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Intermolecular Characterization of Chitosan from the Exoskeleton of Windu Prawn *(Penaeus monodon)* and Mangrove Crab *(Scylla sp.)*

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Abstract

Chitosan $(C_6H_{11}NO_4)_n$ is a chitin-derived polymer that has good biocompatibility, biodegradability, and bioabsorbability properties. This compound can be obtained from exoskeleton waste such as mangrove crabs and Windu prawn. This study aims to evaluate the comparison of chitosan levels based on the degree of deacetylation (%DD) from the waste shell, head, and leg of Windu prawn and mangrove crab shell waste obtained from the coastal area of Pasuruan, East Java. The extraction method was carried out through the stages of deproteinization, demineralization, and deacetylation using an alkaline solution, and then the characterization of molecular structure and %DD was carried out using an FTIR spectrophotometer. The results showed that chitosan from Windu prawn shell had the highest %DD of 73.0%, while mangrove crab shell and prawn head and leg showed lower values of 55.3% and 63.9%. This difference in %DD value indicates a variation in the success rate of deacetylation due to differences in biomaterial composition. The results of SEM testing showed that the use of the type of material in the manufacture of chitosan would affect the results of the surface morphology produced, where the Windu prawn shell produced the flattest surface.

Keywords: Characterization, Chitosan, Exoskeleton, FTIR, SEM

INTRODUCTION

Indonesia has coastal areas with various types of habitat typologies and high biota diversity, one of which is the mangrove forest ecosystem which is the habitat of mangrove crabs (*Scylla sp.*) and Windu prawn (*Penaeus monodon*), both of which are included in the class *Crustacea* that contain chitin in high levels ranging from 20% to 60% depending on the species, stage of development and age of the species. In comparison, the level of chitin in the shell crabs is around 70%, while in prawns the chitin level is around 30% (Rapierna & Mahatmanti, 2012). About 20% of the weight of crabs is meat, so about 80% is crab shell waste that is not utilized correctly and is generally discharged into the environment (Mashuni et al., 2021). In addition to crab shells, prawn shells, and head-leg waste, other waste products also contribute to polluting the environment. These conditions show that the processing of crab and prawn shell waste is not optimal. With these problems, it can open up great opportunities to utilize the chitin content of most marine invertebrates, which researchers can convert into high-value chitosan compounds.

Chitin is a natural linear polymer composed of chains of 2-acetamido-2-deoxy-D-glucopyranose units (or N-acetyl-D-glucosamine), most commonly found in the waste of marine shelled animals belonging to the crustacean and mollusk classes (Anggreini & Sinaga, 2023; Hasanela et al., 2020). Chitosan, on the other hand, is a derivative polymer of chitin characterized by good biocompatibility, biodegradability, and bioresorbability, as well as being non-toxic. Its molecular formula is $[C_6H_{11}NO_4]_n$, and its chemical structure is poly(2-amino-2-deoxy-β-D-glucopyranose). It appears as yellowish-white flakes that are odorless and tasteless (Ningtyas et al., 2020). Chitosan is a type of oligosaccharide compound produced through the deacetylation of chitin at high temperatures. In the chitin molecule, the amino group

(-NH_2) is bound to an acetyl group; therefore, the more acetyl groups are removed (deacetylation) from chitin, the greater the yield of chitosan molecules, with the remaining acetyl groups not exceeding 40-45%. The Degree of Deacetylation (DD) is the extent to which acetyl groups are removed from chitin. Deacetylation is the most critical factor in determining the quality of the resulting chitosan, such as its solubility, chemical reactivity, and biodegradability. Chitosan can be applied as a preservative supplement, food stabilizer, antioxidant, antimicrobial, and antitumor agent (Kusnadi et al., 2022; Sabu et al., 2020; Vimaladevi et al., 2015). Furthermore, chitosan is also utilized in the pharmaceutical and agricultural industries. It is also employed in food processing, pharmaceutical drugs, and environmental wastewater remediation (Chattopadhyay et al., 2019; Kumar et al., 2018; Kusnadi et al., 2022; Rahayu et al., 2020).

