JFBE 6(1): 49-58, 2023

Journal of Food and Bioprocess Engineering

Journal homepage: https://jfabe.ut.ac.ir

Original research

Synbiotic microencapsulation of corncob xylooligosaccharide and in vitro study for bioactivity and stability upon digestion and storage

Hasini Geethma Wanigasinghe[a,*], Niranjalie Perera[a], Nadeesh Adassooriya[b]

[a] *Department of Food Science & Technology, Faculty of Livestock, Fisheries & Nutrition, Wayamba University of Sri Lanka, Makandura, Gonavila, Sri Lanka*
[b] *Department of Chemical & Process Engineering, Faculty of Engineering, University of Peradeniya , 20400 Peradeniya, Sri Lanka*

A B S T R A C T

Xylooligosaccharides (XOS) are emerging prebiotic that may improve the viability of probiotics and gastrointestinal health. XOS derived from corncob was evaluated for its prebiotic activity with three different probiotic strains. The present study focused on XOS to encapsulate *Lactobacillus rhamnosus* (LGG) and explore it's *in vitro* survival & stability upon storage through structural interactive optimization of encapsulation materials. *L. rhamnosus* (LGG) showed the highest viability 9.86 ± 0.04 log CFU/mL upon XOS. Among three different carrier types namely, Sodium alginate (SA), Chitosan & Sodium tripolyphosphate (STPP), Whey protein liquid - maltodextrin complex, the SA showed the highest encapsulation efficiency 87.6 ± 0.1%, yield and cost effectiveness. XOS and SA were used for encapsulation of LGG with different formulations. The stability of free and encapsulated LGG was assessed using gastrointestinal conditions. All the treatments provided better encapsulation efficiency > 80 %. M_2 formulation showed the highest encapsulation efficiency 92 ± 1%, maximum viability in simulated gastric juice 8.7 ± 0.1 log CFU/mL and bile solution 8.6 ± 0.2 log CFU/mL, resulting significantly ($p < 0.05$) improved survival when compared with free bacteria. The microcapsules were then incorporated into yoghurt and the results showed that there was an increased survival of probiotics because of the protection of cells by microencapsulation and the promoting effect of XOS on the probiotics growth. The XOS extracted from corncob was successfully incorporated as a prebiotic encapsulation material for effective delivery of *L. rhamnosus* LGG. The different combinations of wall materials with XOS provided an opportunity to produce beads with better structure and protection.

Keywords: Extrusion; *L. rhamnosus*; Sodium alginate; Chitosan; Whey protein liquid

Received 27 March 2023; Revised 17 May 2023; Accepted 21 May 2023

1. Introduction

"Prebiotics are the compounds that are selectively utilized by host microorganisms to provide health benefits. Prebiotics help to inhibit pathogens, stimulate the immune system, and reduce blood lipid levels and improve the bioavailability of minerals (Pereira et al., 2017)". "Probiotics are microbes that belong to safe species, which provide health benefits to humans and maintain their viability during human digestion (Hill et al., 2014)". They boost the body's natural defenses and lower the risk of gastrointestinal disorders such as diarrhea. Different bacteria and yeasts are used as probiotics in various food items, but *Lactobacillus* is one of the most prevalent with a variety of health claims. Numerous health advantages have been reported for *Lactobacillus rhamnosus* strains (Champagne et al., 2018; Daliri & Byong, 2015; Ranadheera et al., 2018). With biofilms, these strains have been utilized to remove various contaminants from milk and milk products (Assaf et al., 2019; Soltani et al., 2017). The strains can withstand acidic conditions and attach to large intestine epithelial cells. However, the probiotic potentials of *L. rhamnosus* strains are vary depending on their source and viability. Their health advantages are greatly influenced on their survivability in the large intestine. To reap these benefits, *L. rhamnosus* must be viable and accessible, with a fixation rate in the colon ranging from 10^7 to 10^9 CFU/g. The vitality of the product must be maintained during its manufacturing,

*Corresponding author.
E-mail address: hasinigeethma95@gmail.com (H. G. Wanigasinghe).
https://doi.org/10.22059/jfabe.2023.353024.1135

storage, and passage through the gastrointestinal system (GIT). pH, dissolved oxygen level, storage conditions, hydrogen peroxide generation, citric acid concentration, and buffering capability all have an impact on their viability (Bigliardi & Galati, 2013; Cavalheiro et al., 2015; Rascon et al., 2018). Probiotic microencapsulation is an approach for protecting and improving probiotic viability during processing and inside the GIT. The encapsulation matrix protects and sustains the core substance. Extrusion is an excellent method for producing consistent beads with a high yield. Encapsulation materials' intermolecular bonding aids in the formation of a stable matrix structure. Alginate is a polysaccharide-based encapsulating material with excellent gelling properties. With divalent calcium and magnesium ions, it forms a three-dimensional structure. However, the loss of viability, porosity, and sluggish and uncontrolled release are all downsides of alginate. In this instance, the combination of sodium alginate (SA) with XOS may be helpful. Concerns with XOS as an encapsulating material include a low glass transition temperature and water solubility (which results in an unstable matrix) (Rajam & Anandharamakrishnan, 2015). The combination of polymer and oligosaccharide, on the other hand, may assist to solve the limits of each. It has been found that oligosaccharides reduce pore size, increase delivery, and viability inside SA beads. SA, on the other hand, may aid in the reduction of stickiness (Atia et al., 2016; Silva et al., 2018). The proper ratio of these materials may aid in achieving the necessary structural and functional properties. Azam and Saeed (2020) studied combined effect of SA and fructooligosaccharides for improved survival of *L. rhamnosus* with GIT conditions. The suggested study aimed to maximize the combined impact of SA and XOS for increased survival of *L. rhamnosus* under GIT conditions by utilizing corn cob waste XOS extraction. Fruitful and efficient use of XOS in food matrix like yoghurt will be extendedly addressed with the study. Chemo metrics were used to explain the spectrum data.

2. Material and Methods

2.1. Materials

Commercial XOS derived from corn cobs was obtained from Qingdao Century Longlive International Trade Co., Ltd. (Shandong, China) and was > 95% pure. All probiotic strains were obtained from Chr. Hansen, Hønsholm, Denmark. All other chemicals used were of analytical grade. Special reagents used were pepsin, pancreatin, bile salts, Dinitro salicylic acid color reagent.

2.2. Evaluation of prebiotic activity

Three probiotic strains (*Lactobacillus rhamnosus* LGG, *Lactobacillus casei* LCG, and *Bifidobacterium lactis* BB12) were used to evaluate the prebiotic activity of XOS. Carbohydrate-free MRS broth was used as basal growth medium for the probiotic strains. XOS, inulin (prebiotic standard) and glucose (growth control) at the concentration of 2% were added separately to the MRS broth containing 1×10^7 of each selected probiotic strain and incubated for 48 h at 37℃. After the incubation, viable cell numbers in the culture media were determined by pour plate method using MRS agar according to Azmi et al. (2012).

2.3. Preparation and selection of wall materials

XOS nanoparticles will be prepared with ionic gelation of XOS with Chitosan & Sodium tripolyphosphate (STPP) anions. Briefly, different concentrations of XOS will be dissolved in acetic acid solution. A solution of STPP at 0.1 mg/mL will be also prepared. Then, STPP solution will be added dropwise under constant stirring to chitosan - XOS solution. Then it was centrifuged at 5000 rpm for 15 min and the supernatant was collected for further analysis.

