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Short Communication

The Characteristics of Chitosan Derived from Lobster Shells and its Effect on Fungi Activity and Water Stability of Lobster Pellets

Muhsinul Ihsan[1]*, Ali Harris[1], Mukminah[1], Megawati[1], Nining Purwati[1], Handa Muliasari[2], Bayu Priyambodo[3], Clive Jones[4], and Leo Nankervis[4]

[1]Department of Biology Education, Faculty of Teacher and Training, Universitas Islam Negeri Mataram. Indonesia
[2]Department of Pharmacy, Faculty of Medicine and Health Sciences, University of Mataram. Indonesia
[3]Lombok Marine Aquaculture Development Centre. Indonesia
[4]Centre for Sustainable Tropical Fisheries and Aquaculture, James Cook University. Australia

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*) Corresponding author:
E-mail: ihsan@uinmataram.ac.id

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Abstract

Tropical rock lobster aquaculture is a lucrative industry that is currently limited by the lack of appropriate formulated feed. Its nocturnal, benthic feeding behavior necessitates a water-stable feed that maintains integrity under tropical marine conditions without degrading. Chitosan, a biopolymer derived from lobster (*Panulirus homarus*) shells, has potential applications in aquaculture as an antifungal agent and feed binder. We report on the characteristics of chitosan extracted from the exoskeleton of spiny lobsters (*Panulirus homarus*), including its effect on fungal activity and water stability of pellets. Chitosan was produced through three main steps: deproteination, demineralization, and deacetylation. The resulting chitosan was characterized through crude composition (AOAC methods), FTIR spectra, and scanning electron microscope (SEM), while anti-fungal activity was assessed through *in vitro* assays. Chitosan was used to coat lobster feed pellets by immersion method at different concentrations (0%, 0,5%, 1%, 1,5%, and 2%), and its impact on pellet water stability was assessed. There were three replications in fungal activity and water stability test. The yield of chitosan was 5.9 ± 0.01% of the total shell mass, with 96.99% ± 0.01 degree of deacetylation (DD). The resulting product contained 5.94 ± 0.07% moisture, 36.72 ± 0.05% ash and 2.73 ± 0.08% nitrogen. Chitosan morphology was characterized as an irregular shape with dimensions ranging from 157 to 391 µm, with a combination of striated surface textures. Increasing concentration of chitosan increased water stability of pellets up to 1.5% inclusion, while 0.5% optimized *Fusarium* sp. inhibition. These findings suggest that chitosan from lobster shells can be sustainably utilized to enhance feed quality, reducing fungal contamination and nutrient leaching in aquaculture systems.

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1. Introduction

The Spiny Lobster (*Panulirus homarus*) is amongst the highest value seafood products, and has become the basis of a rapidly expanding aquaculture industry in Indonesia (Jones *et al.*, 2019). In 2021, Indonesia exported around 1.96 thousand tons of lobsters, with value 28.62 million USD (Statista, 2024). This production industry has resulted in higher quantities of lobster consumption, and resultant higher volume of waste shells, which pose a disposal issue. These shells are currently disposed of in municipal waste. However, they pose an opportunity for co-product development to add circularity to the lobster supply chain, reducing waste and pollution.

Chitin is the main component in the shells of crustaceans such as shrimp, crab, squid, crayfish, and lobster. It is also can be found in the exoskeleton of mollusks and insects as well as in the cell of walls of some fungi. The derivative of chitin is chitosan. Both of them are natural polysaccharides which contain two monosaccharides, *N*-acetyl *D*-glucosamine and *D*-glucosamine, connected by β-1,4-glycoside bonds. Chitin consists of mainly *N*-acetyl *D*-glucosamine, while chitosan contains mainly D-glucosamine (Bastiaens *et al.*, 2020). The presence of primary and secondary hydroxyl groups and the amine groups in each deacetylated unit make chitosan chemically more active than chitin (Amine *et al.*, 2021). These properties have made chitosan a promising candidate for multiple applications, particularly as an antimicrobial and coating agent in food preservation and aquafeed formulations.

The bioactivity of chitosan is defined as its ability to interact with other compounds, pathogens, or microorganisms. Chitosan and its derivatives can be used as a coating agent and are effective against pathogen in foodstuff (Betchem *et al.*, 2019; Chaudhari *et al.*, 2023; Chouhan and Mandal, 2021; Confederat *et al.*, 2021; Díaz-montes and Castro-muñoz, 2021; Divya *et al.*, 2018; Karamchandani *et al.*, 2022; Li and Zhuang, 2020; Picos-Corrales *et al.*, 2023; Román-Doval *et al.*, 2023; Romanazzi *et al.*, 2018; Romanazzi and Moumni, 2022; Shahbaz *et al.*, 2023; Shrestha *et al.*, 2023; Teixeira-Santos *et al.*, 2021; Zeng *et al.*, 2021). Furthermore, in aquaculture, chitosan and its derivatives also have positive effects against pathogen in *Oreochromis niloticus* (Abdel-Razek, 2019; Aly *et al.*, 2023; Elbahnaswy *et al.*, 2021; Fadl *et al.*, 2020; Ibrahim *et al.*, 2021; Naiel *et al.*, 2020; Shi *et al.*, 2020; Xu *et al.*, 2023), *Barbonymus gonionotus* (Salam *et al.*, 2021), sea cucumber *Apostichus japonicus* (Wang *et al.*, 2023), tropical rohu fish, *Labeo rohita* (Kumar *et al.*, 2022), and Hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *E. lanceolatus* ♂) (Chen *et al.*, 2021).

