin JS, Wagner R, de Menezes CR. Study of viability and storage stability of *Lactobacillus acidophillus* when encapsulated with the prebiotics rice bran, inulin and Hi-maize. Food Hydrocoll. 2019; 95: 238-244. https://doi.org/10.1016/j.foodhyd.2019.04.049

22. Zaeim D, Sarabi-Jamab M, Ghorani B, Kadkhodaee R, Tromp RH. Electrospray assisted fabrication of hydrogel microcapsules by single-and double-stage procedures for encapsulation of probiotics. Food Bioprod Process. 2017; 102: 250-259. https://doi.org/10.1016/j.fbp.2017.01.004

23. Altamirano-Ríos AV, Guadarrama-Lezama AY, Arroyo-Maya IJ, Hernández-Álvarez AJ, Orozco-Villafuerte J. Effect of encapsulation methods and materials on the survival and viability of *Lactobacillus acidophilus*: A review. Int J Food Sci Technol. 2022; 57(7): 4027-4040. https://doi.org/10.1111/ijfs.15779

24. Jantarathin S, Borompichaichartkul C, Sanguandeekul R. Microencapsulation of probiotic and prebiotic in alginate-chitosan capsules and its effect on viability under heat process in shrimp feeding. Mater Today Proc. 2017; 4(5): 6166-6172. https://doi.org/10.1016/j.matpr.2017.06.111

25. Guzik P, Kulawik P, Zając M, Migdał W. Microwave applications in the food industry: An overview of recent developments. Crit Rev Food Sci Nutr. 2022; 62(29): 7989-8008. https://doi.org/10.1080/10408398.2021.1922871

26. Afzaal M, Saeed F, Arshad MU, Nadeem MT, Saeed M, Tufail T. The effect of encapsulation on the stability of probiotic bacteria in ice cream and simulated gastrointestinal conditions. Probiotics Antimicrob Proteins. 2019; 11: 1348-1354. https://doi.org/10.1007/s12602-018-9485-9

27. Mousanejadi N, Barzegar H, Alizadeh Behbahani B, Jooyandeh H. Production and evaluation of a functional fruit beverage consisting of mango juice and probiotic bacteria. J Food Meas Charact. 2023; 17(4): 3240-3253. https://doi.org/10.1007/s11694-023-01862-3

28. Fu Y, Liu L, Zhang J, Wang L, Dong M, McClements DJ, Wan F, Shen P, Li Q. Reinforcing alginate matrixes by tea polysaccharide conjugates or their stabilized nanoemulsion for probiotics encapsulation: Characterization, survival after gastrointestinal digestion and ambient storage. Int J Biol Macromol. 2023; 253: 126828. https://doi.org/10.1016/j.ijbiomac.2023.126828

29. Mahmoud M, Abdallah NA, El-Shafei K, Tawfik NF, El-Sayed HS. Survivability of alginate-microencapsulated *Lactobacillus plantarum* during storage, simulated food processing and gastrointestinal conditions. Heliyon. 2020; 6(3): e03541. https://doi.org/10.1016/j.heliyon.2020.e03541

30. Cheow WS, Hadinoto K. Biofilm-like *Lactobacillus rhamnosus* probiotics encapsulated in alginate and carrageenan microcapsules exhibiting enhanced thermotolerance and freeze-drying resistance. Biomacromolecules. 2013; 14(9): 3214-3222. https://doi.org/10.1021/bm400853d

31. Halim M, Mustafa NAM, Othman M, Wasoh H, Kapri MR, Ariff AB. Effect of encapsulant and cryoprotectant on the

viability of probiotic *Pediococcus acidilactici* ATCC 8042 during freeze-drying and exposure to high acidity, bile salts and heat. LWT. 2017; 81: 210-216. https://doi.org/10.1016/j.lwt.2017.04.009

32. Qi X, Simsek S, Ohm J-B, Chen B, Rao J. Viability of *Lactobacillus rhamnosus* GG microencapsulated in alginate/chitosan hydrogel particles during storage and simulated gastrointestinal digestion: Role of chitosan molecular weight. Soft Matter. 2020; 16(7): 1877-1887. https://doi.org/10.1039/C9SM02387A

33. Yonekura L, Sun H, Soukoulis C, Fisk I. Microencapsulation of *Lactobacillus acidophilus* NCIMB 701748 in matrices containing soluble fibre by spray drying: Technological characterization, storage stability and survival after in vitro digestion. J Funct Foods. 2014; 6: 205-214. https://doi.org/10.1016/j.jff.2013.10.008