The preparation of XOS loaded microparticles was performed according to the method mainly described by Brinques and Ayub (2011) and Shaymaa and Hoda (2020) with modifications according to Azam and Saeed (2020). One gram of sodium alginate was added to 50 mL distilled water (2% w/v) and heated in 70℃ until it formed a gel. After cooling, XOS 1.5 g (3% w/v) was added and stirred until complete dissolve. The formulation was extruded into $CaCl_2$ solution (0.1 M) with magnetic stirring (40 rpm). The beads were kept in the $CaCl_2$ solution (30 min) for hardening. Beads were harvested using Whatman grade 1 filter paper and washed twice using sterile NaCl (9.0 g/L).

Microcapsules preparation was done according to the method described by Na et al. (2011) and Bannikova et al. (2020) with modifications. Whey protein liquid was prepared and a volume of 60 mL whey liquid was homogenized by gentle magnetic stirring at 60–80℃ for 30 min until completely dissolved. Maltodextrin (MD) 10% was dissolved in distilled water (4 g/40 mL) by gentle magnetic stirring at 50–60℃ for 1 h. The wall materials were mixed in ratio 6:4, by gentle magnetic stirring for 1 h. XOS was added to the wall material 10%, at ratio 1:10 and the solution was mixed using a Magnetic Stirrer for 15 min. Then, mixtures were treated by ultrasonication at 53 W, 37- 42℃ for 30 min. Then 0.5% of guar gum solution (10%) was added to the mixture solution under stirring as double wall material. Finally, the microcapsules were centrifuged to separate microcapsules. The supernatant was stored at −18℃ for further analysis.

2.4. Encapsulation efficiency for the wall material

Xylooligosaccharide encapsulation efficiency in different wall materials was evaluated using DNS assay according to Gusakov et al. (2011). Standard XOS solution was pipetted out in the range of 0 to 3 mL in different test tubes and was made up the volume of all test tubes to 3 mL with distilled water concentrations ranging from 0 to 750 mg. DNS reagent was added in 1 mL volume to all the test tubes and were mixed. Extinction was read at 540 mm against the blank. Standard curve was prepared. Three supernatant samples were tested as above and concentrations were determined using standard curve.

2.5. LGG-XOS co-encapsulation

2.5.1. Preparation of the bacterial suspension

The bacterial suspension was prepared following the procedures described by Fritzen-Freire et al. (2012) with some modifications. The final count of LGG in the stock solution was 11.44 ± 0.05 log CFU/mL.

2.5.2. XOS-LGG microcapsule preparation

2.0 g of sodium alginate was added to 48 mL distilled water (4% w/v) and heated in 70℃ for 5 minutes until it formed a gel. XOS was added to distilled water in different amounts (1, 3, and 5% w/v) and was stirred until complete dissolve. Two solutions were used in 1:1 ratio to make a final volume of 200 mL to develop mechanically stable beads. *L. rhamnosus* was used at a fixed concentration of 50 mL (around 10^{10} CFU/mL) in all formulations (M_1, M_2, and M_3). SA 100% + XOS 0% formulation was prepared as M_0 formulation. *L. rhamnosus* (LGG) was used around 10^{10} CFU/mL in all formulations (M_0, M_1, M_2, and M_3). Formulations were extruded using different syringe sizes (21 G, 25 G, and 29 G) into $CaCl_2$ solution (0.1 M) with magnetic stirring (20 rpm).

2.5.3. Encapsulation efficiency of co-encapsulate

The effect of encapsulation on the viability of *L. rhamnosus* was determined before and after encapsulation using the method described by Pinto et al. (2015) with minor modifications related to the number of treatments. Encapsulation efficiency was calculated using following equation.

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Cells released from beads}}{\text{Cells added to the mixture}} \times 100 \quad (1)$$

2.6. Characterization of beads

2.6.1. Diameter and morphology of beads

The beads were analyzed for morphological characters using scanning electron microscopy (Hitachi SU6600 FE-SEM). Images were taken at 15 kV under vacuum of 9.75×10^{-5} Torr at low (≥ × 45) and high magnification (≥ × 1000) (Rajam & Anandharamakrishnan, 2015; Zanjani et al., 2014).

2.6.2. Fourier transform infrared spectrometer (FTIR) analysis of beads

The beads were analyzed using FTIR (Thermo Nicolet 6700, Thermo Electron Corp., Waltham, MA, USA) using the method of Rajam and Anandharamakrishnan (2015). FTIR analysis was carried out using a resolution of 4 cm^{-1} with scan speed of 1 cm/s.

2.7. Comparative stability of free and encapsulated *L. rhamnosus* in GI tract

2.7.1. Simulated gastric juice (SGJ)

Survival of free and encapsulated *L. rhamnosus* LGG was evaluated by adding pepsin (3.0 g/L) in sterile saline solution (0.5% v/v). Final pH of SGJ was adjusted to pH 2.0 with the addition of 1 M HCl. Encapsulated beads (0.5 g) and un-encapsulated cells (0.5 mL) were added into the sterilized SGJ separately. The tubes were incubated for 0, 30, 60 and 120 min at 37℃. The beads were washed and harvested for further enumeration. They were dissolved in 50 mM sodium citrate solution with continuous agitation. The released cells were serially diluted and plated on MRS agar plates. Similarly, the free cells were diluted and plated separately. After 24 h, the results were obtained as log CFU/mL averaging before converting to log (Yasmin et al., 2018).

2.7.2. Bile salt solution

Survival of free and encapsulated *L. rhamnosus* was studied using the protocol of Sathyabama et al. (2014) with adjustments for the number of bead samples. Microcapsules (1 g) and free cells (1 mL) were added separately to 10 mL of bile salt (2%). The tubes were incubated at 37℃ for 0, 40, 80 and 120 min. An aliquot was diluted and plated in MRS agar plates for enumeration. The results were measured free and encapsulated *L. rhamnosus*.

2.7.3. Release rate in simulated intestinal fluid (SIF)

Release rates were determined using the protocol of Wang et al. (2014). Encapsulated *L. rhamnosus* were resuspended in 5 mL of sterile PBS (0.1%) before use. Thereafter, these cells (5 mL) and beads (5 g) were added into SIF (45 mL) pH 6.5 with continuous stirring (450 rpm). Rate of release was calculated using equation.

$$\text{Rate of release (RR\%)} = \frac{V_r}{V_a} \times 100 \quad (2)$$

Vr = Viable cells released from beads in SIF and Va = Viable cells added in beads in SIF.

2.8. Storage stability assay

The storage stability was determined for free and encapsulated *L. rhamnosus* and yoghurt cultured microcapsules at 4℃ for 4 weeks. The encapsulated beads were dissolved in 50 mM sodium citrate solution to release the *L.rhamnosus*. The free and released cells were diluted with saline solution and plated in MRS agar using the pour plate method. The colonies were counted after 0, 7, 14, 21 and 28 days (Martin et al., 2013).

2.9. Co-encapsulated synbiotic yogurt

A set-type yogurt was prepared for the evaluation. Then the samples were incubated at 42℃ and the acidification profile was recorded hourly until a pH of 4.6 was reached. Fermentation was stopped by quick chilling of yogurts. The filled yogurt cups (80 mL) were stored at 5℃, and the CFU/g of yogurt was determined at 1-week intervals for up to 4 weeks.

Table 1. Encapsulation efficiency of different wall material formulations.

