Viability of Encapsulated Bacteria using Gel Particle and Foam-Mat Drying with k-carrageenan/Chitosan and i-carrageenan/ Chitosan

Dwi Setijawati [1,*], Abdul Aziz Jaziri[2], Mohd Azrie Bin Awang[3,4], Muhamad Firdaus[1], Aryanti Diah Ayu Budiutami[2], Alifiani[2], Fenny Amilia Mahara[2], R Adharyan Islamy[5]

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[1] Bioseafood Research Group, Department of Fisheries and Marine Resources Management, Faculty of Fisheries and Marine Science, Brawijaya University, Jl. Veteran, Ketawanggede, District. Lowokwaru, Malang City, East Java 65145, Indonesia
[2] Department of Fishery Product Technology, Department of Fisheries and Marine Resources Management, Faculty of Fisheries and Marine Science, Brawijaya University, Jl. Veteran, Ketawanggede, District. Lowokwaru, Malang City, East Java 65145, Indonesia
[3] Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia
[4] Food Security Research Laboratory, Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia
[5] Aquaculture (Kediri City Kampus), Department of Fisheries and Marine Resources Management, Faculty of Fisheries and Marine Sciences, Brawijaya University, Jl. Pringgodani, Kediri City 64111, East Java, Indonesia

*Corresponding Author:
dwisetyawati@ub.ac.id

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ABSTRACT

The study aimed to determine the effect of using a mixture of kappa-carrageenan/chitosan and iota carrageenan/chitosan as encapsulating materials for *Lactobacillus acidophilus* and *Bifidobacterium bifidum* on viability, encapsulation yield, moisture content, and water activity (A_w) f microcapsules. The research was conducted using an experimental laboratory design, and the data were analyzed using analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) test with Microsoft Excel. The results showed that the use of chitosan with a degree of deacetylation of 83.5% in the i-carrageenan/chitosan mixture yielded the highest viability of *B. bifidum*. The combination of i-carrageenan and chitosan at a concentration ratio of 0.5%:1.5% produced probiotic viability for the mixed culture (*L. acidophilus* + *B. bifidum*) of 6.56 log CFU/g, while the viability of *B. bifidum* alone reached 6.70 log CFU/g. The highest encapsulation yield for the mixed probiotics was 72.85%. The water content of the microcapsules for the mixed probiotics was 8.96%, with a water activity (A_w) of 0.63. These results suggest that further studies should investigate processing factors to optimize probiotic viability.

Keywords: *Eucheuma cottonii*, *Eucheuma spinosum*, Shrimp Shell, Microencapsulation

Introduction

The probiotic segment dominates the functional food market. Scientific studies show that probiotic strains have beneficial effects against various disorders, such as gastro-intestinal diseases, bacterial vaginosis, and urinary tract infections (Krawczyk et al., 2021; Sakandar & Zhang, 2021; Tegegne & Kebede, 2022). Probiotics have been implicated in inhibiting enteric pathogens, maintaining intestinal permeability, modulating the immune system, reducing inflammation, alleviating lactose intolerance, increasing intestinal motility, and decreasing cholesterol concentrations (Cristofori et al., 2021; Krawczyk et al., 2021; Mirzaei et al., 2021). The two types of probiotic bacteria, namely *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, are the most numerous probiotic bacteria in the human body and are distributed throughout the body, especially in the digestive area. Technological aspects related to microbial systems and functional foods are the composition and processing of raw materials, the viability and productivity of the applied starter cultures, and the technological and storage conditions of the

final foods. Among the available techniques, microencapsulation is one of the most effective methods to enhance probiotic survival in the food systems process (Amiri et al., 2022; Cristofori et al., 2021; Krawczyk et al., 2021; Li et al., 2023; Nezamdoost-Sani et al., 2023). Microencapsulation of probiotics such as *L. acidophilus* and *B. bifidum* is a way to protect probiotics from external environmental stresses, including temperature, pH, oxygen, and processing conditions (Afzaal et al., 2020). Moreover, it allows controlled diffusion of lipophilic functional food ingredients. The product resulting from the microencapsulation process is called a "microcapsule" (Zhu et al., 2021).

The selection of of encapsulant materials to protect the two bacteria is essential. Encapsulant materials such as carrageenan and chitosan have been widely used in microencapsulation of *L. acidophilus* and *B. bifidum*, because they are safe, non-toxic, biodegradable, able to form gels, and have high viscosity (Amiri et al., 2022; Cristofori et al., 2021; Koh et al., 2022; Krawczyk et al., 2021). Gel strength and viscosity affect encapsulation methods such as gel particles. Drying microcapsules into granules by foam-mat drying is expected to maintain probiotic viability because it uses drying temperatures ranging from 45-50 ^{o}C (Ermi^{o}, 2021).

Eucheuma sp. is a red hydrocolloid that produces carrageenan. Two essential species in Indonesia are *Eucheuma cottonii* and *Eucheuma spinosum*. Carrageenan is produced through an extraction process using alkali. Extraction with an alkaline solution can increase the strength of carrageenan gel. *E. cottonii* produces kappa carrageenan with a strongly alkaline solution of K^{+}, while *E. spinosum* produces iota carrageenan with an alkaline solution of Ca^{2+} (Altamirano Ríos et al., 2022; Buschmann et al., 2017). The use of different alkaline materials gives different gel properties. The properties of kappa gel are hard, tough, easy to crack, and syneresis (Menaa et al., 2021; Wardhana et al., 2022). In contrast, the properties of iota carrageenan gel are soft, soft, elastic, and not easy to syneresis. This syneresis property can cause changes in moisture content during storage, highlighting the need a composite material that is resistant to syneresis while remaining biocompatible. One of the ingredients that can be used is chitosan, made from shrimp shells through deproteinize and deacetylation processes (Oberoi et al., 2021).