The production of chitin-chitosan compounds can involve processes such as demineralization, deproteinization, and decolorization. In contrast, chitosan production can be carried out through partial hydrolysis via the deacetylation of chitin using the alkali method (Henggu et al., 2022; Salmahaminati, Dyah, et al., 2025). Sartika et al., in 2016 reported that the isolation of chitosan from blue swimming crab shells using the alkali method resulted in a deacetylation degree (%DD) of 70.73%. Meanwhile, research conducted by Salmahaminati, 2022 showed that chitosan derived from mangrove crab shell waste using the microwave irradiation method yielded a deacetylation degree (%DD) of 76.5%. Sam & Putri, 2022 reported that chitosan isolation from prawn shell waste using the alkali method produced a deacetylation degree (%DD) of 83.25%. Sam et al. (2022) reported a deacetylation degree (%DD) of 81.23% from Windu prawn shell waste. Meanwhile, Widyastuti, in 2023 reported that a comparison of chitosan obtained based on the deacetylation degree (%DD) from whiteleg prawn and Windu Prawn Shell waste was 72.63% and 75.44%, respectively, using the alkali method. Therefore, this study aimed to evaluate the comparative chitosan content using the alkali method based on the deacetylation degree (%DD) from Windu Prawn (*Penaeus monodon*) shell, head, and leg waste, as well as Mangrove Crab (*Scylla sp*.) shell waste sourced from the coastal area of Pasuruan City, East Java. However, no comparative study has been conducted on chitosan from these three specific waste sources (shell, head-leg) from the Pasuruan coastal area.

METHODOLOGY

Materials and Instrumentals

The materials used in this study are waste shells, heads, and legs of Windu prawn (*Penaeus monodon*) and mangrove crab shells (*Scylla sp.*) obtained from the coastal areas of Pasuruan City, East Java, distilled water, NaOH, HCl obtained from (CV. Krida Tama Persada), and Universal Indicators (Merck). The tools used in this study are a hotplate magnetic stirrer (IKA RH digital), Oven (Memmert), pH meter (Mediatech), thermometer (GEA S-006), FTIR (Fourier Transform Infra-Red) (Thermo Scientific), glassware (Pyrex), 80-mesh sieve, mortar and blender (Miyako 3 in 1).

Methods

Sample Preparation

This study collected shells, legs, and heads of Windu prawns (*Penaeus monodon*) and mangrove crabs (*Scylla* sp.) from the coastal area of Pasuruan City, East Java, and cleaned them using airflow to remove residual meat and dirt. The study separated the cleaned shells, legs, and heads by species and cut them into small pieces (approximately 1–2 cm) before drying. After drying, the samples were ground in a mortar and blender to obtain a fine powder, then sieved through an 80-mesh sieve for further processing.

Isolation of Chitosan

The isolation of chitosan from the finely ground exoskeleton waste of Windu prawns (*Penaeus monodon*) and mangrove crab shells (*Scylla sp*) was carried out in three stages, based on Arisa et al. (2025) with modifications, as follows:

Deproteinization

Waste powder from the exoskeletons of Windu prawns (*Penaeus monodon*) and mangrove crabs (*Scylla sp.*) with an 80 Mesh size was obtained. 50 g of powder was dissolved in 500 mL of 3N NaOH at a 1:10 (w/v) ratio. The solution was stirred and heated on a magnetic stirrer hot plate at 65 °C for 60 minutes. The residue obtained was filtered and neutralized with distilled water to pH 7. The neutralized powder was dried in an oven at 65 °C for 24 hours, resulting in deproteinized exoskeleton powder from Windu prawns (*Penaeus monodon)* and mangrove crabs (*Scylla sp)* (Arisa et al., 2025).

Demineralization

The deproteinized exoskeleton powder from Windu prawns (*Penaeus monodon*) and mangrove crabs (*Scylla sp*) was dissolved in 1N HCl at a ratio of 1:15 (w/v). The solution was stirred using a magnetic stirrer for 60 minutes at room temperature. The residue obtained was filtered and neutralized with distilled water to pH 7. The neutralized powder was re-ovened

at 65 °C for 24 hours, yielding mineral-free chitin compounds (Arisa et al., 2025).

Deacetylation

The obtained chitin was dissolved in 50% NaOH at a 1:10 (w/v) ratio. The solution was then stirred and heated using a microwave oven. The obtained chitosan residue was filtered and neutralized with distilled water to pH 7. The neutralized chitosan was re-ovened at 80 °C for 4 hours.

Yield of Chitosan

The Yield of chitosan was calculated by equation (1) as follows (Aichayawanich & Saengprapaitip, 2019):

$$Yield\ (\%) = \frac{Weight\ of\ chitosan\ (g)}{Weight\ of\ initial\ dry\ sample\ (g)} x\ 100 \quad (1)$$

Characterization Test: SEM (Scanning Electron Microscopy)

The resulting chitosan surface morphological analysis was performed using Scanning Electron Microscopy (SEM; Hitachi TM-4000 Plus, Japan) at 15 kV, in vacuum, with a magnification of 500×. Before inspection, the chitosan sample is carefully prepared, and uncoated samples were analyzed. Samples were fixed to carbon adhesive tape to improve adhesion and conductivity.