Carrier type	EE%
Maltodextrin-whey liquid	12.4 ± 0.1%
Sodium alginate	87.6 ± 0.1%
Chitosan-STPP	74.6 ± 0.3%

2.10. Statistical analysis

The data were subjected to a two factor factorial with a completely randomized design (CRD) for statistical analysis using SPSS 16.0. Analysis of variance (ANOVA) was used with $p <$

0.05 level of significance. The mean values of triplicates were expressed with their standard deviation (Montgomery, 2008).

3. Results and Discussion

3.1. Encapsulation efficiency for wall materials

The encapsulation efficiency (Table 1) of XOS encapsulated in sodium alginate was significantly higher than that of the chitosan-STPP complex and XOS encapsulated in whey liquid and molto dextrin. It had the highest encapsulation efficiency, yield and cost effectivity in the context of laboratory scale. The difference in the encapsulation efficiency could be due to the differences in wall material and extent of interactions occurring between the wall material and XOS molecules. The very low EE% of XOS encapsulated in whey liquid and maltodextrin may be due to the absence of proper cross-linking between the XOS molecule and the wall material. There was a consistency between the current results and previous reports. Wu and Genyi (2018) had encapsulated *L. plantarum* with SA and arabinoxylan. The calculated EE was > 85% which increased by the addition of prebiotic arabinoxylan from 65 to 85%. The inclusion of prebiotic helped to improve the entrapment and survival of probiotics in simulated gastric conditions.

3.2. Assessment the prebiotic activity of xylooligosaccharide

The viable counts of probiotic strains after incubation for 48 h with 2% of XOS, inulin and glucose are presented in Table 2. Overall, XOS, inulin, and glucose significantly enhanced the growth of the probiotics compared to the negative control. The statistical analysis additionally, revealed that the probiotics viable count incubated with XOS was significantly higher than the probiotics viable count incubated with inulin, while there was no significance difference between the probiotics viable count incubated with XOS and glucose.

3.3. Encapsulation efficiency

The cell count before and after encapsulation for encapsulation efficiency (Table 3) indicated better encapsulation ability (> 80%) of all formulations of SA and XOS except M_4 as it was unable to form proper beads. The results showed that treatments have significant impact ($p < 0.05$) on the encapsulation efficiency. M_2 had maximum encapsulation efficiency while Mo had the minimum. The increase in encapsulation efficiency may be due to the addition of XOS in beads. The interaction between polymers helped to form a network of SA and XOS. This addition helped to increase encapsulation efficiency up to certain level after that it tended to be decreased. This might be due to XOS not being able to maintain proper bead shape. To confer health impacts, probiotics must have better survival rates (10^7-10^9 CFU/mL). All the encapsulation formulations had a much better survival rate of *L. rhamnosus* LGG. M_2 provided optimized conditions for maximum survival.

3.4. Diameter and morphology of beads

Composition of polymers and the needle size of the syringe are the main factors affecting the bead size. The diameter of the beads showed that it varied significantly with needle size and polymer concentration separately. However, their combined effect showed non-significant results. The diameter of beads was decreased as the concentration of SA decreased. The beads of SA were spherical in shape (Mo), while the diameter of beads was reduced with higher concentration of XOS (M_3). The M_4 formulation was unable to form beads due to the water solubility of XOS. Minimum bead size was observed for 24 G needle followed by 25 and 27 G. So, beads of 24 G were selected for further structural analysis.

Table 2. Viable cell count (log CFU/g) of probiotic strains incubated for 48h with XOS and standards.

Supplement	Probiotic strains		
	Lb. casei LCG (log CFU/g)	*Lb. rhamnosus* LGG (log CFU/g)	*Bif. lactis* BB12 (log CFU/g)
Basic MRS	7.18 ± 0.04	7.19 ± 0.05	7.20 ± 0.05
Glucose	9.28 ± 0.04	9.38 ± 0.04	9.26 ± 0.07
Inulin	9.14 ± 0.10	9.24 ± 0.03	9.13 ± 0.02
XOS	9.62 ± 0.10	9.86 ± 0.04	9.77 ± 0.07

Table 3. Encapsulation efficiency of different microcapsule formulations.

Bead formulations	Free cells (log CFU/g)	Cells released (log CFU/g)	Efficiency (%)
M_0	9.7 ± 0.01	8.1 ± 0.01	83 ± 0.01
M_1	9.8 ± 0.10	8.7 ± 0.01	88 ± 0.01
M_2	9.6 ± 0.01	8.9 ± 0.00	92 ± 0.01
M_3	10.4 ± 0.10	8.8 ± 0.00	84 ± 0.10

Table 4. Number of probiotics of synbiotic yoghurt during storage.

Sample	Number of survival cells (log CFU/g)				
	1 d	7 d	14 d	21 d	28 d
N	7.74 ± 0.10^{aA}	7.47 ± 0.08^{bA}	7.22 ± 0.02^{cA}	7.10 ± 0.01^{cA}	6.91 ± 0.01^{cA}
A	9.18 ± 0.05^{aB}	8.85 ± 0.05^{bB}	8.59 ± 0.08^{cB}	8.28 ± 0.07^{dB}	8.10 ± 0.01^{cB}
B	7.79 ± 0.06^{aA}	7.65 ± 0.12^{abA}	7.48 ± 0.03^{cA}	7.28 ± 0.05^{dA}	7.00 ± 0.03^{cA}
C	9.28 ± 0.02^{aB}	8.99 ± 0.06^{bB}	8.84 ± 0.07^{cB}	8.70 ± 0.03^{dB}	8.56 ± 0.01^{cB}
D	10.22 ± 0.02^{aC}	9.93 ± 0.05^{bC}	9.76 ± 0.03^{cC}	9.71 ± 0.02^{dC}	9.65 ± 0.01^{cC}
E	10.29 ± 0.02^{aC}	10.15 ± 0.08^{bD}	9.99 ± 0.02^{cD}	9.93 ± 0.06^{cD}	9.87 ± 0.01^{cD}

Control sample (N), Yogurt with Free LGG and 3% XOS were prepared as sample A and sample B, respectively. Yoghurt with synbiotics 1%XOS-LGG, 3%XOS-LGG and 5%XOS-LGG were set as sample C, D and E respectively. All data were expressed as mean values ± S.D (n = 3). Values followed by different lowercase superscripts in the same row are significantly different for the same yoghurt samples on 1st, 7th, 14th, 21st and 28th day of refrigerated storage ($p < 0.05$). Values followed by different uppercase superscripts in the same row are significantly different for the yoghurt samples on the same day ($p < 0.05$).

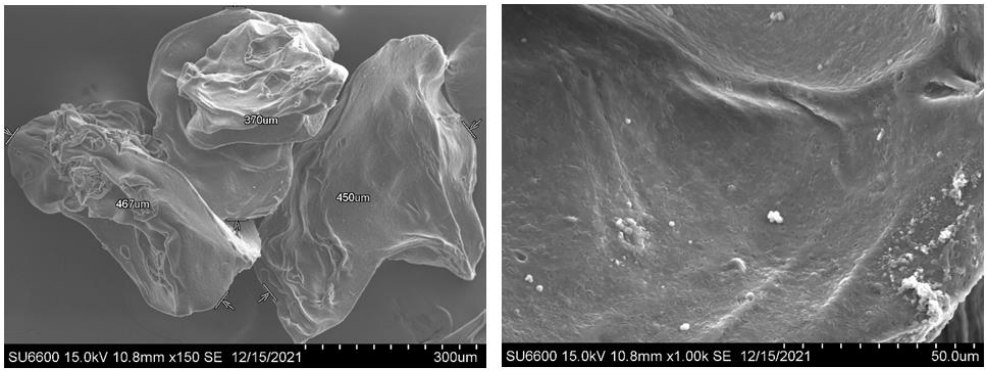

Fig. 1. Encapsulated *L. rhamnosus* LGG beads at (a) low magnification (≥ × 45) and (b) high magnification (≥ × 1000).

