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# Research Article
# Preparation and Characterization of Chitosan from Indonesian Tambak Lorok Shrimp Shell Waste and Crab Shell Waste

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## Abstract

**Background and Objective:** Shrimp shell waste and crab shell waste are causes of environmental pollution and chemical methods can be used to process this waste into chitosan. Chitosan is the second largest natural biopolymer after cellulose and has many applications in food and health products. The aim of this research was to investigate the potential of Tambak Lorok shrimp shell and crab shell waste as an alternative raw material for chitosan production. **Materials and Methods:** Chitosan was prepared by chemically treating shell waste from shrimp in the *Penaeidae rafinesque* family and shell waste from crab in the *Portunidae* family from Tambak Lorok, Indonesia. The chemical structure of chitosan was confirmed by infrared spectroscopy, oswald viscometry and scanning electron microscopy. **Results:** The isolated chitosan from shrimp shell waste showed an 89.6% degree of deacetylation, a 64% degree of crystallinity and a 557 kDa molecular weight. The isolated chitosan from crab shell waste showed an 82.1% degree of deacetylation, an 81% degree of crystallinity and a 690 kDa molecular weight. Chitosan from both shrimp shell waste and crab shell waste had a porous and fibril-like structure. **Conclusion:** It was concluded that shrimp shell waste and crab shell waste are potential alternative raw materials for chitosan production.

Key words: Chitin, chitosan, crab, shrimp, shells

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Chitin is the second largest natural polymer after cellulose[1]. The Chitin structure is similar to cellulose in which the bonding between monomers is coupled by β-(1-4) glycosidic bonds. The difference from the structure of cellulose is that the hydroxyl group associated with the second carbon atom in chitin is replaced by an *acetamide* group ($NHCOCH_2$) such that chitin is an *N-acetylglucosamine*-linked polymer[2]. Chitin has the molecular formula $C_{18}H_{26}N_2O_{10}$[1]. Chitin is a solid substance with an unsolved structure (amorphous), is insoluble in water, dilute inorganic acids, dilute and concentrated alkali solutions, alcohol and other organic solvents and is soluble in concentrated mineral acids[1]. Chitin can be extracted from the cell walls of crustaceans and insects or fungal compounds[3]. Chitin is generally extracted not as a pure substance, but as a combination mixture with other substances such as proteins, calcium carbonates and pigments[2]. Chitin is obtained through demineralization and *deproteination* processes. Demineralization is a mineral removal process using strong acids. The strong acid used is usually hydrochloric acid (HCl)[1]. In addition, the process of demineralization involves the removal of minerals, especially calcium (Ca)[3]. Deproteination involves the removal of proteins by using strong bases. The most commonly used strong base is sodium hydroxide (NaOH)[2]. The demineralization and *deproteination* process used determine the character of the resulting chitin compound, so the process needs to be optimized to obtain the chitin that is free of minerals and protein[1]. The resulting chitin may then undergo a *deacetylation* reaction to form chitosan[2].

Chitosan is also called β-1,4-2-amino-2-dioxy-D-glucose and is a derivative of chitin from *deacetylation*. Chitosan is nontoxic, easy to biodegrade and polyelectrolyte[4]. Chitosan is a multifunctional natural polymer because it contains three groups of compounds: Amino acids, primary hydroxy groups and secondary hydroxy groups[4]. This causes chitosan to have high chemical reactivity[4-6]. Due to the nontoxic nature of chitosan and its high reactivity, chitosan is widely used in health products[5-11] and food[4,12-14]. The process of chitin *deacetylation* to form chitosan is performed by reacting chitin with a strong base. The most commonly used strong base is sodium hydroxide (NaOH). This process produces chitosan with a high degree of *deacetylation*. Chitosan with a high degree of *deacetylation* will have better reactivity than chitosan with a low degree of *deacetylation*.