Besides its tremendous effects as an antimicrobial, chitosan also improves the physical properties of pellets. Previous studies revealed that chitosan-coated microdiet improves significantly water stability and retains more nutrients of feed of *Machrobrachium rosenbergii* (Anas *et al.*, 2008), olive flounder, *Paralichthys olivaceus,* (Cha *et al.*, 2008), *Cherax albidus* (Volpe *et al.*, 2012), and yellow croaker larvae feed (Liu *et al.*, 2022). The ability of chitosan to form edible films is mainly due to its polycationic nature that allows it to work in conjunction with other biopolymers and additives. This synergy causes strong physical interactions between all the components, which reflects the improvement of the physical, mechanical, and permeability properties (Díaz-montes and Castro-muñoz, 2021; Saputra *et al.*, 2022). In addition, the quality of chitosan depends on several aspects, such as concentration, the degree of deacetylation, pH, moisture content, ash, nitrogen, and the species of animals (Goy *et al.*, 2009; Kurniawidi *et al.*, 2022).

Based on previous studies, it can be seen that chitosan has been studied widely as an antimicrobial and an edible coating in foodstuff and artificial feed of aquaculture animals. In addition, some studies also reported the quality of chitosan from several animals. However, there is little information in terms of the quality of chitosan from lobster shells of *Panulirus homarus* and its effects on the lobster feed (pellets). This study aims to measure the quality of chitosan derived from lobster shells of *Panulirus homarus,* and observe its effects on the fungi activities in pellets and water stability of lobster pellets.

2. Materials and Methods

2.1 Materials

2.1.1 The equipments

The research equipments were magnetic stirrer (Guardian 7000 G71HS10C HPS 18 L Hotplate Stirrer - IC-30500611), FTIR spectrophotometer (Nicolet iS50, Thermo Fisher Scientific, Australia), scanning electron microscope, energy-dispersive X-ray spectroscopy (SEM-EDX) (JCM-7000 Benchtop SEM, JEOL, USA), horizontal water bath shaker (Fisherbrand™Isotemp™, Fisher Scientific, USA), and oven (ESCO scientific, Isotherm® Forced Convection Lab Oven). All of these equipments were provided by the Immunobiology Laboratory, Universitas Mataram and Integrated Laboraty, Universitas Islam Negeri Mataram.

2.1.2 The materials

About 1000 g of Spiny Lobster (*Panulirus homarus*) shells were collected from the lobster aquaculture center in Jerowaru, East Lombok, Indonesia. These samples were cleaned with water and dried in an oven at 80°C for 24 hours and then mashed with 60 mesh. The reagents and chemicals were supplied by Sigma Aldrich, Singapore.

2.1.3 Ethical approval

This study does not require ethical approval because it does not use experimental animals.

2.2 Methods

2.2.1 Preparation of chitosan

The chitosan extraction process followed three key steps: deproteination, demineralization, and deacetylation, adapted from previous studies on crustacean-derived chitosan. The first step was performed using 3% NaOH solvent (1:5 w/v) at 70°C using a hot plate under continuous stirring for two hours (Masruriati *et al.*, 2020). The samples were washed with distilled water and dried in an oven. The demineralization was carried out using 1N HCl solvent (1:10 w/v) at 80°C using magnetic stirrer for one hour. The samples were cleaned with distilled water and dried in an oven. Chitin was successfully produced after these steps. Then, chitin was formed into chitosan through a deacetylation process using 50% NaOH in a ratio of 1:15 (chitin to NaOH solution) at 80°C with stirring for one hour. The sample was cleaned using distilled water and dried in an oven at 80°C for eight hours (Azmin *et al.*, 2019).

2.2.2 Characterization of chitosan

The quality of chitosan was assessed by measuring the water, ash, and nitrogen content based on the standard analytical methods (Association of Official Analytical Chemists). FTIR spectrophotometer (Nicolet iS50, Thermo Fisher Scientific, Australia) was used to measure the degree of deacetylation (DD). This is calculated by comparing the absorbance at 1.655 cm^{-1} and 3.450 cm^{-1}. The characterization of chitosan was continued by observing the morphology of chitosan using scanning electron microscope, energy-dispersive X-ray spectroscopy (SEM-EDX) (JCM-7000 Benchtop SEM, JEOL, USA). The yield percentage of chitosan was calculated by the formula:

Yield (%) = Dry Chitosan mass (g) / Dry raw material mass (g) x 100.....(1)