The compatibility of the mixture of ingredients between carrageenan and chitosan is because carrageenan is anionic, while the chitosan is cationic. Using a mixture of k-carrageenan/chitosan and i-carrageenan/chitosan is expected to protect *L. acidophilus* and *B bifidum*, enhancing encapsulation viability and yield. The research problem is whether using a mixture of i-carrageenan/chitosan and k-carrageenan/chitosan can affect the viability and yield of encapsulation. The study aimed to investigate the effect of using a mixture of i-carrageenan/chitosan and k-carrageenan/chitosan on the viability of the probiotics *L. acidophilus* and *B. bifidum* and the resulting encapsulation yield.

Material and Methods

L. acidophilus and *B. bifidum* were obtained from the Microbiology Laboratory, Faculty of Medicine, Brawijaya University, Malang, Indonesia. *E. cottonii* and *E. spinosum* were acquired from Madura Waters, East Java, Indonesia. The chitosan was gained from the Pasuruan shrimp cracker factory in East Java, Indonesia.

Preparation of Carragenan

The k-carrageenan was extracted from *E. cottonii* using the Gel Press method based on previously described methods by Afoakwah et al. (2023), which was modified, namely washing, drying, weighing, extracting in 6% KOH, adding 0.75% KCl, washing with 0.375% KCl 2 times, washing with running water, drying, grinding, and sifting. Then, after it becomes flour, the functional group test was carried out using Fourier Transformer Infra-Red (FTIR).

Conversely, the i-carrageenan was isolated from *E. spinosum* using the KCl Gel Press method based on established methods, which has been modified, namely washing, drying, weighing, extracting in $Ca(OH)^{2}$ 6%, adding $Ca(Cl)^{2}$ 0.75%, washing with 0.375% $Ca(Cl)^{2}$ 2 times, washing with running water, drying in the sun, grinding, and sifting. Then, after it becomes flour, the functional group test was performed using FTIR.

The chitosan was prepared from shrimp shells through two stages: the isolation of chitin and the conversion of chitosan. Isolation of chitin was executed based on earlier investigation (Nowacki et al., 2020), which has been modified to include deproteination and demineralization processes. At the deproteination stage, finely ground shrimp shells were dissolved in 3.5% NaOH with the ratio of the weight of the shrimp shell to the volume of NaOH 1 g: 10 mL. The solution was homogenized at 80-85^{o}C for 30 minutes. The homogeneous solution was washed with running water and filtered until a neutral pH was obtained. The residue was dried in an oven at 105^{o}C for 2 hours. In the demineralization step, the dry residue was dissolved in HCL IN with the ratio of the weight of the residue to the volume of HCL 1 g: 10 mL. The solution was

homogenized at 60-65°C for 60 minutes. The homogeneous solution was washed with running water and filtered until a neutral pH was obtained. The residue was dried in an oven at 105°C for 2 hours to obtain chitin.

The chitosan conversion was carried out based on former studies (Pal et al., 2021; Pellis et al., 2022), which has been modified to include a deacetylation process. At this stage, chitin was dissolved in 50% NaOH with the ratio by weight of chitin to the volume of 1 g: 10 mL NaOH. The solution was homogenized at 90°C for 60 minutes. The homogeneous solution was washed with running water and filtered until a neutral pH was obtained. The residue was dried in an oven at 65°C for 24 hours until chitosan was obtained. Then, functional group testing and DD values were tested using FTIR.

Bacterial Preparation

The culture of *L. acidophilus* bacteria was carried out based on earlier studies (Y. Li et al., 2023; Ren et al., 2022), which has been modified, namely dry starter cultures are inoculated into 5 mL of sterile MRS broth medium and incubated at 37°C for 24 hours under anaerobic conditions. The culture was inoculated into 20 ml MRS broth and incubated under the same conditions for 12 hours. Cells were precipitated from MRS broth media by centrifugation at 15000 rpm for 10 minutes at 4°C. The supernatant was discarded, and the cell precipitate was resuspended with a sterile 0.9% NaCl solution. Cell density was calculated using a spectrophotometer at cÙ 620 nm and ready to use.

The culturing of *B. bifidum* bacteria was carried out based on previous research, which has been modified, namely dry starter cultures inoculated into 10 mL of MRS broth medium with the addition of 0.05% cysteine (w/v) and incubated for 12 hours at 37°C under anaerobic conditions. The culture was inoculated into 100 ml MRS broth with the addition of 0.05% cysteine (w/v) and again incubated under the same conditions until it reached the final logarithmic phase. Cells were precipitated from MRS broth media by centrifugation at 3500 rpm for 15 minutes at 4°C. The supernatant was discarded, and the precipitated cells were washed 2 times with sterile 0.9% NaCl solution until a suspension of bacterial cells was obtained. Cell density was calculated using a spectrophotometer at cÙ 620 nm.

Preparation of Microcapsules

The microcapsule was made from k-carrageenan/ chitosan and i-carrageenan/chitosan using the gel particle method followed by Foam-mat Drying (Khojah, 2020). The first step was to make a chitosan solution with various concentrations of 0%, 0.5%, 1.5%, and 2% (w/v). The chitosan material was prepared and weighed, weighing 0 g, 0.5 g, 1.5 g, and 2 g, which were then dissolved in 100 mL of weak acid solution (CH3COOH).

