Encapsulation of Probiotic by Gellan Gum-Protein Peptide and Polysaccharides

Yanisa Sangkamjan[1], *Wiboon* Riansa-ngawong[1], *Sriwiang* Rittisak[2], *Kriangkrai* Phattayakorn[3], and *Wanticha* Savedboworn[1*]

[1] Department of Agro-Industry Technology and Management, Faculty of Digital Agro-Industry, King Mongkut's University of Technology North Bangkok, Noen Hom, 25230 Prachin Buri, Thailand
[2] Department of Innovation and Product Development Technology, Faculty of Digital Agro-Industry, King Mongkut's University of Technology North Bangkok, Noen Hom, 25230 Prachin Buri, Thailand
[3] Department of Food Technology and Nutrition, Faculty of Natural Resources and Agro-Industry, Kasetsart University, Chiang Khruea, Mueang Sakon Nakhon, 4700 Sakon Nakhon, Thailand

Abstract. Over the years, consumers have become more interested in healthy eating. As a result, the probiotic food market has grown. The development of these products ensures that probiotics remain intact throughout storage. This study examined the survival of the probiotic *Lactobacillus casei* TISTR 1463 through an encapsulation process using extrusion techniques. Encapsulation of probiotics using alginate, gellan gum, sea cucumber peptides and konjac glucomannan coated with chitosan and fucoidan (AGSK-CF) had the highest encapsulation efficiency (%EE) of 99.275%. The results of light microscopy and stereo microscopy showed that gel beads had spherical, elliptical shapes and smooth surface. Free water content values were found in the range of 0.972-0.974. Control exhibited more green than other encapsulants. Hue values of most samples tended to be yellow and chroma values were in the range of 6 – 14. Mechanical characterization analysis of samples showed that AGSK-CF exhibited higher values of Coating Hardness (N), Interior Hardness (N) and Work of cutting/Toughness (N/sec) than other samples.

1 Introduction

Over the past decade, consumer interested in healthy eating has grown steadily, leading to a rise in the availability of probiotic-fortified foods. A major challenge in developing these products have to ensure that probiotics remained viable throughout storage. According to Global Market Insights, the probiotic market was valued at US$5.56 billions in 2023 and is expected to grow at a compound annual growth rate (CAGR) of 6.9% from 2024 to 2032, reaching US$10.28 billions. [1]. One effective solution to improve probiotic stability is encapsulation which protects probiotics from harsh environmental conditions and helps them survive exposure to stomach acid during digestion. Probiotics have gained attention due to their ability to control and maintain microbial ecosystems in the human gastrointestinal tract (GI) [2]. However, the effectiveness and survival rate of probiotics are influenced by several factors including the presence of acids, and bile nutrient depletion, osmotic pressure and oxidative stress [3]. Encapsulation is a technique used to enhance the survival of probiotics by enclosing probiotics cells within a protective material. This barrier helps prevent the degradation of the probiotics, reduces their evaporation rate, and masks any unpleasant tastes, colors, or odors [4]. Encapsulation methods, such as extrusion and emulsification, have been effectively used to protect probiotic cells. These techniques improve cells stability and survival under harsh conditions, including the acidic environment of the stomach, digestive enzymes the presence of bile salts, oxygen, and other microorganisms in the upper gastrointestinal tract [5].

Encapsulants are materials used to protect probiotic strains and support their growth. One of the most commonly used encapsulants is alginate, an edible polymer made up of polysaccharides with strong gelling properties. When alginate interacts with divalent ions such as calcium (Ca^{2+}) or magnesium (Mg^{2+}), it forms a stable three-dimensional gel bead. However, alginate alone is not stable in highly acidic environments, especially at pH levels below 2. To improve its performance under these conditions, it is often combined with other hydrogels, such as proteins or prebiotics, which can enhance the stability and provide additional benefits [6, 7]. Another effective encapsulating material is gellan gum, an anionic polysaccharide that also forms gels in the presence of cations like sodium (Na^{2+}), potassium (K^{+}), and calcium (Ca^{2+}). Gellan gum is more stable than alginate at high temperatures and in acidic environments, and it resists rapid breakdown, making it a strong candidate for probiotic delivery systems [6, 7].

In addition, the incorporation of gellan gum with other polymers enhances gel pellet strength and improves the survival of probiotics in simulated gastric fluid (SGF) [8]. Based on the research of Thinkohkaew et al. [9], encapsulated *Lactobacillus. casei* with a combination of

*Corresponding author: wanticha.s@agro.kmutnb.ac.th

alginate and gellan gum with chitosan coating showed encapsulation efficiency (%EE) of 96.7%. Furthermore, after 6 hours of immersion in intestinal fluid, the gel tablets maintained probiotic levels above 8 log CFU/g even after 30 days of storage.