Indonesia is the largest archipelagic country in the world with a total marine area of 5.9 million $km^2$ comprising 5.9 million $km^2$ of territorial waters and 2.7 million $km^2$ of waters in an exclusive economic zone (ZEE)[15]. As such a large and wide range of territories, Indonesia has a diversity of potential marine resources, both biological and nonbiological, such as fisheries, seaweed and high-value marine resources. According to data from the Ministry of Marine and Fishery, the production and export of marine products, especially shrimp and crabs, continue to increase. The impact of this increase is an increasing amount of waste generated. One area that produces waste from shrimp and crab processing is Tambak Lorok, Central Java. However, the waste generated is very susceptible to microbial decay and disturbs the environment. To deal with this issue, one option is to treat shrimp and crab shell waste to form chitosan compounds. Therefore, the purpose of this research was to investigate the potential of Tambak Lorok shrimp and crab shell waste as an alternative raw material for chitosan production.

## MATERIALS AND METHODS

**Materials:** Shell waste from shrimp in the P*enaeidae Rafinesque* family and shell waste from crab in the *portunidae* family were obtained from fresh shrimp and crab products and processing plants Located in Tambak Lorok, Semarang, Central Java, Indonesia.

**Chemicals and reagents:** Chitosan (commercial), $CH_3COOH$, HCl, NaOH were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents and chemicals were of analytical grade.

**Preparation of raw materials:** Shrimp shell waste and crab shell waste were obtained from fresh shrimp and crab products and processing plants located in Tambak Lorok, Indonesia. The waste was washed thoroughly with tap water and desiccated at room temperature. After drying, the waste was kept at room temperature before use. Next, shell waste was identified in a laboratory of animal systematics to classify it as either shrimp shell waste or crab shells waste.

**Chemical demineralization and deproteinization:** Demineralization and deproteinization were measured according to the method described by Brine and Austin[16] with some modifications. Shrimp shell waste and crab shell waste were treated with 1 N HCl solution for 6 h at room temperature for demineralization process and washed with distilled water until attaining a neutral pH. The decalcified shrimp shell waste and crab shell waste were then treated with 1 N NaOH at 90°C for 6 h for demineralization process, washed with acetone and distilled water until attaining a neutral pH, dried at room temperature.

**Deacetylation of chitin:** Deacetylation of chitin was measured according to the method described by El Knidri *et al.*[17] with modifications. The chitin deacetylation reaction to obtain chitosan was carried out by preparing a mixture of chitin and a solution of 50% NaOH at 100°C for 6 h until the chitin was deacetylated to a chitosan. After the reaction, the crude chitosan was washed several times with distilled water to attain a neutral pH and recovered by drying in a dry heat incubator at 50°C for 12 h.

**Physicochemical characterization:** The ash content (%) (wt/wt) was determined by the AOAC standard method[18]. The protein content was determined, in 3 replicates, according to the Kjeldahl procedure[18]. The mineral content of Ca was determined by an atomic absorption spectrophotometer (ContrAA 300 Analytik Jena, Germany).

**FTIR spectra and degree of deacetylation (DD):** The FTIR spectra of chitosan from the shrimp shell waste and crab shell waste were acquired using an FTIR spectrometer (Nicolet iS10 FTIR spectrometer, Thermo Scienti c, USA) over a wave number range of 4000-400 $cm^{-1}$. Smart iTR was used to collect the horizontal attenuated total reactance (ATR) spectra using a standard ZnSe crystal. Samples were pressed with a Minigrip device to ensure uniform contact between the samples and ATR crystal. The FTIR spectra were recorded by accumulation of at least 64 scans, with a resolution of 4 cm using OMNIC software (Thermo Scienti c). The degree of deacetylation (DD) was assessed by the method of Sabnis and Block[19]. The DD of chitosan deacetylation was determined by the baseline method based on the FTIR spectrum using Baxter's Eq.:

$$DD = 100 - \left[ \frac{A1655}{A3450} \times \frac{100}{1.33} \right]$$

where, $A_{1655}$ is the absorbance at 1655 $cm^{-1}$ of the amide-1 band, $A_{3450}$ is the absorbance at 3450 $cm^{-1}$ of the hydroxyl band and a factor of 1.33 indicates the $A_{3450}/A_{1655}$ ratio for complete degrees of chitosan deacetylation[20].