The next step was the manufacture of microcapsules using the foam mat drying gel particle method based on the published research, which modified kappa (k) and iota (i) carrageenan with concentrations of 0%, 0.5%, 1.5%, and 2% (w/v) dissolved in aqueous solution at 75°C. Then, after dissolving they mixed in chitosan solution with different concentrations. The mixture of k-carrageenan/chitosan and i-carrageenan/chitosan solutions was homogenized with a hot plate stirrer at 1000 rpm and at 97°C. The homogeneous solution was lowered in temperature to 40°C. 100 ml of bacterial cell suspension of *L. acidophilus* and *B. bifidum* (1:1 ratio) was added to 100 ml of a solution of mixed ingredients of k-carrageenan/chitosan and i-carrageenan/chitosan.

After that the mixture was homogenized with stirring at 1000 rpm. The next step was extruding the homogeneous mixture into 75 ml of 3.9 M KCl solution. The microcapsules formed were then filtered using filter paper. Prepared egg white foam weighing 17.5% of the microcapsule weight. Then, the white egg foam was added to the filtered microcapsules. The microcapsules were dried in an oven at 40-45°C for 24-48 hours to obtain dry microcapsules (granules).

Material Characterization

The functional groups of k-carrageenan, i-carrageenan, and chitosan were analyzed using FTIR spectroscopy (Shimadzu 8400S).

Determination of the Degree of Deacetylation of Chitosan

The degree of deacetylation of chitosan was determined by the base line method based on the FTIR spectrum (Weißpflog et al., 2021), with the formula:

$$DD = 100 - \left(\frac{A_{1655}}{A_{3450}} \times \frac{100}{1.33}\right)$$

A1655 shows absorption in the amide band, A3450 indicates absorption in the hydroxyl band, and factor 1.33 reveals the value of the ratio A1655 / A3450 for completely deacetylated chitosan (100%). The absorbance value (A) can be calculated using the formula:

$$A = \log\left(\frac{Po}{P}\right)$$

where Po = % transmittance at baseline, P = % transmittance at peak line, and A = absorbance.

Probiotic Viability and Encapsulation Yield

The encapsulated probiotic viability and encapsulated yield were calculated based on prior research (Afoakwah et al., 2023; Sakoui et al., 2022), which has been modified in that 1 g of microcapsule sample is put into 10 mL of NaFis solution and homogenized with a vortex mixer for 10 minutes. Homogeneous samples were diluted in stages and planted using the pouring method in MRSA media, for *B. bifidum*, 1% sodium thiosulphate was added. Petri dishes were incubated at 370C for 48 hours, for *L. acidophilus* under aerobic conditions and for *B. bifidum* under anaerobic conditions. The number of probiotics was calculated in units of CFU/mL, and the formula calculates the encapsulation yield:

$$EY = (N/N_0)\ x100$$

Where N is the number of live cells after the encapsulation process, and N0 is the number of live cells before the encapsulation process (initial density).

Moisture Content and water activity (A_w) of Microcapsules

The moisture content of the microcapsules was tested based on earlier research (Gallardo et al., 2013), namely by drying the oven (Memmert UN55), while the A_w of the microcapsules was measured using an A_w meter (Testo 650).

Statistical Analysis

The data were analyzed using the variance model (ANOVA) analysis to determine the effect of differences and continued with the Least Significance Difference (LSD) test to determine significant differences between treatments. The data results were expressed as mean ± standard deviation with a 95% confidence interval (á <0.05). The analysis was performed using SPSS 17.0 software.

Results and Discussion

The result of material characteristics of k-carrageenan/chitosan and i-carrageenan/chitosan identification through functional groups was carried out through the FTIR spectrum. The mixture of i-carrageenan/chitosan and k-carrageenan/chitosan was identified using FTIR (Figures 1 and 2).

The FTIR spectra of the i-carrageenan/chitosan mixture indicated that the i-carrageenan functional groups included a sulfate ester at 1222.87 cm{ ¹, a glycosidic bond at 1029.99 cm{ ¹, 3,6-anhydrogalactose at 933.55 cm{ ¹, and D-galactose-2-sulfate at 806.25 cm{ ¹ (Figure 1). In the case of k-carrageenan, the spectra revealed a sulfate ester at 1242.07 cm{ ¹, glycosidic bonds at 1029.92 cm{ ¹, 3,6-anhydrogalactose at 929.63 cm{ ¹, and D-galactose-4-sulfate at 850.55 cm{ ¹ (Figure 2)."

