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Original Article

ISOLATION AND CHARACTERIZATION OF CHITOSAN NANOPARTICLES FROM CRAB SHELL WASTE (*PORTUNUS PELAGICUS*)

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ABSTRACT

Objective: The purpose of this study was to isolate and characterize of chitosan nanoparticles derived from *Portunus pelagicus* shell waste.

Methods: Chitosan was isolated by deproteination, demineralization, and deacetylation methods. Furthermore, nanoparticles (NPs) were made by the ionic gelation method by dissolving chitosan in a mixture of acetic acid and sodium tripolyphosphate. The particle size analyzer and Fourier Transform Infrared Spectroscopy were used to measure the particle size of NPs and determine the functional group and degree of deacetylation.

Results: The yield percentage of chitosan was 90.7%. The size of chitosan nanoparticles based on the highest intensity is 15.05 nm with a polydispersity index (PDI) value of 0.1140 at a concentration of 1%. Based on the degree of deacetylation of chitosan nanoparticles, it was found to be 84.98% at 1% concentration.

Conclusion: The conclusion of this study is the formation of chitosan nanoparticles (1-100 nm) isolated from *Portunus pelagicus* shell waste. Based on the degree of deacetylation, chitosan nanoparticles with high chitosan content (>75%) were obtained.

Keywords: Chitosan, Nanoparticles, *Portunus pelagicus*, Isolation, Characterization

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INTRODUCTION

Chitosan, the second most abundant natural polysaccharide obtained from *crustacean* shells, is a partially deacetylated chitin derivative and a mixture of d-glucosamine linked β-(1-4) and N-acetyl-d-glucosamine [1, 2]. *Portunus pelagicus*, a group of *crustaceans* that produce chitin, generally produces polymorphic α forms [3, 4]. Chitosan has an advantage over other polysaccharides because of the ease of making precise changes at the C-2 position due to its chemical structure [1, 5]. Chitosan has been found to inhibit microbial growth in most of the studies [1, 6]. Its non-toxic, readily biodegradable, and biocompatibility, as well as its antibacterial and anti-inflammatory capabilities, facilitate various applications of chitosan in areas such as pharmaceuticals, tissue engineering, textiles, food packaging, and agricultural products [1, 7, 8].

Chitosan made form of nanoparticles (NPs) has a particle size that meets the requirements in the dimensional range of 1-1000 nm. NPs used as carriers contain materials whose active compounds have been dissolved and encapsulated. The ionic gelation method's principle of nanoparticle formation is the ionic interaction between the positively charged amino groups on chitosan and the negatively charged polyanions. The cross-linking process occurs physically. In this process, organic solvents can be avoided, and the possibility of damage to the active ingredients can be prevented [9, 10]. Currently, chitosan is also valuable in the pharmaceutical field. Several studies have been carried out, such as drug delivery, protein/peptide/vaccine/gene/oligonucleotide delivery, wound dressing, protein binding, bioimaging, antimicrobial substances in food, antibacterials in food packaging materials. and textiles, implants, contact lens components, and cell encapsulation [11].

Chitosan nanoparticles (CS-NPs) are chitosan derivatives with excellent physicochemical properties [12-16]. Nanoparticles (NPs) were considered a delivery system because they have many advantages, such as passing through the smallest capillaries due to their small volume and penetrating cells and tissue gaps. In addition, it can improve the usefulness of drugs and reduce toxic side effects [17] CS-NPs have been prepared using several approaches, such as ionotropic gelation, microemulsion, solvent diffusion emulsification, polyelectrolyte complexes, and inverse micelle methods [14]. Nanoparticles made using various materials and composites have shown antibacterial efficacy against multiple types of bacteria [18, 19]. CS-NPs have conducted increased biological activities such as antimicrobial [12, 20], anticancer [21], anti-inflammatory, antioxidant [22, 23], and anti-nociceptive and immunomodulatory activities [23-28].