**Scanning electron microscopy (SEM):** The morphology of the obtained chitosan was examined by scanning electron microscopy (SEM) (Hitachi Ltd., Japan) with an acceleration voltage of 15 kV and a magnification range of 1000x-10.000x.

**Powder x-ray diffraction (XRD):** The structural characterization and crystallinity of chitosan were studied by using an X-ray diffractometer (D8, Advance Bruker XRD diffractometer, Germany). The sample was placed into a porcelain cup and dried in a drying oven for 1 h at 100°C. The dried samples then were placed in a furnace at 1200°C for 1 h. Subsequently, the sample was removed from the furnace, cooled at room temperature and mashed with mortar. The crystallinity of the soft sample was then observed from the XRD pattern obtained using CuKα radiation ($\lambda = 0.15406$ nm) at 40 kV in the diffractometer. The relative intensity was recorded in the scattering range 2θ of 5-40°. The error of this measurement was ±1°. The XRD instruments were calibrated using standard reference materials (SRMs). The XRD peak intensities on the basis of the constant total peak area were evaluated through a deconvolution procedure using Origin 6.0 (MicroCal Software Inc.). The index of crystallinity could be calculated by the following Eq.:

$$\text{Crystallinity index (\%)} = \frac{Fc}{Fc + Fa} \times 100$$

where, Fc and Fa are the area of the crystalline peaks at $2\theta = 20$ and the amorphous diffraction at $2\theta = 16.\theta$ (rad), which is half the Bragg angle corresponding to the crystalline peak[21].

**Average molecular weight:** The viscosity-average molecular weight, Mv, was determined with an Oswald viscometer at room temperature. The solvent used for chitosan was 0.1 M acetic acid/0.1 M sodium acetat. The viscosity-average molecular weight (Mv) was obtained from the viscosity equation using Mark-Houwink parameters as follows:

$$[\eta] = k\,(M_v)^{\alpha}$$

where, [η] is the intrinsic viscosity and k and α are constants. These parameters were determined for chitin ($k = 0.24$ $cm^3$ $g^{-1}$ and $\alpha = 0.69$) and chitosan ($k = 0.078$ $cm^3$ $g^{-1}$ and $\alpha = 0.76$)[20].

**Statistical analysis:** Data were shown as the mean +/- Standard Deviation (SD) of triplicate determinations.

## RESULTS AND DISCUSSION

**Preparation of raw material:** Taxonomic classification of the shrimp shell waste showed that the waste originated from kingdom Animalia, phylum Arthropoda, subphylum Crustacea, class Malacostraca, ordo Decapoda, subordo Dendrobranchiata, family Penaeidae Rafinesque, 1815. The taxonomy of the crab shell waste showed that the waste came from kingdom Animalia, phylum Arthropoda, subphylum

Table 1: Characterization parameters of chitin extracted from shrimp shell waste and crab shell waste

| Samples | Mass yield (%) | Water content (%) | Protein (%) | Ca (ppm) |
|---|---|---|---|---|
| Shrimp shells waste | 21.12±0.23* | 12.18±0.69* | 10.47±0.32* | 4.49 |
| Crab shell waste | 29.60±0.56* | 10.26±0.28* | 10.58±0.31* | 5.46 |

*Values are given as the Means±SD of the triplicate measurements

Crustacea, class Malacostraca, ordo Decapoda, infraordo Brachyuran, family Portunidae and genus Portunus Weber, 1795.

