JPPIPA 11(1) (2025)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

Optimization of Temperature and Drying Time of Encapsulated Synbiotic Powder on the Characteristics and Viability of Microcapsules

Lintang Dion Pangestu[1*], I Made Dedi Mahariawan[2], Ating Yuniarti[2], Anik Martinah Hariati[2]

[1]Master Program of Aquaculture, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya. Malang, Indonesia
[2]Departement of Aquaculture, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya. Malang, Indonesia

Received: September 15, 2024
Revised: December 10, 2024
Accepted: January 25, 2025
Published: January 31, 2025

Corresponding Author:
Anik Martinah Hariati
a_hariati@ub.ac.id

DOI: 10.29303/jppipa.v11i1.9979

Abstract: The drying process is often a problem in maintaining the stability of probiotics and the quality of microcapsules in the processing of synbiotic microencapsulation. This study aims to optimize the temperature and drying time to maintain the core material's stability and the microcapsules' quality. The research design in this study was a completely randomized design with two factorials, namely drying temperature (30°C, 35°C, and 40°C) and drying time (2, 2.5, and 3 hours). The results showed that the highest viability and efficiency values were obtained in the drying process at 40°C for 2 hours, which were 9.29 log CFU/mL and 98.10%. In addition, the water content and gel strength of the microcapsules obtained also showed optimal conditions. Based on the results obtained, it can be concluded that the drying process at 40°C for 2 hours is the best treatment, with the highest viability and EE values and low water content values.

Keywords: encapsulation efficiency; microencapsulation; synbiotics; viability

Introduction

The application of synbiotics in cultivation plays a major role in maximizing the performance of probiotics in the digestive environment of fish. Synbiotics have also been shown to be more effective when compared to giving probiotics alone (Merrifield et al., 2010). Probiotics play a role in facilitating digestion and improving the immune system through the digestive tract. Probiotics must be sufficient to form colonies and provide benefits; the recommended density is 10^7-10^9 CFU/mL (Mohamadzadeh et al., 2024; Tripathi & Giri, 2014). Research related to oral applications continues to be improved to increase the effectiveness of probiotic use. However, the digestive tract, which has extreme conditions, is still a major obstacle that can reduce the effectiveness of its use (Koh et al., 2022). The diversity of temperature, pH, oxygen, and water content conditions in the digestive system are still variables that affect the survival and viability of bacteria (Razavi et al., 2021).

The application of synbiotic powder has been reported to have excellent advantages in maintaining the stability of probiotics in the digestive environment through sporulation formation. The formation of bacillus bacterial spores has been reported to support the survival of bacteria in less supportive environmental conditions (Mahariawan et al., 2020). However, this application is still limited to bacteria that can form spores only.

Microencapsulation techniques can protect functional materials such as synbiotic powder in digestive environmental conditions. Microencapsulation techniques can prevent decreased viability and maintain stability in the digestive tract

How to Cite:
Pangestu, L. D., Mahariawan, I. M. D., Yuniarti, A., & Hariati, A. M. (2025). Optimization of Temperature and Drying Time of Encapsulated Synbiotic Powder on the Characteristics and Viability of Microcapsules. *Jurnal Penelitian Pendidikan IPA*, *11*(1), 1049–1056. https://doi.org/10.29303/jppipa.v11i1.9979

(Iravani et al., 2015). In addition, synbiotic microencapsulation has also been reported to increase bacterial viability (Do Carmo Alves et al., 2023). However, in the microencapsulation technique, the use of synbiotics as the core material is greatly influenced by the drying process during processing. The drying process is needed to reduce the water content in the microcapsule structure, as water content that is too high can reduce the quality of the microcapsules. However, drying processes such as temperature and drying time can affect the viability of microcapsules, surface structure, and thermal stability.

Therefore, this study aims to determine the optimal drying technique, based on temperature and drying time, to maintain the stability of synbiotic powder in microcapsules and increase its effectiveness in fish farming.

Method

Materials

The matrix material used in this study was Sodium Alginate extracted directly from *Sargassum* sp. from the Balai Besar Perikanan Budidaya Air Payau (BBPBAP) Jepara. Probiotics used in this study were consortia microbial (*Bacillus subtilis, Lactobacillus casei, Nitrosomonas, Nitrobacter,* and *Saccharomyces cerevisiae*), which were fermented with several ingredients such as molasses, brown sugar, rice bran, and additional ingredients, such as ginger, turmeric, temulawak, pineapple, and milk. Synbiotic powder ingredients include rice flour, tapioca flour, coconut water, and garlic. Microencapsulation processing ingredients include 0.9% Na-fis and 0.2 M $CaCl_2$. Bacterial counting ingredients include Plate Count Agar (PCA), 0.9% Na-fis, and distilled water.