2.2.3 Assessment of anti-fungal activity

There were three main steps in this activity, namely: lobster feed preparation, isolation of fungi from lobster feed, and *in vitro* assays of antifungal activity of chitosan. Lobster feed preparation for *in vitro* and *in vivo* assays was based on the previous research developed by Ihsan *et al.* (2020) with little modification in the formula (Table 1). Fungi were isolated from lobster feed by serial dilution and spread plate method. Serial dilution (10^{-1} and 10^{-2}) was made by diluting 10 g of lobster feed in 9 mL of sterile aquadest; 0.1 mL of the suspensions were inoculated in a petri disc containing PCA (plate count agar) and incubated for seven days in room temperature. The identification of fungi was conducted by macroscopic and microscopic observation. The anti-fungal activity of chitosan was determined using diffusion-type assays. Fungi were spread in PCA media using a sterile spreader and 25 µL of chitosan solution which was produced by dissolving chitosan in an added 1% acetic acid. There were three replications and five concentrations of chitosan as treatments, namely: 0%, 0.5%, 1%, 1.5%, and 2%. The inhibition zone was measured after seven days incubation of dishes.

Table 1. Lobster feed formulation.

No	Ingredients	Formula (%/Kg)
1	Fish meal	53
2	Shrimp meal	26.4
3	Wheat	5
4	Soy meal	5
5	ISP	1
6	Fibrisol	0.005
7	Astaxanthin	0.005
8	Vitamin mix	1.4
9	Mineral mix	1
10	Fish Oil	3
11	Agar-agar powder	3.7
Total		100

2.2.4 Water stability test

The water stability test of lobster pellets was conducted through two main phases, namely coating phase and a leaching loss test phase. The pellets were

coated by chitosan using immersion method. Two grams of pellets were immersed for 60 minutes in five different chitosan solutions (0%, 0.5%, 1%, 1.5%, and 2%) as treatments with three replications. These pellets were placed in five glasses containing 100 mL of seawater and shaken by a horizontal water bath shaker (Fisherbrand™Isotemp™, Fisher Scientific, USA) at 100 rpm for 15 minutes at 25°C. The remaining pellets were dried at 105°C for 24 hours and weighed. The physical stability of pellets in terms of dry matter retention was counted by the following formula:

Water Stability (%) = feed remaining (g) / initial feed (g) x100 .....(2)

2.3 Analysis Data

Software SPSS 25 was used to perform one-way ANOVA test, which analyzed the differentiation between the means of treatments in anti-fungal and water stability test. However, post-hoc analysis revealed no significant improvement in stability beyond 1.5% chitosan inclusion, supporting the hypothesis that excessive chitosan cross-linking reduces pellet flexibility. Tukey's test was used to identify the treatments which differ exactly from each other.

3. Results and Discussion

3.1 Results

3.1.1 Characteristics of chitosan

The yield of resulted chitosan was 5.9%, while moisture, ash, and nitrogen content were 5.94%, 36.72%, and 2.73% respectively. Degree of deacetylation (DD) was 96.99% (Table 2). The FTIR spectra of chitosan from lobster shells can be seen in Figure 1 which shows that O-H vibration is detected at band 3377.65 cm^{-1}, while 2790.75 cm^{-1} and 2586.52 cm^{-1} indicate the C-H stretching. The C-O stretching and N-H banding were observed at 1028.92 cm^{-1} and 1419.01 $cm.^{-1}$ respectively.

The morphology of chitosan observed by scanning electron microscope is the irregular shape, 157µm – 391 µm particle size (Figure 2A). The magnification by 2000 times shows that the chitosan is striated with a rough surface (Figure 2B). Energy-dispersive X-ray spectroscopy (EDX) indicates the chitosan consist of carbon and oxygen as the constituent elements, whereas calcium, natrium, magnesium, aluminum, and phosphate are the metal impurities from lobster shells (Figure 2C).

3.1.2 Anti-fungal activity of chitosan

The fungi growing on lobster pellets in this study were identified as *Fusarium* spp. (Figure 3). The antifungal activity of chitosan can be seen in Figure 4. The lowest chitosan concentration (0.5% of chitosan) resulted in the highest zone of inhibition, with decreasing inhibition as chitosan concentration was increased further.

3.1.3 Water stability of lobster pellets

The percentage of water stability of pellets can be seen in Table 3, while the color of water changing before and after water stability test is displayed in Figure 5. Based on Table 3, there is dose-dependent increase in water stability with increasing chitosan concentration, up to 1.5%. This is supported by visual observation of leaching nutrient (Figure 5).

3.2 Discussion

3.2.1 Characteristics of chitosan

The quality of chitosan extracted from Spiny Lobster (*Panulirus homarus*) shells relies on water, ash, nitrogen, and degree of deacetylation (DD). The quality was compared with Protan Laboratory and Food Safety Authority (EFSA) as standards and is displayed in Table 2.

Based on Table 2, the yield of chitosan was slightly higher than previous research conducted by

Table 2. Characteristics of chitosan.

