Low-Cost Production of Chitosan Biopolymer from Seafood Waste: Extraction and Physiochemical Characterization

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Abstract: Chitosan is an abundant natural biopolymer widely used in industrial and pharmaceutical applications. It stands out for its remarkable biodegradability, biocompatibility, and versatility. Herein, we tried to extract chitosan from mud crab (Scylla spp.), a seafood waste abundantly found in Bangladesh's growing crab farming industry, via a simple low-cost production route. At first, chitin was extracted from crab shells through demineralization and deproteinization to eliminate minerals and proteins. The chitosan biopolymer was then obtained by deacetylation of purified chitin. To evaluate its physicochemical properties, the as-prepared chitosan was characterized by different analyses, such as water and fat binding capacity, solubility, viscosity, molecular weight, fourier transform-infrared, thermogravimetric, scanning electron microscopy, and ash content analysis. The results showed that the crab shell contains around 26.8% chitosan by dry weight, making it an excellent raw material for the massive production of the natural biopolymer chitosan. The prepared chitosan showed fat and water binding capacities of 200-300% and ~680.9%, respectively. Furthermore, it was highly soluble in 1% acetic acid and had an ash content of about 33.7%. Convincingly, the produced chitosan showed great physiochemical properties making it suitable for biomass efficiency, sustainable development, revenue generation, and biomedical applications. In addition, the recycling of seafood waste into a valued product is beneficial to help keep the environment clean, which is among the sustainability goals in Bangladesh and globally.

Keywords: Biopolymer, chitin, chitosan, mud crab, seafood waste.

1. INTRODUCTION

Chitin constitutes a linear polysaccharide consisting of randomly distributed ß-(1,4)-linked D-glucosamine and N-acetyl-D-glucosamine [1,2]. It is found in the exoskeleton of crustaceans like crabs, shrimp, lobster, and crayfish, insects' cuticles, fungi cell walls [3], and microalgae [4]. The percentage of different chitins varies depending on the source, and different organisms contain different amounts. Among the crustacean sources, Crayfish (Astacus fluviatilis) contains 36%; Lobster (Homarus vulgaris/Palinurus vulgaris) has 17-25%; while Shrimp (Palñmon Fabricius) holds 22% of chitin of their body weight [5]. Chitin and its derivative (chitosan) sources are renewable because of the large amount of availability in nature. In the world, seafood consumption is increasing year by year. From 1960 to 2014, the global annual consumption of seafood and its products per capita doubles from 10 to 20 kg [6], then it reaches a steady state up to 2021 [7] and starts gradually increasing after that [8]. In most cases, the outer shell of seafood is treated as waste by dumping it in the soil [9]. Interestingly, this is one of the major sources of chitosan production.

Chitosan is a biopolymer composed of randomly distributed β -linked D-glucosamine and N-acetyl-D-glucosamine. It is utilized in various industries due to its unique characteristics including biologically safe, biodegradable, biocompatible, and nontoxic biomaterial [10]. Chitosan biopolymer is widely used in and/or for the textile industry [11], dye removal [12], cosmetic industry [13], food processing industry for food wrapping [14], agricultural industry for plant growth and crop production [15], wastewater treatment [16], methene production [17], biohydrogen production [18], etc. It is also utilized in the biomedical industry for the fabrication of biosensors for the detection of biological molecules, the production of bio-adhesives used for tissue engineering, and the drug delivery system [19-22]. The use of chitosan for the treatment of spinal cord injury has been recently reported [23]. Furthermore, natural biopolymer chitosan has an antimicrobial effect and thereof is used as an antimicrobial agent [24].

Chitosan biopolymer extraction is achieved via the deacetylation process from chitin, a structural component of crustaceans, fungi, and other plants as well as animals. Besides chitin, organisms also have carbohydrates, proteins, and minerals in their outer shells or cell walls. Chitin can be produced by both biological and chemical procedures. In the biological

process of chitin production, deproteinization by lactic acid bacteria and protease enzymes are the major steps [25]. By chemical process, two major steps are employed, i.e., i) demineralization and ii) deproteinization to remove the minerals and proteins. After completion of these two steps thoroughly, the chitosan is obtained by deacetylation process. Some other minor steps are also employed like decolorization and drying [26].