Aliginate Extraction

The Alginate extraction method used refers to the previous research method (Rashedy et al., 2021); extraction was carried out using 25 g of dried seaweed, then impurities were removed using 2% formalin (800 mL) for 24 hours, then continued with soaking using 0.2 M HCl (800 mL) for 24 hours. After that, the sample was washed with deionized water and extracted using 2% sodium carbonate for 3 hours at a temperature of 100°C. The extraction solution was filtered and centrifuged (4,000 rpm) for 30 minutes. After that, 2.5% Sodium Hypochloride was added to the filtrate to be bleached until it was yellowish-white. The bleaching results were precipitated with 95% ethanol, three times the sample volume. The collected sodium alginate was washed twice with 100 mL of acetone and dried at 60°C.

Probiotic preparation

Culture was carried out using the streak plate method on prepared Tryptic Soy Agar and Nutrient Agar media. After that, incubation was carried out at 37°C for 24 hours to grow probiotics. After the probiotics were refreshed on the agar media, a scale-up was carried out on liquid media. The bacterial culture process on liquid media was carried out using Tryptic Soy Broth media for *Nitrosomonas* and *Nitrobacter*, Nutrient Broth media for *B. subtilis* and *L. casei*, and 0.9% NaCl for Yeast *S. cerevisiae*. After planting, the culture process was carried out using a shaker incubator at 37°C for 24 hours at a speed of 175 rpm.

Probiotic Fermentation

Probiotic fermentation was carried out according to previous research based on (Fernanda & Hariani, 2021). Additional ingredients such as red ginger, turmeric, and temulawak were each ground with water (w:v) and heated with an additional 11 L of water. Furthermore, 1 kg of brown sugar and 400 g of bran were added and reheated until boiling. Then, the temperature was lowered to around 60-70°C to add 600 g of pineapple, 1 L of milk, and 1 L of molasses and reheated at a stable temperature. After completion, the ingredients were left to room temperature, and then Probiotics such as *Nitrosomonas, Nitrobacter, B. subtilis* and *L. casei* were added, each given 100 mL and Yeast *S. cerevisiae* 24 mL, with a density of 10^8 CFU/mL each (Tripathi & Giri, 2014). Furthermore, the fermentation process was carried out for two weeks to increase its nutritional value.

Synbiotic Preparation

Synbiotics are made by combining probiotics and prebiotics that have been made. Synbiotic powder is made by mixing (spray method) 100 mL of probiotic fermentation solution into 100 g of tapioca flour and 100 g of sterile rice flour. After that, a mixture of 20 mL of coconut water and 1.2 g of garlic is added. After mixing, the ingredients are dried in a dehydrator at about 35°C for 48 hours.

Microcapsule Processing

The encapsulation technique used in this study is the extrusion method. The encapsulation process is carried out by dissolving a 2.25% alginate matrix into 60 mL of 0.9% NaCl and heating until the ingredients are dissolved. Then, let the matrix solution stand until it is at 40°C, and mix the synbiotic powder into it. The suspension obtained is then put into a syringe, and microcapsule granules are formed by pouring it into 0.2M $CaCl_2$. The formed microcapsules are then filtered and rinsed again with 0.9% sterile NaCl and dried in a dehydrator with a temperature treatment of 30°C, 35°C,

40°C, and 2, 2.5, and 3 hours of drying time, with three repetitions for each treatment.

Research Parameters

Viability of Consortium

Bacterial viability is carried out to determine the number of probiotic bacteria in the sample to be taken and to identify changes in viability that occur before and after treatment. This bacterial cell calculation is carried out by taking 1 g of microcapsules as a sample and then suspending them in 9 mL of 0.9% NaCl. The next step is to dilute and calculate bacterial cells by taking 100 µL of the dilution results into agar media and then incubating them for 48 hours at 37°C (Pupa et al., 2021).

Encapsulation Efficiency

Encapsulation efficiency is calculating the efficiency value of using microcapsules to determine bacterial viability before and after encapsulation. This parameter determines how efficiently the material protects the core material (bacteria) from other factors. The efficiency of microcapsules can be calculated using the Formula 1 (Sun et al., 2022):

$$EE\ (\%) = \frac{N_t}{N_0} \times\ 100\% \quad (1)$$

Description:
EE : Encapsulation Efficiency
N_t : Viability after encapsulation (log CFU/mL)
N_0 : Viability before encapsulation (log CFU/mL)

Gel Strength

Gel strength measures the matrix material's ability to form a gel to protect the core material. Gel strength testing uses a Brookfield texture analyzer at the Food Quality and Safety Testing Laboratory, Faculty of Agricultural Technology, Universitas Brawijaya, Malang. Gel strength is the main parameter that can be used to determine the effectiveness of microcapsules in protecting core materials from environmental factors.

