RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2023.084

ScienceAsia 49 (2023): **813–818**

Survival improvement of *Enterococcus faecalis* HZNU S1 by encapsulating in flaxseed milk-alginate microbeads

Bi-Lin Sun[a,†], Xuan-Ya Huang[a,†], Hang-Jie Chen[a], Zhen-Xing Tang[b,*], Hai Zhou[a], Zhong-Bao Jiang[a], Shan-Shan Fang[a], Lu-E Shi[a,*]

[a] College of Life and Environmental Sciences, Hangzhou Normal University, Zhejiang 310016 China
[b] School of Culinary Art, Tourism College of Zhejiang, Zhejiang 311231 China

*Corresponding authors, e-mail: tangzhenxing@126.com, shilue@126.com
† These authors contributed equally to this work and should be regarded as co-first authors.

Received 24 Mar 2023, Accepted 2 Sep 2023
Available online 22 Nov 2023

ABSTRACT: The viability and high survival rate of probiotics through human gastrointestinal tract are very important to the host health. The present research was investigated to explicate the influence of encapsulation and wall materials on the survival of *Enterococcus faecalis* HZNU S1. Purposely, the microbeads were prepared using sodium alginate (SA) and flaxseed milk (FM) by extrusion method. The viability of investigated probiotics was accessed in simulated gastric juice (SGJ). Free cells exhibited a poor survival rate in SGJ. However, encapsulation improved the probiotic viability in the same circumstance. Encapsulated probiotics exhibited higher viability when they were exposed to pH 2.5 SGJ and 1.0 and 2.0% bile salt solutions. Furthermore, encapsulation could improve the storage stability of probiotics. The viable number of encapsulated cells was 10.01 Log CFU/g at 0 day and decreased to 9.6 Log CFU/g (4 °C) and 9.2 Log CFU/g (25 °C) after 8 days. Full encapsulated cells could be liberated after the microbeads were exposed to simulated intestine juice (SIJ) within 120 min. This study indicates that FM-SA microbeads can be suggested as good agents for encapsulating probiotics.

KEYWORDS: encapsulating, flaxseed, survival, *Enterococcus faecalis*, probiotics, sodium alginate

INTRODUCTION

Probiotic bacteria are recognized as alive microbe and can offer positive benefits to the host when they are ingested in sufficient amounts [1, 2]. In order to achieve the health effects of bacterial cells, the viable numbers varying from 10^6 to 10^8 CFU/ml are generally recommended in many studies [3, 4]. Unfortunately, the stability of probiotic bacteria is easily affected by adverse circumstances in human gastrointestinal environments, for instance low pH and high bile salt content [5]. Thus, in order to enhance the survival of probiotics, many approaches, for example the co-encapsulation with prebiotics, structure improvement of food matrix, and encapsulation of probiotics, have been investigated [6, 7]. Among these approaches, encapsulation may be described as the best way to protect probiotic bacteria in gastrointestinal tract environments [8–11].

Encapsulation methods such as emulsion, freeze drying, extrusion, spray drying, and coacervation, etc. have been widely studied to reduce the loss of probiotic bacteria numbers in gastrointestinal tract [3, 5, 10–14]. Among these methods, the extrusion method may be one of the gentlest approaches for probiotic encapsulation [10, 11]. The choice of an appropriate material for probiotic bacteria encapsulation is one of crucial issues [3, 15]. Among the employed encapsulating materials, sodium alginate (SA) is the most commonly used polymer for encapsulating probiotics due to its non-toxic property [4]. However, SA cannot offer effective protection for probiotics under high acid environments because of the porosity of SA microbeads [13, 16–18]. To overcome this limitation, the blend or coating SA with other hydrocolloid compounds such as gums and proteins has been highly suggested [4, 8, 19]. The combination of SA with other polymers can improve the protection ability for probiotics under common storage and gastric conditions and obtain acceptable release characteristic in intestinal conditions [20]. Recently, milk and milk proteins have been widely employed as wall materials for encapsulating bioactives due to their good physicochemical characteristics, for instance good buffering ability and excellent emulsification ability, etc [21, 22]. Various reports have demonstrated that the blending of SA and milk proteins is able to effectively protect probiotics from being destroyed in digestive process [21–24]. Carrageeenan-locust bean gum coated milk microbeads and SA-milk microspheres were developed in our group [10, 11, 25]. These milk-based agents could enhance the viable numbers of *Lactobacillus bulgaricus* against the adverse gastrointestinal and storage conditions. However, only limited studies regarding the influence of SA-plant-based milk agents on the encapsulation of probiotics have been reported. In our group, we used soy protein-SA to encapsulate *E. faecalis* HZNU P2 and found that the developed soy milk-SA microspheres were good agents for protecting *E. faecalis* HZNU P2 from damaging by unfavorable