Numerous studies have demonstrated that incorporating proteins enhanced the efficiency of probiotic encapsulation. Sea cucumber peptides (SCPs), derived from the skin of sea cucumbers, are formed through the self-aggregation of small peptides into fibrous structures, which can bind solvents and form gels. Researches indicated that both temperature and acid concentration significantly influence the quality of sea cucumbers, as the rigid wall structure of sea cucumbers becomes more flexible under heat and acidic conditions, eventually forming a gel-like texture [10]. de Farias et al. [11], suggested that polysaccharides when combined with gelling agents like sea cucumbers, enhanced the protective matrix around probiotics and contributed to positively effect on gastrointestinal health.

In addition, the preservation of encapsulated probiotics to a high survival number can be improved by coating a layer of gel pellets, of which chitosan is one of the most widely used materials. This was probably because a positive electrostatic charge that can be encapsulated with negatively charged of probiotics. This technique has advantages to protect probiotics in the stomach system, severe pH and heat treatment [12]. Fucoidan is polysaccharides located in the cell walls of algae, soluted in water [13]. and known for its high stability under acidic and alkaline conditions. Fucoidan also has a strong ability to bind to compounds [14].

A major challenge in developing encapsulation techniques is ensuring that post-encapsulation probiotics remain survive throughout storage. This research explored the use of alginate and gellan gum as encapsulants, both of which have the ability to create strong gel and are resistant to acidic environments. The incorporation of sea cucumbers in food products presents an interesting option for the future. Sea cucumber peptides enhance the encapsulation efficiency and flexibility of gel beads, which in turn enhances the viability of probiotics under gastrointestinal conditions. In addition, the coating of survival rate more than one layer increases the survival rate of probiotics under inappropriate conditions [10, 13].

The objective of this research was to evaluate the survival of *L. casei* TISTR 1463 encapsulated with sea cucumber peptides and different polysaccharides including konjac glucomannan, yeast β-glucan and *Lycium barbarum* and coated with chitosan - fucoidan. The determination of physical properties including size, a_w, color values, and mechanical characteristics were also conducted.

2 Materials and methods

2.1 Preparation of probiotic *L. casei* TISTR 1463

A culture of *L. casei* TISTR 1463 was grown in MRS broth at 37°C for 30 hr. The culture was then centrifuged at 4000 rpm for 15 min, yielding approximately 5 mL of cell pellets. The drop plate method was used to determine the initial microbial load, which was approximately 10 log CFU/g. The pellet was then mixed with the encapsulating agent as described in section 2.2 [1].

2.2 Preparation of encapsulation probiotics

Solutions of 1.5% sodium alginate (w/v) (Aldrich, Iceland) and 0.5% gellan gum (w/v) (MySkinRecipes,Thailand) were prepared, along with a 1% sea cucumber peptide solution (w/v) (MySkinRecipes,Thailand). Encapsulant formulations were also prepared by combining each with 1% (w/v) of various polysaccharide, as shown in Table 2-1. The mixtures were thoroughly blended, and 5 mL obtained from section 2.1 were added. Mixtures were then transferred into a 20G syringe and dropped into 100 mL of 0.2 M $CaCl_2$ solution under constant stirring to form gel beads.

The resulting gel beads were soaked on and stirred gently for 30 minutes, then washed with 100 mL of 0.1% (w/v) sterile peptone solution under gentle agitation for 10 seconds. Encapsulated beads were coated using chitosan and fucoidan solutions. Coating solutions of 0.3% chitosan (w/v) (Aldrich, Iceland) and 0.3% fucoidan (w/v) (MySkinRecipes, Thailand) were dissolved in 100 mL of water. The beads were soaked in the chitosan solution and stirred gently for 30 minutes to form the first coating layer.

A second layer was then applied using fucoidan under the same condition. After coating, the gel beads were collected and the encapsulation efficiency (%EE) of each encapsulant formulation was calculated [1, 15].

Table 2-1. Encapsulated probiotic treatments.