According to the literature [27], crab shells are one of the richest sources of chitin, which contain about 20-30% chitin, 30-40% protein, and 30-50% calcium carbonate. However, in Bangladesh, they mostly regard these shells as worthless waste and discard them in the soil. Herein, we aimed to convert this waste into a valuable product, chitosan. Besides, recycling seafood waste is useful in helping to keep the environment clean, which is one of the sustainability goals in Bangladesh and even globally. Chemical extraction procedures were employed in this work to extract and purify the chitosan. Afterward, the physiological property characterizations, including water and fat binding capacity, solubility, viscosity, molecular weight, fourier transform infrared, thermogravimetric, scanning electron microscopy, and ash content analysis, were used to confirm the product quality.

2. EXPERIMENTAL SECTION

2.1. Materials and Instruments

Hydrochloric acid (HCl), sodium hydroxide (NaOH), acetic acid (AA), sodium dodecyl sulfate (SDS), and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Aldrich. Whatman filter paper (0.22 μm) was purchased from a local vendor (LabTex, Bangladesh). All aqueous solutions were prepared with ultrapure water from 18.2 MΩ.cm Millipore Milli-Q water purification equipment.

The infrared (IR) spectra were recorded using an IR-Presttige-21 Shimadzu Fourier Transform-Infrared (FT-IR) 4200 Spectrometer. The Thermogravimetric Analysis (TGA) data were acquired using a Thermogravimetric Analysis (TGA) analyzer (TGA 5500, TAinstruments, USA). The morphologies of produced chitosan were imaged by the JCM7000 Scanning Electron Microscopy (SEM).

2.2. Production of Chitin

2.2.1. Sample Collection and Preparation

The crab (*Scylla serrata*) shells were collected from a crab processing industry located in Nolvog, sector 12, Uttara, Dhaka, Bangladesh. The crab farmers, from Khulna and Satkhira, Bangladesh, send their crabs to the crab processing industry, where the crab flesh and shells are separated. As the separated shells are considered waste, they are either disposed of in the ground or soil to the chicken feed processing industry. After the collection, the samples were thoroughly washed and dried in the air. Once properly dried, the shells were ground into a fine powder using a grinder (WBL-15SMG6, Walton, Bangladesh).

2.2.2. Deproteinization and Demineralization

Deproteinization and demineralization steps were adapted from the reported work with modification [25]. First, fifty grams of crab shell powder were placed in a conical flask and then added with 250 mL of 4.0% (w/v) NaOH. The mixture was boiled while stirring and refluxed for 1 h to denature and remove the proteins from the crab shell. After an hour, the crab shells were washed with distilled water consecutively to get rid of the excess sodium hydroxide until the protein residue from the sample reached a neutral pH of 7.0. Finally, the sample was dried overnight at 50 °C in a dryer. The sodium hydroxide catalyzes proteins in the following mechanism.

$$R\text{-}CO\text{-}NH\text{-}R + NaOH \rightarrow RCOO\text{-}Na^+ + RNH_2$$
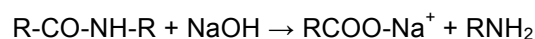
Where R-CO-NH-R is the common structure of proteins. The $R\text{-}CO^-$ of the protein binds with NaO^- of NaOH.

The deproteinized sample was added to 250 mL of 4.0% (w/w) HCl and then stirred overnight to remove the minerals. Afterward, it was washed successively with distilled water until pH reached 7.0. Hydrochloric acid is dissociated into be H^+ cation and Cl^- anion in the aqueous solution, where Cl^- anion binds to the minerals and then separates them from the chitin. The collected sample was dried in the dryer.