gastrointestinal environments such as low pH and bile salt solution [26]. FM enriched in flaxseed protein has various functional characteristics, for instance solubility, oil/water interface ability, and emulsifying activity among others, which makes it a great potential as an encapsulation agent [27–29]. As far as our knowledge goes, the employment of SA-FM as wall material for probiotic protection has not been studied.

Enterococci are natural microorganisms in our gut and have been used as probiotics in some countries [30, 31]. A strain of *Enterococcus faecalis* HZNU S1 exhibited high resistance to intestinal circumstances and adhesion ability to gut cells. However, it showed poor survival when exposed to simulated gastric juice (SGJ) and bile salt solutions. Accordingly, to enhance the stability of *E. faecalis* HZNU S1 in gastrointestinal tract conditions, in present investigation, encapsulated cells employing the blending of SA and FM were studied. The viable amounts of encapsulated cells in SGJ and the liberating property of encapsulated cells exposed to simulated intestine juice (SIJ) were carried out. In addition, the survival of encapsulated probiotics at 4 and 25 °C was determined.

MATERIALS AND METHODS

E. faecalis HZNU S1 culture activation

The pure culture, *E. faecalis* HZNU S1, was obtained from Hangzhou Normal University, which was isolated from peacock stool. The obtained culture was activated through culturing it in MRS (Man Rogosa Sharpe) liquid medium at 37 °C for 24 h. Afterwards, the harvested probiotics were obtained through centrifuging at 4 °C, 4670 g for 10 min. The harvested probiotics were rinsed by sterile water and then suspended in 0.85% saline solution. The probiotic content was made at above 10^{11} CFU/ml and employed for further investigations.

Encapsulation process

E. faecalis HZNU S1 was encapsulated as described in our previous studies with slight modifications [10, 11, 18, 32]. Briefly, FM (Hangzhou Tianlong Group Co., Ltd., Zhejiang, China; 3.2% protein concentration) and SA (Sigma Aldrich, Shanghai, China) were autoclaved at 110 °C for 10 min and 121 °C for 15 min, respectively. After being cooled, the prepared solutions (FM/SA = 2:1, 2.0% SA) were mixed with probiotic cell culture (10^{11} CFU/ml) at 9:1 (v/v) ratio. For extrusion method, the obtained mixture was added dropwise into 100 mM $CaCl_2$ using a syringe with gentle agitation at 100 rpm. The formed microbeads were solidified in $CaCl_2$ with the rotating speed of 100 rpm for 30 min. Afterwards, the obtained microbeads were filtered, rinsed by distilled water, and then preserved at 4 °C till the following work.

The determination of viable numbers of free and encapsulated *E. faecalis* HZNU S1

The microbeads were randomly chosen and broken after being exposed to 50 mM sodium citrate solution. The viable encapsulated probiotics were determined using the pour plate technique. Briefly, the disintegrated microbead suspension was appropriately diluted by 0.85% saline solution. Then, 100 µl of dilute solution was added onto the plates with MRS agar. Released cell amounts were determined after the plates with MRS agar were cultured at 37 °C for 24 h.

Free cells were also determined using the pour plate technique. Free cell suspensions were suitably diluted by 0.85% saline solution, and 100 µl of cell solutions were added onto the plates with MRS agar. Cell numbers were calculated according to the above method of encapsulated cells.