Abbreviation	Materials	1% Sea cucumber peptide	1% Polysaccharides	Chitosan - Fucoidan coating
Control	1.5%Alginate + 0.5%Gellan gum	✖	✖	✖
AGS-CF		✔	✖	✔
AGSK-CF		✔	Konjac glucomannan	✔
AGSY-CF		✔	Yeast β – glucan	✔
AGSL-CF		✔	*Lycium barbarum*	✔

Note: Control: 1.5%Alginate + 0.5%Gellan gum; **AGS-CF:** 1.5%Alginate + 0.5%Gellan gum + 1%Sea cucumber peptide (Coating with Chitosan and Fucoidan); **AGSK-CF:** 1.5%Alginate + 0.5%Gellan gum + 1%Sea cucumber peptide + 1% Konjac glucomannan (Coating with Chitosan and Fucoidan); **AGSY-CF:** 1.5%Alginate + 0.5%Gellan gum + 1%Sea cucumber peptide + 1%Yeast β – glucan (Coating with Chitosan and Fucoidan); **AGSL-CF:** 1.5%Alginate + 0.5%Gellan gum + 1%Sea cucumber peptide + 1% *Lycium barbarum* (Coating with Chitosan and Fucoidan)

2.3 Probiotic cell encapsulation efficacy test

0.5 gram sample of gel beads were added to 4.5 mL of 2% sodium citrate solution and allowed to stand for 10 minutes to release the encapsulated cells. The suspension was then diluted with 0.1% (w/v) steriled peptone solution. The number of viable cells were determined using the drop plate method on MRS agar (Himedia, India) containing 0.5% calcium carbonate. The plates were incubated at 37°C for 30 hr. The results were reported as log CFU/g. Encapsulation efficiency was calculated using the following equation.

$$\text{Encapsulation efficiency (\%)} = \frac{xt}{x0} \times 100 \qquad (1)$$

Assign X_0 as the number of viable cells prior to encapsulation (log CFU/g)
X_t is the number of viable cells after encapsulation (log CFU/g)

2.4 Free water content measurement (a_w)

The water activity (a_w) value was measured using an AquaLab 3TE a_w meter. Two grams of gel beads were placed in a sample container for each measurement. Three measurements were taken to collect consistent analysis results.

2.5 Color measurement

Color analysis was performed using a ColorFlex colorimeter (HunterLab, USA). Two grams of gel beads were placed in a sample container, and the color was measured using the CIELAB color system (CIELAB). The results were displayed on a computer screen, and three measurements were taken to ensure consistency.

2.6 Measuring the size of gel beads

Ten gel beads were randomly selected, and their sizes were measured in millimetres (mm) using a digital vernier calliper. The average size was then calculated from the measured values.

2.7 Gel beads characteristics by a conventional optical microscope and a stereo microscope

Gel bead were placed on a glass slide and examined under a microscope (NIKON, Japan) using standard lighting. The microscope was set to 40X magnification by combining a 4X objective lens with a 10X ocular lens. The image was adjusted for clarity, and then saved. Gel beads were on the stereo microscope (Motic, China) saucer and placed a black pad under the gel saucer.

2.8 Mechanical characterization analysis

One gel tablet was placed in the Texture Analyser (T. A. XT. plus, Thailand) to determine its pressure resistance (MPa). A selected indenter was mounted on the device and applied vertical pressure from top to bottom. During compression, the force exerted on the indenter was recorded. The results were then averaged across the tested samples [16].

2.9 Statistical analysis

A factorial experiment was planned to test the differences in different encapsulants by analyzing the variance by ANOVA at a confidence level of 95%, and analyzing the differences in the means by means (Duncan's new Multiple Range Test, DMRT) using the statistical SPSS version 28.0 (IBM SPSS, USA).