2.3. Production of Chitosan from Chitin

2.3.1. Deacetylation Process of Chitin

Chitosan is a deacetylated derivative of chitin. The yielded chitin undergoes a deacetylation process by concentrated base treatment. Scheme 1 demonstrates the conversion of chitin into chitosan via the deacetylation process. At first, the chitin was added to 250 mL of 50.0% (w/v) NaOH heated at 100 °C with continuous stirring and refluxed for 2 h. Then, the solution was cooled down to 25 °C and subsequently washed several times using concentrated sodium hydroxide (50.0%). Afterward, the deacetylated sample was washed with distilled water until the sample solution reached a neutral pH (7.0). The crude chitosan obtained as a cream-colored wet cake was dried. The as-prepared crude chitosan contains some heavy
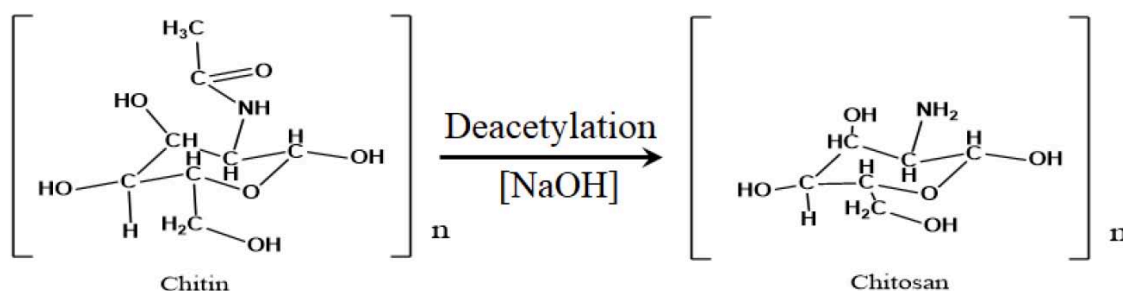
Scheme 1: Conversion of chitin into chitosan by deacetylation method.

metal ions and insoluble dust. To obtain the purified chitosan, this crude chitosan should go through a purification process [28].

2.3.2. Purification of Produced Chitosan

The produced chitosan might contain some insoluble dust. In the first step of purification, the as-produced chitosan was kept in a conical flask containing 500 mL of 5.0% (v/v) CH_3COOH. Then, it was stirred at normal speed until a homogeneous solution was obtained. Next, the solution was filtered with Whatman filter paper to trap the insoluble dust in the pores of the filter paper. Afterward, the pH of the collected chitosan solution was adjusted to 8.5 using the diluted sodium hydroxide solution. At a pH of 8.5, the solubilized chitosan became insoluble and then it was centrifuged to collect the chitosan from the solution. Finally, it was washed several times with distilled water and dried.

To remove the heavy metal ions from the chitosan, the sodium dodecyl sulfate (SDS) and ethylenediaminetetraacetic acid (EDTA) treatments were done after the removal of dust. The dried chitosan was added with 250 mL of 10.0% (w/v) SDS and then stirred overnight. The SDS neutralizes the heavy metals by absorbing them forming a complex. Five hundred fifty milliliters of 5.0% (w/v) EDTA were added to the stirred chitosan solution then let it for 2 h. The EDTA acts as a chelating agent which helps to precipitate the heavy metals from the solution. Afterward, the solution was filtered using Whatman filter paper with 0.22 μm pore and then the chitosan was recovered by centrifuging the filtered solution at 8000 rpm for 5 min. The recovered chitosan was washed several times to remove the excess SDS and the EDTA. Finally, the purified chitosan was dried at 50 °C. In this stage, the produced chitosan was ready for further physicochemical property analyses.

2.4. Physiochemical Property Analyses of Produced Chitosan

2.4.1. Degree of Deacetylation

The deacetylation degree was estimated by an acid-base titration method. To do that, 0.1 g chitosan was dissolved in 30 mL of 0.1 M (v/v) HCl. The solution mixture was stirred using a magnetic stirrer at 25 °C to prepare a homogeneous solution. Then, 30 mL of the indicator methyl orange was added to the solution. It was known that methyl orange indicates the neutral position of the solution by its color change from red to orange. After that, 0.1 M NaOH was prepared for the titration. It was placed in the burette since the volume of NaOH solution is required to measure the degree of deacetylation that can be estimated using Equation 1.