A study by Alqahtani *et al.*, reported that CS-NPs have a potential antimicrobial, characterized by inhibiting *Neisseria gonnorrhoeae* with the minimum inhibitory concentration of 0.16-0.31 mg/ml [29]. Furthermore, a study by Luthfiyana *et al.*, demonstrated that CS-NPs derived from mangrove crab shells (*Scylla sp.*) had antimicrobial activity against *S. aureus* with a concentration of 1% [30]. *S. aureus* is a bacteria triggering severe infection, including pneumonia, sepsis, and toxic shock syndrome. However, *S. aureus* could develop into *Methicillin*-resistant *Staphylococcus aureus* (MRSA) that resists several antibiotics (*beta-lactam*) and is more challenging to treat. Therefore, a compound from natural sources was required to treat infection caused by *S. aureus* [31]. Based on this background, a study was conducted to isolate and characterize chitosan nanoparticles derived from *Portunus pelagicus* shells.

MATERIALS AND METHODS

Raw material

The blue swimming crab (*Portunus pelagicus*) shell waste was obtained from fishermen from Pangkah Kulon village, Ujungpangkah sub-district, Gresik regency, East Java, Indonesia, whose meat was sold to canned crab meat export companies. The samples were cleaned with clean water flow, dried in an oven at 80 °C temperature, and then mashed with 60 mesh. All other solvents and chemicals used were of analytical grade. The research tools were provided by the Pharmaceutical Technology Laboratory Pharmacy Study Program Hang Tuah University, Microbiology Laboratory Faculty of Medicine Hang Tuah University, Growth Center Laboratory Hang Tuah University, Lembaga Ilmu Hayati, Teknik, dan Rekayasa (LIHTR) Laboratory Airlangga University, Chemistry Laboratory Institut Teknologi Sepuluh Nopember (ITS), Energy and Environment Laboratory ITS.

Preparation of chitosan

The isolation of chitosan from *Portunus pelagicus* shell waste was made by deproteination, demineralization, and deacetylation with several modifications [30, 32, 33]. 1000 g wet *Portunus pelagicus* shells are dried and ground into powder to obtain 677 g. The deproteinization process uses 1N Sodium hydroxide solution (ratio 1:100 w/v), with constant stirring for approximately 24 h or 3% Sodium hydroxide 1:6 weight of solute per volume of solution (w/v) heated for 30 min at 85 °C neutralized with distilled water to Potential Hydrogen (pH) 7 and then filtered with a Buchner filter, in the oven at 35 °C for 24 h. The result is a solid free of protein. The demineralization process used 1N Hydrochloric acid (ratio 1:15 w/v), with an immersion temperature range of-20 °C to 22 °C. Soaking with constant stirring for 1 h at room temperature is done to minimize the hydrolysis of the polymer chains. In addition, you can use 1N Hydrochloric acid with a ratio of 1:10, stir it at 75 °C for 1 h, neutralize it to a neutral pH of pH 7 and filter it and heat it at 35 °C for 24 h. The result of the isolation is chitin. This process was repeated two times to remove the remaining Calcium carbonate ($CaCO_3$). Deacetylation. This process. make deacetylation variations such as 5% Sodium hydroxide, 150 °C, 24 h; 40% Sodium hydroxide, 100 °C, 1 h. After the deacetylation process, Sodium hydroxide is dried, and pure chitosan is washed with deionized water until neutral and dried in an oven at 60 °C or Sodium hydroxide 45% 1:20 (w/v) heated at 110 °C within 1 h, then neutralized to pH 7 filtered and then in the oven at 80 °C for 24 h. The result was calculated by weighing the resulting chitosan and then dividing it by the weight of processed dry raw material with the equation below [32].

$$\text{Yield\%} = \frac{\text{Dry Chitosan mass (g)}}{\text{Dry raw material mass (g)}} \times 100\%$$

The quality of chitosan was assessed by analyzing the water, ash, and nitrogen content based on the Association of Official Analytical Chemists (AOAC) [33]. Fourier Transform Infrared Spectroscopy (FTIR) spectra were used to calculate chitosan yield using wavelengths ranging from 4,000-400 cm^{-1}. The percentage degree of deacetylation (DD%) was calculated from the ratio between the absorbance at 1,655 cm^{-1} and 3,450 cm^{-1} with the equation below [30].

$$\text{DD\%} = 100 \left[\left(\frac{A_{1,655}}{A_{3,450}}\right) \times \frac{100}{1,33}\right]$$

Preparation of nanochitosan

We used an ionic gelation method to produce chitosan nanoparticles from *Portunus pelagicus*. Chitosan solutions with concentrations of 0.1% and 0.2% were made by dissolving 0.1 gram of chitosan in a 1% acetic acid solution of 100 ml, and then stirred constantly at 1000 rpm at room temperature until dissolved. Furthermore, a 100 ml of sodium tripolyphosphate solution was made with a concentration of 0.1% and 0.2% and stirred until dissolved as a crosslinker. The crosslinker solution was added to the mixture of chitosan solution in acetic acid drop by drop until the crosslinker solution ran out and stirred at 1000 rpm for 2 h. The nanoparticle suspension was centrifuged at 6000 rpm for 5 min. The precipitate was washed three times with distilled water. After that, it was dried using freeze drying for 47 h [10, 30, 34, 35]. The dried nanoparticles were physically characterized through several tests, such as particle size.