Viable numbers of free and encapsulated *E. faecalis* HZNU S1 in SGJ

Free and encapsulated cells were subjected to SGJ (0.20% NaCl, pH 2.0 and 2.5). SGJ was prepared using 0.20% NaCl, and 0.1 M HCl was used to adjust pH to desired pH (2.0 and 2.5). The tolerance of free and encapsulated cells was calculated using the same method as in our previous studies with some modifications [10, 11, 18, 32]. Then, 0.50 g of microbeads/0.50 ml of cell suspension was suspended in SGJ. Free and encapsulated cells were mixed with SGJ at 37 °C with the vibration speed of 100 rpm. The viable numbers of free and encapsulated cells were measured at certain times of 0, 30, 60, 90, and 120 min. After specific time intervals, 100 µl of SGJ with free cells was drawn to measure the viable numbers. The harvested microbeads were determined for the amounts of viable cells using the pour plate technique promptly.

Stability of free and encapsulated *E. faecalis* HZNU S1 in bile salt solution

Free and encapsulated cells were exposed to 1.0 and 2.0% bile salt solutions (Sigma-Aldrich, Shanghai, China). The stability of free and encapsulated cells in bile salt solutions was determined based on our previous studies with some modifications [10, 11, 18, 32]. Briefly, 0.50 g of microbeads/0.50 ml of cell suspension was exposed to 4.5 ml bile salt solution and cultured at 37 °C for 1 and 2 h. At each investigated time interval, the samples were taken out to measure the viability of probiotics in bile salt solution using the pour MRS plate technique.

Release profile of encapsulated *E. faecalis* HZNU S1 exposed to SIJ

The liberating profile of encapsulated cells in SIJ (pH 6.8, 50 mM KH_2PO_4) was investigated employing the methods showed in our previous studies [10, 11, 18, 32]. Briefly, 0.50 g of the microbeads was

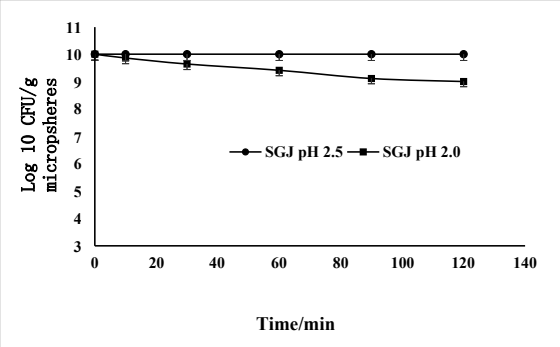

Fig. 1 Survival of free and encapsulated *E. faecalis* HZNU S1 in pH 2.0 and 2.5 SGJ.

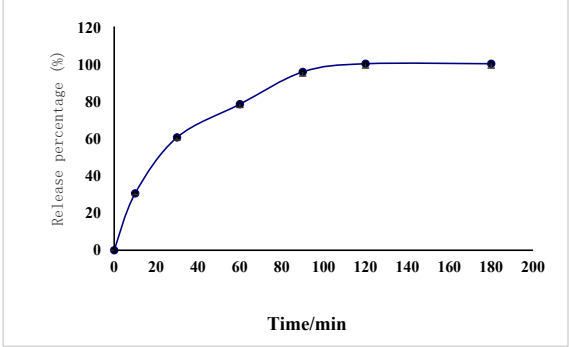

Fig. 2 The release profile of encapsulated *E. faecalis* HZNU S1 in SIJ.

Table 1 Stability of free and encapsulated *E. faecalis* HZNU S1 exposed to 1.0 and 2.0% bile salt solution for 1 and 2 h (Log CFU/ml or g microbeads).

Condition/time	Bile content (%)		
	0	1	2
Free cell (1 h)	10.01 ± 0.12	0	0
Encapsulated cell (1 h)	10.01 ± 0.11	9.81 ± 0.12	9.54 ± 0.08
Free cell (2 h)	10.02 ± 0.09	0	0
Encapsulated cell (2 h)	10.01 ± 0.13	9.12 ± 0.09	8.53 ± 0.11

mixed with 4.5 ml SIF and placed in shaking incubator (100 rpm) at 37 °C for 2 h incubation. Then, 100 µl of the supernatant was taken out at various time intervals (0, 10, 30, 60, 90, and 120 min) and appropriately diluted with 0.85% saline solution. To substitute the withdrawn solution, 100 µl of fresh medium was used. The viability of probiotics was measured through employing the previously described method.