3 Results and discussions

3.1 Encapsulation efficiency of probiotics with various encapsulants

Encapsulation efficiency (EE) indicates the quantity of probiotics contained with in a particle or structure [4]. According to Figure 3-1, it can be seen that AGSK-CF added with konjac glucomannan has the highest encapsulation efficiency of 99.275%, and control showed the lowest encapsulation efficiency of 97.502%. This was probably because control treatment did not contain polysaccharides as an adjunct supplemented in encapsulation and peptides from sea cucumbers to reinforce the encapsulant spaces or pores. The supplementation of konjac glucomannan with other polymers helps form elastic gels, promotes the growth of beneficial bacteria, and also serves as a prebiotic source [17-19]. This is consistent with the experimental results of Phumsombat et al. [20], that *L. rhamnosus* L34 and *L. acidophilus* LA5 encapsulated with konjac glucomannan exhibited a maximum survival rate of 97.92% and 88.94%, respectively. AGSY-CF, AGSL-CF and AGS-CF supplemented with polysaccharide, sea cucumber peptides, and yeast β-glucan had the encapsulation efficiency lower than konjac glucomannan with the encapsulation efficiency of 98.068%, 98.115% and 98.188%, respectively, with no statistically significant difference ($p \geq 0.05$). Polysaccharide and yeast β - glucan are substances influenced the growth of beneficial bacteria. Polysaccharide is a source of nutrients, allowing probiotics to be used in intestinal growth. Polysaccharide also has a surface suitable for probiotic adhesion and increases transmission live probiotics to the small intestine [21]. In addition, the structure of yeast β - Glucan is porous. This makes probiotics easily, attached to the mesh structure, increasing the survival rate of probiotics [22]. Several studies have confirmed that both temperature and acid concentration influence the quality of sea cucumber peptides. When exposed to heat and acid, these peptides gradually transform into a flexible gel structure [23]. This gel is effective in encapsulating probiotics and significantly enhances their survival rate after encapsulation. In this study, the control treatment was encapsulated using both alginate and gellan gum without the addition of polysaccharide and coating material. Employing alginate solely for cell encapsulation produced highly porous gel beads [10]. However, when encapsulated with other polymers, the gel was formed with calcium ions (Ca^{2+}) and other cations of sodium alginate. Therefore, the structure was similar to "egg box junction", making the structure stronger than alginate alone [22]. Encapsulating more than 0.1% alginate stabilized the gel beads and increased their strength [22]. In addition, the two-layer coating (layer by layer) also increased the efficiency of encapsulation. The first layer of the coating was chitosan, and fucoidan was used as the second layer. Kraithong et al. [24] proposed that fucoidan may occupy spaces between the bonds of encapsulated substances, there by enhancing encapsulation efficiency. Upon analyzing the statistical results, the comparison of the survival efficacy among the five probiotic samples revealed no statistically significant difference.

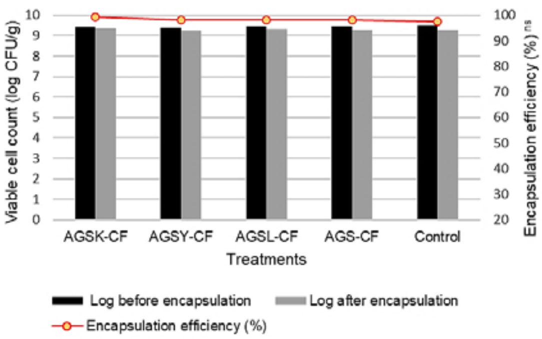

Fig. 3-1. Viable cell count and encapsulation efficiency of encapsulated probiotics with various encapsulants.
Note: ns Revealed no statistically significant difference. ($p \geq 0.05$).

3.2 Size of gel beads

Measurements of the gel granule size, following encapsulation via the extrusion technique, were conducted to assess their consistency. As shown in Table 3-2, the results revealed that sizes of AGSK-CF, AGSY-CF and AGSL-CF ranged from of 3.330 to 3.340 mm, which was not significantly difference ($p \geq 0.05$). In addition, gel tablets with polysaccharides showed a slightly larger size than controland AGS-CF. The larger gel pellet size and higher viscosity were observed in the samples containing polysaccharides due to the distinct properties of the polysaccharides used as encapsulants. These polysaccharides form a more robust gel matrix compared to control and AGS-CF samples. Without polysaccharides added, prepared gel solution with lower viscosity was observed [9]. This may come from the sample being very viscous with a slow flow rate through the needle tips, resulting in a large size of gel pellets. It has a positive effect on the survival of probiotic cells. This is because when the gel pellets are large, the surface area will be less exposed to various conditions, reducing the death of probiotics. While small gel pellets have a large surface area exposed to various conditions, increasing the death of probiotics [25].

Table 3-2. Size of microcapsule pellets.

Samples	Size of encapsulated beads ns (mm) ± SD
Control	3.300±0.240
AGS-CF	3.310±0.197
AGSK-CF	3.330±0.195
AGSY-CF	3.340±0.143
AGSL-CF	3.330±0.258

Note: ns Revealed no statistically significant difference.($p \geq 0.05$).

3.3 Physical analysis under light microscope and stereo microscope of gel beads

Structural characterization of gel pellets using conventional light microscopy and stereo microscopy revealed structural features. As shown in Figure 3-2, the gel beads were spherical, elliptical shapes, and smooth under a light microscope and a stereo microscope. Using alginate alone has a disadvantage, such as large porosity which affects cell encapsulation [9]. The addition of gellan gum effectively affects the strength of the gel and the survival of probiotic cells. This aligns with the findings of Koh et al. [26], which indicated that the combination of alginate and gellan gum enhances biological function and the flexible modulus of gel beads, thereby promoting structural cohesion.