Water Content

Water content is a parameter used to measure the value of water composition in a material. Water content measurement is carried out to determine changes in the composition of water content in microcapsules. The water content testing method used is the Gravimetric method (Cai et al., 2020), with the Formula 2.

$$Water\ content\ (\%) = \frac{B-C}{B-A} \times 100\% \quad (2)$$

Description:
A : Weight of empty cup (g)
B : Cup weight + Initial sample (g)
C : Weight of cup + dry sample (g)

Result and Discussion

Water Content of Microcapsule

Based on the statistical analysis obtained, the drying process with different temperatures and drying times showed significantly different results on the water content of the microcapsules with a p-value of 0.000 ≤ 0.050. Based on Duncan's further test analysis, the drying process at 35°C and 40°C has no different water content results (p-value 0.136 ≥ 0.050) (Figure 1); this indicates that the use of alginate matrix in microcapsules can maintain the core material from environmental influences. The cross-linking condition of the alginate gel structure helps to keep the core molecules inside the matrix; the more calcium ions are bound to the alginate gel structure, the less space is left for water molecules to escape into the matrix (Łabowska et al., 2023).

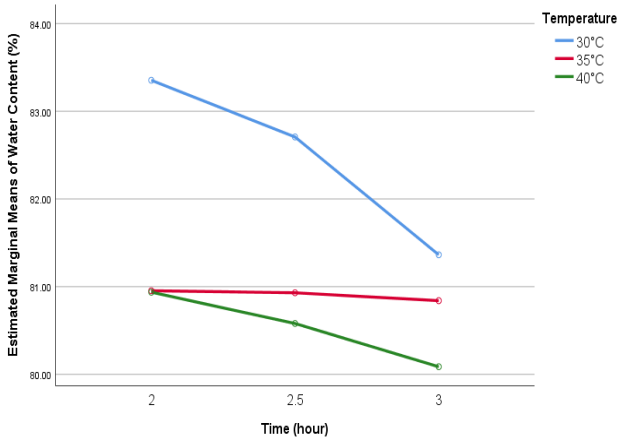

Figure 1. Water content of microcapsule

The results show that the water content value in all microcapsule treatments is ≥80%. Nezamdoost-Sani et al. (2023), explained that alginate hydrogels can wrap core materials with high water content, up to more than 90% of water in free, bound, and semi-bound forms. These results are also supported by the research of (Li et al., 2023), who used alginate material with several combination methods, resulting in a 92.1-95% water content. In this study, the lowest water content results were obtained in the drying process at 40°C for 3 hours, which amounted to 80.09%. Meanwhile, the highest water content of 83.35% was obtained at the lowest drying temperature, 30°C, for 2 hours. Based on these results, it can be explained that the higher the temperature and the longer the drying time, the lower the water content. This drying process aims to dry the microcapsule surface without damaging the core material in the microcapsules. The drying process at 35°C for 2 hours shows the optimal drying point on the microcapsule surface, as evidenced by the results that are not significantly different in the higher temperature

and drying duration treatments given. The water content on the microcapsule surface will quickly evaporate during the drying process. However, the water content in the microcapsules will be maintained due to the capture process. Too high water content on the microcapsule surface can affect the microcapsule's shelf life quality. Water content on the microcapsule surface can lead to a high potential for contamination due to the attachment of contaminant microorganisms to the moist surface. Zhu et al. (2022), also explained that high water content can cause microbiological damage to microcapsules. High water content can increase the water-holding capacity in the matrix so that the cross-linking of gel particles weakens and causes structural damage (Qi et al., 2020).

Microcapsule Gel Strength

Based on the ANOVA test obtained shows that the temperature and duration of the microcapsule drying process have a significant effect on the gel strength of the microcapsules (p-value 0.000 ≤ 0.050). The longer the drying time, the greater the gel strength of microcapsules at 30°C and 35°C. Conversely, at 40°C, it tends to decrease the longer the drying process is carried out. These results are also shown in the interaction graph (Figure 2), which shows an interaction in the length of drying time for 3 hours between temperatures of 30°C and 40°C, which shows a decrease in gel strength at a temperature of 40°C and an increase at a temperature of 30°C. The highest gel strength in this study was obtained at 35°C with a drying time of 3 hours with 25.6 N, while the lowest gel strength value was 16.7 N at 30°C for 2 hours. The gel strength results obtained in this study are more significant than the alginate gel strength values in several previous studies, namely 9.40 - 11.86 N (Qi et al., 2020), and 10.97 - 15.51 N/cm^2 (Abka-khajouei et al., 2022).

Based on the results obtained at 35 ° C, treatment is also the optimal condition for drying the water content in the microcapsule surface (Figure 1). Dry conditions in the microcapsule surface can tighten the molecular bonds, thus strengthening the gel surface of the microcapsules. In addition, drying temperatures that are too high can have a negative impact and reduce the strength of the gel in the microcapsules. Temperature significantly affects the gel-forming properties of alginate (Altamirano-Ríos et al., 2022). Too high a drying temperature can decrease the microcapsules' gel strength (Liu et al., 2022).