The beads of SA showed a good ability to form round shape beads as compared to XOS. XOS had high stickiness probably due to the low glass transition temperature. Solubility of XOS increases with hot water (60℃). The loss of water from the beads resulted in deformed beads due to their solubility. Therefore, SA beads were more spherical as compared to XOS beads. On the other hand, beads with higher amounts of XOS showed better structure due to better polymeric distribution, which helped to fill the pores of alginate beads. The presence of dents was probably the result of freeze-drying of the beads. Loss of water during freeze-drying resulted in loss of shape. However, the synergistic effect of alginate and XOS helped to overcome cavities and resulted in homogenous, smooth and compact structures. Yasmin et al. (2019) had explained the dents in beads made of whey protein, alginate and pectin for microencapsulation of *Bifidobacterium longum*. Shriveling and dents were the results of freeze-drying before SEM. Freeze drying resulted in dehydration of beads, which eventually led to wrinkled beads. Spray drying can help to reduce the structural loss and maintained structural identity of the polymeric microcapsules. SEM images were consistent with the efficient encapsulation with better protection of *L. rhamnosus* LGG in all formulations. Atia et al. (2016) observed similar results. They had encapsulated three probiotic strains with prebiotic matrix (inulin) and alginate. The addition of prebiotic to alginate beads improved the structure and polymeric distribution. In another study, Silva et al. (2018) encapsulated *L. paracasei* with an alginate, gelatin and FOS based matrix. The addition of FOS led to the reduction in structural pores of alginate beads and gelatin helped to thicken the walls.

Probiotic product development prefers the beads with smaller diameter to provide suitable sensorial attributes (Yee et al., 2019). The beads produced from 25 and 27 G were larger, which are not desirable in getting maximum cell loading. The diameter depends upon different parameters including polymer and calcium chloride concentration, needle size, flow rate and falling distance of beads from needle to $CaCl_2$ solution. The size of beads decreased with the gelling time and increased concentration of calcium. This decrease in size was due to the crosslinking between the polymer and calcium solution (Huang & Yung, 2017). The present study results were consistent with the results of Darjani et al. (2016). They had reported similar results for the diameter of inulin-alginate-chitosan coated beads using extrusion for *Lactobacillus casei*. The mean diameter of alginate-inulin beads was 2.5 mm and chitosan-alginate-inulin beads 2.9 mm.

SEM showed structural identities of beads with specific concentrations of SA and XOS. The addition of XOS significantly ($p < 0.05$) contributes towards the reduction in porosity of beads. Two micrographs (Fig. 1) were taken to cover general and detailed structure. Sphericity and porosity of bead layers decreased with increased concentration of XOS. The beads with higher concentration of alginate (Mo and M_1) showed better spherical shapes. The beads with lower concentration of SA (M_2 and M_3) had shriveled beads. The dents were observed in each bead formulation.

3.5. *FTIR spectral analysis*

The spectral analysis of encapsulation beads was done to detect functional groups and presence of the SA and XOS. FTIR spectra from M_2 sample (Fig. 2). FTIR spectra of SA beads (Mo) indicated bands of aliphatic groups C-H (2900- 2700 cm^{-1}) and OH stretching vibrations (3600-3000 cm^{-1}). The symmetric and asymmetric stretching of COO- corresponded to 1415 and 1592 cm^{-1}, respectively. These bands were distinctive among alginate and its conjugated products. Pyranosyle ring and functional groups of C-O and C-C-H deformations were found at 1021 and 817 cm^{-1}. The XOS showed distinct bands with stretching and deformations of oligosaccharides C-O, C-C, C-O-H and C-O-C. Similarly, bands between 3500-3400 and 1100-900 cm^{-1} were considered as the fingerprints of oligosaccharides (Rajam & Anandharamakrishnan, 2015). Spectral overlapping was observed in the region of 1700-1500 cm^{-1}, which may be due to the interaction between OH group of XOS and COO- group of alginate. FTIR results showed the presence of XOS in the beads. The three spectral regions -O-H, C-C and C-O stretching of oligosaccharides) were present between 3000-600 cm^{-1} as indicators of the presence of XOS. The interconnected network formation of SA and XOS was due to the formation of homogenous and heterogeneous alginate monomers. These monomers bind with calcium ions due to the gelation of alginate. The results of spectral data showed that SA and XOS are retained after encapsulation. Stojanovic et al. (2011) had found similar results encapsulating thyme extract in alginate-inulin beads. Atia et al. (2016) studied prebiotic based encapsulation material for targeted delivery of three probiotic stains; *Pediocucus acidilactici*, *L. reuteri* and *L. salivarius*. SA and inulin were used as encapsulation material. The FTIR spectral analysis showed the presence of aliphatic groups (C-H) and hydroxyl groups at 3500-300 and 1200-900 cm^{-1}. Likewise, the corresponding functional groups at 3600-2700 cm^{-1} were the presence of alginate in beads.

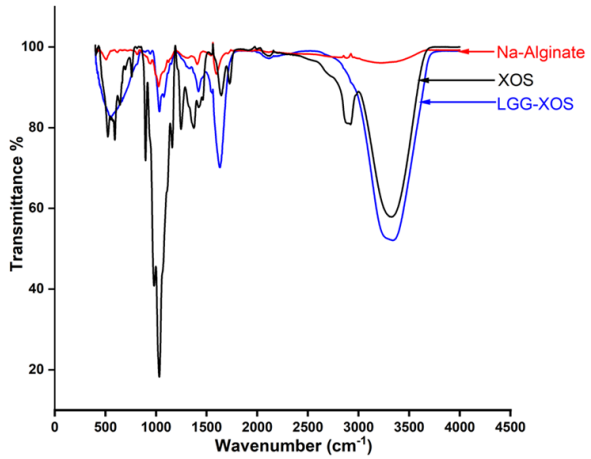

Fig. 2. FTIR Spectral data for M_2 29 G bead formulation with *L. rhamnosus* LGG M_2= 2% SA + 3% XOS.