Evaluation of free and encapsulated *E. faecalis* HZNU S1 under various storage circumstances

The survival numbers of free and encapsulated cells were determined at 4 and 25 °C for certain time intervals. At each interval of storage, the microbeads were broken after being exposed to 50 mM sodium citrate solution. Afterwards, the suitable dilution and the pour MRS plate technique were used to measure the viable numbers of probiotic bacteria.

Statistical analyses

Each value was determined after taking the mean of at least three replicates, and the values were expressed as mean ± standard deviations (SD). All the obtained results were subjected to analysis of variance using Origin 8.0 for Windows, and the mean values were followed by Student's *t*-test at the level of 95% confidence interval.

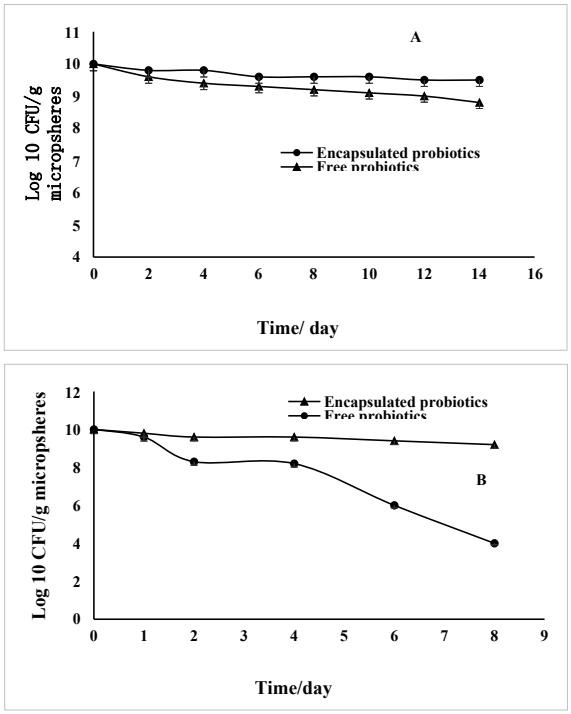

Fig. 3 Storage stability of free and encapsulated *E. faecalis* HZNU S1 at 4 °C (A) and 25 °C (B).

RESULTS AND DISCUSSION

The viable cell numbers of free cells decreased significantly, which dropped to non-detectable level (< 10 CFU/ml) within 30 min of the incubation period (results not shown). The results indicated that *E. faecalis* HZNU S1 was susceptible to acid condition of SGJ and thus brought the challenges to its applications in food industries. Likewise, Prasanna and Charalampopoulos found that the viable numbers of free *Bifidobacteria longum* subsp. infantis CCUG were markedly reduced within 90 min of the incubation period and dropped

to non-detectable amounts after 120 min when free cells were incubated in SGJ [21]. The present research indicated that encapsulation could provide significant protection for *E. faecalis* HZNU S1 in SGJ (Fig. 1). Full survival of encapsulated cells was able to be remained after 2 h of exposure in pH 2.5 SGJ. Only 1 Log CFU/g was reduced after encapsulated *E. faecalis* HZNU S1 was in pH 2.0 SGJ for 2 h incubation. The excellent protection of FM-based microbeads for encapsulated probiotics may be because of the high buffering ability of flaxseed protein. Additionally, the formed filling materials through the interaction of flaxseed proteins and SA can reduce the porous structure of FM-SA microbeads. The obtained results are also consistent with the results reported in various investigations. It was also reported that the resistance of bifidobacteria to pH 2.0 SGJ could be enhanced through using an encapsulation agent of SA, goat milk, and inulin [22]. The viable numbers of encapsulated bifidobacteria were reduced from 9.44 Log CFU/g to 8.44 Log CFU/g after being exposed to pH 2.0 SGJ for 2 h. The authors indicated a good protection for bifidobacteria was due to the high buffering capacity of goat milk. Prasanna and Charalampopoulos reported that the viable counts of SA-cow milk and SA-goat milk microcapsules were 6.37 Log CFU/g and 5.19 Log CFU/g, respectively, after being incubated in pH 2.0 SGJ for 120 min [21]. The authors demonstrated that SA-goat milk (and -cow milk) microbeads with dense structure showed better performances in SGJ than SA microbeads. Annan et al also found that SA-coated gelatin microbeads could enhance the survival of probiotics exposed to SGJ because of the buffering ability of gelatin [33]. The decrease of 3.45 Log CFU/mL for free cells, 2.55 Log CFU/g for gelatin microbeads, and 1.21 Log CFU/g for SA-coated gelatin microbeads was observed during exposure to pH 2.0 SGJ for 120 min. Chen et al reported that the viable counts of encapsulated *Lactobacillus bulgaricus* in whey protein–SA microbeads decreased less than 1.0 Log CFU/g for 2 h incubation in pH 2.0 SGJ due to whey protein buffering capacity [16].