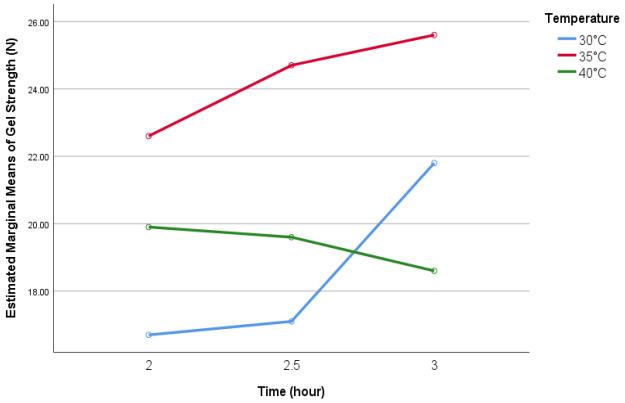

Figure 2. Microcapsule gel strength

Drying at high temperatures can remove calcium ions that play a role in the crosslinking process to form alginate microcapsule gels. Reduced crosslinking of calcium ions in alginate may reduce the strength of the microcapsule gel. Previous studies have also reported that ionic crosslinking in alginate gels and the drying process affect each other (Łabowska et al., 2023). Da Silva Fernandes et al. (2018) also explained that degradation can occur due to low crosslinking of calcium ions in the alginate matrix. This is also supported by (Qi et al., 2020), who explained that microgel deterioration can be caused by weakened ionic crosslinking in the particles. Therefore, the higher the temperature used, the lower the gel strength in the microcapsules, but this also depends on the size of the crosslinks that occur; if the gel formation is balanced by increasing the crosslinks in the structure, the microcapsules formed will be more resistant to higher temperatures.

In addition, the 30°C drying process has a low average gel strength value compared to other treatments. The hydrophilic nature of alginate can easily absorb water while forming the encapsulation solution (Cokrowati et al., 2022), so the resulting water content is relatively high. It impacts the capacity of alginate molecules that crosslink with calcium ions in the crosslinking solution. The more water molecules trapped can widen the distance of the binding molecules and reduce the binding capacity. The drying process at 30°C has a higher water content than the other treatments (Figure 1), so these results also impact the lower gel strength values in these treatments (Figure 2). This condition is also explained in research (Kusuma et al., 2022) on kappa carrageenan and glucomannan polymers, which explains that a large enough water content can cause the ability to withstand the load to be smaller so that the strength of the gel can also be weakened because the particle ratio of the dressing material is lower than the water content in the microcapsules.

Viability of Consortium

Based on the results obtained, drying temperature and time significantly affected the viability value of probiotics in microcapsules (p-value 0.000 ≤ 0.050). Previous research also concluded that the viability value of encapsulated probiotics is strongly influenced by the temperature and time of the microcapsule drying process (Avila-Reyes et al., 2014). The temperature of 40°C had the highest viability value at a drying time of 2 hours, which amounted to 9.29 log CFU/mL. However, the longer the drying process, the viability at 40°C tends to decrease to 8.97 log CFU/mL at a drying time of 3 hours; this result is also directly proportional to the resulting gel strength value, where the longer the drying process at 40°C can reduce the gel strength value so that it has an impact on the protection process of the core material. Figure 2 also shows an interaction in the drying process for 2.5 hours between 40°C and 35°C due to the decrease in viability that occurs at 40°C the more extended the drying process and the increase in viability at 35°C. At 35°C, the viability value increased along with the length of drying time. The viability values obtained were 9.03, 9.11, and 9.12 log CFU/mL, respectively. In contrast to 40°C and 35°C, the treatment at 30°C tended to experience slow growth and decreased after 3 hours of drying.

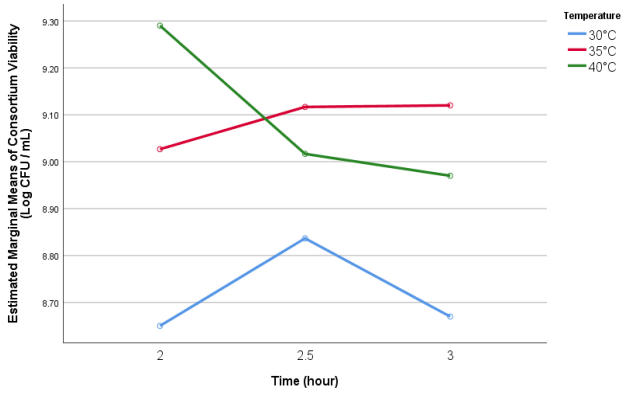

Figure 3. Viability of Consortium

Temperature is an environmental parameter that significantly affects the viability of a consortium (Ni et al., 2023). Temperature conditions that exceed or fall below the optimal limit can interfere with the cellular activity of the consortium cells and can even damage the cell membrane, thereby reducing the viability value (Mbye et al., 2020; Homayouni-Rad et al., 2021; Okfrianti et al., 2018). Lactobacillus bacteria can grow at high temperatures with an optimum limit of 42°C (Tefara et al., 2024). Therefore, a reasonably high viability value can occur at a drying temperature of 40°C for 2 hours. However, 40°C is the maximum limit of growth tolerance in other probiotics. It can interfere with the growth of probiotics, so prolonged exposure to 40°C can inhibit growth and reduce the viability value of probiotics. Bacillus subtilis grows optimally at 35-37°C (Vehapi et al., 2023), S. cerevisiae at 30-35°C (Yang et al., 2023), while nitrifying bacteria are reported to grow highly at 30°C, but at temperatures around 40°C their growth rate starts to decrease (Clements et al., 2024)..