$$DD = \frac{Ma \times Va - Mb \times Vb}{M \times 0.0994} \times 0.016$$

$$\%DD = \frac{Ma \times Va - Mb \times Vb}{M \times 0.0994} \times 0.016 \times 100\% \qquad \text{Eq. 1}$$

Where Ma is the concentration of acid (HCl), Mb is the concentration of base (NaOH), Va is the volume of acid, Vb is the volume of base needed in the titration from burette, and M is the weight of chitosan. The value of 0.0994 is the proportion of the NH_2 group by weight in chitosan. The corresponding weight of the NH_2 group in 1 mL of normal 1.0 M HCl solution is 0.016 (g).

2.4.2. Yield of Produced Chitosan

A yield is the productivity of a product, which is among the most important characterizations for the production of material. Crab waste is a high-yielding source of chitosan. In this work, the yield of the chitosan was measured by Equation 2.

$$\%Yield = \frac{\text{Product weight found after extraction and purification (g)}}{\text{Sample weight taken for extraction and purification (g)}} \times 100\%$$

$$\text{Eq. 2}$$

2.4.3. Water Binding Capacity of Produced Chitosan

To measure the water binding capacity (WBC) of chitosan, first, 0.25 g of chitosan was placed in the tube, and then added 5 mL of distilled water. The solution was then vortexed for 1 min. After that, the tube was placed in the shaker incubator at 30 °C for 30 min. Next, the solution was centrifuged at 6000 rpm for 10 min. At last, the supernatant was discarded, and the wet chitosan was collected and weighed. The WBC of the chitosan was measured by Equation 3.

$$\%WBC = \frac{\text{Wet Chitosan weight (g)}}{\text{Initial chitosan weight (g)}} \times 100\% \qquad \text{Eq. 3}$$

2.4.4. Fat Binding Capacity of Produced Chitosan

Fat binding capacity (FBC) is an important characteristic of chitosan. Three edible oils, i.e., soyabean oil, mustard oil, and olive oil were used for the characterization of chitosan's FBC. Five milliliters of different oils were put in different vials followed by the addıtıon with 0.25 g produced chitosan for each vial. Afterward, the vials were vortexed for 1 minute and kept in the shaker incubator at 30 °C for 30 min. The mixture was centrifuged and then the oil was discarded. Meanwhile, the extra oil was removed. The wet chitosan was weighted and the FBC was measured for different oils using Equation 4.

$$\%FBC = \frac{\text{Weight of oil bound chitosan (g)}}{\text{Initial chitosan weight (g)}} \times 100\% \qquad \text{Eq. 4}$$

2.4.5. Solubility of Produced Chitosan

The chitosan's solubility was measured in various solvents, including methanol, acetone, sodium hydroxide solution, water, and acetic acid. Five milliliters of each solvent were taken in separate vials, and 50 mg of chitosan was added to each vial. The different solvents containing chitosan were stirred at medium speed for 30 min at 25 °C. The solutions were then immersed in boiling water for 10 min and then allowed to cool back down to 25 °C. Subsequently, 3 ml of each solution was moved to quartz tubes, and the optical density of the solutions was recorded in triplicates using a spectrophotometer at a wavelength of 600 nm. The solubility was measured using different concentrations of 0.1, 0.5, 0.8, 1.0, and 1.2% acetic acid, following the described procedure. Clear solutions absorb less light and exhibit lower optical density values, whereas dark solutions show higher optical density values. According to this principle, the solubility of chitosan in different solvents was determined.