Parameters	Results ± SD (%)	Standards	
		Protan Laboratory	EFSA
Yield	5.9 ± 0.01	-	-
Moisture	5.94 ± 0.07	≤ 10	≤ 10
Ash	36.72 ± 0.05	≤ 2	≤ 3
Nitrogen	2.73 ± 0.08	-	≤ 6
Degree of deacetylation (DD)	96.99 ± 0.01	≥ 70	≥ 90

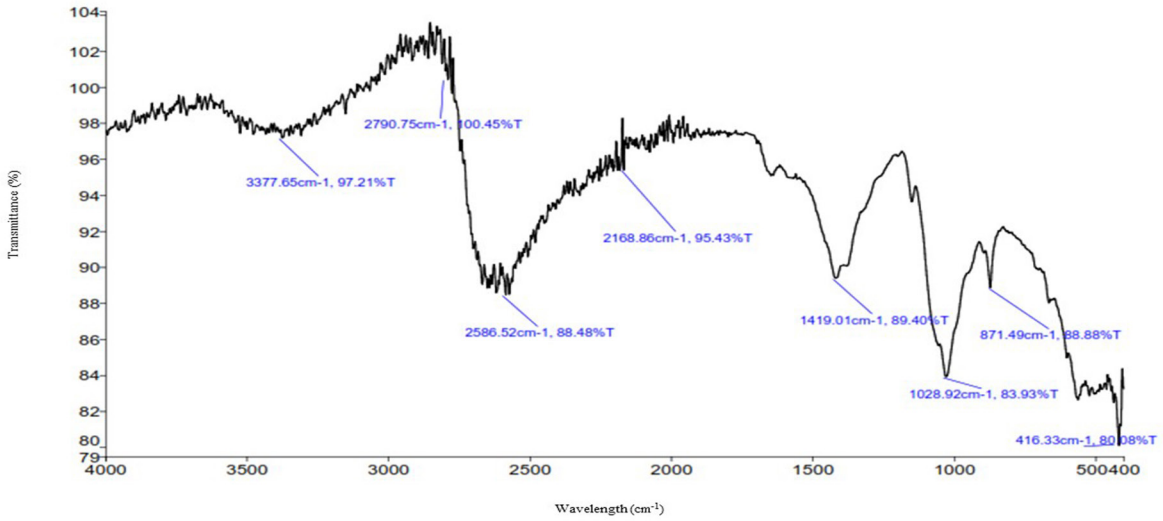

Figure 1. FTIR spectrum of the lobster shells (*Panulirus homarus*).