The stability of free and encapsulated cells in bile salt solution for 1 and 2 h exposure is presented in Table 1. The viable numbers of free cells exhibited a significant reduction within 1 h exposure. The survival of free cells was completely lost in 1.0 and 2.0% bile salt solutions after 1 h exposure (Table 1). The interaction of bile salt and free cells may damage cell wall integrity, leading to the leakage of cellular compounds and thus causing the decline of cells survival [3, 21, 34]. Similarly, Shi et al reported that the survival of free cells completely lost after being exposed to 2.0% bile salt solution at 37 °C after 1 h [25]. Encapsulation offered excellent protection for probiotics in bile salt solutions. The initial viable amounts of encapsulated cells dropped from 10.01 to 9.81 Log CFU/g after 1 h exposure and then reduced to 9.54 Log CFU/g after 2 h exposure in 1.0 % bile salt solution. Likewise, a minor reduction in the survival of encapsulated probiotics after being exposed to 2.0% bile salt solution for 1 and 2 h was observed. Milk-based microbeads could provide effective protection for the cells in bile salt solution. It was because the matrices formed through the interaction of milk ingredients and SA could inhibit the contact of bile salt with probiotics in the microbeads [21]. Likewise, SA-milk based encapsulation agents showed a great potential to protect *Lactobacillus bulgaricus* and *E. faecalis* HZNU P2 in bile salt solutions [10, 11, 21].

The liberated ability of encapsulated cells in SIJ at 37 °C during a 3-h time period is shown in Fig. 2. FM-SA microbeads exhibited a fast release profile in SIJ. Around 60% of encapsulated cells were able to be liberated within 30 min incubation. Encapsulated cells were completely released in 120 min. The survival of free cells suggested that once released, *E. faecalis* HZNU S1 would be able to survive in SIJ conditions. Various investigations have reported the release property of encapsulated probiotic bacteria in SIJ [10, 11, 25, 26]. Khan et al found that once legume protein isolate-SA microbeads were added into SIJ, a rapid liberation of ∼5.0 Log CFU/g microbeads (at time 0), followed by a more progressive release of ∼6.5 Log CFU/g microbeads within 2 h, was observed [35]. Shi et al demonstrated that *E. faecalis* HZNU P2 entrapped in milk-SA was completely liberated in SIJ within 1 h [25]. Likewise, embedded *E. faecalis* HZNU P2 in soy milk-SA microbeads was also fully released in 1 h. About 50% of encapsulated cells in soy milk-SA microbeads could be released within 10 min [26]. Therefore, our studies indicated that FM-SA microbeads exhibited a rapid release profile within SIJ. It indicates that the developed microbeads have the potential for targeted delivery of probiotics to intestinal tract, where they have the potential to colonize, providing health benefits to the host.

Fig. 3 shows the survival of free and encapsulated probiotics over the storage periods at 4 and 25 °C. The amounts of free cells dropped significantly from 10.01 to 8.8 Log CFU/g (Fig. 3A) after 2-week storage, demonstrating that free cells were not stable under the refrigerated storage circumstance. At the same conditions, only 0.50 Log CFU/ml reduction of encapsulated cells was observed. The viability of encapsulated cells slowly decreased over time while the survival of free cells declined rapidly. After the storage of 8 days at 25 °C (Fig. 3B), the survival of free cells exhibited more than 6.43 Log CFU/ml decline (from 10.01 to 3.58 Log CFU/ml); however, the survival of encapsulated cells only showed 0.81 Log CFU/ml decrease (from 10.01 to 9.2 Log CFU/ml). According to these results, it was obviously indicated that encapsulation was able to enhance the survival of *E. faecalis* HZNU S1 during the investigated storage. The excellent protection