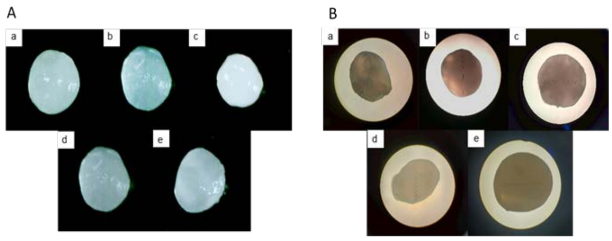

Fig. 3-2. Gel beads under a light microscopy (A) and a stereo microscopy (B)
Note: a: AGSK-CF; b: AGSY-CF; c: AGSL-CF; d: AGS-CF; e: Control

3.4 Free water content (a_w) of the gel beads

The a_w value is one of the most important variables indicating the shelf life of probiotics. The samples had free water contents ranged from of 0.972 to 0.974, which was not significant difference ($p \geq 0.05$) (Table 3-3). This is consistent with the reporting of Rather et al. [27], stated that the presence of prebiotics could protect the probiotics from moisture and had no effect on the a_w value. Moreover, alginate helps encapsulate probiotics to pass well into the intestines. It is estimated to have a pore size of less than 17 nm [28].

https://doi.org/10.1051/e3sconf/202567901023

Table 3-3. Free water contents of various microcapsules

Samples	Free water content ns (a_w)± SD
Control	0.972±0.001
AGS-CF	0.973±0.002
AGSK-CF	0.973±0.002
AGSY-CF	0.972±0.002
AGSL-CF	0.974±0.001

Note: ns Revealed no statistically significant difference. (p≥0.05).

3.5 Colorimetric measurement

Color measurement of gel beads was shown in Table 3-4. Control sample showed a value of –a* greater than other samples. The mixture of alginate and gellan gum causes green color more than other encapsulated beads, and also exhibited the highest brightness. However, AGSK-CF has a lower brightness value than control sample, and the a* value tended to be more yellow than the control sample. The +b* value tended to be less green than the other treatments, resulting in a pale yellow-green color for the gel beads. In AGSY-CF and AGS-CF, a similar brightness and the -a* and +b* values were found, and tended to have same direction resulted in a yellow-green color of gel beads. AGSY-CF showed a slightly darker color. However, AGSL-CF has a lower brightness value than other samples because *Lycium barbarum* has a reddish-brown color with a tendency of +a* value indicating redness and +b* value indicating yellowness. Hue value indicated the color tone or the color of the sample (0° or 360° = red, 90° = yellow, 180° = green, and 270° = blue). Most samples tended to be yellow with hue values in the range of 95°- 106°. Chroma value is an indicator of the color intensity of or the color saturation. Chroma values of encapsulated beads were in the range of 6-14. Sample with a high chroma value exhibited more vivid or saturated [29]. All samples were green due to the color of fucoidan, which is consistent with the experimental results of Morsy et al. [30], that *Lactobacillus rhamnosus* GG was encapsulated with alginate and anthocyanin, which gave the structure of the gel granules a red color caused by the anthocyanin pigment.

3.6 Mechanical characterization analysis of gel beads

The use of different encapsulating agents may affect the properties of the gel beads. The mechanical strength of the gel beads indicated the encapsulation efficiency of the probiotic. From the experimental results in Table 3-5, it was found that AGSK-CF samples had the highest values of Coating Hardness (N), Interior Hardness (N), and Work of cutting/Toughness (N/sec) than other encapsulating agents, resulting in higher gel compressive force than the other samples. Konjac glucomannan was encapsulating agent which showed the highest pressure resistance. The addition of konjac glucomannan together with gellan gum can improve the flow characteristics and the formation of gels with better elasticity [17, 18]. This is consistent with the experimental results of Lia et al. [31], that alginate enhances the hardness of the gel granules, with an average hardness of 131.1 ± 9.7 N. However, adding pectin, gellan gum, and κ-carrageenan decreased the hardness while increasing the elasticity of the gel granules.

AGS-CF sample had higher Coating Hardness (N), Interior Hardness (N), and Work of cutting/Toughness (N/sec) values than control sample. This is because the sea cucumber peptides are formed by the self-assembly of small peptides into fibers, which can bind to the solvent and form a gel. By using sea cucumber peptides as an encapsulating agent, many reports have confirmed that heating and increasing the concentration of acid will help sea cucumber peptides form gels. The inflexible sea cucumber surface wall will gradually change to a flexible gel structure when exposed to heat and acid [11]. As a result, the samples with sea cucumber peptides were more flexible than the control sample, and required higher compression force.