Previous studies have reported that high-temperature conditions can accelerate the exponential phase in bacteria (Mahariawan et al., 2020); the higher the ambient temperature, the faster bacterial metabolism increases, but metabolism decreases sharply at higher temperatures (Schulte, 2015). Increased metabolism can occur at higher temperatures, but prolonged exposure to the maximum temperature of cell life can inhibit metabolic activity in the cell. Temperature affects microbial growth and metabolism through enzyme activity in the cell. Cell metabolism is supported by the provision of nutrients from outside, which is assisted by enzymes that process large nutrient molecules through hydrolysis (Subagiyo et al., 2015). Environmental temperature conditions strongly influence the structure of amino acids in this enzyme; temperatures above the optimal limit can damage the folding structure of enzyme proteins. It can impact the metabolic process and the survival of bacterial cells (Armiliandi, 2024).

Encapsulation Efficiency

The EE value is directly proportional to the viability obtained; the higher the viability, the higher the EE, and vice versa. The results show that the EE value during the drying process is relatively high at more than 90%, indicating that the capture process occurs well. The drying process at different temperatures and drying times significantly affected the resulting EE value (p-value 0.000 ≤ 0.050). However, the drying process at 35°C at each drying time based on Duncan's further test had values that were not significantly different (p-value 0.065 ≥ 0.050). These results show that the temperature of 35°C is still optimal during drying.

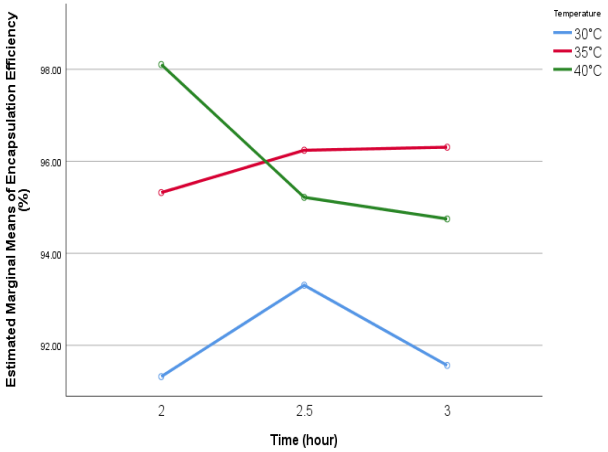

Figure 4. Encapsulation Efficiency

Although the 35°C treatment had a relatively high average EE at each drying duration, the highest EE value was obtained at 40°C with a drying duration of 2 hours, which amounted to 98.1%. The results also show that the drying duration of 2 hours is optimal at 40°C because the longer the drying duration is carried out, the decrease in EE value is shown. Temperature significantly affects the function of intracellular and extracellular enzymes of microorganisms; optimal temperature conditions can increase enzyme activity in bacterial cells (Saravanan et al., 2021), which can help accelerate cellular metabolic processes. Meanwhile, the drying temperature of 30°C tends to have a lower EE value compared to other treatments. Temperatures below the optimum conditions can inhibit membrane function and nutrient absorption (Nie et al., 2021; Subagiyo et al., 2015). In addition, this result is also related to the gel strength values obtained (Figure 2). The low value of gel strength in the 30°C drying treatment is one factor that interferes with the stability of probiotics in microcapsules. This result is also in line with previous research that shows a straight comparison between gel strength and the efficiency value of pure alginate hydrogel, in the study showing that the lower the gel strength produced, the efficiency value will decrease and vice versa (Karakas et al., 2022).

Conclusion

Based on the results obtained, it can be concluded that the drying process at 40°C for 2 hours can produce the highest viability and EE values among the treatments tested, with values of 9.29 log CFU/mL and 98.10%. A high enough temperature can accelerate cell metabolic processes so that viability can be optimally maintained, but prolonged exposure to high temperatures can reduce the viability value of probiotics. Therefore, a drying temperature of 40°C for 2 hours is an appropriate treatment to maintain the viability of the consortium in microcapsules. The water content value obtained in this treatment was also not significantly different compared to the lowest water content; the value obtained was 80.94%. In addition, the gel strength value obtained in this treatment amounted to 19.9 N, which is relatively high and proved to be good at protecting the core material based on the value of viability, EE, and maintained water content.