Particle size analyser (PSA)

A particle size analyzer (PSA) was used to determine the chitosan nanoparticles size by BIOBASE BK-802N with a HAMAMATSU photo-multiplier detector (5-90°C). The nano chitosan was dissolved in distilled water and then put into a cuvette for measurement [36]. The analyzed nano chitosan can be viewed on a monitor, and the magnification was adjusted [30]. All measurements were performed in triplicate and reported as the means±standard deviation (SD).

Fourier transform infrared spectroscopy (FTIR)

The chitosan samples were characterized by an infrared spectrometer (Shimadzu). Chitosan samples were made into potassium bromide (KBr) Pellets to obtain a transmittance infrared spectrogram, which is scanned in the range of 400-4000 cm^{-1} [37].

RESULTS AND DISCUSSION

Characterization of chitosan

In the early stages of the study, chitosan was isolated from crab shell waste (*Portunus pelagicus*) by carrying out three steps, namely deproteination, demineralization, and deacetylation with an initial sample weight of *Portunus pelagicus* shells of 1000 g. After being mashed, the weight becomes 677 g. The results of chitosan isolation produced after sifting mesh 100 and 60 were 70 g. Details of the chitosan isolation results are presented in table 1.

Table 1: Observation results of chitosan isolation

	Sample	Deproteination	Demineralization 1	Demineralization 2	Deacetylation
Weight (g)	677	394	298	193	175
pH	-	7	7	7	7
Result	-	Protein free	Chitin	Chitin	Chitosan
% Yield	-	58.2%	75.6%	64.8%	90.7%

Determination of the degree of deacetylation (DD) from the chitosan isolation was determined by the percentage of the number of acetyl groups that are lost and turned into amine groups. The higher the degree of deacetylation, the fewer acetyl groups present in chitosan and the better the quality of chitosan [38]. Based on the Fourier Transform Infrared Spectroscopy (FTIR), the DD is calculated as 77.5% and these results meet the quality standards of chitosan in Indonesian National Standards (SNI) 7949:2013 because a reasonable degree of deacetylation of chitosan must be ≥75% [39].

The ratio of Hydrochloric acid (HCl) samples in the demineralization process affects the DD of the resulting chitosan. A higher amount of HCl indicates a higher DD. The DD is a critical parameter affecting chitosan characteristics, such as biological, physicochemical, and mechanical properties [40]. Several studies reported that the degree of deacetylation (%DD) is influenced by factors such as base concentration, reaction time, and reaction temperature during deacetylation [41]. However, %DD cannot reach 100% because there is a copolymer between N-acetylglucosamine and glucosamine, which supports the creation of biocompatible, biodegradable, and adsorption properties [42].

The chitosan obtained was prepared as chitosan nanoparticles using the ionic gelation method, a complexation of polyelectrolytes between positively charged chitosan and negatively charged tripolyphosphate. Chitosan solutions were made at concentrations of 1% and 2% with a sodium TPP ratio of 0.1% and 0.2%, constant stirring was carried out at 1000 rpm for 2 h, after which they were stored in the refrigerator for 24 h, then centrifuged at 6000 rpm for 10 min. The sediment was taken and stored in the freezer for 24 h. Then, it was dried using freeze drying for 47 h. The results of the two chitosan nanoparticle formulas produced have the physical characteristics shown in table 2.

Based on particle size analyzer (PSA) measurements, it was obtained that the size of nano chitosan with the highest intensity was 15.05 nm with a polydispersity index (PDI) value of 0.1140 at 1% chitosan concentration. The size of this nano chitosan indicates that nanoparticles have been formed. A particle is considered a nanoparticle if the particle size is 1-100 nm [43]. In addition, the PDI value can describe the particle size distribution. A good PDI value indicates good long-term stability and a small PDI value indicates nanoparticle size stability [30].