Characterization Test: FTIR (Fourier Transform Infra-Red)

Analysis of the structure and degree of deacetylation using the FTIR spectrophotometer for the determination of typical functional groups in chitosans, such as hydroxyl (-OH), amine (-NH_2), and acetyl (-$COCH_3$) groups. The spectra were recorded over 4000 cm^{-1} –500 cm^{-1} with a resolution of 4 cm^{-1}, averaged over 32 scans. The analysis of these intermolecular characteristics can be a determinant of the quality of the chitosan produced based on the %DD (Degree of Deacetylation) obtained.

Data analysis

The determination of %DD (Degree of Deacetylation) using FTIR spectroscopy is calculated based on its absorbance ratio. In this study, Sabnis and Block's equation (equation 2) was used, namely by using the absorbance ratio of 1655 cm^{-1} and 3450 cm^{-1} with the following calculation (Mahardika et al., 2020):

$$\%DD = 97.67 - \left[\left(\frac{A1655}{A3450}\right) x\ 26.486\right] \quad (2)$$

RESULTS AND DISCUSSION

Chitosan Isolation from Windu Prawn and Mangrove Crab Shell Waste

Isolation of chitin from waste shells, heads, and legs of Windu prawn (*Penaeus monodon*) and mangrove crab shells (*Scylla sp*) through the stages of deproteinization, demineralization, and deacetylation. The isolation of chitin aims to separate and convert the acetamide group (-$NHCOCH_3$) in chitin into an amine group (-NH_2) using a high concentration of a strong base, such as protein and calcium carbonate, so that chitin is obtained (Dompeipen et al., 2016). The yields obtained from each stage are shown in Table 1.

Table 1. Yield and Texture/Visual Characteristics of Chitosan Synthesis

Process Stages	MCS	WPS	WPHL	Texture/ Visual
Deproteinization	33.29 g (66.6%)	22.51 g (45.0%)	113.64 g (27.3%)	Solid residue, slightly coarse, pale brown residue
Demineralization	9.06 g (18.1%)	10.77 g (21.5%)	5.94 g (11.9%)	Fragile, yellowish -white, CO_2 foam formed during reaction
Deacetylation	6.77 g (13.5%)	10.31 g (20.6%)	5.45 g (10.9%)	Fine powder, yellowish -creamy white, more porous

Note: Mangrove Crab Shell (MCS); Windu Prawn Shell (WPS); Windu Prawn Head and Leg (WPHL).

Deproteinization

The deproteination stage aims to break the bond between proteins and chitin found in mangrove crab shell powder and Windu prawn shells, heads, and legs by adding NaOH solution, so that the bound proteins will dissolve in the base. At this stage, the solution is brown and viscous, indicating that the protein binds to chitin and to water-soluble Na^+ ions, forming sodium proteinate (Aldila et al., 2020), while chitin settles as a solid because it is insoluble in water. Proteins associate with chitin primarily through non-covalent interactions, including hydrogen bonds, electrostatic interactions, and van der Waals forces. Stirring and heating under alkaline conditions (NaOH) denature the

proteins and weaken non-covalent interactions, thereby enabling the release of proteins from the chitin matrix. This process facilitates protein dissociation by altering their secondary and tertiary structures rather than directly breaking covalent bonds (Rinaudo, 2006; Younes & Rinaudo, 2015). The stirring and heating process is carried out to accelerate the binding of protein chain ends to NaOH, thereby enabling optimal breakdown and deposition of proteins (Silalahi et al., 2020).

The results obtained show that the mangrove crab shell (MCS) sample had the highest deproteinization yield of 66.6%. In contrast, the lowest deproteinization yield was obtained from the Windu prawn head and leg (WSHL) sample, which was 27.3%. This is because the mangrove crab shell (MCS) sample contains high minerals, namely $CaCO_3$, $Ca_3(PO_4)_2$, and relatively low protein, so that the resulting residue is more than that of the Windu prawn head and leg, which are rich in protein and other organic compounds that dissolve in the base solvent used. The reaction that occurs makes the structure of the residue obtained harder and stiffer, as some protective proteins are broken, resulting in a complex texture of the residue at the deproteinization stage.

Demineralization

The demineralization stage aims to remove inorganic minerals, such as $CaCO_3$ and $Ca_3(PO_4)_2$, from mangrove crab and Windu prawn shells, including the heads and legs. At this stage, HCl is added gradually because it reacts with $CaCO_3^-$ to form CO_2 gas bubbles and $CaCl_2$, a soluble salt in water (Mahardika et al., 2020). The formation of CO_2 gas during the demineralization stage indicates the reaction of hydrochloric acid with mineral salts contained in the waste shells of mangrove crabs and the shells, heads, and legs of windu shrimps (Figure 1). During this process, protein, fat, phosphorus, magnesium, and iron are discarded, and calcium compounds react with hydrochloric acid dissolved in water (Dompeipen et al., 2016). The chitin powder obtained from the demineralization of the three samples showed different colors (Figure 1).