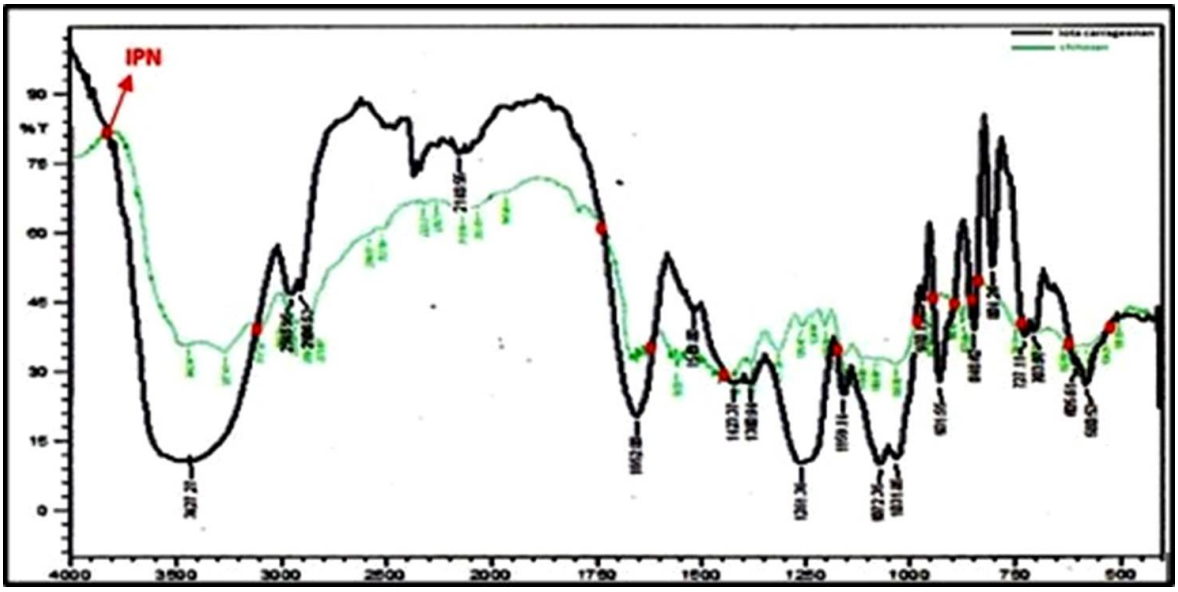

Figure 1. FTIR spectrum results of a mixture of ingredients i-carrageenan/chitosan.

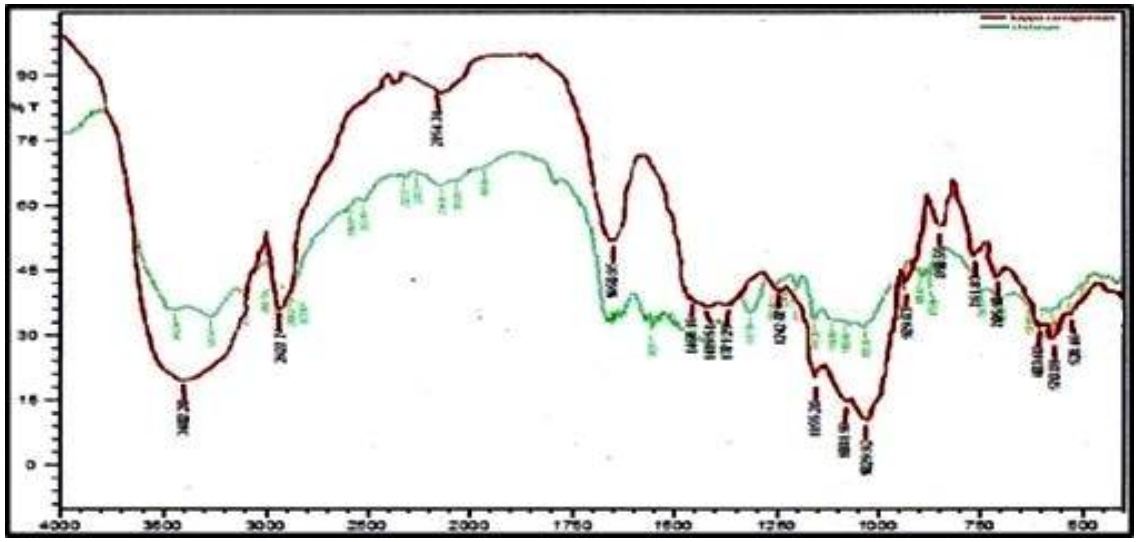

Figure 2. FTIR spectrum results of a mixture of k-carrageenan/chitosan ingredients.

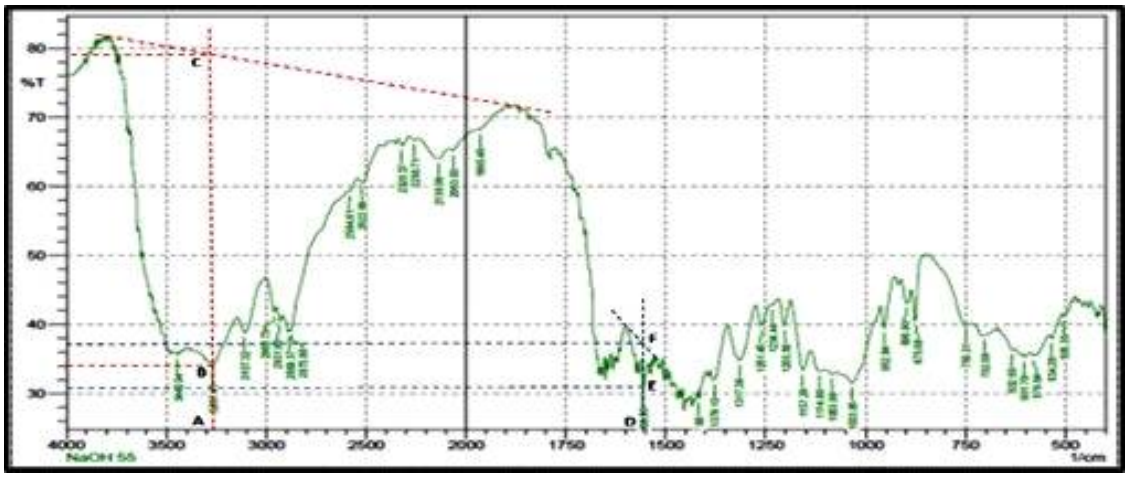

Figure 3. Determination of the degree of chitosan deacetylation from the FTIR spectrum.

