The Potential Development of Shrimp Shell Waste Into Chitosan Originating from Pacitan Coast, Indonesia

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Abstract. Shrimp is one of the biggest comodities at the Pacitan coast area that was taken its meat to be processed into many food products. This could be due to the accumulation of shrimp shell waste abundantly and has no selling value. The conversion of shrimp shell waste into chitosan is the one breakthrough to increase the value of the shrimp shell waste. The objective of this research is to convert shrimp shell waste into chitosan and characterized the quality of chitosan including the deacetylation degree, crystalinity, and its morphology. This research has successfully isolated chitosan that extracted from shrimp shell waste obtained from Sudimoro coast, Pacitan, Indonesia. Chitosan was isolated through three steps of reaction including deproteination, demineralization, and deacetylation. The chitosan produced had the first deacetylation degree at 75% with the second deacetylation degree at 82% and the total of shrinkage from the raw material is at 84%. The synthesized chitosan also showed the decreasing of its crystalinity and had flakes-type morphology that observed by scanning electron microscopy (SEM).

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1. INTRODUCTION

Chitosan is a natural biopolymer compound derived from chitin compounds. Chitin is the second most abundant polysaccharide after cellulose with an N-acetylglucosamine structure. Presently, commercial chitin is widely isolated from crustacean shells, such as crabs, shrimps, and lobsters [1,2]. Crustacean shell composition consists of protein (30–40%), minerals (30–50%), and chitin (20–30%) [3]. Chitosan with empirical formula $(C_6H_{11}NO_4)n$ is a yellowish-white amorphous solid with the polyelectrolyte characteristic. Chitosan or poly-(2-amino-2-deoxy-β-1,4-D-glucopyranose) can be synthesized by removing some of the acetyl groups from chitin by deacetylation using a high concentration of an alkaline solution. Chitin can be categorized as chitosan if it has a degree of deacetylation (DD) above 50% [4]. Chitosan is commonly solvable in aqueous acids with a pH of about 4 – 6.5 and insolvable at lower/higher pH. The degree of solubility can be influenced by the molecular weight and the DD [5]. Chitosan has been widely applied in various fields such as industry, medicine/biomedical, pharmaceutical, water treatment, and food processing [6]. Chitosan is increasingly popular because it has biodegradable properties, is easily chemically modified, has reactive groups and is conductive in acid solutions, is non-toxic, can be formed into thin films, is easy to obtain, and is relatively inexpensive [7]. In addition, the cationic character of chitosan can act as an antibacterial or antiviral agent [8]. These reasons make chitosan has a high selling value.

Shrimp is one of the most dominant fishery products cultured in the world, especially in Asian countries, because of its growth of characteristics, economic value, and high nutritional value [9]. The world of shrimp aquaculture production is about 5 million tons, accounting for 52.9% of the world's total shrimp aquaculture [2]. The higher the amount of production, the possibility of the formation of production waste also increases. The by-products remaining from the consumption of seafood, particularly shrimp shells, account for about 40–50% of the total mass, and this waste is a major ecological challenge as it degrades gradually, causing the accumulation of garbage in the sea, and decomposition. The seafood processing industry produces about 6-8 million tons of crab, prawn, and lobster shells global annually. According to TÜİK data, the total quantity of shellfish harvested in Turkey was 4570.4 tons and almost 99% of that value consists of prawn [3].

Pacitan, East Java, Indonesia is one of the regions in Indonesia that has the most shrimp ponds. The shrimp harvested in Pacitan are usually sold fresh, either sold to large restaurants or the food processing industry, and only the meat is used. Thus, the shrimp shell waste produced is very abundant and its utilization is not optimal. Based on these reasons, this study aims to develop the potential of shrimp shell waste by converting it into chitosan which has a high selling value by determining its characteristics, the degree of deacetylation, crystallinity, and morphology of chitosan with shrimp shell waste treatment process includes three main stages, namely deproteination, demineralization, and deacetylation.

2. MATERIALS AND METHODS

In this study, chitosan was extracted according to the method used in previous studies [10,11]. Extraction of chitosan involves 3 main steps of reactions including deproteination, demineralization, and deacetylation.