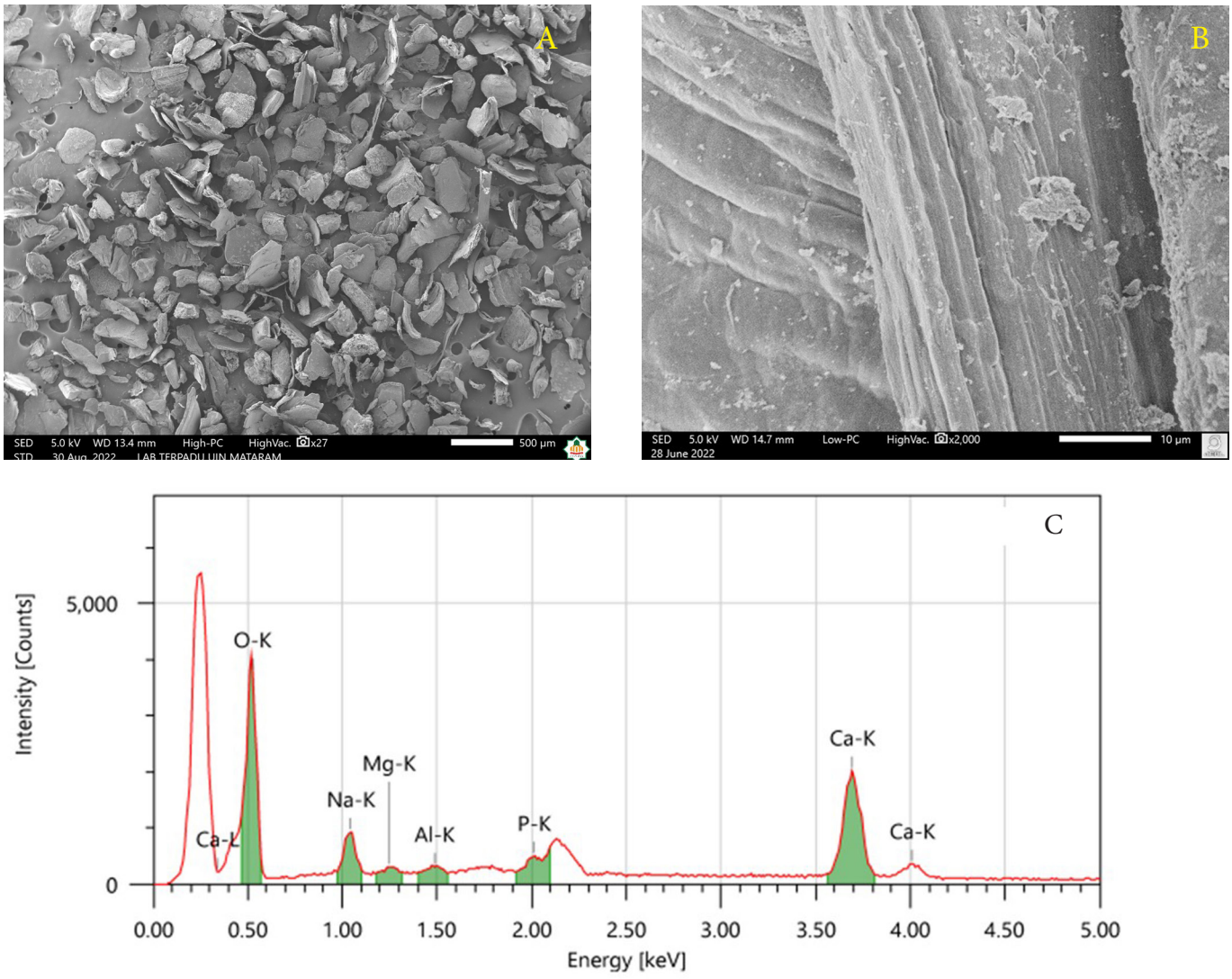

Figure 2. Scanning electron microscope-Energy Dispersive X-Ray (SEM-EDX) (A, B); composition of chitosan (C).

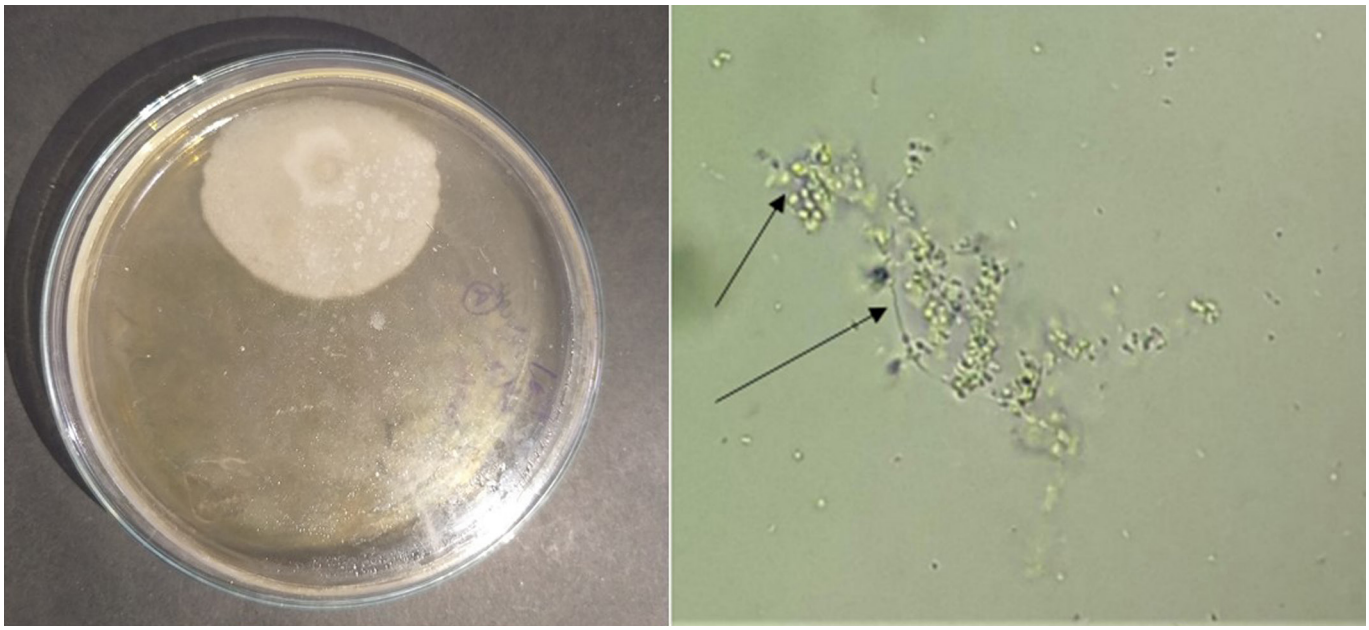

Figure 3. *Fusarium* spp. Isolated from lobster pellets.