Table 1. Wave number of functional groups of i-carrageenan and carrageenan

Functional Groups	Wave Number (cm^{-1})					
	K-carrageenan		I-carrageenan		Chitosan	
	Sigma Standard *	Research Result	Sigma Standard **	Research Result	Standard***	Research Result
Sulfate Esters	1257.59	1242.07	1220-1240	1261.36		
Glycosidic Bond	1072.42	1029.92	-	1031.85-1072.35		
3,6-anhydrogalactose	925.83	929.63	930	931.55		
D-galactose-4-sulfate	848.68	850.55	-	-		
D-galactose-2-sulfate	-	-	803-805	804.26		
O-H and N-H					3500-3300	3442.94
C-O-C					1150	1157.29
C-N					1317	1317.33

Note: * (Distantina et al., 2011)
** (Freile Pelegrín & Robledo, 2007)
*** (Dounighi et al., 2012)

Table 2. Effect of coating material concentration on probiotic viability and encapsulation yield

K-carrageenan (%)	I-carrageenan (%)	Chitosan (%)	Viability of L. acidophilus (A) (Log CFU/g)	Viability of *B. bifidum* (B) (Log CFU/g)	Viability (L+B) (Log CFU/g)	Encapsulation Yield (%)
2		0	4.36	5.06	4.71±0.02[a]	52.37±0.17[a]
1.5		0.5	4.39	5.39	4.89±0.01[ab]	54.37±0.06[ab]
0.5		1.5	4.60	5.42	5.01±0.16[b]	55.67±1.74[b]
0		2	4.35	5.10	4.73±0.01[a]	52.56±0.12[a]
	2	0	5.94	5.10	5.52±0.05[c]	61.37±0.50[c]
	1.5	0.5	4.35	6.13	5.24±0.38[b]	58.26±4.23[b]
	0.5	1.5	6.42	6.70	6.56±0.02[d]	72.85±0.17[d]
	0	2	4.21	6.06	5.14±0.02[b]	57.07±0.17[b]

Note: Notations accompanied by the same letters are not significantly different

Table 3. Effect of coating material concentration on moisture content and microcapsule AW

K-carrageenan (%)	I-carrageenan (%)	Chitosan (%)	Types of Probiotics	Water Content (%)	AW
2		0	L+B	8.86±0.06[b]	0.63±0.00[b]
1.5		0.5	L+B	7.44±0.56[a]	0.63±0.01[b]
0.5		1.5	L+B	8.89±0.01[b]	0.64±0.01[c]
0		2	L+B	7.29±0.60[a]	0.63±0.01[b]
	2	0	L+B	8.91±0.02[b]	0.63±0.00[b]
	1.5	0.5	L+B	7.63±0.58[a]	0.62±0.00[a]
	0.5	1.5	L+B	8.96±0.02[b]	0.63±0.00[b]
	0	2	L+B	7.92±0.99[a]	0.63±0.00[b]

The FTIR analysis of i-carrageenan and k-carrageenan was supported by standard carrageenan from Sigma products (Table 1). The absorption intensities corresponding to sulfate esters, galactose, and glycosidic bonds were classified as very strong. The presence of the galactose-2-sulfate functional group distinguished i-carrageenan, while the galactose-4-sulfate functional group was characteristic of k-carrageenan.

Table 1 shows that the observed functional groups in this study corresponded with those of standard carrageenan from Sigma, confirming that the materials used were iota carrageenan (Figure 1) and kappa carrageenan (Figure 2). Chitosan functional group analysis revealed O–H and N–H bonds at 3442.94 cm{ ¹, C–O–C bonds at 1157.29 cm{ ¹, and C–N bonds at 1317.33 cm{ ¹. These results were consistent with previously published data (Table 1), confirming the presence of chitosan in the materials used.

The combination of i-carrageenan–chitosan and k-carrageenan–chitosan formed interpenetrating polymer network (IPN) crosslinks, as shown in Figure 4. The IPNs are physically formed networks resembling woven patterns due to the combination of two or more polymers. An increased number of IPN crosslinks improved the quality of the microcapsule matrix by reducing material porosity and forming a tighter membrane structure. The i-carrageenan–chitosan mixture exhibited a higher number of IPN crosslinks (14 IPNs, Figure 1) compared with the k-carrageenan–chitosan mixture (9 IPNs, Figure 2). This difference was presumably due to the varying electrolyte content, with anionic i-carrageenan and cationic chitosan promoting the formation of ionic crosslinks, which enhanced IPN formation. Therefore, the i-carrageenan–chitosan mixture was considered to produce a microcapsule matrix of superior quality based on the larger number of IPN crosslinks formed. In addition, the analysis of the FTIR spectra also allowed identification of specific functional group regions by examining the wave numbers reported in the spectra.

