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Physicochemical and Functional Properties of Nano-Chitosan Derived from Green Mussel (*Perna viridis*) Shells

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Abstract

Increasing stocking density in catfish cultivation is part of efforts to increase production to meet catfish needs nationally and internationally. The obstacles faced by household Green mussels (*Perna viridis*) are a major fishery commodity in Indonesia, producing substantial shell waste. Green mussel shell waste can be used as shrimp feed because of its high calcium content. Nano-chitosan, derived from chitin extracted from green mussel shells, offers enhanced bioavailability and functional properties, making it a promising additive for feed applications. Nano chitosan has the advantage of being biocompatible, biodegradable, and non-toxic. The goal of this study is to create a nano chitosan extract from green mussel shells. Green mussel shells are extracted during the stages of deproteination, demineralization, and deacetylation. The obtained chitosan extract is then combined with Sodium Tripolyphosphate (NaTTP) to yield nano chitosan particles. The nano chitosan particles were then measured and analyzed for the compounds they contained. The extracted chitosan appeared white, with a yield of 71%, which aligns with previous studies on shell-derived chitosan. FTIR analysis confirmed the presence of characteristic chitosan functional groups, including C=O and O-H, at 343 nm. This study successfully synthesized nano-chitosan from green mussel shells that have biocompatibility and high calcium content.

INTRODUCTION

Feed is one of the most expensive components in aquaculture, accounting for 50-70% of total production costs (Santanumurti *et al*., 2022). Limited feed availability or inadequate nutritional content can significantly affect cultivation success.

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Therefore, alternative feed sources are needed to reduce costs while maintaining nutritional quality. One potential alternative is green mussels (*Perna viridis*), which are rich in protein and minerals, making them a suitable feed ingredient for aquatic organisms such as shrimp.

Green mussel is a widely cultivated aquaculture commodity in Indonesia, but its high production also generates large quantities of shell waste that pose environmental concerns. This waste, however, offers valuable potential as a raw material for chitosan extraction. Chitosan derived from green mussel shells can function as a bioactive feed additive that enhances specific growth rate and protein retention in vannamei shrimp (*Litopenaeus vannamei*) (Ekaputri, 2018). Furthermore, the calcium content in mussel shells contributes to exoskeleton formation, which may reduce molting-related mortality (Chamidah *et al.*, 2019). The chitosan yield from green mussel shells has been reported at 81.33%, reflecting efficient extraction (Arsyi *et al.,* 2018), and consists primarily of N-acetyl-D-glucosamine and D-glucosamine linked by β-1,4-glycosidic bonds—structures that support its biocompatibility and functional benefits in aquaculture applications (Logithkumar *et al.*, 2016; Hameed *et al.*, 2016).

Chitosan has been further processed into nano-chitosan due to its biocompatible, biodegradable, and non-toxic properties (Sandeep *et al.,* 2013). Chitosan is soluble in acidic solvents such as citric acid, formic acid, acetic acid, and lactic acid, but not in water (Karsli *et al.*, 2019). Nano-chitosan can be synthesized using the ionic gelation method, in which chitosan interacts with tripolyphosphate (TPP) anions to form nanoparticles (Bodmeier *et al.*, 1989; Shofura *et al.,* 2017). Ionic gelation is a method that has been widely developed for nano chitosan extraction due to its simplicity of the extraction procedure. The ionic glass method is carried out by adding a tripolyphosphate solution to the chitosan acid solution with constant stirring (Arsyi *et al.*, 2018). Nano-chitosan derived from green mussel shells can be a potential supplement for vannamei shrimp. Its bioactive properties may help enhance immune response, reduce mortality during the molting phase, and improve survival and growth rates.

METHODOLOGY

Ethical Approval

No animals were harmed or improperly treated in this study.

Place and Time

The research was carried out from July 18 to August 10, 2023, at the Aquaculture Laboratory of the Aquaculture Study Program, Faculty of Agriculture, Muhammadiyah University of Gresik, and the Biosciences Laboratory, Brawijaya University, Malang.

Research Materials

The materials used in this research include green mussel shells, 1% NaTTP solution prepared at a weight-to-volume ratio of 7:10 (w/v), commercial vanamei shrimp feed, distilled water, 3.5% NaOH solution prepared at a weight-to-volume ratio of 1:5 (w/v), 1 N HCl prepared at a weight-to-volume ratio of 1:5 (w/v), and 10% glacial acetic acid prepared at a weight-to-volume ratio of 1:5 (w/v). The equipment used included a hot plate, Petri dishes, a digital balance, a magnetic stirrer, 500 mL Erlenmeyer flask, 500 mL (Pyrex, USA), Beaker Glass (Pyrex, USA), 1000 mL Measuring Glass (Pyrex, USA), an 80 mesh sieve, an Oven, a particle size analyzer, an FTIR 8400S Spectrophotometer (Shimadzu, Japan), and scanning electron microscopy (SEM) with SEI (secondary electron) and QBSD (backscattered electron) detectors.