ability of encapsulation could be illustrated because of the dense layer of the microbeads formed through the interaction of FM and SA. Similarly, many studies have indicated that milk-based encapsulation can enhance the survival of probiotics under storage circumstances [10, 11, 26]. Prasanna and Charalampopoulos reported that 3.67 Log CFU/g reduction in the viable numbers of free bifidobacteria at 4 °C over a period of 28 days was observed, whereas the cell concentration in SA-goat milk-inulin microcapsules did not decrease below the recommended level at the same storage conditions [22]. In the same group, the authors found that SA-cow milk (and -goat milk) microbeads were able to offer better protection for bifidobacteria within the refrigerated storage period compared to SA microcapsules. The viable numbers above 6.0 Log CFU/g in SA-cow milk (and -goat milk) microbeads after 28 days of storage were maintained. The authors speculated that the denser layer of alginate-dairy microcapsules could protect probiotics from harsh environments within the storage period [21]. Shi et al showed that SA-milk microbeads offered a good protection for the embedded *E. faecalis* HZNU P2, compared to free cells after the storage of 2 weeks at 4 °C [25].

Encapsulation of probiotics is a fast-developing research field. The potential applications of encapsulated probiotics are mainly in dairy (yogurt, cheese, etc.) and non-dairy sections (fermented sausages, juices, biscuits, etc.). Storage stability is the vital importance for the products with probiotics [9, 14, 36]. Encapsulation can not only improve the survival of probiotics exposed to gastrointestinal conditions, but also improve the storage stability of probiotics. However, even if valuable and exciting studies have been reported in the past few years, and various kinds of probiotic-based foods are being on the market, in order to investigate the compatibility of encapsulated probiotics with food matrix, more investigations should be carried out regarding the applications of encapsulated probiotic bacteria in foods in the future.

CONCLUSION

In this study, *E. faecalis* HZNU S1 was successfully encapsulated through the extrusion technique using FM and SA. The encapsulated cells exhibited better performance when being exposed to low pH (pH 2.0 and 2.5 SGJ), bile salt solutions (1.0 and 2.0%), and the investigated storage conditions compared to free cells. Encapsulated cells in SIF were with a rapid releasing property. In short, the encapsulation using FM and SA as wall materials could be used to effectively enhance the stability of free cells under hostile conditions.

Acknowledgements: The work was supported by the projects of Scientific Innovation Teams in Tourism College of Zhejiang (No. 2023TDDS07) and the Post-supported project for Excellent Research Achievements of Tourism College of Zhejiang (No. 2023HQZD03).