3.6. *Survival of free and encapsulated L. rhamnosus in SGJ*

The survival of *L. rhamnosus* LGG was important in SGJ to claim the health benefits of probiotics. Encapsulation materials have significant effects on the survival of *L. rhamnosus* LGG in SGJ with different incubation times (Fig. 3). Higher survival rates were observed in M_1 and M_2, with the fewest free was observed in free cells. Maximum survival was observed in M_2 with a minimum log reduction of 1.4 log CFU/mL after 120 min and with no encapsulation (NE) a maximum log reduction of 7.5 log CFU/mL. The decrease in viability was observed as a function of time. The viability was not maintained even after 40 min of incubation while encapsulated *L. rhamnosus* LGG was maintained with less log reduction after 40 min in each formulation. The rate of decrease was maximum after 80 min and minimum after 120 min of incubation. The free *L. rhamnosus* LGG viability decreased as the acidic conditions disrupted their outer layer and resulted in loss of viability. The decrease in viability of encapsulated *L. rhamnosus* LGG was due to the diffusion of SGJ into cracks of the microbeads. The SGJ entered into the beads of SA (Mo) due to large pores in their structure. Similarly, the exposure of beads in SGJ resulted in more reduction of viable cells. Increasing the concentration of XOS (M_1, M_2) filled the pores of alginate beads and resulted in increased survival rate. On the other hand, further increases in concentration of XOS resulted in decreased survival rate. XOS with less SA was unable to withstand simulated gastric conditions and the least survival rate was observed in M_3. However, M_1 and M_2 were found to be the optimum formulations for encapsulation and survival in the acidic conditions of SGJ. Arslan-Tontul and Mustafa (2017) had observed similar results working with gum Arabic and β-cyclodextrin for the encapsulation of different probiotic strains (*Saccharomyces boulardii*, *B. bifidum* and *L. acidophilus*). Their survival increased in SGJ with increased concentration of polymers in SGJ. There was a difference between the viability of free and encapsulated probiotics. Santos et al. (2019) had used inulin as an encapsulation material for *L. acidophilus*. Relatively, beads of inulin showed less log reduction as compared to un-encapsulated probiotics. However, there was 2.0 log reduction of encapsulated probiotics with spray drying. Chitosan, alginate and inulin were used for encapsulation of *L. rhamnosus*. According to their results, 27% of the bacterial population survived in acidic conditions and maximum loss in survival was observed in non-encapsulated *L. plantarum* (Gandomi et al., 2016).

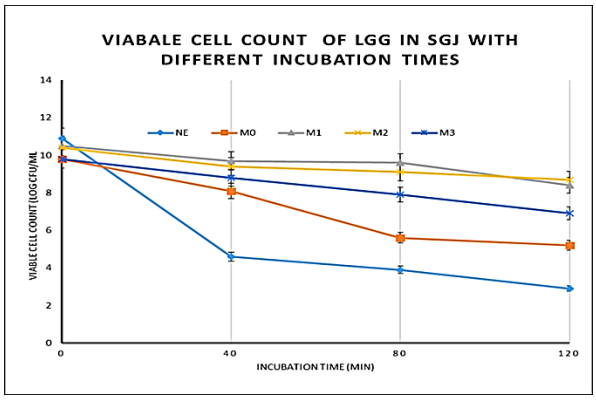

Fig. 3. Viable cell count of *L. rhamnosus* LGG in SGJ.

3.7. *Survival of free and encapsulated L. rhamnosus LGG in bile salt solution*

The viability of *L. rhamnosus* LGG (Fig. 4) was changed with and without encapsulating materials. Mo, M_1 and M_3 showed better protection for viable cells of *L. rhamnosus* LGG from bile salt (> 10^6 CFU/mL), which can colonize in large intestine. The optimized concentration of both wall materials provided better protection and

prevented penetration of bile salt into the beads. The wall materials had a significant effect on the viability of *L. rhamnosus* LGG in bile salt at varying incubation times. The viability of isolates increased with the addition of XOS in SA beads as compared to SA beads. Similarly, the non-encapsulated *L. rhamnosus* LGG were unable to maintain their viability to an effective level. Crosslinking of alginate and inulin prevented the diffusion of bile salt. Comparatively, encapsulation prevented the loss in viability as compared to non-encapsulated probiotics. The decrease in viability of encapsulated *L. rhamnosus* LGG was probably due to the poor protection of XOS alone and maximum survival was due to optimum linkage of SA and XOS. Incubation time was an important factor, which affects the viability of *L. rhamnosus* LGG. Maximum survival was observed after 30 min and minimum was observed after 120 min of incubation time. Incubation time influenced the survival of *L. rhamnosus* LGG in bile salt solution as longer incubation times resulted in less survival. The interaction of incubation time and bead formulation significantly affected the viable cell count of *L. rhamnosus* LGG. Rodrigues et al. (2015) had found similar results. They had used natural polymers for the survival of *L. casei* with gastrointestinal conditions. The addition of XOS improved the survival of *L. casei* with simulated gastrointestinal conditions as compared to alginate beads. The free probiotics were unprotected from bile salt and resulted in lower viability. However, the XOS beads did not have the capability to withstand the gastrointestinal conditions. The beads of alginate had intramolecular spaces inside, which allowed the diffusion of bile salt into beads, which may cause the swelling of alginate beads. The addition of XOS in alginate beads reduced the chances of structural collapse of beads. Sathyabama et al. (2014) co-encapsulated probiotics *Staphylococcus succinus* and *Enterococcus fecium* with SA and prebiotics (sugar beet and chicory root). The addition of prebiotics improved the survival of probiotics in bile salt solution. Prebiotics improved the survival in 2 and 4% of bile salt at higher rates as compared to the alginate beads. Haghshenas et al. (2015) investigated the addition of fenugreek and inulin in alginate-psyllium beads for survival of *Enterococcus durans*. The survival rate of *E. durans* was maximum (79%) in alginate-psyllium with fenugreek as the prebiotic in the bile salt solution. The addition of inulin or fenugreek increased the survival rate 19-32% compared to the alginate beads.

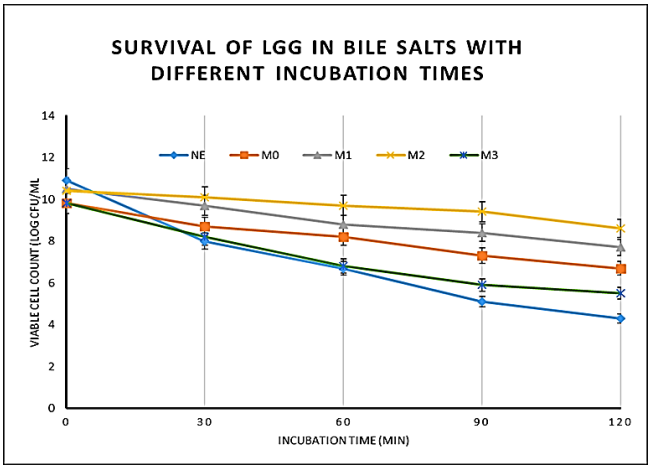

Fig. 4. Survival of *L. rhamnosus* LGG in bile salt with different incubation times.