Acknowledgments
This work was supported by Universitas Brawijaya, Indonesia

Author Contributions
Conceptualization, Anik Martinah Hariati, I M. D. M., and L. D. P.; methodology, L. D. P. and I M. D. M.; software, L. D. P.; validation, A. M. H., A. Y., and I M. D. M.; writing—original draft preparation, L. D. P.; writing—review and editing, L. D. P. and I M. D. M.; visualization, L. D. P.; supervision, A. M. H.; project administration, I M. D. M.; funding acquisition, A. M. H. The authors listed in this article have read and agree to the published version of the manuscript.

Funding
Professor Grant Research Scheme, Universitas Brawijaya, with contract number: 3709/UN10.F06/KS/2024

Conflicts of Interest
The authors declares no conflict of interst

References

Abka-khajouei, R., Tounsi, L., Shahabi, N., Patel, A. K., Abdelkafi, S., & Michaud, P. (2022). Structures, Properties and Applications of Alginates. *Marine Drugs*, *20*(6), 364. https://doi.org/10.3390/md20060364

Altamirano-Ríos, A. V., Guadarrama-Lezama, A. Y., Arroyo-Maya, I. J., Hernández-Álvarez, A., & 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 & Technology*, *57*(7), 4027–4040. https://doi.org/10.1111/ijfs.15779

Armiliandi, R. (2024). Pengaruh Inkubasi Suhu dalam Memproduksi Enzim Xilanase dari Konsorsium Trikultur Bakteri Termofilik. *Jurnal Pendidikan Tambusai,* *8*(1), 15912–15921. https://doi.org/10.31004/jptam.v8i1.14641

Avila-Reyes, S. V., Garcia-Suarez, F. J., Jiménez, M. T., San Martín-Gonzalez, M. F., & Bello-Perez, L. A. (2014). Protection of L. rhamnosus by spray-drying using two prebiotics colloids to enhance the viability. *Carbohydrate Polymers*, *102*, 423–430. https://doi.org/10.1016/j.carbpol.2013.11.033

Cai, C., Ma, R., Duan, M., Deng, Y., Liu, T., & Lu, D. (2020). Effect of starch film containing thyme essential oil microcapsules on physicochemical activity of mango. *LWT*, *131*, 109700. https://doi.org/10.1016/j.lwt.2020.109700

Clements, E., Nahum, Y., Pérez-Calleja, P., Kim, B., & Nerenberg, R. (2024). Effects of temperature on nitrifying membrane-aerated biofilms: An experimental and modeling study. *Water Research*, *253*, 121272. https://doi.org/10.1016/j.watres.2024.121272

Cokrowati, N., Prasedya, E. S., Ilhami, B. T. K., Hariadi, H., Jumat, M., Jayusri, Waang, D. C. S., & Qoriasmadillah, W. (2022). Introduksi Teknologi Budidaya Sargassum sp. Di Gerupuk Kabupaten Lombok Tengah. *Jurnal Pengabdian Magister Pendidikan IPA*, *5*(4), 343–348. https://doi.org/10.29303/jpmpi.v5i4.2538

Da Silva Fernandes, R., De Moura, M. R., Glenn, G. M., & Aouada, F. A. (2018). Thermal, microstructural, and spectroscopic analysis of Ca2+ alginate/clay nanocomposite hydrogel beads. *Journal of Molecular Liquids*, *265*, 327–336. https://doi.org/10.1016/j.molliq.2018.06.005

Do Carmo Alves, A. P., Do Carmo Alves, A., Ferreira Rodrigues, R. A., Da Silva Cerozi, B., & Possebon Cyrino, J. E. (2023). Microencapsulation of *Bacillus subtilis* and oat β-glucan and their application as a synbiotic in fish feed. *Journal of Microencapsulation*, *40*(7), 491–501. https://doi.org/10.1080/02652048.2023.2220394

Fernanda, D. A., & Hariani, D. (2021). Pengaruh Pemberian Sinbiotik dan Enzim dengan Berbagai Konsentrasi pada Pakan terhadap Pertumbuhan Benih Ikan Nila GIFT (Oreochromis sp.). *LenteraBio : Berkala Ilmiah Biologi*, *9*. https://doi.org/10.26740/lenterabio.v9n3.p239-249

Homayouni-Rad, A., Mortazavian, A. M., Mashkani, M. G., Hajipour, N., & Pourjafar, H. (2021). Effect of Alyssum homolocarpum mucilage and inulin microencapsulation on the survivability of Lactobacillus casei in simulated gastrointestinal and high-temperature conditions. *Biocatalysis and Agricultural Biotechnology*, *35*, 102075. https://doi.org/10.1016/j.bcab.2021.102075

Iravani, S., Korbekandi, H., & Mirmohammadi, S. V. (2015). Technology and potential applications of probiotic encapsulation in fermented milk products. *Journal of Food Science and Technology*, *52*(8), 4679–4696. https://doi.org/10.1007/s13197-014-1516-2