At this stage, the resulting residue experienced a significant decrease in mass, especially in the MCS (Mangrove Crab Shell) sample, which initially had a yield mass of 33.29 g, decreasing to 9.06 g (27.2%). This indicates that the MCS sample contains very high minerals, especially $CaCO_3$. Meanwhile, the yield of the WSS sample was 21.5% higher than that of the MCS sample, indicating a higher chitin content and lower mineral content. In addition, the mineral content in the WPS (Windu Prawn Shell) and WSHL (Windu Prawn Head and Leg) samples was easier to separate from protein because it was physically bound (Agustina et al., 2008). The WSHL sample had the lowest yield because the head and leg samples of Windu prawns were composed not only of chitin and minerals but also of protein, pigments, and lipids. During the deproteinization stage, most of the protein dissolves in the base. In contrast, during the demineralization stage, other organic compounds, such as carotenoid pigments and organic phosphates, dissolve along with inorganic minerals in the acid treatment (Shaqsi et al., 2020). The texture of the residue after demineralization is more brittle and lighter than the residue during the deproteinization stage because the inorganic minerals that support the structure have been broken down.

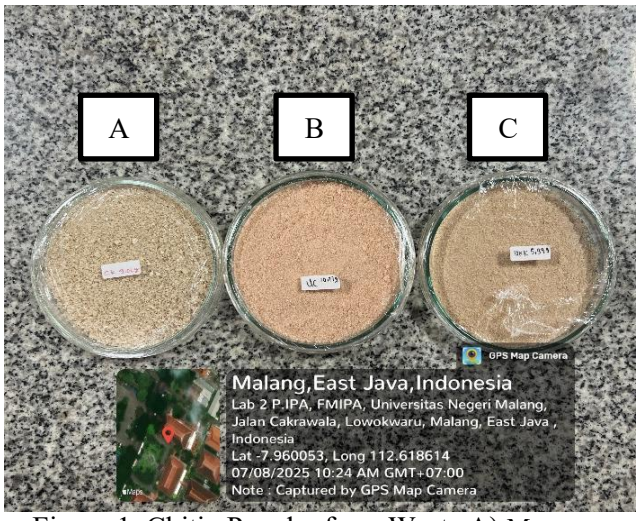

Figure 1. Chitin Powder from Waste A) Mangrove Crab Shell (MCS); B) Windu Prawn Shell (WPS); C) Windu Prawn Head and Leg (WPHL)

Deacetylation

The deacetylation stage converts the acetyl group ($-NHCOCH_3$) in chitin into a free amine group ($-NH_2$), thereby forming chitosan. The cleavage of acetyl bonds generally requires a base, so in this study, a strong base of 50% NaOH was heated using a microwave oven to accelerate the release of the acetyl group ($-NHCOCH_3$) to form chitosan with a free amine group ($-NH_2$) (Shaqsi et al., 2020). The concentration of OH- ions also dramatically affects the process of releasing acetyl groups from chitin acetamide groups. The higher the concentration of OH- ions (the more basic), the faster the process of breaking the acetyl groups from chitin acetamide groups (Dompeipen et al., 2016). In this case, microwave heating can provide high, even heat energy quickly, allowing the basic hydrolysis reaction

to occur more efficiently than with conventional methods (Salmahaminati, 2022). At this stage, physical changes also occur in the resulting chitosan, which turns yellowish-white to cream in color, develops a smooth texture, and becomes more porous due to the release of acetyl groups, which create space between the polymer chains.

The WPS (Windu Prawn Shell) sample produced the highest chitosan yield of 10.31 g, or approximately 20.6%, while the lowest chitosan yield was obtained from the WSHL sample, which was 5.45 g, or approximately 10.9%. These results confirm that Windu prawn shells are the most promising source of chitin among the samples in this study. Additionally, this is influenced by the sequence of the deproteinization and demineralization stages in chitin isolation. Salmahaminati in 2022 reported that chitin isolation via the demineralization-deproteinization stage yields a higher yield than that via the deproteinization-demineralization stage. This is because minerals form a rigid shield on the shells of crabs and prawns, whereas proteins do not. Therefore, by removing the minerals first, the alkaline deproteinization stage can be optimized for removing proteins, as the mineral *shield* has been removed.

Characterization of Chitosan with SEM

The results of SEM characterization at 500x magnification on the surface of chitosan derived from a) Mangrove Crab Shell (MCS); b) Windu Prawn Shell (WPS); c) Windu Prawn Head and Legs (WPHL) are shown in Figure 2. Figure 2 reveals that the resulting surface morphology depends on the body part and the animal species utilized for chitosan production. The surface differences between chitosan from prawn shells and crab shells primarily arise from variations in the raw material composition and the physical structure of the shells (Rahman & Maniruzzaman, 2023).