3.8. *Release of encapsulated L. rhamnosus LGG in SIF*

The release profile of encapsulated *L. rhamnosus* LGG was affected by different bead formulations and incubation time (Fig. 5). The release percentage was significantly affected by the release time. Maximum release was observed after 60 min of incubation and minimum was observed for 20 min of incubation. Both the bead formulations and incubation time interacted significantly. The release rate of M_3 was the fastest as compared to the other treatments as *L. rhamnosus* LGG was fully released after 120 min of incubation in SIF. After 60 min of incubation, more than 60% of *L. rhamnosus* LGG were released in all formulations. The release of *L. rhamnosus* LGG from SA and XOS beads was due to an ion exchange process. Whenever the SA-XOS beads were exposed to SIF, the carboxylic group of sodium-calcium started an ion exchange process, which ultimately resulted in the collapse of beads. The interconnected network started degrading and resulted in release of *L. rhamnosus* LGG. However, the XOS and SA beads were more resistant to collapse as compared to SA or XOS beads alone. The addition of XOS in alginate beads filled the interspaces and prevents the diffusional process of SIF into the beads. The increase in XOS in beads increased the release rate of *L. rhamnosus* LGG from beads. Likewise, the SA beads have a higher release rate due to the presence of interspaces in their structure. It was concluded that the addition of XOS in SA beads helped to reduce the pores present in their structure and helped to control the release process of *L. rhamnosus* LGG from beads. The beads with better protection were slower to release the probiotics. These results were consistent with the results of Wu and Genyi (2018), who had stated that higher concentration of FOS provided less protection but improved the release rate of encapsulated *L. plantarum* in SA and prebiotic arabinoxylan. The addition of arabinoxylan in SA beads improved the protection of *L. plantarum* from intestinal conditions and decreased the rate of release. The XOS addition in the beads improved the interlinking and ultimately the structure of beads. The resulting beads provided better protection with reduced release rate of probiotics. Peredo et al. (2016) had used prebiotics Plantago psyllium and inulin with alginate for protection of *L. casei* and *L. plantarum*. The porous structure and solubilization of SA resulted in lower viability of the probiotics. The addition of prebiotics such as inulin and Plantago psyllium improved the structure of beads.

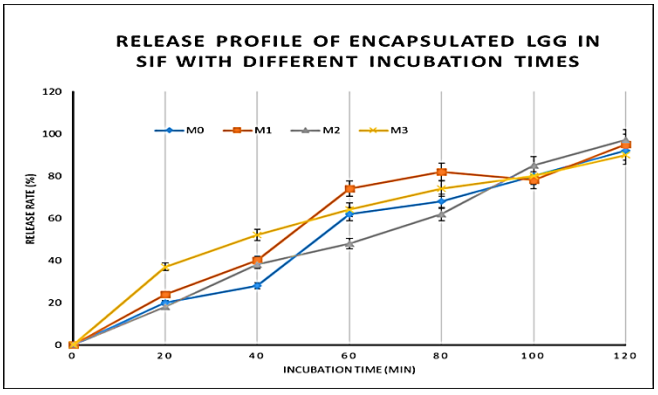

Fig. 5. Release profile of encapsulated *L. rhamnosus* LGG in SIF.

3.9. *Storage stability of encapsulated L. rhamnosus LGG*

The viability for *L. rhamnosus* LGG (Fig. 6) was studied for one month. Wall materials significantly affected the viability of *L. rhamnosus* LGG and all the formulations retained viability up to 7 log CFU/mL. The increase in concentration of XOS significantly increased the viability of *L. rhamnosus*. Storage time significantly decreased the viability of *L. rhamnosus* LGG. M_2 has the maximum storage stability with minimum loss. Non-encapsulated *L. rhamnosus* LGG were more prone to the environmental stress. Their viability significantly decreased as compared to the encapsulated *L. rhamnosus*. This was due to the production of different metabolites that ultimately decreased their viability. Storage at 4℃ helped to maintain the viability and storage at higher temperature would decrease the viability. The free cells of *L. acidophilus* had significant reduction in viability after one month of storage as compared to the encapsulated *L. acidophilus*. Albertini et al. (2010) had encapsulated *L. acidophilus* and *B. lactis* with varying concentration of SA and xanthan gum. The number of viable cells decreased during storage but the required level of probiotics was maintained at storage of 4℃. However, the loss in viability of encapsulated probiotics was significantly less than the free cells. Ivanovska et al. (2010) had encapsulated *L. casei* with prebiotic inulin enriched with oligofructose. The decrease in viability was observed after one month of storage in cold conditions. The beads with prebiotic had a better survival rate as compared to the other beads. Therefore, the current study suggested the inclusion of prebiotics in encapsulation of beads for better survival rates during storage. The encapsulated probiotics should be used in various food matrices to investigate their survival. These probiotic food products need to be evaluated for sensory analysis using consumer evaluation. The inclusion of probiotics imparts different flavors and taste to the products (Mituniewicz-Malek et al., 2019; Torres et al., 2017).

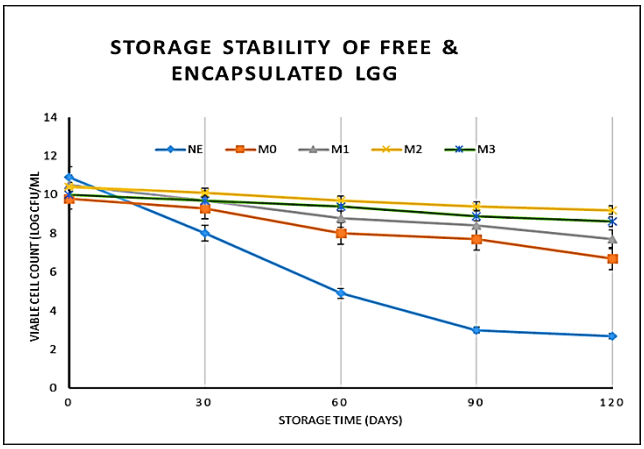

Fig. 6. Storage stability of free and encapsulated *L. rhamnosus* LGG.

3.10. *Synbiotic yoghurt characterization*

The prepared yogurts were evaluated over a 6 weeks' storage period for change in the viable cell count. There was a decline of about 4.0 log over a period of 4 weeks in the cell numbers of *L. rhamnosus* when incorporated as free cultures, whereas there was only a 1.0 log cycle decrease in viable cell count in co-encapsulated cells. The pH in the yogurts decreased from 4.6 at the initial of storage to 4.1 after 6th week of storage. The generation of lactic acid along with the low pH of yogurt might be responsible for the reduced viability of free probiotic cells in yogurt. There was a significant increase ($p < 0.05$) in the viable counts of *L. rhamnosus* in alginate co-encapsulated beads compared with the free cells. Survival of probiotics in alginate-XOS beads had been improved during refrigerated storage in yogurt. We have shown the positive role of incorporation of prebiotic (XOS) into the alginate mix during encapsulation.

Changes in viability of probiotic cells in the symbiotic yoghurt are shown in Table 4. The viability of the probiotic strain in all samples had been decreased during the storage period, but the viable numbers of encapsulated probiotic cells were higher than the therapeutic value (> 10^7 CFU/g). After 28 days of storage, the reduction logs of yoghurts with 1, 3 and 5% XOS-LGG addition (sample C, D and E) were 0.72, 0.57 and 0.42 respectively, which were significantly lower than compared with the control N (0.83 logs reduction) sample A (1.08) and B (0.79 logs reduction). The microcapsules with and without XOS addition were added into the yoghurt and the properties of synbiotic yoghurt were studied. At the end of storage, sample E presented the highest number of viable probiotics (9.95 log CFU/g). This was partly because the presence of XOS in the microcapsules, which promoted the growth of lactic acid bacteria, and similar results were observed on the sample B and C. On the other hand, the protection of microcapsules on probiotics improved their survival rate in low pH environment. Previous studies have shown that incorporation of encapsulated probiotics into yoghurt will sustain a better survivability compared to the free strains (Ribeiroetal et al., 2014). A reduction of viability of *L. acidophilus* Lac-04 encapsulated with pectin and casein in yoghurt made from buffalo milk was 3.19 logs lower than free strains (Shoji et al., 2013).