**Preparation chitin:** Chitin in crustaceans is associated with calcium carbonate, protein, lipids and pigments. Chitin is isolated by removing calcium with dilute HCl solution and removing protein with dilute NaOH[22]. In this study, the chitin prepared from shrimp shell waste had a 21.12±0.23% mass yield and contained 12.18±0.69% water, 10.47±0.32% protein and 4.49 ppm Ca minerals. The chitin from crab shell waste had a 29.60±0.56% mass yield and contained 10.26±0.28% water, 10.58±0.31% protein and 5.46 ppm Ca minerals (Table 1).

In the research of Hajji *et al.*[21], chitin was produced from shrimp waste with a mass yield of 20% and from crab shells with a mass yield of 10%. In the present study, more chitin was generated than in the research by Hajji *et al.*[21], especially chitin derived from crab shell waste. Differences in yield can be affected by the type of shrimp and crab that were used. According to Hajji *et al.*[21], the shrimp *Penaeus kerathurus* and the crab *Carcinus mediterraneus* were used.

Benhabiles *et al.*[20] mentioned that chitin from shrimp waste had a protein content of 3.14% (bt/bt) and an ash content of 0.22% (bt/bt). The physical and chemical properties of chitin are not constant because they were influenced by organisms physiology, seasonal variations and species differences[23,24]. The yield of chitin produced was strongly influenced by the deproteination process. Proteins are bound by covalent bonds with chitin, forming a stable complex that made it difficult to obtain 100% yield. The almost complete removal of proteins is desirable because the presence of protein affects the solubility of chitosan. Chitosan solubility increases as the degree of deproteination increases[20].

**Preparation of chitosan:** Chitosan is obtained by the partial deacetylation of chitin and is composed of glucosamine and N-acetylglucosamine. The process of deacetylation involves the conversion of acetyl groups to $-NH_2$ groups on C2 glucosamine[21]. In this study, chitosan prepared from shrimp shell waste had a 16.70±0.41% mass yield and contained 7.60±0.36% water and 0.40±0.04% ash. Chitosan prepared from crab shell waste had a 5.90±0.61% mass yield and contained 8.14±0.02% water and 0.45±0.10% ash.

Hajji *et al.*[21], reported that chitosan was produced from shrimp waste with a mass yield of 14.9% and chitin was produced from crab shell with a mass yield of 5.3%. Similar to chitin, the chitosan yield was also influenced by the type of shrimp and crab used and the extraction process. Kumari *et al.*[25], reported that chitosan extracted from shrimp and crab waste had a protein content of 8 and 10% and an ash content of 0.03 and 2.5%, respectively. In addition, according to Benhabiles *et al.*[20], chitosan from shrimp waste had a protein content of 1.08% (bt/bt), while the ash content was 0.18% (bt/bt). No and Meyers[26] stated that high quality chitosan was associated with an ash content of less than 1%. The chitosan produced in the present study had an ash content of 0.40±0.04 and 0.45±0.10% when produced from shrimp and crab waste, respectively. Therefore, it could be stated that the resulting chitosan was high quality.

**FTIR analysis:** Infrared spectroscopy was used to determine the chitosan structure. The spectra were observed at 3287-3355 $cm^{-1}$, which corresponds to the stretching and vibration of aliphatic O-H groups. The absorption peak at 2871 $cm^{-1}$, indicates the C-H vibrations of $CH_3$ groups, the absorption at 1644 $cm^{-1}$, a carbonyl group, the absorption at 1586 $cm^{-1}$, the N-H bending vibrations of $R-NH_2$ groups, the absorption at 1375 $cm^{-1}$, amide groups, and the absorption at 1026 and 1061 $cm^{-1}$, C-O stretching. The results also showed an absorption loss at 1311 $cm^{-1}$, indicating the loss of acetyl groups. Figure 1 shows that absorption patterns of the spectrum are similar to those of commercial grade chitosan.