Figure 2.a shows that the surface morphology of chitosan originating from the Mangrove Crab Shell has a rougher surface with some cracks and fibers coming out of the surface. This is because crab chitosan tends to have a denser, rougher structure due to its chitin and mineral content (Daramola et al., 2021). So that the solubility value of chitosan derived from crab is lower, and in the process of dissolving, it makes the surface rougher (Sultana et al., 2020).

Figures 2b and 2c show that the chitosan from windu prawn shells has a smoother surface and is more homogeneous. This is because the surface of the windu prawn shell (WPS) is a tightly layered structure (Bradic, 2020). This fiber is also in line with previous research conducted by Priyangga et al. (2023), which states that chitosan produced from prawn shows a subtle tendency due to the concentration and reaction time. The difference between the two is apparent in the consistency of flatness produced. Basically, the structure of the prawn shell consists of several layers (such as epicuticle - exocuticle, exocuticle - endocuticle, and endocuticle - membrane) where the more this layer is eroded, the rougher the surface produced (Rahman & Maniruzzaman, 2023).

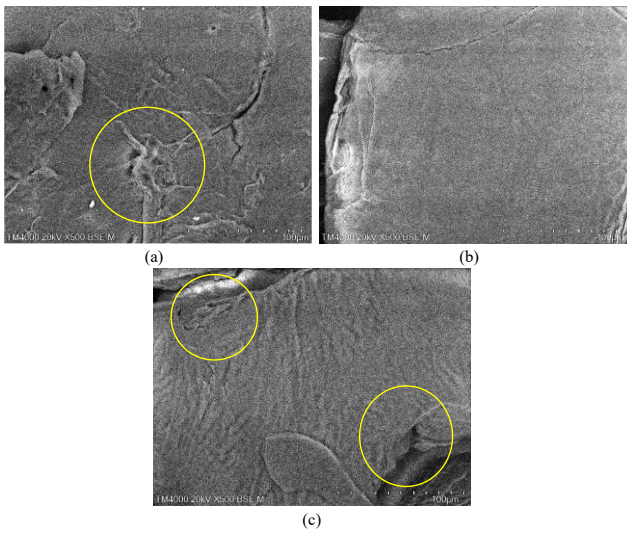

Figure 2. Chitosan surface morphology of (a) Mangrove Crab Shell (MCS); (b) Windu Prawn Shell (WPS); (c) Windu Prawn Head and Leg (WPHL) with scale bar 500 times

Previous research has also revealed that the presence of specific elements, as indicated by FTIR analysis—such as carbon (C), nitrogen (N), and oxygen (O) with intensities corresponding to their concentrations in each body part—also influences the solubility capability of prawn chitosan. Additionally, characteristic phosphorus (P) peaks and trace amounts of calcium (Ca) were observed only in chitin and chitosan, indicating the successful removal of nearly all minerals from prawn shells through chemical treatment (Sachs et al., 2006).

Characterization of Chitosan with FTIR

The FTIR (*Fourier Transform Infra-Red)* spectrum functional group analysis aims to identify functional groups in chitosan isolated from mangrove crab shells (MCS), Windu prawn shells (WSS), and Windu prawn head-leg (WSHL) samples. Based on comparative IR spectrum data of standard chitosan from the Industrial Research and Standardization Center (Dziedzic, 2023; Weißpflog et al., 2021), it was found that the functional groups at each wavenumber

were not significantly different from the standard, suggesting that the compounds isolated from mangrove crab shell, Windu prawn shell, and Windu prawn head-leg waste samples produced chitosan that met the standard (Table 2).

Table 2. FTIR Spectrum Absorption Peaks of Chitosan

Functional Group	Wavenumber (cm^{-1})			
	Standard*	MCS	WPS	WPHL
v (O-H)	3428-3251	3431	3420	3430
v (O-H) or (N-H)	3349-3215	3255	3254	3254
v (C-H)	2935-2864	2874	2876	2876
v (C=O); amide I	1652-1648	1652	1652	1652
δ (N-H); amide II	1594-1551	1553	1553	1553
δ (C-H)	1453-997	1375	1375	1375
v (C-H); amide III	1323-946	1308	1308	1308
v (C-O-C)	1155-895	1007	1007	1007

Note: *Institute for Industrial Research and Standardization; v= stretching vibration; δ=bending vibration; Mangrove Crab Shell (MCS); Windu Prawn Shell (WPS); Windu Prawn Head and Leg (WPHL).