Degree of Chitosan Deacetylation

The degree of deacetylation of chitosan was determined from the FTIR spectrum using the baseline method, as shown in Figure 3. This method relies on the influence of hydroxyl and amide groups. The hydroxyl group exhibited absorption at 3367 cm{ ¹, while the C=O group of the amide appeared at 1651 cm{ ¹. In Figure 3, the absorption peak for the hydroxyl group was observed at 3287.41 cm{ ¹, and the amide

absorption peak appeared at 1556.55 cm{ ¹. The degree of deacetylation of chitosan was calculated as 83.74%, indicating that 83.74% of the acetyl groups had been removed from chitin. Since chitin with a deacetylation degree greater than 50% is classified as chitosan, the material used in this study was confirmed as chitosan.

Viability of Probiotics and Yield of Encapsulation

The results of the statistical analysis showed that all treatments had significantly different effects ($P < 0.05$). Differences between treatments for probiotic viability and microcapsule encapsulation yield were further analyzed using the LSD test, as presented in Table 2.Table 2 shows that the highest probiotic viability and encapsulation yield were obtained in the i-carrageenan–chitosan treatment at a concentration ratio of 0.5%:1.5%, with an average of 6.56 log CFU/g (6.42 log CFU/g for *L. acidophilus* and 6.70 log CFU/g for *B. bifidum*) and 72.85% encapsulation yield. These results indicate that the combination of i-carrageenan and chitosan at these concentrations produced higher-quality microcapsules that better protected the viability of both probiotic strains.

The improved microcapsule quality in the i-carrageenan–chitosan 0.5%:1.5% treatment is likely due to the formation of strong microcapsule membranes during gelation. Polyelectrolyte complexation occurred between positively charged chitosan (due to amino groups) and negatively charged i-carrageenan (due to high sulfate content), resulting in a stable PEC (poly-electrolyte complex) coating. The PEC membranes were more resistant to pH changes and more effective in preventing the release of encapsulated material.

Additionally, i-carrageenan was expected to provide nutritional support for *B. bifidum* due to its calcium ion (Ca^2z) content. The Ca^2z ions are essential for the growth of Bifidobacteria and support the morphological transition from filamentous to rod-shaped (bacilli) cells, which are more resistant to freezing. However, for long-term storage, chitosan's antimicrobial properties could reduce probiotic viability. The antimicrobial activity of chitosan is influenced by its degree of deacetylation; higher deacetylation levels (up to 97.5%), which is increases positive charge density, strengthening electrostatic interactions and enhancing antimicrobial effects.

Despite this limitation, chitosan as a coating material offers advantages, including storage stability and controlled release of the core material. The microcapsules coated with chitosan exhibited low water vapor absorption and low solubility at neutral pH. To mitigate the antimicrobial effect, chitosan is often used in combination with other coating materials as co-protectors or within a double microencapsulation system, serving as the outer coating. Supporting these observations, previous research reported that using chitosan as an outer coating with Ca^2z ions in a double microencapsulation system based on alginate increased the adhesion of microparticles to the negatively charged intestinal mucosa (Oberoi et al., 2021).

Moisture Content and Water Activity (A_w) of Microcapsules

The results of the statistical analysis showed that all treatments had significantly different effects ($P < 0.05$). Differences between treatments for microcapsule moisture content and AW were further analyzed using the LSD test, as presented in Table 3. The Table 3 shows that the lowest microcapsule moisture content (7.29%) was obtained in the k-carrageenan:chitosan 0%:2% treatment. However, this value was only slightly lower than those observed in the k-carrageenan:chitosan 1.5%:0.5%, i-carrageenan:chitosan 1.5%:0.5%, and i-carrageenan:chitosan 0%:2% treatments, which exhibited moisture contents of 7.44%, 7.63%, and 7.92%, respectively. Similarly, the microcapsule A_W values were comparable across all treatments. These results indicate that the type and concentration of coating materials had minimal effect on the moisture content and A_W of the microcapsules.

The water content and A_W of microcapsules are generally influenced by the drying method and temperature. Spray drying at an inlet temperature of approximately 130 °C has been reported to produce probiotic powders with low moisture while maintaining high bacterial viability (Ghandi et al., 2012; Afzaal et al., 2020). For example, Ghandi et al. (2012) demonstrated that spray drying of *Lactococcus lactis ssp. cremoris* at an inlet of 130 °C and outlet of 65 °C yielded powders with reduced moisture content and stable probiotic survival. Other studies similarly reported that spray drying above 120 °C achieved moisture levels of 4–5% and A_W values near 0.26, while maintaining approximately 90% probiotic viability (Ghandi et al., 2012; Kakuda et al., 2023). These findings support the present results, where low water content and A_W values were observed, indicating that spray drying under controlled inlet temperatures effectively produced stable probiotic microcapsules suitable for long-term storage.

Conclusion

The use of chitosan with a degree of deacetylation of 83.5% in the k-carrageenan mixture resulted in the highest *B. bifidum* viability, as well as in the *L. acidophilus* + *B. bifidum* (L+B) mixture. The combination of i-carrageenan and chitosan as coating materials at a concentration ratio of 0.5%:1.5% produced the highest probiotic viability, with L+B reaching 6.56 log CFU/g and *B. bifidum* reaching 6.70 log CFU/g. The highest probiotic encapsulation yield for L+B was 72.85%. The microcapsules exhibited a water content of 8.96% (L+B) and a A_w of 0.63.