Table 3-4. Color values of encapsulated gel with different encapsulants

Samples	Color values of encapsulated pellets				
	L*	a*	b*	Hue	Chroma
Control	64.290±0.360[a]	-1.907±0.067[a]	8.743±0.117[c]	102.323±0.331[b]	8.947±0.126[c]
AGS-CF	62.867±0.015[c]	-1.503±0.046[b]	9.633±0.031b[c]	98.893±0.237[c]	9.750±0.036b[c]
AGSK-CF	64.187±0.189[a]	-1.817±0.012[a]	6.180±0.053[d]	106.387±0.117[a]	6.443±0.057[d]
AGSY-CF	63.317±0.025[b]	-1.117±0.035[c]	10.730±0.075[b]	95.927±0.166[c]	10.783±0.076[b]
AGSL-CF	59.407±0.029[d]	0.203±0.061[d]	14.343±0.117[a]	89.187±0.244[d]	14.343±0.117[a]

Note: The superscripts (a-d) in each column mean statistical significant difference ($p<0.05$)

Table 3-5. Strength values of encapsulated pellets with different encapsulants

Samples	Strength values of encapsulated pellets		
	Coating Hardness (N)	Interior Hardness (N)	Work of cutting/Toughness (N.sec)
Control	0.521±0.018[d]	0.521±0.018[d]	0.142±0.003[d]
AGS-CF	0.523±0.014[c]	0.523±0.014[c]	0.147±0.002[c]
AGSK-CF	0.527±0.018[a]	0.527±0.018[a]	0.153±0.003[a]
AGSY-CF	0.526±0.024[ab]	0.526±0.024[ab]	0.150±0.002[ab]
AGSL-CF	0.526±0.025[ab]	0.526±0.025[ab]	0.150±0.006[ab]

Note: The superscripts (a-d) in each column mean statistical significant difference ($p<0.05$)

4 Conclusion

An encapsulation experiment of *Lactobacillus casei* TISTR 1463 was conducted using the extrusion technique. AGSK-CF showed the highest encapsulation efficiency of 99.275%, which positively affects the survival of probiotics. The gel bead sizes were in the range of 3.300-3.340 mm and the size of the gel beads with different compositions were not significantly different (p≥0.5). Gel beads were spherical, elliptical, and smooth under light microscopy and stereo microscopy. The free water content values were in the range of 0.972-0.974. Prebiotic supplementation could protect the probiotics from moisture. Therefore, it did not affect the change of a_w value. The color analysis of the samples showed that AGSK-CF were green and the brightest. AGSL-CF is less bright than the other samples. The hue values of most samples tended to be yellow with values in the range of 95°-106°. Chroma values of samples were in the range of 6-14. In addition, the mechanical analysis showed that AGSK-CF has higher Coating Hardness (N), Interior Hardness (N), and Work of cutting/Toughness (N/sec) values than the other coated samples. The addition of konjac glucomannan together with gellan gum (AGSK-CF) can help in the formation of gels with better elasticity, resulting in requiring more pressure to compress the gel beads than other samples. Based on all experimental results, AGSK-CF exhibited higher survival efficiency and also showed higher Coating Hardness (N), Interior Hardness (N), and Work of cutting/Toughness (N/sec) values than the other coated samples. However, the study of the number of probiotics that survive through the gastrointestinal tract and storage needs to be further studied.

References

1. K. Pulidindi, K. Ahuja, Probiotics Market Size - By Ingredients (Lactobacilli, Bifidobacterium, Streptococcus, Bacillus), By End-Use (Human, Animal), By Application (Food and Beverages, Dietary Supplements, Animal Feed) & Forecast. 2024 – 2032 (2023). https://www.gminsights.com/toc/details/probiotics-market.
2. P. Apiwattanasiri, S. Jantrasee, R. Charoen, W. Savedboworn, S. Rittisak, Co-encapsulation efficiency of silk sericin-alginate-prebiotics and the effectiveness of silk sericin coating layer on the survival of probiotic Lactobacillus casei, Food Bioscience. **46**, 101576 (2022). https://doi.org/10.1016/j.fbio.2022.101576
3. C. Sun, M. Zhang, D. Feng, S. Wang, M. Li, Effects of dietary D-mannose supplementation on growth performance, intestinal digestive capacity, gut microbiota, and ammonia tolerance of largemouth bass

micropterus salmoides, Aquaculture Reports **36**, 102054 (2024). https://doi.org/10.1016/j.aqrep.2024.102054