The spectrum data show similarities in the absorption patterns of the three samples (MCS, WSS, and WSHL) with that of the standard chitosan. The absorption peak that appears at a wave number of around 3431 cm-1 in the three samples indicates the presence of hydroxyl group (O-H) stretching vibration, which is a characteristic group of the chitosan structure, which is a polysaccharide with many hydroxyl groups (Ardean et al., 2021; Tanasale et al., 2016; Weißpflog et al., 2021). The absorption band in the region around 3254-3255 cm^{-1} indicates the presence of stretching vibrations from hydroxyl (O-H) or ammine (N-H) groups. In this region, the absorption *band* appears *broad* due to the formation *of hydrogen bonds* between molecules, which causes overlap between the O–H and N-H stretching vibrations (Lorenz-Fonfria, 2020; Salmahaminati, Putri, et al., 2025). Both of these groups contribute to the hydrophilic characteristics of chitosan and are often difficult to distinguish clearly because of their close absorption distances (around 3000-3500 cm^{-1})(Gieroba et al., 2023). Furthermore, absorption in the range of 2874-2876 cm^{-1} indicates the stretching vibration of the C-H group (Keikhaei et al., 2019), while the band at 1652 cm^{-1} represents the carbonyl group (C=O) of amide I, which is an important indicator in determining the degree of chitosan deacetylation (Weißpflog et al., 2021). Meanwhile, amide II and amide III were consistently detected in all three samples at approximately 1553 cm^{-1} for amide II and 1308 cm^{-1} for amide III (Ghimire, 2017; Gieroba et al., 2023). In addition, several other characteristic chitosan groups, namely the C-H group appearing at a wavenumber of 1375 cm^{-1} and the C-O-C group at a wavenumber of 1008 cm^{-1}, indicate the presence of glycosidic bonds as a key feature of the polysaccharide structure (El-Fawal & El-Shamy, 2019; Kabir et al., 2022; Triandani et al., 2024).

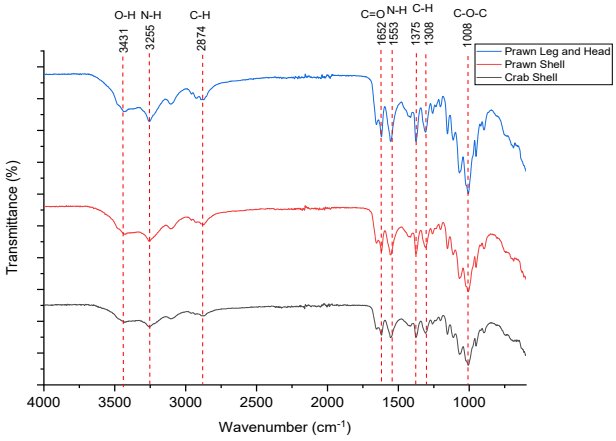

Figure 3. FTIR Spectrum from MCS, WPS, and WPHL Samples

The absorption peaks observed are consistent with the literature standard chitosan spectrum (Dziedzic, 2023; Weißpflog et al., 2021). This indicates that the three chitosans obtained have chemical structures similar to those of chitosan in general. The slight differences in intensity and wavenumber positions between the three samples, as seen in Figure 3, are likely influenced by variations in the raw material sources (crab shells, prawn shells, and prawn heads and legs) and also the purity level of the resulting chitosan (Fatima, 2020). Thus, the FTIR data confirm that the chitin deacetylation process was successful, producing chitosan in the three samples: mangrove crab shells (MCS), Windu prawn shells (WSS), and Windu prawn head-leg (WSHL). Furthermore, the absorption peaks in the 3430-3420 cm^{-1} (O-H) and 1652 cm^{-1} (C=O; amide I) were used as references in calculating the degree of deacetylation (DD), because the intensity of these two bands reflects the change from amide groups to amine groups during the deacetylation process from chitin to chitosan.

Measurement of the Deacetylation Degree (%DD) of Chitosan

The deacetylation degree (%DD) is a crucial parameter that determines the success of the

deacetylation process in converting chitin into chitosan (Pérez-Álvarez et al., 2018). The degree of chitosan deacetylation is determined based on the quantity of acetyl groups lost from chitin, which transform into amine groups (–NH_2). The deacetylation process of chitin yields chitosan, a compound characterized by its solubility in dilute acetic acid. In this context, one can determine the deacetylation degree by measuring the absorbance at 1655 cm^{-1} (C=O group, amide I) and 3450 cm^{-1} (O–H group) using FTIR (Fourier Transform Infrared) spectroscopy. Pharmaceutical preparations identify chitosan by specific characteristics: a white to yellowish color, powder form, pH 6.5-7.5, deacetylation degree of 70-95%, moisture content ≤ 10%, ash content ≤ 2%, nitrogen content ≤ 5%, tastelessness, and odorlessness (Sartika et al., 2016). Furthermore, a higher chitosan deacetylation degree corresponds to a lower acetyl group content, thereby strengthening ionic interactions and hydrogen bonding within the polymer (Dompeipen et al., 2016). The following presents the deacetylation degree values for the three test samples (Table 3) and their deacetylation absorbance spectra (Figure 5).