4. Conclusion

In this work, prebiotic biopolymer based beads were prepared with extrusion for the encapsulation of *L. rhamnosus* LGG and further in vitro investigations and storage stability assays were done. XOS which was extracted from corn cob was evaluated for its prebiotic properties. With the obtained results from preliminaries, biopolymeric gel systems (XOS + SA) were prepared using natural, cost effective, non-toxic and food grade materials. High encapsulation efficiency was observed due to compatibility of encapsulation materials and *L. rhamnosus* LGG. The addition of XOS improved the structural integrity of beads and the viability of encapsulated probiotics. SEM showed the effect of XOS addition to improve structural defects in beads compared to pre-tested alginate beads. Spectral analysis effectively showed the comparative presence of encapsulation materials in the bead formulation. The optimization of both encapsulation materials provided better protection in gastric acidic conditions and bile salt as compared to free cells. Comparatively, free cells and SA beads were more prone to acidic and bile salt than composite beads (SA-XOS). Encapsulated *L. rhamnosus* LGG was more stable in SGF, SIF, bile salt, refrigerated storage and in yoghurt formulations as compared to un-encapsulated cells. The viability of *L. rhamnosus* LGG was maintained at more than the recommended therapeutic level in order to confer health benefits. The SA-XOS based prebiotic beads can serve as an encapsulation matrix for survival and storage of *L. rhamnosus* LGG. SA-XOS beads were effective in protection and targeted delivery of *L. rhamnosus* LGG and this can be incorporated into different food products. Corn cob waste can be

sustainably and effectively utilized as an encapsulation material for probiotics.

Acknowledgment

We greatly acknowledge the support provided by the Faculty of Livestock Fisheries and Nutrition, Wayamba University of Sri Lanka for the technical support during the research period. We also thank the staff members of the Sri Lanka Institute of Nanotechnology, for their support for the completion of this research study.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Albertini, B., Vitali, B., Passerini, N., Cruciani, F., Di Sabatino, M., Rodriguez, L., & Brigidi, P. (2010). Development of microparticulate systems for intestinal delivery of *Lactobacillus acidophilus* and *Bifidobacterium lactis*. *European Journal of Pharmaceutical Sciences*, *40*(4), 359–366. https://doi.org/10.1016/j.ejps.2010.04.011

Gusakov, A. V., Kondratyeva, E. G., & Sinitsyn, A. P. (2011). Comparison of two methods for assaying reducing sugars in the determination of carbohydrase activities. *International Journal of Analytical Chemistry*, 2011. https://doi.org/10.1155/2011/283658

Assaf, J. C., Khoury, A. El, Chokr, A., Louka, N., & Atoui, A. (2019). A novel method for elimination of aflatoxin M1 in milk using *Lactobacillus rhamnosus* GG biofilm. *International Journal of Dairy Technology*, *72*(2), 248–256. https://doi.org/10.1111/1471-0307.12578

Atia, A., Gomaa, A., Fliss, I., Beyssac, E., Garrait, G., & Subirade, M. (2016). A prebiotic matrix for encapsulation of probiotics: Physicochemical and microbiological study. *Journal of Microencapsulation*, *33*(1), 89–101. https://doi.org/10.3109/02652048.2015.1134688

Azam, M., Saeed, M., Pasha, I., & Shahid, M. (2020). A prebiotic-based biopolymeric encapsulation system for improved survival of *Lactobacillus rhamnosus*. *Food Bioscience*, *37*, 100679.

Azmi, A. F. M. N., Mustafa, S., Hashim, D. M., & Manap, Y. A. (2012). Prebiotic activity of polysaccharides extracted from Gigantochloalevis (Buluhbeting) shoots. *Molecules*, *17*(2), 1635–1651. https://doi.org/10.3390/molecules17021635

Bannikova, A., Zyainitdinov, D., Evteev, A., Drevko, Y., & Evdokimov, I. (2020). Microencapsulation of polyphenols and xylooligosaccharides from oat bran in whey protein-maltodextrin complex coacervates: In-vitro evaluation and controlled release. *Bioactive Carbohydrates & Dietary Fibre*, *23*, 100236.

Bigliardi, B., & Galati, F. (2013). Innovation trends in the food industry: The case of functional foods. *Trends in Food Science & Technology*, *31*(2), 118–129. https://doi.org/10.1016/j.tifs.2013.03.006

Champagne, C. P., Gomes da Cruz, A., & Daga, M. (2018). Strategies to improve the functionality of probiotics in supplements and foods. *Current Opinion in Food Science*, *22*, 160–166. https://doi.org/10.1016/j.cofs.2018.04.008

Daliri, E. B.-M., & Lee, B. H. (2015). New perspectives on probiotics in health and disease. *Food Science & Human Wellness*, *4*(2), 56–65. https://doi.org/10.1016/j.fshw.2015.06.002

Darjani, P., Hosseini Nezhad, M., Kadkhodaee, R., & Milani, E. (2016). Influence of prebiotic and coating materials on morphology and survival of a probiotic strain of *Lactobacillus casei* exposed to simulated gastrointestinal conditions. *LWT - Food Science & Technology*, *73*, 162–167. https://doi.org/10.1016/j.lwt.2016.05.032

De Castro-Cislaghi, F. P., Carina Dos Reis, E. S., Fritzen-Freire, C. B., Lorenz, J. G., & Sant'Anna, E. S. (2012). *Bifidobacterium* Bb-12 microencapsulated by spray drying with whey: Survival under simulated gastrointestinal conditions, tolerance to NaCl, and viability during storage. *Journal of Food Engineering*, *113*(2), 186-193. https://doi.org/10.1016/j.jfoodeng.2012.06.006

Gandomi, H., Abbaszadeh, S., Misaghi, A., Bokaie, S., & Noori, N. (2016). Effect of chitosan-alginate encapsulation with inulin on survival of *Lactobacillus rhamnosus* GG during apple juice storage and under simulated gastrointestinal conditions. *LWT - Food Science & Technology*, *69*, 365–371. https://doi.org/10.1016/j.lwt.2016.01.064

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., Scott, K., Stanton, C., Swanson, K. S., Cani, P. D., Verbeke, K., & Gregor R. (2017). Expert consensus document: The international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology*, *14*(8), 491–502. https://doi.org/10.1038/nrgastro.2017.75

Brinques, G. B., & Ayub, M. A. Z. (2011). Effect of microencapsulation on survival of *Lactobacillus plantarum* in simulated gastrointestinal conditions, refrigeration, and yogurt. *Journal of food engineering*, *103*(2), 123-128. https://doi.org/10.1016/j.jfoodeng.2010.10.006

Haghshenas, B., Nami, Y., Haghshenas, M., Barzegari, A., Sharifi, S., Radiah, D., Rosli, R., & Abdullah, N. (2015). Effect of addition of inulin and fenugreek on the survival of microencapsulated *Enterococcus durans* 39C in alginate-psyllium polymeric blends in simulated digestive system and yogurt. *Asian Journal of Pharmaceutical Sciences*, *10*(4), 350–361. https://doi.org/10.1016/j.ajps.2015.04.001

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C., & Sanders, M. E. (2014). Expert consensus document: The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*, *11*(8), 506–514. https://doi.org/10.1038/nrgastro.2014.66

Huang, J., Wang, Q., Li, T., Xia, N., & Xia, Q. (2017). Nanostructured lipid carrier (NLC) as a strategy for encapsulation of quercetin and linseed oil: Preparation and in vitro characterization studies. *Journal of Food Engineering*, *215*, 1–12. https://doi.org/10.1016/j.jfoodeng.2017.07.002