**Degree of deacetylation of chitosan:** The DD of chitosan is vital because it influences the physical, chemical and biological properties of chitosan[22,26]. The N-acetyl groups bound of chitin are difficult to remove and their removal requires a high concentration of NaOH at high temperature. The DD of chitosan also depends on the preparation method and crustacean spesies used. The DD of chitosan ranges from 56-99%, with an average of 80%. In this study, 50% NaOH was used to remove the N-acetyl groups at 100°C. The degree of deacetylation of chitosan was calculated by FTIR

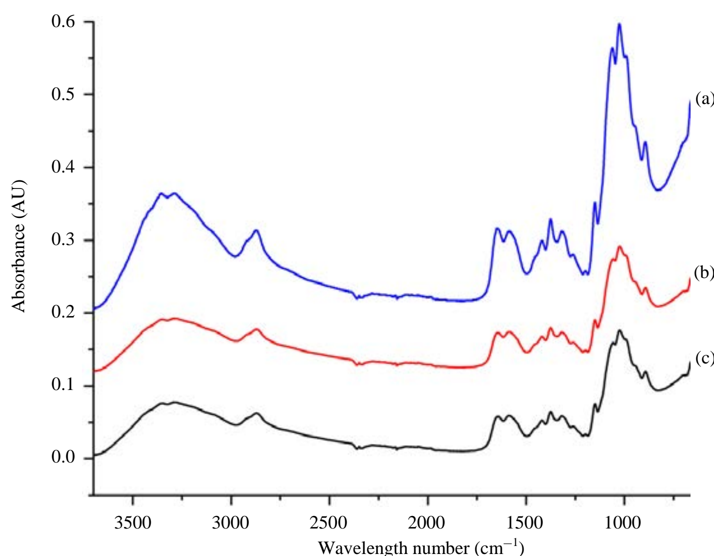


Fig. 1 (a-c): FTIR spectra of (a) chitosan extracted from crab shell waste, (b) chitosan extracted from shrimp shell waste and (c) commercially chitosan (siqma)

Table 2: Characterization parameters of chitosan extracted from shrimp shell waste and crab shell waste

| Samples | Mass yield (%) | Water content (%) | Ash content (%) | DD (% by IR) | Mv (kda) |
|---|---|---|---|---|---|
| Shrimp shell waste | 16.70±0.41* | 7.60±0.36* | 0.40±0.04* | 89.6 | 557 |
| Crab shell waste | 5.90±0.61* | 8.14±0.02* | 0.45±0.10* | 82.1 | 690 |

*Values are given as the Means±SD of the triplicate measurements

methods[22,25] and the values were 89.6% for shrimp shell waste and 82.1% for crab shell waste, respectively. It was found that crab DD was lower than shrimp. Research conducted by Kumari *et al.*[25] also showed that the DD of crab-derived chitosan is lower than the DD of shrimp-derived chitosan Table 2.

**Average molecular weight:** The viscosity-average molecular weight, Mv, of chitosan obtained in this study was determined with an Oswald viscometer at room temperature and was calculated using Mark-Houwink parameters[20]. The viscosity average molecular weight was determined to be 557 kDa for shrimp shell waste and 690 kDa for crab shell waste. The molecular weight of chitosan depend on the sources of material and the preparation method. In a previous study of shell waste from the shrimp species *P. longirostris* by Benhabiles *et al.*[20], the molecular weight was 12.000 Da with a DD of 80%. The shrimp was processed in the present study resulted in chitosan with a higher molecular weight than the chitosan produced from *P. longirostris* waste.