Research Design

The research design used in this study was experimental. Experimental research is an activity of data collection, data processing, analysis, and presentation carried out using systematic and planned experimental methods to prove the truth of a theory. The goal of this study is to create a

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nano chitosan extract from green mussel shells. The study of nano chitosan from green mussel has been conducted by previous studies and this research has never been conducted in the Gresik area (Fadhilah and Firmani, 2024).

Work Procedure

Preparation of Green Mussel Shells

Green mussel shell samples were obtained from Banyuurip Village, Ujungpangkah District, Gresik. The shell was separated from the meat and washed using running water to remove debris. Next, the shells are dried in the sun for 8 hours and crushed to a fine powder using a blender. The shell powder was then sieved through an 80-mesh sieve.

Chitosan Extraction

The first step in chitosan extraction was the deproteination stage. The fine powder of green mussel shells was put into an Erlenmeyer flask and 3.5% NaOH solution (w/v; 1:5) was added. The mixture was then macerated at 70°C for 2 hours on a hot plate with constant stirring. It was then filtered and washed with distilled water until it reached a neutral pH. The obtained solid was dried at 50°C until a constant weight was achieved. This extracted chitosan will serve as the raw material for the synthesis of nano-chitosan in the next stage.

The Second step was demineralization. The second step was demineralization. The green mussel shell extract was treated with 1 N HCl (w/v; 1:5) and left to soak at room temperature. The mixture was then heated at 75°C for 1 hour. After heating, it was filtered, and the residue was collected. The residue was washed with distilled water until a neutral pH was achieved, then dried at 50°C for 12 hours. At this stage, chitin powder was obtained. This chitin will be further processed into chitosan through the deacetylation stage, which is essential for the production of nano-chitosan.

The final stage was deacetylation. The resulting chitin powder was placed in an Erlenmeyer flask and treated with 10% glacial acetic acid (w/v; 1:5) at 90°C for 3 hours with continuous stirring. The mixture was then filtered and washed with distilled water until a neutral pH was achieved. The obtained solid was dried in an oven at 60°C until a constant weight was reached. At this stage, chitosan was successfully obtained. To produce nano-chitosan, the extracted chitosan was further processed using the ionic gelation method with NaTTP to form nanoparticles.

Making Nano Chitosan Ionic Gelation Method

The chitosan was then macerated with 1% NaTTP solution, with electrostatic interaction between positively charged chitosan and negatively charged NaTTP (w/v; 7:10), respectively, with a stirring speed of 900 rpm for 1 hour. This procedure, modified from the method described by Arsyi *et al.* (2018), resulted in the formation of chitosan nanoparticles as a dispersed solid.

Preparation of Feed and Repelleting

At this stage, feed treatment was conducted by mixing chitosan nanoparticles with commercial vannamei shrimp feed containing >50% protein. The nano chitosan was weighed and dissolved in distilled water (w/v; 1:3), then evenly sprayed onto the feed. The coated feed was left to absorb the solution at room temperature before undergoing a drying process in an oven at 50°C for 5 hours. After drying, the feed was finely ground to match the size of the shrimp larvae' openings. The final product was stored in plastic containers at 4°C to maintain its quality.

Characterization of Nanochitosan Particle Size Analysis

The particle size of nano chitosan was determined using a particle size analyzer (SA) from a 1 g sample. The analysis measured the average particle diameter and distribution in 343 nanometers (nm). A 100 mg sample was analyzed using an FT-IR 8400S Spectrophotometer (Shimadzu, Japan) to identify functional groups characteristic of chitosan. The presence of

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hydroxyl (-OH) at ~3200–3400 cm^{-1}, carbonyl (C=O) at ~1650 cm^{-1} and amine (-NH_2) at ~1550 cm^{-1} was observed to confirm nano chitosan formation. The microstructure of nano chitosan was examined using SE1 (secondary electron) and QBSD (backscattered electron) detectors. The analysis was performed at varying magnifications to assess surface morphology, particle uniformity, and porosity.

Data Analysis

The research data were analyzed descriptively by presenting the results of nano chitosan characterization (particle size, FTIR spectra, SEM images) and evaluating the effectiveness of nano chitosan-supplemented feed on shrimp growth and survival. The data is presented in the form of tables and figures to illustrate differences across treatments.