34. Kim J, Hlaing SP, Lee J, Saparbayeva A, Kim S, Hwang DS, Lee EH, Yoon I-S, Yun H, Kim M-S. Exfoliated bentonite/alginate nanocomposite hydrogel enhances intestinal delivery of probiotics by resistance to gastric pH and on-demand disintegration. Carbohydr Polym. 2021; 272: 118462. https://doi.org/10.1016/j.carbpol.2021.118462

35. Guo Z, Liu M, Xiang X, Wang Z, Yang B, Chen X, Chen G, Kan J. Effects of inulins with various molecular weights and added concentrations on the structural properties and thermal stability of heat-induced gliadin and glutenin gels. LWT. 2021; 149: 111891. https://doi.org/10.1016/j.lwt.2021.111891

36. Deng X, Huang H, Huang S, Yang M, Wu J, Ci Z, He Y, Wu Z, Han L, Zhang D. Insight into the incredible effects of microwave heating: Driving changes in the structure, properties and functions of macromolecular nutrients in novel food. Front Nutr. 2022; 9: 941527. https://doi.org/10.3389/fnut.2022.941527

37. Dai F, Zhuang Q, Huang G, Deng H, Zhang X. Infrared spectrum characteristics and quantification of OH groups in coal. ACS Omega. 2023; 8(19): 17064-17076. https://doi.org/10.1021/acsomega.3c01336

38. Akram W, Garud N, Joshi R. Role of inulin as prebiotics on inflammatory bowel disease. Drug Discov Ther. 2019; 13(1): 1-8. https://doi.org/10.5582/ddt.2019.01000

39. Mahadevaiah R, Lalithamba HS, Shekarappa S, Hanumanaika R. Synthesis of Nα-protected formamides from amino acids using MgO nano catalyst: Study of molecular docking and antibacterial activity. Sci Iranica. 2017; 24(6): 3002-3013. https://doi.org/10.24200/SCI.2017.4491

40. Zaeim D, Sarabi-Jamab M, Ghorani B, Kadkhodaee R, Liu W, Tromp RH. Microencapsulation of probiotics in multiple-polysaccharide microcapsules by electro-hydrodynamic atomization and incorporation into ice-cream formulation. Food Struct. 2020; 25: 100147. https://doi.org/10.1016/j.foostr.2020.100147

41. Rao Y, Deng J, Zhang C, Song Y, Liu L. Probiotics encapsulated by calcium pectin/chitosan-calcium pectin/sodium alginate-pectin-whey through biofilm-based microencapsulation strategy and their preventive effects on ulcerative colitis. Food Hydrocoll. 2025; 158: 110501. https://doi.org/10.1016/j.foodhyd.2024.110501

42. da Silva Menegazzi G, Ribeiro ES, de Farias BS, da Luz GdQ, Oliveira GM, Junior TRSAC, de Almeida Pinto LA, Diaz PS. Microencapsulation of *Lacticaseibacillus casei* CSL3 using cheese whey, fructo-oligosaccharide and xanthan gum by spray drying. Food Biosci. 2023; 56: 103348. https://doi.org/10.1016/j.fbio.2023.103348

Research Article

pISSN: 2345-5357
eISSN: 2423-4214

OPEN ACCESS

APPLIED FOOD BIOTECHNOLOGY, 2025, 12 (1): e10
Journal homepage: www.journals.sbmu.ac.ir/afb

بیونانوکامپوزیت پوشش داده شده با O-کربوکسی متیل کیتوزان برای افزایش زنده‌مانی زیست-یار در شرایط هضم در دستگاه گوارش، نگهداری و تیمار حرارتی

محمد صادق محمدزاده[۱]، ابراهیم واشقانی-فرهانی[۲]، احمد فاضلی[۱]، سید عباس شجاع الساداتی[۱*]

۱- گروه بیوتکنولوژی، دانشکده مهندسی شیمی، دانشگاه تربیت مدرس، تهران، ایران
۲- بخش مهندسی پزشکی، دانشکده مهندسی شیمی، دانشگاه تربیت مدرس، تهران، ایران

تاریخچه مقاله

دریافت ۱۴ فوریه ۲۰۲۵
داوری ۱۰ آوریل ۲۰۲۵
پذیرش ۱۸ آوریل ۲۰۲۵

واژگان کلیدی

- بیونانوکامپوزیت
- *لاکتوباسیلوس روتری*
- O-کربوکسی متیل کیتوزان
- کمک زیست¬یار
- پایداری