Table 3. Degree of Deacetylation (%DD) of Chitosan

Sample	Functional Group	Wavenumber (cm^{-1})	Absorbance Value	%DD
MCS	v (O-H)	3450	0.0451	63.9
	v (C=O); amide I	1655	0.0574	
WPS	v (O-H)	3450	0.06157	73.0
	v (C=O); amide I	1655	0.0573	
WPHL	v (O-H)	3450	0.0765	62.3
	v (C=O); amide I	1655	0.1021	

Note: Mangrove Crab Shell (MCS); Windu Prawn Shell (WPS); Windu Prawn Head and Leg (WPHL).

Table 3 presents the wavenumber and absorbance intensity data for the main functional groups of chitosan, namely the hydroxyl (O–H) and amide carbonyl (C=O) groups, used in determining the deacetylation degree (%DD). The absorbance ratio of the amide I (C=O) to hydroxyl (O–H) peaks reflects the extent of acetyl group removal during deacetylation. Therefore, a lower intensity of the amide I band relative to the O–H band indicates a higher %DD value of the resulting chitosan.

In the Windu Prawn Shell (WPS) sample, the O–H absorbance of 0.06157 and C=O absorbance of 0.05730 result in a lower amide ratio compared to the other two samples. This condition indicates a more efficient acetyl group conversion, reflected in the highest %DD of 73.0%, which falls within the moderate category according to commercial chitosan quality standards. The Windu Prawn Head and Legs (WPHL) sample shows an O–H absorbance of 0.07975 and a C=O absorbance of 0.1018, with a relatively higher amide I intensity. This ratio suggests a significant number of residual acetyl groups, resulting in a %DD of 63.9% (low–medium category). Meanwhile, the Mangrove Crab Shell (MCS) sample has an O–H absorbance of 0.04737 and a C=O absorbance of 0.0758, displaying the highest dominant amide I intensity among all samples. This indicates the least efficient deacetylation process, yielding a %DD value of only 55.3%, which falls into the low category.

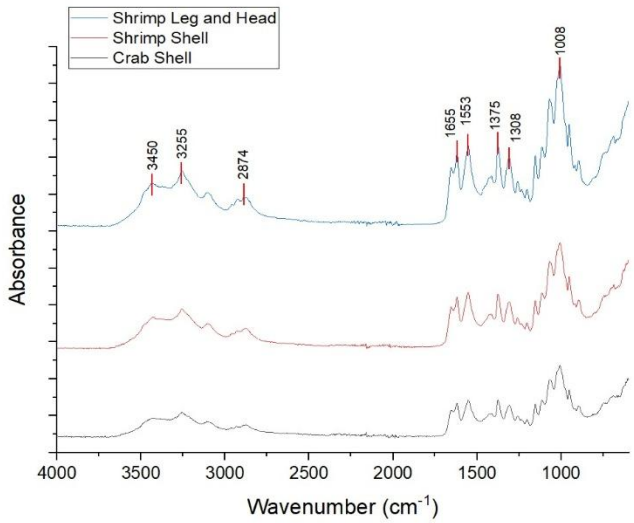

Figure 5. Absorbance Spectrum of Deacetylation Degree from MCS, WPS, and WPHL Samples

In the Windu Prawn Shell (WPS) sample, the O–H absorbance of 0.06157 and C=O absorbance of 0.05730 result in a lower amide ratio compared to the other two samples. This condition indicates a more efficient acetyl group conversion, reflected in the highest %DD of 73.0%, which falls within the moderate category according to commercial chitosan quality standards. The Windu Prawn Head and Legs (WPHL) sample shows an O–H absorbance of 0.07975 and a C=O absorbance of 0.1018, with a relatively higher amide I intensity. This ratio suggests a significant number of residual acetyl groups, resulting in a %DD of 63.9% (low–medium category). Meanwhile, the Mangrove Crab Shell (MCS) sample has an O–H absorbance of 0.04737 and a C=O absorbance of 0.0758, displaying the highest dominant amide I intensity among all samples. This indicates the least efficient deacetylation process, yielding a %DD value of only 55.3%, which falls into the low category.

The degree of deacetylation will also affect the properties of the resulting chitosan, including solubility, chemical reactivity, and biodegradability. According to He et al. (2016), in general, DD values between 55% and 70% are categorized as low degree of deacetylation chitosan, 70%–85% as medium, 85%–95% as high, and 95%–100% as ultra-high. Based on the results obtained (Table 3), the degree of deacetylation (%DD) of chitosan from the three samples, namely Mangrove Crab Shell (MCS), Windu Prawn Shell (WPS), and Windu Prawn Head and Leg (WPHL), only the Windu prawn shell (WSS) sample met the standard for the degree of deacetylation percentage, which was 73.0% in the medium category. Meanwhile, the mangrove crab shell (MCS) and Windu prawn head-leg (WSHL) samples had a low degree of deacetylation, ranging from 62.3% to 63.9%, respectively.