RESULTS AND DISCUSSIONS

The initial stage of making nano chitosan in this research was processing green mussel shells obtained from Banyu Urip Village, Ujung Pangkah Gresik District. Figure 1 shows a waste pile of various types of shells, but only green mussel shells were used in this research. When collecting green mussel shells at waste locations, the shells were directly sorted as suitable for use.

Figure 1. Piles of waste from green mussel shells in Banyu Urip Village, Ujung Pangkah, Gresik.

The sorted green mussel shells were processed to obtain fine powder suitable for chitosan extraction. This involved washing to eliminate surface impurities, followed by oven drying at 60 °C for 24 hours to reduce moisture content and inhibit microbial growth. The dried shells were then ground using a blender, and the resulting powder was sieved through an 80-mesh sieve to ensure uniform particle size. This standardized preparation was essential to support consistent chitosan yield and quality. The controlled drying conditions and hygienic handling also minimized the risk of contamination, thereby improving the reliability of subsequent extraction steps. The picture of the processing stages of green mussel shells is presented in Figure 2.

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Figure 2. Processing of green mussel shells into shell powder.

The finely ground green mussel shell powder was processed into chitosan through three main stages: deproteination, demineralization, and deacetylation, each designed to remove proteins, minerals, and acetyl groups, respectively, to isolate chitosan. At the end of the extraction process, chitosan powder was obtained with a yield of 51%. The yield was calculated using the formula: Yield (%) = (Final chitosan weight / Initial shell powder weight) × 100%.

Based on this calculation, 1540 g of chitosan was produced from 3000 g of shell powder. This yield is consistent with previous studies that reported chitosan yields ranging from 45–60%, depending on species, processing conditions, and shell composition. The chitosan extraction steps and the resulting powder are presented in Figure 3.

Figure 3. Chitosan extraction process and the resulting chitosan powder.

After the chitosan extraction stage, nano chitosan was synthesized using the ionic gelation method, which involved two key steps: (1) dissolving chitosan in acetic acid and (2) cross-linking with sodium tripolyphosphate (NaTPP). This process produced nano chitosan with a yield of 70%, calculated using the formula: Yield (%) = (Final nano chitosan weight / Initial chitosan weight) × 100%.

Fourier-transform infrared (FTIR) spectroscopy was then used to identify the functional groups present in the nano chitosan granules derived from green mussel shells. The synthesis process is illustrated in Figure 4.

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Figure 4. Nano chitosan was synthesized from chitosan using the ionic gelation method. Chitosan was first dissolved in a 10% acetic acid solution at a ratio of 1:10 (chitosan: acetic acid) to protonate the amino groups and enhance solubility. Then, 0.1% sodium tripolyphosphate (NaTPP) was added at a 7:1 ratio (chitosan solution: NaTPP). As a crosslinking agent, NaTPP facilitated ionic interactions between the positively charged chitosan and negatively charged phosphate groups, resulting in the formation of nano chitosan as a dispersed solid.

The deacetylation process of chitin into chitosan was carried out using sodium hydroxide (NaOH), which acts as a strong alkaline agent. This process involves hydrolyzing the acetyl (-$COCH_3$) groups from the acetamide (-$NHCOCH_3$) groups in chitin, converting them into free amine (-NH_2) groups, which characterize chitosan. The extent of acetyl group removal is quantified as the Degree of Deacetylation (DD), a crucial parameter influencing chitosan's solubility, bioactivity, and functional properties. The higher the DD, the greater the proportion of amine groups, resulting in enhanced reactivity and solubility. According to Bastaman (1989), NaOH is the most effective alkaline agent for achieving high DD. The deacetylation process typically involves heating chitin in a concentrated NaOH solution under controlled conditions. Key parameters such as NaOH concentration, temperature, and reaction duration influence the final DD and purity of the chitosan obtained.

The FTIR analysis results (Table 1) confirmed the presence of key functional groups characteristic of chitosan. The broad absorption band around ~3400 cm^{-1} corresponds to O–H stretching vibrations, indicating hydroxyl groups. The peak near ~1080 cm^{-1} is attributed to C–O stretching, associated with the polysaccharide backbone. Additionally, the presence of a peak around ~1650 cm^{-1} (amide I) indicates residual chitin, while the peak near ~1550 cm^{-1} corresponds to N–H bending vibrations, confirming the presence of primary amine groups (–NH_2) resulting from successful deacetylation.