4. J. M. Bae, Prophylactic efficacy of probiotics on travelers' diarrhea: An adaptive meta-analysis of randomized controlled trials. Epidemiol, Health. **40**, (2018). https://doi.org10.4178/ epih.e2018043
5. F. J. Rodrigues, M. F. Cedran, J. L. Bicas, H. H. Sato, Encapsulated probiotic cells: relevant techniques, natural sources as encapsulating materials and food applications-a narrative review. Food Res Int. **137**, 1016 (2020). https://doi.org/10.1016/ j.foodres. 2020.109682
6. D. Gomes, J. P. Batista-Silva, A. Sousa, L. A. Passarinha, Progress and opportunities in gellan gum-based materials: A review of preparation, characterization and emerging applications, Carbohydrate Polymers, **311**, 120782 (2023). https://doi.org10.1016/ j.carbpol.2023.120782
7. S. Rittisak, R. Charoen, S. Seamsin, J. Buppha, T. Ruangthong, S. Muenkhling' W. Riansa ngawong, S. Eadmusik, K. Phattayakorn, S. Jantrasee, W. Savedboworn, Effects of incorporated collagen/prebiotics and different coating substances on the survival rate of encapsulated probiotics, Food Sci Biotechnol, **34**, 1383–1399 (2025). https://doi.org/ 10.1007/s10068-024-01794-8
8. A. Nag, K.S. Han, H. Singh, Microencapsulation of probiotic bacteria using pH-Induced gelation of sodium caseinate and gellan gum, Int. Dairy J. **21**, 247–253 (2011). https://doi.org/ 10.1016/j.idairyj.2010.11.002
9. K. Thinkohkaew, V. Jonjaroen, N. Niamsir,A. Panya, I. Suppavorasatit, P. Potiyaraj, Microencapsulation of probiotics in chitosan-coated alginate/gellan gum: Optimization for viability and stability enhancement, Food Hydrocolloids. **151**, 109788 (2024). https://doi.org/10.1016/j.foodhyd.2024.109788
10. W. J. Li, L. Li, W. Y. Zhen, L. F. Wang, M. Pan, J. Qian Lv, F. Wang, Y.F.Yao, S. P. Nie, M.Y. Xie, Ganoderma atrum polysaccharide ameliorates ROS generation and apoptosis in spleen and thymus of immunosuppressed mice, Food Chem Toxicol. **99**, 199–208 (2017). https://doi.org/10.1016/j.fct.2016.11.033
11. T. G. S. de Farias, H. F. L. Ladislau, T. C. M. Stamford, J. A. C. Medeiros, B. L. M. Soares, T. M. S. Arnaud, T. L. M. Stamford, Viabilities of Lactobacillus rhamnosus ASCC 290 and Lactobacillus casei ATCC 334 (in free form or encapsulated with calcium alginate-chitosan) in yellow mombin ice cream, LWT. **100**, 391-396 (2019). https://doi.org10.1016/ j.lwt.2018.10.084
12. Y. Zhu, Z. Wang, L. Bai, J. Deng, Q. Zhou, Biomaterial-based encapsulated probiotics for biomedical applications : current status and future perspectives, Materials and Design. **210**, 110018 (2021). https://doi.org/10.1016/ j.matdes.2021.110018
13. C. L. Okolie, B. Mason, A. Mohan, N. Pitts, C. C. Udenigwe, Extraction technology impacts on the structure-function relationship between sodium alginate extracts and their in vitro prebiotic activity, Food Bioscience. **37**, (2020). https://doi.org/10.1016/j.fbio.2020.100672
14. R. Sakthivel, K. P. Devi, Antioxidant, anti-inflammatory and anticancer potential of natural bioactive compounds from seaweeds, Studies in Natural Products Chemistry. **63**, 113–160 (2019). https://doi.org/10.1016/B978-0-12-817901-7.00005-8
15. H. Michida, S. Tamalampudi, S. S. Pandiella, C. Webb, H. Fukuda, A. Kondo, Effect of cereal extracts and cereal fiber on viability of Lactobacillus plantarum under gastrointestinal tract conditions, Biochem Eng J. **28**, 73-78 (2006). https://doi.org/10.1016/j.bej.2005. 09.004
16. A. Kistaubayeva, M. Abdulzhanova, S. Zhantlessova, I. Savitskaya, T. Karpenyuk, A. Goncharova, Y. Sinyavskiy, The Effect of encapsulating a prebiotic-based biopolymer delivery system for enhanced probiotic survival, Polymers. **15**, 1752 (2023). https://doi.org10.3390/polym15071752
17. Y. Yuan, L. Wang, R.J. Mu, J. Gong, Y. Wang, Y. Li, J. Ma, J. Pang, C. Wu, Effects of konjac glucomannan on the structure, properties, and drug release characteristics of agarose hydrogels, Carbohydrate Polymers. **190**, 196–203 (2018). https://doi.org/10.1016/ j.carbpol. 2018.02.049
18. D. Qiao, W. Shi, M. Luo, F. Jiang, B. Zhang, Poly vinyl alcohol inclusion can optimize the sol-gel, mechanical and hydrophobic features of agar/konjac glucomannan system, Carbohydrate Polymers. **277**, 118879 (2022). https://doi.org/10.1016/j.carbpol.2021.118879
19. R. A. Rastall, V.Maitin, Prebiotics and synbiotics: towards the next generation, Curr Opin Biotechnol, **13**, 490-496 (2002). https://doi.org/ 10.1016/ S0958-1669(02)00365-8
20. P. Phumsombat, S. Lekhavat, C. Borompichaichartkul, S. Devahastin, N. Chiewchan, Enhancing probiotic encapsulation with konjac glucomannan hydrolysate, IJFST. **59**, 7887–7896 (2024). https://doi.org/ 10.1111/ijfs.17084
21. H. Liu, M. Xie S. Nie, Recent trends and applications of polysaccharides for microencapsulation of probiotics, Food Frontiers. **1**, 45–59 (2020). https://doi.org/10.1002/fft2.11