Karakas, C. Y., Ordu, H. R., Bozkurt, F., & Karadag, A. (2022). Electrosprayed chitosan-coated alginate–pectin beads as potential system for colon-targeted delivery of ellagic acid. *Journal of the Science of Food and Agriculture*, *102*(3), 965–975. https://doi.org/10.1002/jsfa.11430

Koh, W. Y., Lim, X. X., Tan, T.-C., Kobun, R., & 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

Kusuma, V. R. A. G., Syahputraningrat, G. R., Rahman, H. M., & Fadilah, F. (2022). Pemanfaatan Polimer Alam Kappa-Karagenan dan Glukomanan untuk Mikroenkapsulasi Extra Virgin Olive Oil. *Equilibrium Journal of Chemical Engineering*, *6*(1). https://doi.org/10.20961/equilibrium.v6i1.58249

Łabowska, M. B., Skrodzka, M., Sicińska, H., Michalak, I., & Detyna, J. (2023). Influence of Cross-Linking Conditions on Drying Kinetics of Alginate Hydrogel. *Gels*, *9*(1), 63. https://doi.org/10.3390/gels9010063

Li, Q., Lin, H., Li, J., Liu, L., Huang, J., Cao, Y., Zhao, T., McClements, D. J., Chen, J., Liu, C., Liu, J., Shen, P., & Zhou, M. (2023). Improving probiotic (Lactobacillus casei) viability by encapsulation in alginate-based microgels: Impact of polymeric and colloidal fillers. *Food Hydrocolloids*, *134*, 108028. https://doi.org/10.1016/j.foodhyd.2022.108028

Liu, W., Pan, W., Li, J., Chen, Y., Yu, Q., Rong, L., Xiao, W., Wen, H., & Xie, J. (2022). Dry heat treatment induced the gelatinization, rheology and gel properties changes of chestnut starch. *Current Research in Food Science*, *5*, 28–33. https://doi.org/10.1016/j.crfs.2021.12.004

Mahariawan, I. M. D., Ariffin, N. B., Kusuma, W. E., Yuniarti, A., Beltran, M. A. G., & Hariati, A. M. (2020). Effect of different carbon doses of tapioca (Manihot esculenta) flour on vegetative cells and spore production of Bacillus megaterium. *IOP Conference Series: Earth and Environmental Science*, *441*(1), 012106. https://doi.org/10.1088/1755-1315/441/1/012106

Mahariawan, I. M. D., Kusuma, W. E., Yuniarti, A., & Hariati, A. M. (2020). Effect of temperature and pH combination on vegetative cell growth of Bacillus megaterium. *Journal of Physics: Conference Series*, *1665*(1), 012013. https://doi.org/10.1088/1742-6596/1665/1/012013

Mbye, M., Baig, M. A., AbuQamar, S. F., El-Tarabily, K. A., Obaid, R. S., Osaili, T. M., Al-Nabulsi, A. A., Turner, M. S., Shah, N. P., & Ayyash, M. M. (2020). Updates on understanding of probiotic lactic acid bacteria responses to environmental stresses and highlights on proteomic analyses. *Comprehensive Reviews in Food Science and Food Safety*, *19*(3), 1110–1124. https://doi.org/10.1111/1541-4337.12554

Merrifield, D. L., Dimitroglou, A., Foey, A., Davies, S. J., Baker, R. T. M., Bøgwald, J., Castex, M., & Ringø, E. (2010). The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquaculture*, *302*(1–2), 1–18. https://doi.org/10.1016/j.aquaculture.2010.02.007

Mohamadzadeh, M., Fazeli, A., & Shojaosadati, S. A. (2024). Polysaccharides and proteins-based bionanocomposites for microencapsulation of probiotics to improve stability and viability in the gastrointestinal tract: A review. *International Journal of Biological Macromolecules*, *259*, 129287. https://doi.org/10.1016/j.ijbiomac.2024.129287

Nezamdoost-Sani, N., Khaledabad, M. A., Amiri, S., & Mousavi Khaneghah, A. (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

Ni, F., Luo, X., Zhao, Z., Yuan, J., Song, Y., Liu, C., Huang, M., Dong, L., Xie, H., Cai, L., Ren, G., & Gu, Q. (2023). Enhancing viability of Lactobacillus plantarum encapsulated by alginate-gelatin hydrogel beads during gastrointestinal digestion, storage and in the mimic beverage systems. *International Journal of Biological Macromolecules*, *224*, 94–104. https://doi.org/10.1016/j.ijbiomac.2022.10.106

Nie, E., He, P., Zhang, H., Hao, L., Shao, L., & Lü, F. (2021). How does temperature regulate anaerobic digestion? *Renewable and Sustainable Energy Reviews*, *150*, 111453. https://doi.org/10.1016/j.rser.2021.111453

Okfrianti, Y., Darwis, D., & Pravita, A. (2018). Bakteri Asam Laktat Lactobacillus Plantarum C410LI dan Lactobacillus Rossiae LS6 yang Diisolasi dari Lemea Rejang terhadap Suhu, pH dan Garam Empedu Berpotensi sebagai Prebiotik. *Jurnal Ilmu dan Teknologi Kesehatan*, *6*(1), 49–58. https://doi.org/10.32668/jitek.v6i1.108