2.1 Instrumentation

Chitosan was characterized by Fourier Transform Infra Red (FTIR, 8400S Shimadzu), X-Ray Diffraction (XRD, Philips X-Pert MPD), and Scanning Electron Microscopy (SEM, Zeiss Evo MA), hot plate, oven, blender, and 100 mesh sieving.

2.2 Materials

The materials that were used for extraction consisted of shells of shrimp obtained from shrimp ponds on the coast of Sudimoro, Pacitan, East Java, Indonesia, ninhydrin (p.a., Merck), NaOH pellets (p.a., Merck), 37% HCl solution (p.a., Merck), and aquadest.

2.3 Extraction of Chitosan

2.3.1 Preparation of Shrimp Shell Powder

The shrimp shell and head were separated from the flesh, cleaned with running water, then dried. The dried shrimp shells were mashed with a blender, then sieved through a 100 mesh sieve.

2.3.2 Deproteination Stage

The shrimp shell powder was weighed with a certain weight, then mixed into a 3.5 wt.% NaOH at 65 °C and for 2 h. The precipitate was separated from the mixture and washed with the aquadest until the pH around 7-8. The precipitate was then dried in an oven for 6 h to obtain a dried powder. The dried powder was weighed and the percentage of the results was calculated from the initial weight.

2.3.3 Demineralization Stage

The demineralization process was carried out at 65 °C with 1 M HCl solution for 30 min. The mixture was then filtered and the obtained precipitate was washed with the aquadest until the pH around 6-7. The precipitate was then dried using an oven for 6 h to obtain the chitin powder.

2.3.4 Deacetylation Stage

The demineralized dry chitin powder was mixed with 50 wt.% NaOH and heated at 120 °C for 4 h. The resulting slurry was filtered and washed with aquadest until the pH around 7-8. The slurry was dried in an oven for 6 h and obtained the chitosan powder.

2.4 Chemical Testing and Characterizations

2.4.1 Ninhydrin Test

The ninhydrin test was carried out to determine that the deproteinized powder no longer contained protein by adding 10 drops of 0.1% ninhydrin solution to the sample, heating for

1-2 minutes, then observing the color's change. The sample still contains protein if the solution turns purple.

2.4.2 Functional Group Characterization

In this study, all materials in the form of solid powder were characterized by FTIR to determine the presented functional groups. Before the FTIR analysis, the samples were added with KBr. Both were mixed and grounded to form a homogeneous fine powder. Furthermore, the powder was formed into a thin pellet and analyzed at the wavenumber range of 4000-400 cm^{-1}.

2.4.3 Crystallinity Study

Crystal field and crystallinity level of chitin and chitosan were analyzed by XRD. The analyzed sample must be dry and then placed on the pin stub holder. The analysis was carried out at a short angle of 2θ = 5-60° with Cu Kα radiation (λ = 0.154056 Å). The crystallinity of each sample was determined by comparing the peak intensities of the samples before and after modifications so that the degree of crystallinity (%).

2.4.4 Morphological Characterization

Chitosan that has been synthesized in this study was characterized by SEM to determine the morphology of chitosan. Chitosan samples before being analyzed were coated with gold particles so that the samples became conductive and detected by the instrument. The sample was then placed in the sample holder and detected at a magnification of 2000×, working distance: 10.5 mm and 10 kV. This analysis was also used to confirm the specific particle size of chitosan.

3. RESULT AND DISCUSSION

3.1 Ninhydrin Chemical Testing

The stage of protein removal in shrimp shell powder or deproteination was carried out by adding NaOH with a certain concentration and temperature that led to the effectiveness of protein removal and accelerated the deacetylation process. The protein content in the shrimp shell itself ranges from 20-40% [12]. The occurrence of a deproteination reaction was physically observed by the appearance of foam and a solution that changed its color to dark brown and had a distinctive pungent odor. The deproteinized powder was tested qualitatively by dripping 0.1% ninhydrin solution and heated. The test result showed that the deproteinized powder did not change its color indicating it contained no protein anymore however if the color changes to purple then the powder still contains protein. Removal of protein from shrimp shells is very important to reduce the contamination in chitosan. The chemical reaction that occurred between amino acids from protein and ninhydrin produced the main compound Ruhemann's purple which gives a purple color effect and the by-products are carbon dioxide (CO_2) and aldehyde (R-CHO) [13]. The ninhydrin test and its chemical reaction are shown in Fig. 1 and 2.