**Crystallinity:** Figure 2 presents the XRD data for chitosan extracted from shrimp shell waste and crab shell waste. The X-ray diffraction patterns showed strong reflections at 2 approximately 9-10° and 2 approximately 20-21°. The chitosan sample extracted from shrimp shell waste displayed several reflections at 9.49° and 19.59°, with a crystallinity index of 64%. The chitosan extracted from crab shell waste displayed several reflections at 10.35°, 19.81° and 22.10° with a crystallinity index of 81%. El Knidri *et al.*[17] reported that the peak characteristics of chitosan derived using conventional methods exhibit two characteristic peaks at 2 = 10.21° and 2 = 19.98° with a crystallinity index 64.91%. From this analysis, it can be observed that the XRD pattern of the chitosan isolated from shrimp shell waste in the present study was more closely matches commercial-grade chitosan than does the chitosan derived from crab shell waste.

**Scanning electron microscopy (SEM) analysis:** The morphology of the chitosan samples was studied using a scanning electron microscope. SEM is a visualization method used to study sample morphology[27]. SEM images obtained at different magnifications and of different areas of the chitosan samples derived from shrimp and crab shells are presented in Fig. 3. In the study by Kumari *et al.*[25], SEM photographs showed that chitin from shrimp waste and crab waste displayed fibrils and granular surfaces. The chitosan samples in the present study had a very smooth surface. The particles

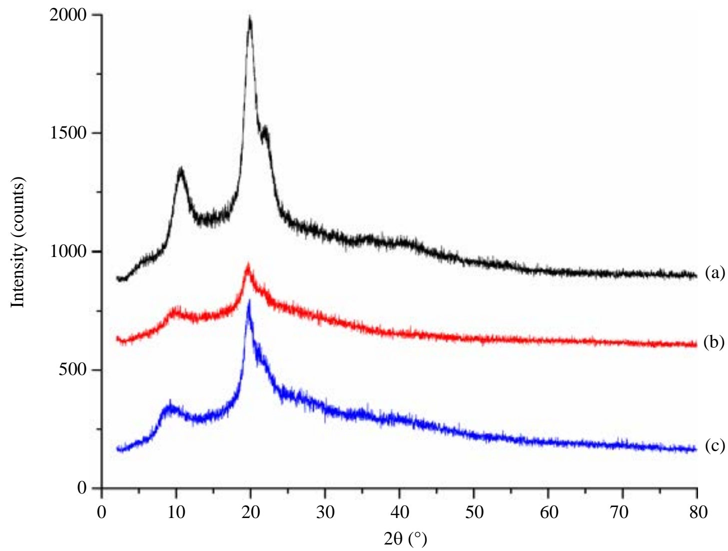


Fig. 2 (a-c): X-ray diffraction patterns of (a) Chitosan extracted from crab shell waste, (b) Chitosan extracted from shrimp shell waste and (c) Commercially chitosan (siqma)