22. A. Shah, A. Gani, M. Ahmad, B. A. Ashwar, F. A. Masoodi, β-Glucan as an encapsulating agent: Effect on probiotic survival in simulated gastrointestinal tract, Int. J. Biol. Macromol. **82**, 217-222 (2016). https://doi.org/10.1016/j.ijbiomac.2015.11.017
23. X. Song, L. Si, X. Sun, X. Zhu, Z. Li, Y. Li, Y. Wang, H. Hou, Rheological properties, thermal stability and conformational changes of collagen from sea cucumber (Apostichopus japonicas), Food Chemistry. **389**,133033 (2022). https://doi.org/10.1016/j.foodchem. 2022.133033
24. S. Kraithong, A. Theppawong, Y. Liu, P. Sangsawad, R. Huang, Fucoidan as an encapsulant material: applications and advantages in active agents and probiotic encapsulations, Food Chemistry. **477**, 143570 (2025). https://doi.org10.1016/ j.foodchem. 2025.143570
25. A. Kikuchi, M. Kawabuchi, M. Sugihara, Y. Sakurai, T. Okano, Pulsed dextran release from calcium-alginate gel beads, J. Contr. Release, 47, 1 (1997). https://doi.org10.1016/S0168-3659(96)01612-4.
26. W.Y. Koh, X. X. Lim, T.C. Tan, R. Kobun, B. Rasti, Encapsulated probiotics: potential techniques and coating materials for non-dairy food applications, Applied Sciences. **12**, 19 (2022). https://doi.org/10.3390/app121910005
27. S. A. Rather, R. Akhter, F. A. Masoodi, A. Gani, S. M. Wani, Effect of double alginate microencapsulation on in vitro digestibility and thermal tolerance of Lactobacillus plantarum NCDC201 and Lactobacillus casei NCDC297, LWT. **83**, 50–58 (2017). https://doi.org/10.1016/j.lwt.2017.04.036
28. P. Smrdel, M. Bogataj, A. Zega, O. Planinsek, A. Mrhar, Shape optimization and characterization of polysaccharide beads prepared by ionotropic gelation, J. Microencapsul. **25**, 90-105 (2008). https://doi.org/10.1080/02652040701776109
29. C. Saldanha do Carmo, C. Maia,ab Joana Poejo, I. Lychko,ab P. Gamito, I. Nogueira, M. R. Bronze, Ana Teresa Serra, Catarina M. M. Duarte, Microencapsulation of α-tocopherol with zein and β-cyclodextrin using spray drying for colour stability and shelf-life improvement of fruit beverages, Rsc Advances. **7**, 32065, (2017). https://doi.org/10.1039/C6RA25946D
30. M. K. Morsy, O.M. Morsy, M. A. Abdelmonem, R. Elsabagh, Anthocyanin Colored Microencapsulation Effects on Survival Rate of Lactobacillus rhamnosus GG, Color Stability, and Sensory Parameters in Strawberry Nectar Model. FBT. **15**, 352-367 (2022). https://doi.org/10.1007/s11947-022-02758-8
31. Q. Lia, H. Lina, J. Lia, L. Liua, J. Huanga, Y. Caoa, T. Zhaoa, D. J. McClementsb, J. Chenc, C. Liuc, J. Liud, P. Shenb, M. Zhoua, Improving probiotic (Lactobacillus casei) viability by encapsulation in alginate-based microgels: Impact of polymeric and colloidal fillers, Food Hydrocolloids. **138**, (2023). https https://doi.org/10.1016/j.foodhyd.2022.108028