نویسنده مسئول

سید عباس شجاع الساداتی

تلفن: ۹۸۲۱۸۲۸۸۳۳۴۱+
نمابر: ۹۸۲۱۸۲۸۸۳۳۴۱+
پست الکترونیک:
shoja_sa@modares.ac.ir

چکیده

سابقه و هدف: بهبود زنده‌مانی زیست‌یارها[۱] در شرایط هضم و نگهداری برای صنایع غذایی و دارویی چالش‌برانگیز است. مطالعه حاضر با هدف افزایش زنده‌مانی سویه زیست‌یار ریزپوشانی‌شده *لاکتوباسیلوس روتری* ATCC 23272 در بیونانوکامپوزیت پوشش داده شده با O-کربوکسی متیل کیتوزان انجام شد. از O-کربوکسی متیل کیتوزان برای پوشش‌دهی بیونانوکامپوزیت حاوی کمک‌زیست‌یارهای[۲] پکتین و اینولین در حضور نانوذرات اکسید منیزیم استفاده شد.

مواد و روش ها: پکتین و اینولین به‌عنوان کمک‌زیست‌یار به همراه نانوذرات اکسید منیزیم برای بهبود ساختار میکروژل و O-کربوکسی متیل کیتوزان برای پوشش‌دهی ریزپوشینه‌ها[۳] با هدف افزایش زنده‌مانی و پایداری زیست‌یارها استفاده شدند. راندمان اکستروژن، زنده‌مانی پس از خشک‌کردن در مایکروویو، زنده‌مانی در شیره‌های گوارشی شبیه‌سازی شده، زنده‌مانی پس از عملیات حرارتی و میزان زنده‌مانی در نگهداری طولانی‌مدت در دمای ۴ و ۲۵ درجه سلسیوس پس از ۴۲ روز مورد تجزیه و تحلیل قرار گرفت. بهینه‌سازی اینولین، پکتین و O-کربوکسی متیل کیتوزان در بیونانوکامپوزیت برپایه آلژینات پوشش داده شده با O-کربوکسی متیل کیتوزان با استفاده از نرم‌افزار Design-Expert و طراحی ترکیبی شبکه‌ای سیمپلکس[۴] انجام شد.

یافته‌ها و نتیجه‌گیری: فرمولاسیون بهینه با استفاده از بسپار[۵] پوشش‌دهنده کیتوزان O-کربوکسی متیل (۶۸٪ w/v)، اینولین (۲۹/۴٪ w/v) و پکتین (۲/۶٪ w/v) با نانوذرات اکسید منیزیم در غلظت ثابت به‌دست آمد. نتایج، راندمان ریزپوشانی (۹۶/۴۳٪)، بقا پس از خشک شدن در مایکروویو (۹۹/۴۵٪) و بقا در شرایط شبیه‌سازی شده دستگاه گوارش (۸۸/۹۵٪) را نشان داد. زنده‌مانی زیست‌یارهای پوشینه‌دار شده در ریزپوشینه‌های پوشش داده شده با کیتوزان O-کربوکسی متیل در دمای ۸۰ درجه سلسیوس به مدت ۵ دقیقه، ۱/۴۶ $\log CFU.g^{-1}$ کاهش یافت. علاوه بر این، بیونانوکامپوزیت پوشش داده شده با O-کربوکسی متیل کیتوزان، پایداری زیست‌یارها را در دمای ۴ و ۲۵ درجه سلسیوس پس از ۴۲ روز، در مقایسه با دانه‌های آلژینات، به ترتیب $\log CFU.g^{-1}$ ۲/۹۳ و ۳/۲۵ بهبود بخشید. علاوه بر این، مشاهده شد که پوشش O-کربوکسی متیل کیتوزان، پایداری زیست‌یارهای پوشینه‌دار شده در دانه‌های بیونانوکامپوزیت را افزایش می‌دهد. نتایج نشان داد که بیونانوکامپوزیت پوشینه‌دار شده با O-کربوکسی متیل کیتوزان، به‌عنوان یک ریزپوشینه‌دار جدید، می‌تواند در مقایسه با دانه‌های آلژینات، ماندگاری و زنده‌مانی *لاکتوباسیلوس روتری* در شرایط سخت مختلف را به‌طور قابل توجهی افزایش دهد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

۱ Probiotics
۲ Prebiotics غذاهای غیرقابل‌هضمی که باعث تحریک رشد ریزاندامگان‌های پس‌روده/ کولون می‌شوند
۳ microcapsules
۴ simplex lattice mixture design
۵ polymer