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References

Afoakwah, N. A., Komla, M. G., Ali, A., & Ahmed, S. (2023). Extraction, structural properties, and applications of carrageenan gum. In *Elsevier eBooks* (pp. 647–668). https://doi.org/10.1016/b978-0-323-99468-2.00024-3

Afzaal, M., Saeed, F., Hussain, S., Mohamed, A. A., Alamri, M. S., Hussain, M., Ateeq, H., Tufail, T., & Hussain, M. (2020). Survival and storage stability of encapsulated probiotic under simulated digestion conditions and on dried apple snacks. *Food Science and Nutrition*, *8*(10), 5392–5401. https://doi.org/10.1002/fsn3.1815

Altamirano Ríos, A. V., Guadarrama-Lezama, A. Y., Arroyo Maya, I. J., Hernández-Álvarez, A. J., & Orozco-Villafuerte, J. (2022). Effect of encapsulation methods and materials on the survival and viability of *Lactobacillus acidophilus*: A review. *International Journal of Food Science and Technology*, *57*(7), 4027–4040. https://doi.org/10.1111/ijfs.15779

Amiri, S., Nezamdoost-Sani, N., Mostashari, P., McClements, D. J., Marsza³ek, K., & Khaneghah, A. M. (2022). Effect of the molecular structure and mechanical properties of plant-based hydrogels in food systems to deliver probiotics: an updated review. *Critical Reviews in Food Science and Nutrition*, 1–27. https://doi.org/10.1080/10408398.2022.2121260

Buschmann, A. H., Camus, C., Infante, J., Neori, A., Israel, Á., Hernández-González, M. C., Pereda, S. V., Gómez Pinchetti, J. L., Golberg, A., Tadmor-Shalev, N., & Critchley, A. T. (2017). Seaweed production: overview of the global state of exploitation, farming and emerging research activity. *European Journal of Phycology*, *52*(4), 391–406. https://doi.org/10.1080/09670262.2017.1365175

Cristofori, F., Dargenio, V. N., Dargenio, C., Miniello, V. L., Barone, M., & Francavilla, R. (2021). Anti-Inflammatory and immunomodulatory effects of probiotics in gut inflammation: a door to the body. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/fimmu.2021.578386

Distantina, S., Wiratni, Fahrurrozi, M. F. M., & Rochmadi. (2011). Carrageenan Properties Extracted From Eucheuma cottonii, Indonesia. *World Academy of Science, Engineering and Technology, International Journal of Chemical, Molecular, Nuclear, Materials and Metallurgical Engineering*, *5*(6), 501–505. https://doi.org/10.5281/zenodo.1333328

Dounighi, N. M., Eskandari, R., Avadi, Zolfagharian, H., Sadeghi, A., & Rezayat, M. (2012). Preparation and in vitro characterization of chitosan nanoparticles containing Mesobuthus eupeus scorpion venom as an antigen delivery system. *Journal of Venomous Animals and Toxins Including Tropical Diseases*, *18*(1), 44–52. https://doi.org/10.1590/s1678-91992012000100006

Ermiº, E. (2021). A review of drying methods for improving the quality of probiotic powders and characterization. *Drying Technology*, *40*(11), 2199–2216. https://doi.org/10.1080/07373937.2021.1950169

Freile Pelegrín, Y., & Robledo, D. (2007). Carrageenan of Eucheuma isiforme (Solieriaceae, Rhodophyta) from Nicaragua. *Journal of Applied Phycology*, *20*(5), 537–541. https://doi.org/10.1007/s10811-007-9270-8

Gallardo, G., Guida, L., Martínez, V., López, M. C. S., Bernhardt, D., Blasco, R., Pedroza Islas, R., & Hermida, L. G. (2013). Microencapsulation of linseed oil by spray drying for functional food application. *Food Research International*, *52*(2), 473–482. https://doi.org/10.1016/j.foodres.2013.01.020

Ghandi, A., Powell, I. B., Chen, X. D., & Adhikari, B. (2012). The Effect of Dryer Inlet and Outlet Air Temperatures and Protectant Solids on the Survival of Lactococcus lactis during Spray Drying. *Drying Technology*, *30*(14), 1649-1657. https://doi.org/10.1080/07373937.2012.703743

Kakuda, L., Jaramillo, Y., Niño-Arias, F. C., Souza, M. F., Conceição, E. C., Alves, V. F., Almeida, O. G. G., De Martinis, E. C., & Oliveira, W. P. (2023). Process development for the spray-drying of probiotic bacteria and evaluation of the product quality. *Journal of Visualized Experiments*, (194). https://doi.org/10.3791/65192

Khojah, S. M. (2020). Bio-based Coating from Fish Gelatin, K-Carrageenan and Extract of Pomegranate Peels for Maintaining the Overall Qualities of Fish Fillet. *Journal of Aquatic Food Product Technology*, *29*(8), 810–822. https://doi.org/10.1080/10498850.2020.1718261

Koh, W. Y., Lim, X. X., Tan, T., Rovina, K., & Rasti, B. (2022). Encapsulated Probiotics: Potential techniques and coating materials for Non-Dairy Food Applications. *Applied Sciences*, *12*(19), 10005. https://doi.org/10.3390/app121910005

Krawczyk, B., Wityk, P., Ga³êcka, M., & Michalik, M. (2021). The Many Faces of Enterococcus spp.—Commensal, Probiotic and Opportunistic Pathogen. *Microorganisms*, *9*(9), 1900. https://doi.org/10.3390/microorganisms9091900

Li, S., Fan, L., X, S., Di, Q., Zhang, H., Li, B., & Liu, X. (2023). Validation of Layer-By-Layer Coating as a Procedure to Enhance *Lactobacillus plantarum* Survival during In Vitro Digestion, Storage, and Fermentation. *Journal of Agricultural and Food Chemistry*, *71*(3), 1701–1712. https://doi.org/10.1021/acs.jafc.2c07139