REFERENCES

1. Bevilacqua A, Speranza B, Santillo A, Albenzio M, Gallo M, Sinigaglia M, Corbo MR (2019) Alginate-microencapsulation of *Lactobacillus casei* and *Bifidobacterium bifidum*: performances of encapsulated microorganisms and bead-validation in lamb rennet. *LWT Food Sci Technol* **113**, 108349.
2. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, et al (2014) Expert consensus document: The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastro Hepat* **11**, 506–514.
3. Li H, Li Y, Zhang T, Liu T, Yang J, Luo X, Li H, Xue C, et al (2021) Co-encapsulation of *Lactobacillus paracasei* with lactitol in caseinate gelation cross-linked by *Zea mays* transglutaminase. *LWT Food Sci Technol* **147**, 111535.
4. Silva HL, Balthazar CF, Silva R, Vieira AH, Costa RG, Esmerino EA, Freitas MG, Cruz AG (2018) Sodium reduction and flavor enhancer addition in probiotic prato cheese: contributions of quantitative descriptive analysis and temporal dominance of sensations for sensory profiling. *J Dairy Sci* **101**, 8837–8846.
5. Silva KKP, Queiros MS, Ribeiro APB, Gigante ML (2022) Modified milk fat as encapsulating material for the probiotic microorganism *Lactobacillus acidophilus* LA3. *Int Dairy J* **125**, 105237.
6. Tolve R, Galgano F, Caruso MC, Tchuenbou-Magaia FL, Condelli N, Favati F, Zhang Z (2016) Encapsulation of health-promoting ingredients: applications in foodstuffs. *Int J Food Sci Nutr* **67**, 888–918.
7. Zeashan M, Afzaal M, Saeed F, Ahmed A, Tufail T, Ahmed A, Anjum FM (2020) Survival and behavior of free and encapsulated probiotic bacteria under simulated human gastrointestinal and technological conditions. *Food Sci Nutr* **8**, 2419–2426.
8. Apiwattanasiri P, Charoen R, Rittisak S, Phattayakorn K, Jantrasee S, Savedboworn W (2022) Co-encapsulation efficiency of silk sericin-alginate-prebiotics and the effectiveness of silk sericin coating layer on the survival of probiotic *Lactobacillus casei*. *Food Biosci* **46**, 101576.
9. Reque PM, Brandelli A (2021) Encapsulation of probiotics and nutraceuticals: applications in functional food industry. *Trends Food Sci Tech* **114**, 1–10.
10. Shi LE, Li ZH, Li DT, Xu M, Chen HY, Zhang ZL, Tang ZX (2013) Encapsulation of probiotics *Lactobacillus bulgaricus* in alginate-milk microspheres and evaluation of survival in simulated gastrointestinal conditions. *J Food Eng* **117**, 99–104.
11. Shi LE, Zhang ZL, Song YQ, Zhou ML, Yu WM, Tang ZX (2013) Encapsulation of *Lactobacillus bulgaricus* in carragenan-locust bean gum coated milk microspheres with double layer structure. *LWT Food Sci Technol* **54**, 147–151.
12. Dong QY, Chen MY, Xin Y, Qin XY, Cheng Z, Shi LE, Tang ZX (2013) Alginate-based and protein-based materials for probiotics encapsulation: a review. *Int J Food Sci Technol* **48**, 1339–1351.