Ismail, S. A., El-Sayed, H. S., & Fayed, B. (2020). Production of prebiotic chitooligosaccharide and its nano/microencapsulation for the production of functional yoghurt. *Carbohydrate polymers*, *234*, 115941. https://doi.org/10.1016/j.carbpol.2020.115941

Martin, M. J., Lara-Villoslada, F., Ruiz, M. A., & Morales, M. E. (2013). Effect of unmodified starch on viability of alginate-encapsulated *Lactobacillus fermentum* CECT5716. *LWT - Food Science & Technology*, *53*(2), 480–486. https://doi.org/10.1016/j.lwt.2013.03.019

Mituniewicz–Małek, A., Zielinska, D., & Ziarno, M. (2019). Probiotic monocultures in fermented goat milk beverages – Sensory quality of final product. *International Journal of Dairy Technology*, *72*(2), 240–247. https://doi.org/10.1111/1471-0307.12576

Moreno, F. J., Corzo, N., Montilla, A., Villamiel, M., & Olano, A. (2017). Current state and latest advances in the concept, production and functionality of prebiotic oligosaccharides. *Current Opinion in Food Science*, *13*, 50–55. https://doi.org/10.1016/j.cofs.2017.02.009

Pasqualin Cavalheiro, C., Ruiz-Capillas, C., Herrero, A. M., Jimenez-Colmenero, F., Ragagnin de Menezes, C., & Martins Fries, L. L. (2015). Application of probiotic delivery systems in meat products. *Trends in Food Science & Technology*, *46*(1), 120–131. https://doi.org/10.1016/j.tifs.2015.09.004

Peredo, A. G., Beristain, C. I., Pascual, L. A., Azuara, E., & Jimenez, M. (2016). The effect of prebiotics on the viability of encapsulated probiotic bacteria. *LWT - Food Science & Technology*, *73*, 191–196. https://doi.org/10.1016/j.lwt.2016.06.021

Pereira, G. A., Arruda, H. S., Molina, G., & Pastore, G. M. (2018). Extraction optimization and profile analysis of oligosaccharides in banana pulp and peel. *Journal of Food Processing & Preservation*, *42*(1), 1–10. https://doi.org/10.1111/jfpp.13408

Pinto, S. S., Fritzen-Freire, C. B., Benedetti, S., Murakami, F. S., Petrus, J. C. C., Prudêncio, E. S., & Amboni, R. D. (2015). Potential use of

whey concentrate and prebiotics as carrier agents to protect *Bifidobacterium*-BB-12 microencapsulated by spray drying. *Food Research International*, *67*, 400-408. https://doi.org/10.1016/j.foodres.2014.11.038

Rajam, R., & Anandharamakrishnan, C. (2015). Microencapsulation of *Lactobacillus plantarum* (MTCC 5422) with fructooligosaccharides as wall material by spray drying. *LWT - Food Science & Technology*, *60*(2), 773–780. https://doi.org/10.1016/j.lwt.2014.09.062

Ranadheera, C. S., Naumovski, N., & Ajlouni, S. (2018). Non-bovine milk products as emerging probiotic carriers: Recent developments and innovations. *Current Opinion in Food Science*, *22*, 109–114. https://doi.org/10.1016/j.cofs.2018.02.010

Rascon, M. P., Huerta-Vera, K., Pascual-Pineda, L. A., Contreras-Oliva, A., Flores-Andrade, E., Castillo-Morales., & Gonzalez-Morales, I. (2018). Osmotic dehydration assisted impregnation of *Lactobacillus rhamnosus* in banana and effect of water activity on the storage stability of probiotic in the freeze-dried product. *LWT - Food Science & Technology*, *92*, 490–496. https://doi.org/10.1016/j.lwt.2018.02.074

Rodrigues, F. J., Omura, M. H., Cedran, M. F., Dekker, R. F. H., Barbosa-Dekker, A. M., & Garcia, S. (2017). Effect of natural polymers on the survival of *Lactobacillus casei* encapsulated in alginate microspheres. *Journal of Microencapsulation*, *34*(5), 431–439. https://doi.org/10.1080/02652048.2017.1343872

Sathyabama, S., Ranjith kumar, M., Bruntha devi, P., Vijayabharathi, R., & Brindhapriyadharisini, V. (2014). Co-encapsulation of probiotics with prebiotics on alginate matrix and its effect on viability in simulated gastric environment. *LWT - Food Science & Technology*, *57*(1), 419–425. https://doi.org/10.1016/j.lwt.2013.12.024

Silva, K. C. G., Cezarino, E. C., Michelon, M., & Sato, A. C. K. (2018). Symbiotic microencapsulation to enhance *Lactobacillus acidophilus* survival. *LWT - Food Science & Technology*, *89*, 503–509. https://doi.org/10.1016/j.lwt.2017.11.026

Soltani, M., Hekmat, S., & Ahmadi, L. (2018). Microbial and sensory evaluation of probiotic yoghurt supplemented with cereal/pseudo-cereal grains and legumes. *International Journal of Dairy Technology*, *71*, 141–148. https://doi.org/10.1111/1471-0307.12389

Stojanovic, R., Belscak-Cvitanovic, A., Manojlovic, V., Komes, D., Nedovic, V., & Bugarski, B. (2012). Encapsulation of thyme (*Thymus serpyllum* L.) aqueous extract in calcium alginate beads. *Journal of the Science of Food & Agriculture*, *92*(3), 685–696. https://doi.org/10.1002/jsfa.4632

Wang, J., Korber, D. R., Low, N. H., & Nickerson, M. T. (2014). Entrapment, survival and release of *Bifidobacterium adolescentis* within chickpea protein-based microcapsules. *Food Research International*, *55*, 20–27. https://doi.org/10.1016/j.foodres.2013.09.018

Wu, Y., & Zhang, G. (2018). Synbiotic encapsulation of probiotic *Latobacillus plantarum* by alginate-arabinoxylan composite microspheres. *LWT*, *93*, 135-141. https://doi.org/10.1016/j.lwt.2018.03.034

Xavier dos Santos, D., Casazza, A. A., Aliakbarian, B., Bedani, R., Saad, S. M. I., & Perego, P. (2019). Improved probiotic survival to *in vitro* gastrointestinal stress in a mousse containing *Lactobacillus acidophilus* La-5 microencapsulated with inulin by spray drying. *LWT - Food Science & Technology*, *99*, 404–410. https://doi.org/10.1016/j.lwt.2018.10.010

Yasmin, I., Saeed, M., Pasha, I., & Zia, M. A. (2019). Development of whey protein concentrate-pectin-alginate based delivery system to improve survival of *B. longum* bl-05 in simulated gastrointestinal conditions. *Probiotics & Antimicrobial Proteins*, *11*(2), 413–426. https://doi.org/10.1007/s12602-018-9407-x

Yee, W. L., Chan, L. Y., Nyam K. L., & Pui, L. P. (2019). Microencapsulation of *Lactobacillus acidophilus* NCFM incorporated with mannitol and its storage stability in mulberry tea. *Ciencia e Agrotecnologia*, *43*, e005819. https://doi.org/10.1590/1413-7054201943005819

Zanjani, M. A. K., Tarzi, B. G., Sharifan, A., & Mohammadi, N. (2014). Microencapsulation of probiotics by calcium alginate-gelatinized starch with chitosan coating and evaluation of survival in simulated human gastro-intestinal condition. *Iranian Journal of Pharmaceutical Research*, *13*(3), 843–852.