The low degree of deacetylation is due to various factors, including the difficulty of removing the acetyl group bound to chitin (Moosa et al., 2016). To improve the quality of the chitosan produced, high NaOH concentrations and elevated temperatures are required during processing; however, excessively high NaOH concentrations can also reduce the quality of the chitosan product. Therefore, optimal NaOH concentrations and temperatures are necessary during the deacetylation procedure (Safitri et al., 2022). This phenomenon is caused by the use of an excessively high NaOH dose, which can remove the acetyl group from chitin. As a result, the remaining OH- ions will attack the chitin ring, causing the chitin complex to degrade.

The degree of chitosan deacetylation also depends on the crustacean species used and the preparation method, resulting in a range of 56% to 99% with an average of 80% (No & Hur, 1998). For example, in Kumari et al.'s 2017 study, using 40% KOH in the deacetylation process resulted in low deacetylation levels across all tested sources (fish fins, prawn shells, and crab shells). In addition, Ismail et al., (2023) revealed that the deacetylation reaction rate is directly proportional to the deacetylation temperature in chitin. This is because intermolecular mobility increases with higher deacetylation temperature, thereby accelerating the removal of the acetyl group.

The deacetylation degree of chitosan from the Windu Prawn Shell (WPS) sample in this study exceeds the value reported by Wahyuni & Khaeruni, (2014) for the same sample, namely 68.60%. Meanwhile, Pristiwani (2023) reports deacetylation degrees of 49.76% and 54.32% for Mangrove Crab Shell and blue swimming crab shell samples, respectively, using the same method as this study. These results indicate that the deacetylation degree achieved for the Mangrove Crab Shell (MCS) sample in our research is more optimal than previous findings. However, the deacetylation degrees for the Mangrove Crab Shell (MCS) and Windu Prawn Head and Legs (WPHL) samples fall below the standard. Several factors influence this outcome, including the NaOH concentration, temperature, and duration of the heating process during deacetylation. A higher NaOH concentration increases the reaction rate, generating a greater amount of OH^- ions. This elevated concentration leads to the release of more CH_3COO^- groups, consequently producing a higher number of amide ($-NH_2$) groups (Cahyono, 2018). The chitin powder from the last process showed different colors in the three samples (Figure 4).

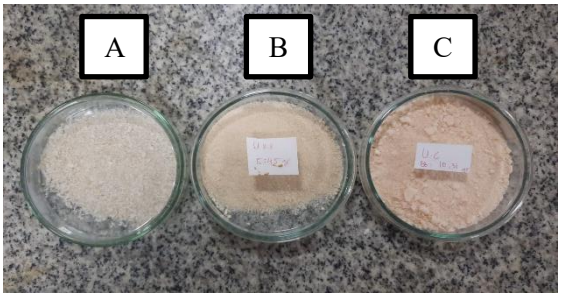

Figure 4. Chitin Powder from Waste A) Mangrove Crab Shell (MCS); B) Windu Prawn Shell (WPS); C) Windu Prawn Head and Leg (WPHL)

Based on the results (Figure 4), the three samples exhibit different chitosan colors that do not conform to the pharmaceutical formulation specifications for white or yellowish. This discrepancy occurs because this study did not include a decolorization step prior to chitosan isolation. Consequently, all three samples contain red-orange astaxanthin, a type of carotenoid pigment naturally present in chitin.

CONCLUSION

Based on the results of this study, it is shown that the exoskeleton waste from Windu prawns and mangrove crabs can be used as a source of biomaterial for chitosan production via alkaline processing. Variation in waste sources influenced the quality of the chitosan produced. Windu Prawn Shell samples produced chitosan with a 73% degree of deacetylation, which met the medium category standard. These findings confirm that Windu Prawn Shell waste is the most potential source for the production of chitosan based on the alkaline method. Based on the results of the SEM test, it was found that the type of base material

used would affect the morphology of the surface of the chitosan produced, where Windu Prawn Shells have a flatter chitosan surface than Windu Prawn Heads and Legs, as well as Mangrove Crab Shells. This is the first comparative study of chitosan derived from three different waste sources in the Pasuruan coastal area. Limitations of this study include the lack of optimization of extraction parameters and the absence of application testing. Future research should focus on: (1) optimizing NaOH concentration and temperature to achieve higher %DD (>85%), (2) conducting application tests for specific uses, and (3) economic feasibility analysis.

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