The spectral of FTIR of nano chitosan from *Portunus pelagicus* shell waste is shown in fig. 2 and 3.

Table 2: Nano chitosan particle size results

Formulas	Replication	Particle size (nm)	mean±SD	PDI	mean±SD
Chitosan 1 %	1	15.05	10.84±3.92	0.1140	0.2250±0.1172
	2	10.25		0.2136	
	3	7.23		0.3476	
Chitosan 2 %	1	13.95	10.51±3.11	0.2040	0.1564±0.0464
	2	7.89		0.1114	
	3	9.71		0.1538	

*Results are expressed as a mean±SD, n=3, The size distribution of nano chitosan at concentration 1% as shown in fig. 1.

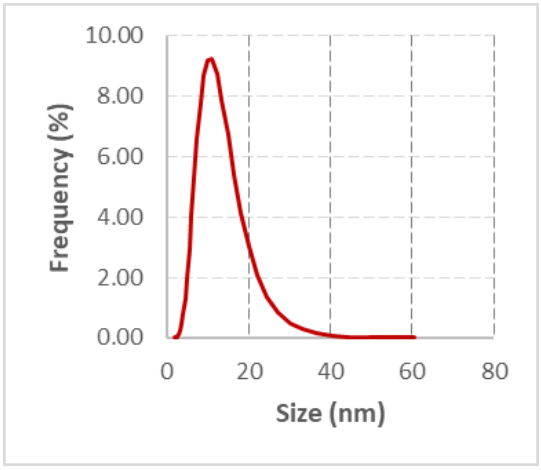

Fig. 1: Size distribution of nanochitosan concentration 1%

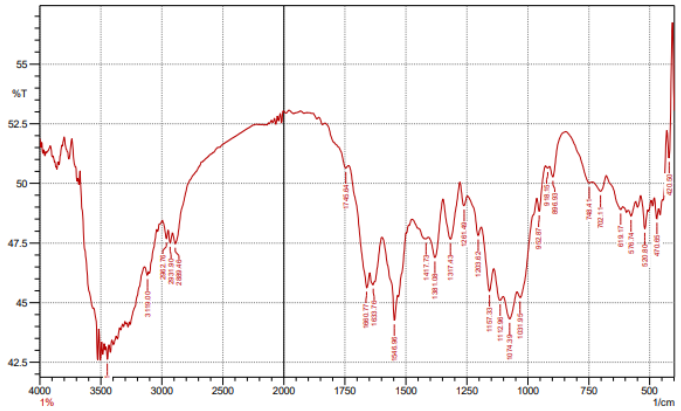

Fig. 2: Nano chitosan 1% with FTIR spectra of *Portunus pelagicus* shell waste

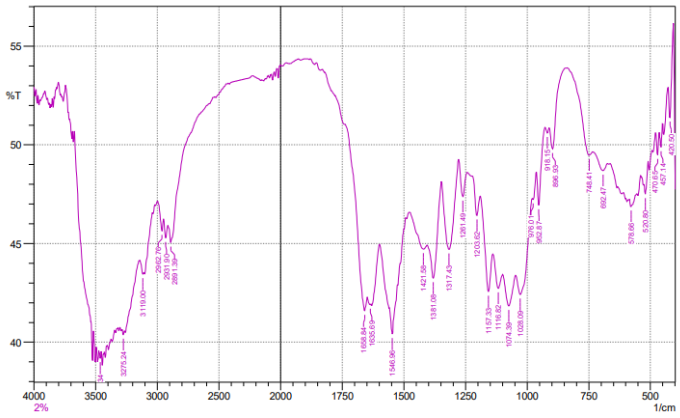

Fig. 3: Nano chitosan 2% with FTIR spectra of *Portunus pelagicus* shell waste

Based on the FTIR results above, the percentage of DD of *Portunus pelagicus* chitosan nanoparticles was calculated as 84.98% for 1% nano chitosan and 77.47% for 2% nano chitosan. A study by Luthfiyana *et al.*, and Ahyat *et al.*, reported that FTIR vibration patterns from crabs *Portunus pelagicus* also showed stretching of hydroxyl (O-H), amine (N-H) and carbonyl (C-O) groups indicated the presence of chitosan [30, 32]. Characteristic of nano chitosan from FTIR spectra was shown in table 3.