The resulting nano chitosan appeared as a fine, white, low-density powder. Its preparation involved three main steps. First, deproteination was conducted using NaOH, which hydrolyzes peptide bonds to effectively remove proteins from the green mussel shell matrix. This was followed by demineralization using 10% HCl to dissolve calcium carbonate ($CaCO_3$), producing soluble calcium chloride ($CaCl_2$) and releasing CO_2 gas, observable as air bubbles during the reaction, as described by Ramírez *et al*. (2017). Finally, the deacetylation step converted chitin into chitosan by removing acetyl groups (–$COCH_3$), thereby increasing the free amine group content.

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Table 1. FT-IR test measurement results of nano chitosan in this study.

Wavelength (cm^{-1})	Functional Group	Peak Characteristics
3277.43	H bonds with NH_2 and -OH bonds	Wide
3070.63	C-O and O-H groups	Medium
2962.24-2930.86	CH and aliphatic group CH_2	Medium
1627.31	C=O amide group	Sharp
1513.21	C-C aromatic (ring)	Sharp
1447.60-1337.79	CH aliphatic group	Medium
1230.82	C-N amide group III	Sharp
1169.49-1026.87	C-O-C polysaccharide	Medium
821.50	Inorganic carbonate bond –C=O	Wide

The chitosan yield in this study was 50%, which is higher than the research of Ögretmen *et al*. (2022), namely 18.82%, which extracted chitosan from pink shrimp shells. This result is also higher than the research of Ait *et al*. (2018), namely 2.1% and 4.4%. The high yield of chitosan in this study may be due to the extraction method. The research results of Sedaghat *et al*. (2017) compared three different methods (traditional, microwave, and autoclave) for extracting chitosan from shrimp shells, and found that the highest yield of chitosan was obtained by the autoclave method.

In addition, Hossain and Iqbal (2014) reported that the low concentration of HCl used in the demineralization stage could not remove minerals from shrimp. The low chitosan yield may also be caused by depolymerization of the chitosan polymer, loss of mass/weight due to excessive removal of acetyl groups from the polymer during the deacetylation process, and loss of chitosan particles during washing. The increased yield of chitosan could also be caused by the high concentration of NaOH solution used in the deacetylation process (Fatima, 2020). The difference in chitosan yield may be related to its effectiveness in removing minerals and proteins attached during the deproteination and demineralization processes.

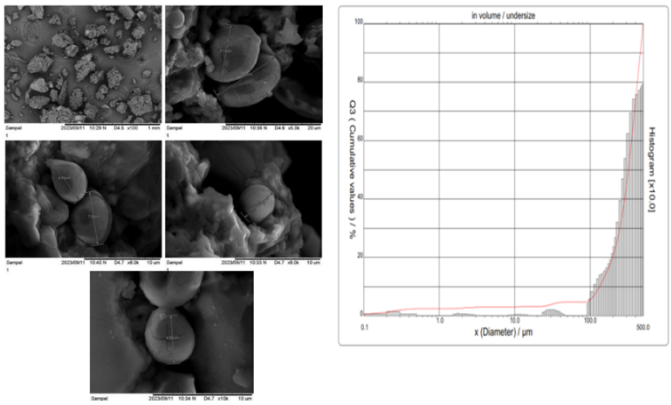

Figure 5. Nanoparticle chitosan shape analysis using SEM-EDX (left), Particle size of Nano Chitosan (right).

The SEM characterization of nano chitosan (Figure 5) revealed the presence of spherical and wrinkled particles, which are typical of chitosan nanoparticles synthesized using acid-base solvents. The measured particle size was <500 μm, though Mohanraj and Chen (2006) define nanoparticles as having a size between 10–106 nm. The successful production of nano chitosan from green mussel shells in this study represents a first step toward its application as a feed additive for vannamei shrimp (Davis *et al*., 1993).

A previous study (Wang *et al*., 2015) have reported the use of chitosan in vannamei shrimp feed, but limited research has focused on green mussel-derived nano chitosan. Further studies are needed to

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analyze the protein content of nano chitosan and conduct feeding trials to evaluate its effects on shrimp growth and health.

CONCLUSION

The research results showed that the yield value of nano chitosan was 71%. The FTIR test results found the presence of C-O and OH groups, which are chitosan molecular groups. Nano chitosan was successfully extracted and can be used as a supplementation ingredient in shrimp feed.

CONFLICT OF INTEREST

No conflict of interest among all authors upon writing and publishing the manuscript.

AUTHOR CONTRIBUTION

The first author contributed to writing the proposal, research, data analysis, and drafting the article. The second author contributed to nanoparticle chitosan extraction and characterization, including drafting the article. The third author contributed to drafting the article.

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