Pupa, P., Apiwatsiri, P., Sirichokchatchawan, W., Pirarat, N., Muangsin, N., Shah, A. A., & Prapasarakul, N. (2021). The efficacy of three double-microencapsulation methods for preservation of probiotic bacteria. *Scientific Reports*, *11*(1), 13753. https://doi.org/10.1038/s41598-021-93263-z

Qi, X., Simsek, S., Chen, B., & Rao, J. (2020). Alginate-based double-network hydrogel improves the viability of encapsulated probiotics during simulated sequential gastrointestinal digestion: Effect of biopolymer type and concentrations. *International Journal of Biological Macromolecules*, *165*, 1675–1685. https://doi.org/10.1016/j.ijbiomac.2020.10.028

Rashedy, S. H., Abd El Hafez, M. S. M., Dar, M. A., Cotas, J., & Pereira, L. (2021). Evaluation and Characterization of Alginate Extracted from Brown Seaweed Collected in the Red Sea. *Applied Sciences*, *11*(14), 6290. https://doi.org/10.3390/app11146290

Razavi, S., Janfaza, S., Tasnim, N., Gibson, D. L., & Hoorfar, M. (2021). Microencapsulating polymers for probiotics delivery systems: Preparation, characterization, and applications. *Food Hydrocolloids*, *120*, 106882. https://doi.org/10.1016/j.foodhyd.2021.106882

Saravanan, A., Kumar, P. S., Vo, D.-V. N., Jeevanantham, S., Karishma, S., & Yaashikaa, P. R. (2021). A review on catalytic-enzyme degradation of toxic environmental pollutants: Microbial enzymes. *Journal of Hazardous Materials*, *419*, 126451. https://doi.org/10.1016/j.jhazmat.2021.126451

Schulte, P. M. (2015). The effects of temperature on aerobic metabolism: Towards a mechanistic understanding of the responses of ectotherms to a changing environment. *Journal of Experimental Biology*, *218*(12), 1856–1866. https://doi.org/10.1242/jeb.118851

Subagiyo, S., Margino, S., Triyanto, T., & Ari Setyati1,2, W. A. (2015). Effects Of pH, Temperature And Salinity In Growth And Organic Acid Production Of Lactic Acid Bacteria Isolated From Penaeid Shrimp Intestine. *ILMU KELAUTAN: Indonesian Journal of Marine Sciences*, *20*(4), 187. https://doi.org/10.14710/ik.ijms.20.4.187-194

Sun, W., Nguyen, Q. D., Sipiczki, G., Ziane, S. R., Hristovski, K., Friedrich, L., Visy, A., Hitka, G., Gere, A., & Bujna, E. (2022). Microencapsulation of Lactobacillus plantarum 299v Strain with Whey Proteins by Lyophilization and Its Application in Production of Probiotic Apple Juices. *Applied Sciences*, *13*(1), 318. https://doi.org/10.3390/app13010318

Tefara, S. F., Begna Jiru, E., & G/Meskel Bairu, A. (2024). Optimization of fermentation condition for production of lactic acid from khat ("Catha edulis") waste by using immobilized Lactobacillus plantarum. *Biomass Conversion and Biorefinery*, *14*(5), 6637–6647. https://doi.org/10.1007/s13399-022-02797-3

Tripathi, M. K., & Giri, S. K. (2014). Probiotic functional foods: Survival of probiotics during processing and storage. *Journal of Functional Foods*, *9*, 225–241. https://doi.org/10.1016/j.jff.2014.04.030

Vehapi, M., İnan, B., Kayacan-Cakmakoglu, S., Sagdic, O., & Özçimen, D. (2023). Optimization of Growth Conditions for the Production of Bacillus subtilis Using Central Composite Design and Its Antagonism Against Pathogenic Fungi. *Probiotics and Antimicrobial Proteins*, *15*(3), 682–693. https://doi.org/10.1007/s12602-021-09904-2

Yang, P., Wu, W., Chen, J., Jiang, S., Zheng, Z., Deng, Y., Lu, J., Wang, H., Zhou, Y., Geng, Y., & Wang, K. (2023). Thermotolerance improvement of engineered Saccharomyces cerevisiae ERG5 Delta ERG4 Delta ERG3 Delta, molecular mechanism, and its application in corn ethanol production. *Biotechnology for Biofuels and Bioproducts*, *16*(1), 66. https://doi.org/10.1186/s13068-023-02312-4

Zhu, J., Li, X., Liu, L., Li, Y., Qi, B., & Jiang, L. (2022). Preparation of spray-dried soybean oil body microcapsules using maltodextrin: Effects of dextrose equivalence. *LWT*, *154*, 112874. https://doi.org/10.1016/j.lwt.2021.112874