Table 3: Characteristics of nano chitosan FTIR spectra

Functional group prediction	λ range	The length λ is obtained
O-H Alcohol	3600-3100 cm^{-1}	3446.91 cm^{-1}
		3119.00 cm^{-1}
N-H Amine	3500-3100 cm^{-1}	3446.91 cm^{-1}
		3119.00 cm^{-1}
Aliphatic C-H Stretch	3000-2850 cm^{-1}	2962.76 cm^{-1}
		2889.46 cm^{-1}
C=O Stretch Aldehydes	1750-1625 cm^{-1}	1745.64 cm^{-1}
C=O Secondary Amines	1670-1600 cm^{-1}	1660.77 cm^{-1}
		1633.76 cm^{-1}
		1546.96 cm^{-1}
C-H Alkyl	1450-1375 cm^{-1}	1417.73 cm^{-1}
C-O Eter	1300-1000 cm^{-1}	1381.08 cm^{-1}
		1317.43 cm^{-1}

Materials in nanometric size are used as diagnostic instruments or even to deliver therapeutic compounds to specific target regions in a controlled manner in nanoparticles and nano-delivery systems, which is a relatively young but rapidly growing discipline. Nanotechnology provides many advantages in treating chronic diseases in humans by having drugs that are accurate to defined locations and targets. Nanomedicines (including chemotherapeutic drugs, biological agents, immunotherapeutic agents, etc.) have recently seen several important uses in treating various diseases. Through careful examination of the discovery and use of nanomaterials in improving the effectiveness of new and old drugs (such as organic products) and preferential diagnosis through disease marker substances [44].

Ultimately, the use of nanoparticles will evolve as our understanding of disease increases at the cellular scale or reflects the identification of biomarkers equivalent to the nanomaterial-subcellular scale to open up new pathways for diagnosis and treatment. The search for more accurate treatment and diagnosis is a growing trend worldwide, and the development of nanoparticles and nano-drug delivery systems seems promising. The creation of nanodevices that work in tissue diagnostics and repair with complete external control methods has attracted much attention. Therefore, a thorough examination of the potential short- or long-term harmful consequences of new nanomaterials to humans and the environment is necessary. The accessibility of nanomedicine will be another study topic that requires more study input as it becomes more widespread [44].

The application of nanotechnology in medicine, especially for drug delivery, is expected to increase. Pharmaceutical science has been using nanoparticles to reduce drug toxicity and side effects for many years. It was not known until recently that the carrier system itself could pose a danger to patients. Further to the typical risks presented by compounds in the delivery matrix, new risks are added with the use of nanoparticles for drug delivery. Unfortunately, there is currently no scientific framework for potential (adverse) reactions of nanoparticles, and we know little about the fundamentals of how nanoparticles react with living organisms, tissues, and animals. For the future sustainable development and application of nanomaterials in drug delivery, a conceptual understanding of the biological response to nanoparticles is necessary [44].

Several studies have developed chitosan nanoparticle preparations such as Lactobionic acid conjugated nanoparticles that show great potential as targeted drug delivery systems and possess remarkable beneficial properties not seen in other delivery modules. Some properties that make it a suitable carrier are high drug loading, good targeting, and excellent biocompatibility. The proposed targeting strategy increases the therapeutic index of conventional anticancer drugs and reduces their cytotoxic effect on normal cells, resulting in a potential drug delivery system [17]. In addition, there are studies on nano chitosan, which can heal wounds on the skin [45], and nano chitosan, which shows potential as a promising antibacterial agent [30].

CONCLUSION

The conclusion of this study is chitosan from crab shell waste (*Portunus pelagicus*) has a DD value of 77.5% with a yield of 90.7%. The DD value meets the quality standards of chitosan according to SNI 7949:2013 because a reasonable degree of deacetylation must be ≥75%. The value of the degree of deacetylation indicates the quality of a chitosan related to its purity; the higher the DD value of a chitosan, the better the quality. The preparation of chitosan nanoparticles from *Portunus pelagicus* crab shell waste will be developed into a topical pharmaceutical preparation as a gel. It is expected to be used for gangrene wound patients to accelerate wound recovery. Therefore, the results of this study will be followed by other preliminary research to determine the effectiveness of chitosan nanoparticles of crab shell waste as antibacterial and anti-inflammatory.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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