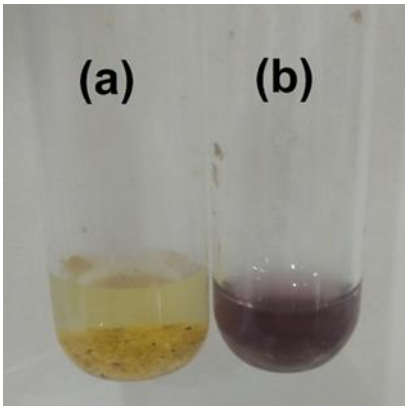

Fig. 1. (a) Deproteination result and (b) shrimp shells

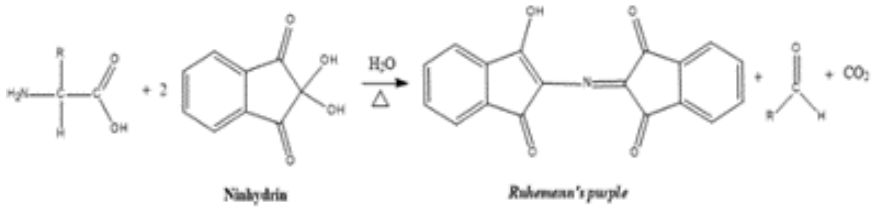

Fig. 2. The chemical reaction between amino acid and ninhydrin

3.2 Chitosan Functional Group Analysis

The result of chitosan extraction can be analyzed using FTIR qualitatively to determine the functional groups contained in it. The FTIR spectra of shrimp shells showed the absorption at the wavenumbers of 3410 cm^{-1} (stretching of -OH group), 2926 cm^{-1} (-CH_3 group), 1636 cm^{-1} (stretching of -C=O group), 1413 cm^{-1} (stretching of -C-N group), and 1068 cm^{-1} (stretching of -C-O group). These uptakes indicate several functional groups in shrimp shells. These functional groups were detected because of the presence of protein in the shrimp shell consisting of alkyl groups (-R), carboxyl groups (-COOH), and -C-N groups [14]. Meanwhile, the FTIR of chitosan showed some of the absorption peaks shifted at certain wavenumbers. The -OH group in chitosan shifted to a larger wavenumber, namely 3423 cm^{-1} with a wider absorption peak. This is due to the reduction of the -NH group in -$NHCOCH_3$ which initially overlaps with the -OH absorption. The reduced intensity of the absorption band -C=O and amide I in chitosan indicated that the shrimp shell has been deacetylated. These shifts can be influenced by 3 stages of chemical reactions, namely deproteination, demineralization, and deacetylation [15]. The absorption of chitosan functional groups can be seen in Table 1. And the FTIR of shrimp shells and chitosan are shown in Fig. 3.

Table 1. FTIR of shrimp shell and chitosan.

Functional group	Type of vibration	Wavenumber (cm^{-1})	
		Shrimp shells	Chitosan
-O-H	Stretch	3410	3423

-C-H	Stretch	2926	2885
-C=O	Stretch	1636	1597
-C-N	Stretch	1413	1381
-C-O	Stretch	1068	1095
-N-H	Swish	873	895