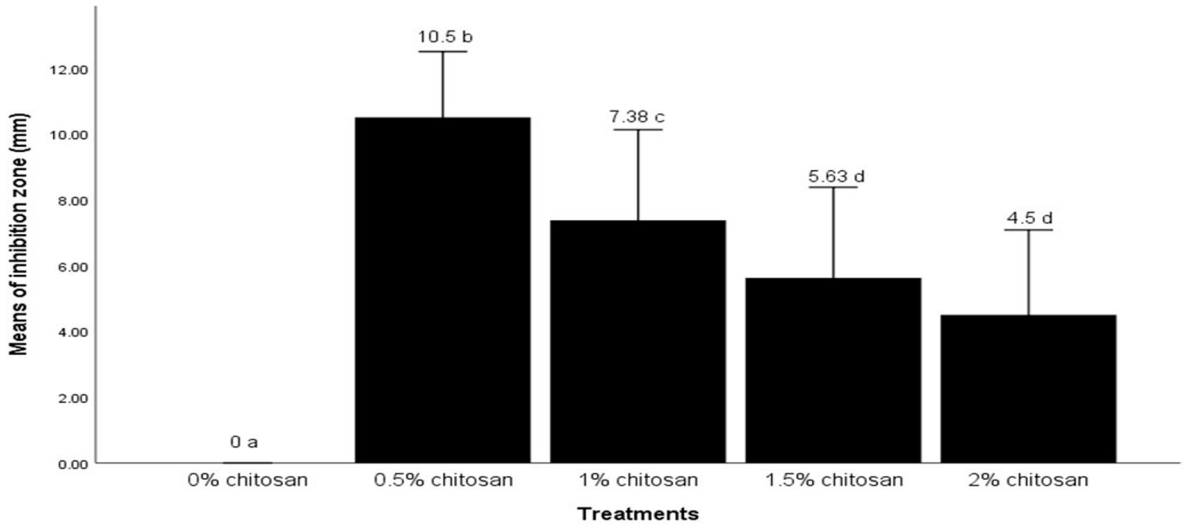

Figure 4. Inhibition zone of chitosan against *Fusarium* sp. Note: Different alphabets show significant differences ($p < 0.05$). The data were expressed as mean ± standard deviation (SD) of three replications.

Table 3. Mean of water stability of experimental pellets.

Treatments	Mean initial weight ± SD (g)	Mean final weight ± SD (g)	Mean water stability ± SD (%)
0% chitosan	1.39 ± 0.00	1.14 ± 0.01	82.33 ± 0.30^{a}
0.5% chitosan	1.39 ± 0.00	1.27 ± 0.01	90.80 ± 0.20^{b}
1% chitosan	1.39 ± 0.00	1.35 ± 0.01	97.00 ± 0.10^{c}
1.5% chitosan	1.39 ± 0.00	1.36 ± 0.01	97.48 ± 0.08^{d}
2% chitosan	1.39 ± 0.00	1.37 ± 0.01	97.75 ± 0.05^{d}

Note: Different alphabets show significant differences ($p < 0.05$). The data were expressed as mean ± standard deviation (SD) of three replications.

Bolat *et al.* (2010) and Luthfiyana *et al.* (2022) who respectively reported that the yield of chitosan from mangrove crab shells was 4.65% and 5.82%. These differentiations probably affected several factors such as concentration of NaOH, temperature, time of extraction as well as the environment of each species. The higher concentration of NaOH, the lower of yield chitosan (Luthfiyana *et al*., 2022). However, in the case of Cuttlefish (*S.pharaonis* sp.), the percentage of yield chitosan in higher NaOH concentration (12.5 M) was higher than 7.5 M of NaOH (Hazeena *et al.*, 2022). These findings suggest that NaOH is not a single factor affecting the yield of chitosan. it depends also on the species of animals. In addition, Bolat *et al.* (2010) stated that species and their environment affect the chitin production from crab shells.

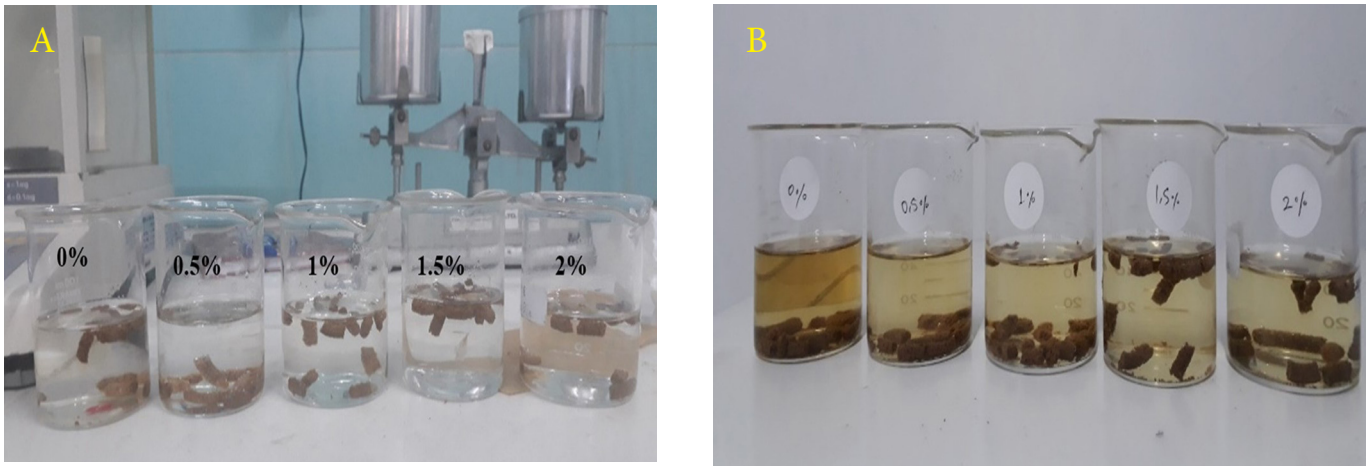

Figure 5. The color of water changing before (A) and after (B) experiment.