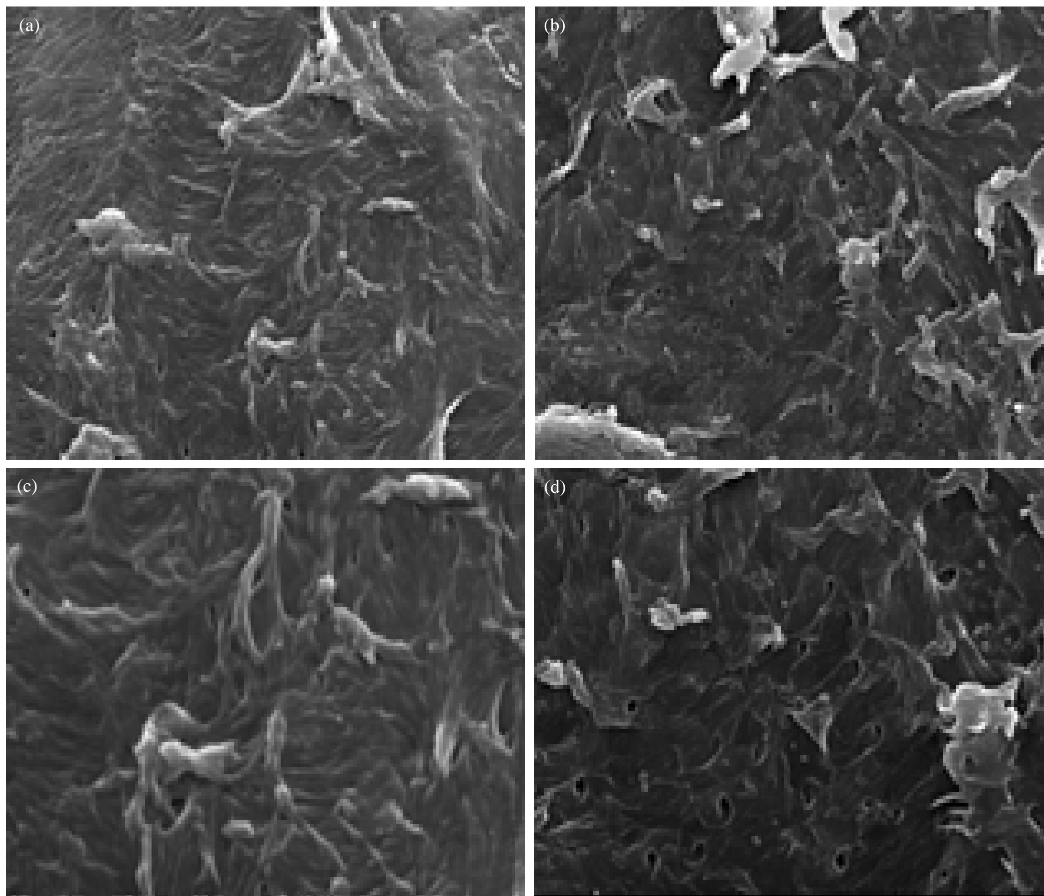


Fig. 3 (a-d): SEM photographs of (a) Chitosan extracted from shrimp shell waste magnifications at 5000x, (b) Chitosan extracted from crab shell waste magnifications at 5000x, (c) Chitosan extracted from shrimp shell waste magnifications at 10.000x and (d) Chitosan extracted from crab shell magnifications at 10.000x

composing upper surface of the chitosan samples were fine and a fractured nanoscale structure was observed. In addition, Kaya *et al.*[28] stated that chitin and chitosan derived from five species of insects had a nanofiber structure. Paulino *et al.*[29] prepared chitin and chitosan from the silkworm head. In the present work, an SEM photograph shows that the chitin structure appears to be composed of finely united leaves and similar results were obtained previously with chitin isolated from crustaceans. Chitosan prepared from silkworm chitin had a highly porous structure. In the present research, the chitosan biopolymer derived from shrimp and crab shell waste had a porous and fibril-like structure. The resulting chitosan was high quality.

## CONCLUSIONS

Chitosan was isolated from shrimp shell waste and crab shell waste by treatment with dilute NaOH solution for deproteinization, HCl solution for demineralization and NaOH at high temperature for deacetylation. The results show that the degree of deacetylation estimated by FTIR methods of the chitosan derived from shrimp shell waste is higher than that from crab shell waste. The XRD patterns confirm that the index of crystallinity of shrimp shell waste derived chitosan is lower than that of crab shells waste derived chitosan. The average molecular weight of chitosan from shrimp shell waste is lower than that of chitosan from crab shell waste. Chitosan from both shrimp shell waste and crab shell waste showed a porous and fibril-like structure. The results suggest that shrimp shell waste and crab shell waste are interesting sources of chitosan.

## SIGNIFICANCE STATEMENT

The results of this study show that shrimp shell waste and crab shell waste are potent sources of chitosan. This study describes the characterization of chitosan from shrimp shell waste and crab shell waste. This study provides information on the degree of deacetylation, degree of crystallinity, molecular weight and morphology of chitosan, which may be beneficial for increasing the value of shrimp shell waste and crab shell waste.

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