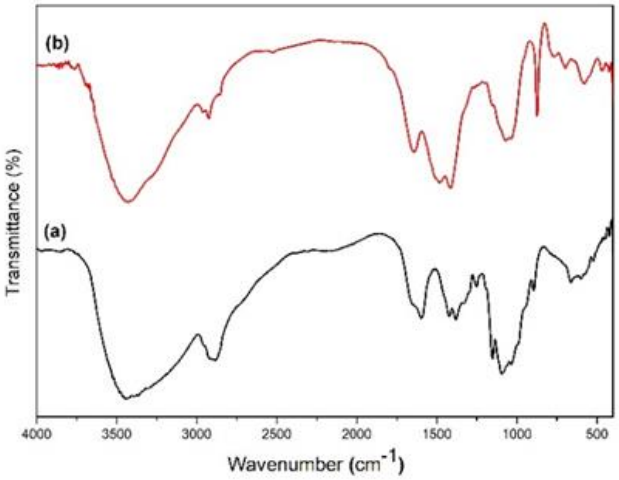

Fig. 3. FTIR of (a) chitosan and (b) shrimp shell

3.3 Chitosan Crystallinity Analysis

The crystallinity of chitin and chitosan was analyzed by XRD to determine the crystallinity changes that occurred during the chemical modification process from chitin to chitosan. Typical diffraction peaks of chitin appear at 2θ = 9°, 20°, and 26° with sharp and high intensity. This indicates the presence of a crystal structure of chitin with a high degree of crystallinity. In chitosan, these peaks (2θ = 9°, 20°, and 26°) were also detected with the lower and wider peaks indicating the low crystallinity level. The low level of crystallinity has a positive impact on chitosan being more soluble in a solvent. This is due to the reduced rigidity of the crystal lattice in chitosan so the crystal lattice is irregular and tenuous (a lot of space) [16]. The level of crystallinity of chitin and chitosan is shown in Table 2. Meanwhile, the diffractogram of chitin and chitosan is shown in Fig. 4.

Table 2. The degree of crystallinity of chitin and chitosan.

Sample type	Crystallinity degree (%)
chitin	30.06
chitosan	24.05

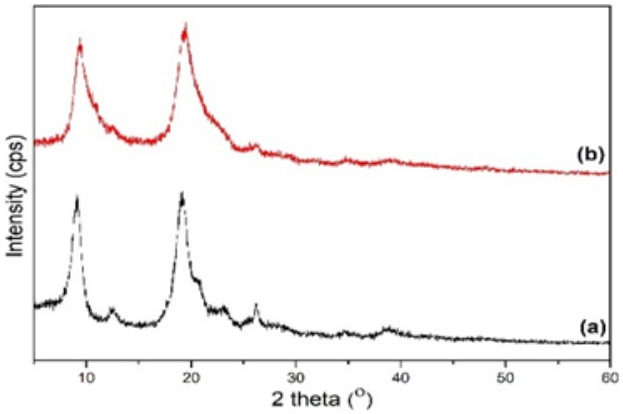

Fig. 4. Diffractogram of (a) chitin and (b) chitosan

3.4 Chitosan Morphology

The morphological characteristics of chitosan were observed using SEM. SEM micrographs showed that chitosan has a structure with a smooth surface, non-porous, and shaped like a chip. The average particle size for chitosan produced from this synthesis is 55 µm. A previously reported study [17] showed that pure chitosan powder had a size of about 100 µm. The micrograph also showed that chitosan has an irregular and wavy shape which was influenced by the deacetylation process in chitin [18]. In addition, the effect of concentration and reaction time used can also affect the morphology and size of the chitosan particles [17]. The morphology of chitosan is shown in Fig. 5.

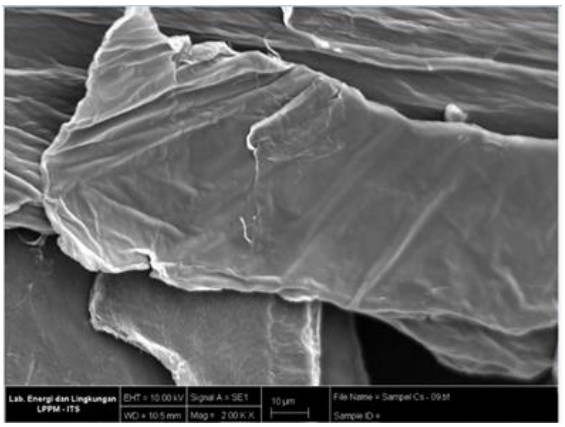

Fig. 5 The morphology of chitosan

3.5 Chitosan Quality

The quality of chitosan can be determined by the value of the DD. The higher of DD, the higher the quality of chitosan. Determination of DD can be determined through FTIR with the baseline method [19]. In this study, the first DD of chitosan produced from shrimp shell waste was 75% and the second process showed the increasing of its deacetylation to 82%. The high value of DD can affect the reactivity of chitosan in a solvent. This is due to