The moisture content was 5.94 ± 0.07%, which complied with Protan Laboratory and EFSA standards. This moisture was low because chitosan contains acetyl groups that are hydrophobic or dislike water. The chitosan, therefore, does not bind the water (Cui *et al.*, 2016; Onwuka *et al.*, 2019).

The ash content of chitosan was 36.72 ± 0.05% which is higher than Protan Laboratory and EFSA standards. The ash content is strongly caused by the concentration of HCl, temperature, and the length of time of demineralization (Luthfiyana *et al.*, 2022). In previous research, the ash content of chitin produced with 6N HCl and temperature 33°C was lower than 2N HCl and 4N HCl. This is because of the higher concentration of HCl, and the higher reactivity rate between the HCl and minerals. Furthermore, the reaction will be faster at a higher temperature (Nugroho *et al.*, 2020).

High ash content in chitosan can have adverse effects on fungal activity and water stability. High levels of ash can interfere with chitosan's ability to bind to fungal cell walls and interfere with their nutrient absorption. This can reduce the effectiveness of chitosan in inhibiting fungal growth (Ke *et al.*, 2021) in lobster feed. Furthermore, some types of minerals in ash, such as calcium and magnesium, can act as nutrients for mold (Lübeck *et al.*, 2022). This can encourage mold growth in lobster feed, especially if the water content is also high. In terms of water stability, high ash content can reduce chitosan's ability to absorb and hold water (Adamczuk *et al.*, 2021). This can cause shrimp feed to dry out and break down more easily, making it more difficult for shrimp to consume. In addition, ash dissolved in water can increase the turbidity (Brito *et al.*, 2021) of cultivation water. This can disrupt plankton photosynthesis and reduce shrimp visibility, which can have negative consequences for their health and growth.

Based on this result, the nitrogen content of chitosan was in accordance with the EFSA standards. This result is in line with the previous research which stated that the mangrove crab shells also have 1.32% of nitrogen content. Sodium hydroxide (NaOH) is one kind of reagent that disrupts the bond of chemicals between chitin and protein (Younes and Rinaudo, 2015).

The degree of deacetylation (DD) is a parameter that highly affects the biological, physicochemical, and mechanical properties of chitosan (Metin *et al.*, 2019). Based on this study, the degree of deacetylation was 96.99 ± 0.01%, which complies with the EFSA standards. Concentration of NaOH and temperature of deacetylation highly affect the degree of deacetylation (Mathaba and Daramola, 2020). Moreover, hydrolysis

of the biopolymer causes a decrease in the molecular weight of chitosan (Younes and Rinaudo, 2015).

The degree of deacetylation (DD) is an important parameter in chitosan which measures the percentage of acetyl groups removed from the chitin chain. Chitosan with high DD has fewer acetyl groups and more free amino groups. This provides several benefits in relation to fungal activity and water stability in shrimp feed. Chitosan with high DD has a stronger ability to bind to fungal cell walls and interfere with their nutrient absorption. This can increase the effectiveness of chitosan in inhibiting fungal growth (Ke *et al.*, 2021) in shrimp or lobster feed. Moreover, chitosan with high DD has been proven to be effective against various types of fungal pathogens that are often found in shrimp feed, including *Aspergillus flavus*, *Fusarium moniliforme*, and *Penicillium* sp. (Ke *et al.*, 2022; Karamchandani *et al.*, 2022; Gong *et al.*, 2024) Regarding water stability, chitosan with high DD has better ability to absorb and retain water (Jin *et al.*, 2017). This can help keep the shrimp or lobster feed moist and prevent it from drying out and falling apart, making it easier for the shrimp or lobster to consume. Furthermore, Chitosan with high DD has a higher absorption capacity for heavy metals and other organic compounds (Hsu *et al.*, 2024) found in cultivation water. This can help improve water quality and reduce water pollution.

The pattern of FTIR vibrations are in accordance with the FTIR spectra of *Portunus pelagicus,* commercial chitosan, and mangrove crab shells conducted by Ahyat *et al.* (2017), Gharaie *et al.* (2018), and Luthfiyana *et al.* (2022), respectively. The SEM-EDX result is the same as the morphology of chitosan from mangrove crab shells reported by other researchers (Liu *et al.*, 2022; Luthfiyana *et al.*, 2022). The data of FTIR and SEM-EDX are strong evidence that chitosan is successfully manufactured.