13. Santos MAS, Machado MTC (2021) Coated alginate-chitosan particles to improve the stability of probiotic yeast. *Int J Food Sci Technol* **56**, 2122–2131.
14. Pourjafar H, Noori N, Gandomi H, Basti AA, Ansari F (2020) Viability of microencapsulated and nonmicroencapsulated lactobacilli in a commercial beverage. *Biotechnol Rep* **25**, e00432.
15. Hosssain MN, Ranadheera CS, Fang Z, Ajlouni S (2021) Impact of encapsulating probiotics with cocoa powder on the viability of probiotics during chocolate processing, storage, and *in vitro* gastrointestinal digestion. *J Food Sci* **86**, 1629–1641.
16. Chen MY, Zheng W, Dong QY, Li ZH, Shi LE, Tang ZX (2014) Activity of encapsulated *Lactobacillus bulgaricus* in alginate-whey protein microspheres. *Braz Arch Biol Technol* **57**, 736–741.
17. Afzaal M, Saeed F, Hussain S, Mohamed AA, Alamri MS, Ahmad A, Ateeq H, Tufail T, et al (2020) Survival and storage stability of encapsulated probiotic under simulated digestion conditions and on dried apple snacks. *Food Sci Nutr* **8**, 5392–5401.
18. Pan LX, Fang XJ, Yu Z, Xin Y, Liu XY, Shi LE, Tang ZX (2013) Encapsulation in alginate-skim milk microspheres improves viability of *Lactobacillus bulgaricus* in gastrointestinal conditions. *Int J Food Sci Nutr* **64**, 380–394.
19. Eratte D, McKnight S, Gengenbach TR, Dowling K, Barrow CJ, Adhikari BP (2015) Co-encapsulation and characterisation of omega-3 fatty acids and probiotic bacteria in whey protein isolate-gum arabic complex coacervates. *J Funct Food* **19**, 882–892.
20. Krasaekoopt W, Watcharapoka S (2014) Effect of addition of inulin and galactooligosaccharide on the survival of microencapsulated probiotics in alginate beads coated with chitosan in simulated digestive system, yogurt and fruit juice. *LWT Food Sci Technol* **57**, 761–766.
21. Prasanna PHP, Charalampopoulos D (2018) Encapsulation of *Bifidobacterium longum* in alginate-dairy matrices and survival in simulated gastrointestinal conditions, refrigeration, cow milk and goat milk. *Food Biosci* **21**, 72–79.
22. Prasanna PHP, Charalampopoulos D (2019) Encapsulation in an alginate-goats' milk-inulin matrix improves survival of probiotic *Bifidobacterium* in simulated gastrointestinal conditions and goats' milk yogurt. *Int J Dairy Technol* **72**, 132–141.
23. Ranadheera CS, Evans CA, Adams MC, Baines SK (2015) Microencapsulation of *Lactobacillus acidophilus* LA-5, *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Propionibacterium jensenii* 702 by spray drying in goat's milk. *Small Ruminant Res* **123**, 155–159.
24. Saeed F, Afzaal M, Ahmad A, Aamir M, Aziz M, Aslam S, Ateeq H, Hussain M (2022) Enhanced viability of microencapsulated lyophilized probiotics under *in vitro* simulated gastrointestinal conditions. *J Food Process Pres* **46**, e16543.
25. Shi LE, Zheng W, Zhang Y, Tang ZX (2016) Milk-alginate microspheres: protection and delivery of *Enterococcus faecalis* HZNU P2. *LWT Food Sci Technol* **65**, 840–844.
26. Shi LE, Zheng W, Zhang Y, Liu XY, Tang ZX (2015) Soy milk-based microspheres as potential carriers for the protection of *Enterococcus faecalis* HZNU P2. *J Funct Food* **18**, 487–491.
27. Mohamed RS, Fouda K, Akl EM (2020) Hepatorenal protective effect of flaxseed protein isolate incorporated in lemon juice against lead toxicity in rats. *Toxicol Rep* **7**, 30–35.
28. Sharma M, Saini CS (2021) Postharvest shelf-life extension of fresh-cut guavas (*Psidium guajava*) using flaxseed protein-based composite coatings. *Food Hydrocolloid Health* **1**, 100015.
29. Ye XP, Xu MF, Tang ZX, Chen HJ, Wu DT, Wang ZY, Songzhen YX, Hao J, et al (2022) Flaxseed protein: extraction, functionalities and applications. *Food Sci Technol* **42**, e22021.
30. Kouhi F, Mirzaei H, Nami Y, Khandaghi J, Javadi A (2022) Potential probiotic and safety characterisation of enterococcus bacteria isolated from indigenous fermented motal cheese. *Int Dairy J* **126**, 105247.
31. Zheng W, Zhang Y, Lu HM, Li DT, Zhang ZL, Tang ZX, Shi LE (2015) Antimicrobial activity and safety evaluation of *Enterococcus faecium* KQ 2.6 isolated from peacock feces. *BMC Biotechnol* **15**, 30.
32. Tang ZX, Huang XQ, Baxi S, Chambers JR, Sabour PM, Wang Q (2013) Whey protein improves survival and release characteristics of bacteriophage Felix O1 encapsulated in alginate microspheres. *Food Res Int* **52**, 460–466.
33. Annan NT, Borza AD, Hansen LT (2008) Encapsulation in alginate-coated gelatin microspheres improves survival of the probiotic *Bifidobacterium adolescentis* 15703T during exposure to simulated gastro-intestinal conditions. *Food Res Int* **41**, 184–193.
34. Bron PA, Marco M, Hoffer SM, Van Mullekom E, De Vos WM, Kleerebezem M (2004) Genetic characterization of the bile salt response in *Lactobacillus plantarum* and analysis of responsive promoters *in vitro* and *in situ* in the gastrointestinal tract. *J Bacteriol* **186**, 7829–7835.
35. Khan NH, Kober DR, Low NH, Nickerson MT (2013) Development of extrusion-based legume protein isolate-alginate capsules for the protein and delivery of the acid sensitive probiotic, *Bifidobacterium adolescentis*. *Food Res Int* **54**, 730–737.
36. Priscilla MR, Adriano B (2021) Encapsulation of probiotics and nutraceuticals: applications in functional food industry. *Trends Food Sci Tech* **114**, 1–10.