Li, Y., Cao, Z., Yu, Z., Zhu, Y., & Zhao, K. (2023). Effect of inoculating mixed starter cultures of Lactobacillus and Staphylococcus on bacterial communities and volatile flavor in fermented sausages. *Food Science and Human Wellness*, *12*(1), 200–211. https://doi.org/10.1016/j.fshw.2022.07.010

Menaa, F., Wijesinghe, U., Thiripuranathar, G., Althobaiti, N. A., Albalawi, A. E., Khan, B. A., & Menaa, B. (2021). Marine Algae-Derived Bioactive Compounds: a new wave of nanodrugs? *Marine Drugs*, *19*(9), 484. https://doi.org/10.3390/md19090484

Mirzaei, R., Bouzari, B., Hosseini Fard, S. R., Mazaheri, M. A., Ahmadyousefi, Y., Abdi, M., Jalalifar, S., Karimitabar, Z., Teimoori, A., Keyvani, H., Zamani, F., Yousefimashouf, R., & Karampoor, S. (2021). Role of microbiota-derived short-chain fatty acids in nervous system disorders. *Biomedicine & Pharmacotherapy*, *139*, 111661. https://doi.org/10.1016/j.biopha.2021.111661

Nezamdoost-Sani, N., Khaledabad, M. A., Amiri, S., & Khaneghah, A. M. (2023). Alginate and derivatives hydrogels in encapsulation of probiotic bacteria: An updated review. *Food Bioscience*, *52*, 102433. https://doi.org/10.1016/j.fbio.2023.102433

Nowacki, K., Stêpniak, I., Langer, E., Tsurkan, M. V., Wysokowski, M., Petrenko, I., Khrunyk, Y., Fursov, A., Bo, M., Bavestrello, G., Joseph, Y., & Ehrlich, H. (2020). Electrochemical Approach for Isolation of Chitin from the Skeleton of the Black Coral Cirrhipathes sp. (Antipatharia). *Marine Drugs*, *18*(6), 297. https://doi.org/10.3390/md18060297

Oberoi, K., Tolun, A., Altýntaþ, Z., & Sharma, S. (2021). Effect of Alginate-Microencapsulated Hydrogels on the Survival of Lactobacillus rhamnosus under Simulated Gastrointestinal Conditions. *Foods*, *10*(9), 1999. https://doi.org/10.3390/foods10091999

Pal, K., Bharti, D., Sarkar, P., Anis, A., Kim, D., Cha³as, R., Maksymiuk, P., Stachurski, P., & Jarzêbski, M. (2021). Selected applications of Chitosan Composites. *International Journal of Molecular Sciences*, *22*(20), 10968. https://doi.org/10.3390/ijms222010968

Pellis, A., Guebitz, G. M., & Nyanhongo, G. S. (2022). Chitosan: Sources, processing and modification techniques. *Gels*, *8*(7), 393. https://doi.org/10.3390/gels8070393

Ren, H., Deng, Y., & Wang, X. (2022). Effect of a compound starter cultures inoculation on bacterial profile and biogenic amine accumulation in Chinese Sichuan sausages. *Food Science and Human Wellness*, *11*(2), 341–348. https://doi.org/10.1016/j.fshw.2021.11.009

Sakandar, H. A., & Zhang, H. (2021). Trends in Probiotic(s)-Fermented milks and their in vivo functionality: A review. *Trends in Food Science and Technology*, *110*, 55–65. https://doi.org/10.1016/j.tifs.2021.01.054

Sakoui, S., Derdak, R., Pop, O. L., Vodnar, D. C., Addoum, B., Teleky, B., Elemer, S., Elmakssoudi, A., Suharoschi, R., Soukri, A., & Khalfi, B. E. (2022). Effect of encapsulated probiotic in Inulin-Maltodextrin-Sodium alginate matrix on the viability of Enterococcus mundtii SRBG1 and the rheological parameters of fermented milk. *Current Research in Food Science*, *5*, 1713–1719. https://doi.org/10.1016/j.crfs.2022.09.027

Tegegne, B. A., & Kebede, B. (2022). Probiotics, their prophylactic and therapeutic applications in human health development: A review of the literature. *Heliyon*, *8*(6), e09725. https://doi.org/10.1016/j.heliyon.2022.e09725

Wardhana, Y. W., Aanisah, N., Sopyan, I., Hendriani, R., & Chaerunisaa, A. Y. (2022). Gelling Power Alteration on Kappa-Carrageenan Dispersion through Esterification Method with Different Fatty Acid Saturation. *Gels*, *8*(11), 752. https://doi.org/10.3390/gels8110752

Weißpflog, J., Vehlow, D., Müller, M., Kohn, B., Scheler, U., Boye, S., & Schwarz, S. (2021). Characterization of chitosan with different degree of deacetylation and equal viscosity in dissolved and solid state – Insights by various complimentary methods. *International Journal of Biological Macromolecules*, *171*, 242–261. https://doi.org/10.1016/j.ijbiomac.2021.01.010

Zhu, Y., Wang, Z., Bai, L., Deng, J., & Zhou, Q. (2021). Biomaterial-based encapsulated probiotics for biomedical applications: Current status and future perspectives. *Materials & Design*, *210*, 110018. https://doi.org/10.1016/j.matdes.2021.110018