3.2.2 In vitro assay of anti-fungal activity of chitosan

Fusarium spp. are fungal pathogens responsible for plant diseases and also act as agents of infections in humans and animals. These fungi are ubiquitous and can colonize various organic substances, such as fish and crustacean pellets, due to their remarkable adaptability to diverse environmental conditions (Ekwomadu and Mwanza, 2023). The Fusarium colonies observed in this study resemble those of *Fusarium solani*, characterized by their white to cream coloration (Leslie and Summerell, 2006).

Based on *in vitro* assay, chitosan has a significant effect as a fungicidal in lobster pellets, with 0.5% of chitosan showing the best concentration. This result aligns with previous findings, demonstrating that chitosan and its derivatives effectively inhibit *Rhizoctonia solani, Fusarium oxysporum, Collectotrichum acutatum, and Phytophthora infestans* (Divya *et al.*, 2018), *Colletotrichum gleosporioides* (Wu *et al.*, 2023), *Candida albicans* (Gamil *et al.*, 2024), *Aspergillus niger* (El-Araby*et al.*, 2022), *Alteraria alternate* (Guo *et al.*, 2020), *and Colletotrichum truncatum* (Gowda and Sriram, 2023)

The fungicidal effects of chitosan operate through an intracellular mechanism, penetrating pathogenic cells or accumulating on cell walls, membranes, or septa. This process disrupts cell membrane permeability by causing ion exchange, particularly K^+ or cytoplasmic content loss, resulting in the leakage of cellular components like proteins and nucleic acids, ultimately leading to the breakdown of cell structure (Guo *et al.*, 2020; Ke *et al.*, 2021; Singh *et al.*, 2008). The zone of inhibition was decreased for chitosan levels of 1% or more, which may relate to the incomplete demineralization of our process.

3.2.3 Water Stability of lobster pellets

The quality of lobster diets depends not only on their nutritional composition but also on their physical properties, particularly water stability. Lobster pellets must remain intact to prevent disintegration and the loss of water-soluble nutrients when exposed to water or during consumption. Unlike fast-feeding fish species such as salmon, catfish, and tilapia, which require pellets with short water stability, lobsters need pellets with extended stability due to their slow and selective feeding habits. Using their chelate pereiopods, lobsters handle and masticate the feed outside the buccal cavity before ingestion. Consequently, a formulated lobster diet must be well-bound to endure feeding manipulation, reduce feed waste, and maintain water quality (Chouljenko *et al.*, 2024).

Chitosan serves as an effective binder and coating agent to enhance the water stability of lobster pellets. The acetyl groups in chitosan facilitate the formation of hydrogen bonds, which stabilize the crystalline structure of the pellets and reduce nutrient leaching. Moreover, chitosan, with higher degree of deacetylation, exhibits even greater resistance to fracture (Cui *et al.*, 2016). The resulted water stability in this research align with previous research, which demonstrated that chitosan-containing pellets maintained a highly stable size distribution during water immersion tests (Volpe *et al.*, 2012).

4. Conclusion

Chitosan morphology was identified as an irregular shape with striated surface textures. The yield of chitosan was 5.9 ± 0.01% of the total shell mass, with 96.99% ± 0.01 degree of deacetylation (DD). The resulting product contained 5.94 ± 0.07% moisture, 36.72 ± 0.05% ash and 2.73 ± 0.08% nitrogen. . This research has highlighted two major roles of chitosan in lobster feeds; at 0.5% inclusion, chitosan optimally inhibits fungi (*Fusarium* sp.), while higher inclusion (1.5%) was required to optimize water stability. Chitosan from lobster shells has strong antimicrobial properties, as well as effectively binding pellets and reducing nutrient losses into the water. These properties are expected to maintain pellet integrity and microbial status for increased nutrient delivery and health of farmed lobsters. In addition, this research demonstrated an effective circular use of existing waste products that can increase sustainability of the lobster supply chain. It is likely that mold inhibition and water holding capacity of chitosan can be further improved through optimized demineralization, while future research should explore long-term dietary effects, digestibility, and growth performance in lobsters to optimize its commercial viability in aquaculture. By integrating waste valorization with functional feed improvements, this study supports circular economy principles in seafood production.

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Authors' Contribution

All authors made significant contributions to the manuscript. MI and Mg were responsible for data collection and drafting the manuscript. AH and Mk contributed to data analysis and figure design. NP, HM, BP, CJ, and LN were involved in conceptualizing the main idea and providing critical revisions to the article.

Conflict of Interest

The authors declare that they have no competing interests.

Declaration of Artificial Intelligence (AI)

The author(s) acknowledge the use of ChatGPT for language refinement in preparing this manuscript. All AI-generated content was rigorously reviewed, edited, and validated to ensure accuracy and originality. Full responsibility for the manuscript's final content rests with the author(s). To ensure transparency and support the review process, a comprehensive delineation of the tool's application is furnished in the "Introduction" or "Materials and Methods" section of this manuscript in compliance with the publisher's ethical guidelines.

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