the partial substitution of the acetyl group (-$COCH_3$) by the amine group (-NH_2). The amine group plays a role in increasing the reactivity of chitosan. In addition, the steric factor possessed by the acetyl group is greater when compared to the amine group so the more amine groups possessed by chitosan can reduce the steric factor of the polymer chain. The reduced steric factor in the chitosan polymer chain has an impact on increasing the reactivity of chitosan in a certain solvent [20]. Quantitative measurement of the degree of deacetylation is shown in Fig. 6 and 7.

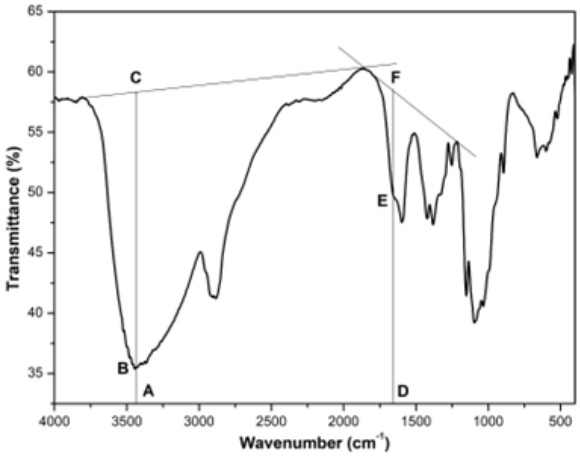

Fig. 6. The first deacetylation degree of chitosan

The percentage of shrinkage from the initial mass is shown in Table 3. The shrinkage that occurs is the impact of the loss of protein content from shrimp shells, minerals, and acetyl groups when the 3 reaction steps occur. The total shrinkage that occurred was 84% so the yield of chitosan obtained was 16%. The resulting yield was not much different from the yield of chitosan extracted from oyster shells of 18.33% [21].

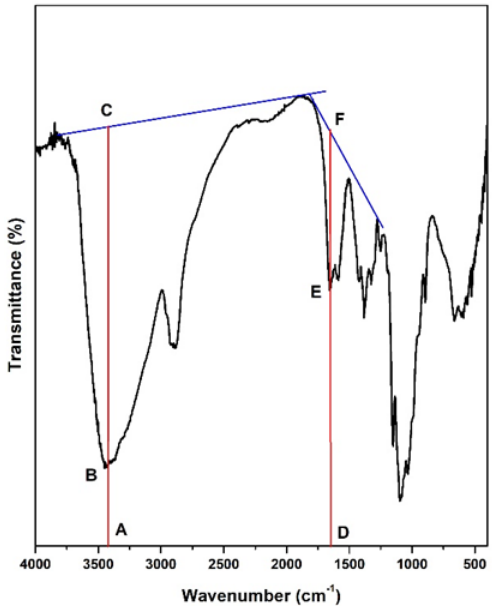

Fig. 7. The second deacetylation degree of chitosan

Table 3. The percentage of shrinkage

Step of reaction	Final mass (g)	Rendemen (%)
Shrimp shell	1500	-
Deproteination	1000	66,67
Demineralization	280	18,67
Deacetylation	240	16

3.6 Conclusion

In this study, chitosan isolated from the shrimp shell waste was produced with the first and second deacetylation degree of 75% and 82%, respectively with a total shrinkage of 84% of the initial shrimp shell waste. The crystallinity of chitosan also decreased to 24.05% from 30.06% of chitin. In addition, the resulting morphology is a 3-dimensional chip structure with a particle size of 55 μm.

Conflict of Interest

The authors declare that there is no conflict of interest.

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