2.4.6. Determination of Viscosity

The produced chitosan was prepared in acetate buffer (0.5 M AcOH-0.2 M NaOAc). To calculate the viscosity, 0.01% (w/v) chitosan was dissolved in the buffer solution. The reduced viscosity of the solutions was measured using a standard size A, borosilicate glass Ostwald U-tube viscometer that was calibrated and clamped into a leveled water bath. The measurements were performed at 25 ± 1 °C employing the mean flow time of three determinations of the solutions. The viscosities of the solvent and the polymer solutions were used for the calculation of the relative viscosity, specific viscosity, and reduced viscosity employing Equations 5, 6, 7, and 8, respectively [25].

$$\text{Relative viscosity } (\eta_{rel}) = \frac{\text{Time Efflux of the ploymer}}{\text{Time Efflux of the solvent}}$$

$$\eta_{rel} = \frac{T}{Ts} \qquad \text{Eq. 5}$$

$$\text{Specific viscosity } = \frac{\text{Time Efflux of the polymer}}{\text{Time Efflux of the solvent}} - 1$$

$$\eta_{sp} = \frac{T}{Ts} - 1 \qquad \text{Eq. 6}$$

$$\text{Reduced viscosity } (\eta red) = \frac{\eta_{sp}}{c} \qquad \text{Eq. 7}$$

$$\text{Intrinsic viscosity } ([\eta]) = (\eta_{red})\ c \rightarrow O \qquad \text{Eq. 8}$$

Where T is the mean flow time of polymer solution, Ts indicates the solvent's mean flow time, and C stands for the viscometer constant. A plot of reduced viscosity vs. polymer concentration (Huggin's plot) on extrapolation to infinite dilution gives the polymer's intrinsic viscosity. The intrinsic viscosity is measured using the Huggins equation, η sp/c=[η]+K[η]2c. The specific viscosity can be calculated by employing a viscometer and the above equation was used for the calculation.

2.4.6. Determination of Molecular Weight

The solvent was introduced into the reservoir of a thoroughly cleaned viscometer, which was held in an erect position inside a constant temperature of the water bath that was maintained at 25 ± 0.1 °C. It was then blown up into the upper viscometer bulb using a borosilicate glass viscometer and the time of the efflux of the solvent was noted with the help of a stopwatch. This process was repeated several times. The bulb of the viscometer was emptied and then it was thoroughly dried. After that, a solution was introduced into the viscometer reservoir and the time of efflux of the solution was recorded in the same way as for solvent. The molecular weight (MW) for the measurement of viscosity-average MW (Dalton) and the intrinsic viscosity (η) of the polymer were used. From the intrinsic viscosity, the MW was measured using the Mark-Houwink equation (Equation 9) [29].

$$[\eta] = K \times Ma \qquad \text{Eq. 9}$$

Where M is the viscosity-average MW; K and a are constants, where their values are dependent on the polymer type and the selected solvent. For the chitosan and solvent (0.5 M AcOH-0.2 M NaOAc), these constants are 3.5×10^{-4} and 0.76, respectively which are not dependent on the degree of deacetylation.

Calculation of molecular weight:

The limiting viscosity number [Ɲ] can be calculated using Equation 10.

$$[\eta] = \frac{\frac{\eta_{sp}}{C}}{1 + K\eta \, \eta_{sp}} \qquad \text{Eq. 10}$$

$K\eta$ = 0.28, C = 0.0208652 mm^2/s^2. The viscosity-average MW of the polymer calculates the limiting viscosity number in Equation 11.

$$[\eta] = K'M^a \qquad \text{Eq. 11}$$

Where K' = 18.5 × 10^{-3} ml/g and a = 0.75.

2.4.7. Fourier Transform-Infrared Spectroscopy

The infrared (IR) spectra were recorded in KBr discs on an IR-Presttige-21 Shimazu FTIR 4200 spectrometer under dry air. The number of scans per sample was 50 and the FT-IR spectra were recorded in the wavelength number region of 4000-400 cm^{-1}. The KBr pellets were prepared with 1.0 mg chitosan with 200 mg KBr at a ratio of 1:200 and stabilized under controlled relative humidity before acquiring the spectrum. The spectra indicate the absorbance/transmittance of a material as a function of wavelength number.

2.4.8. Thermogravimetric Analysis

The thermal analysis of produced chitosan was done using a thermogravimetric analyzer over a temperature ranging from 25 to 500 °C at a scan rate of 10 °C/min under the dry nitrogen environment.

2.4.9. Scanning Electron Microscopy

The microscopic structure of produced chitosan was imaged using the VEGA3 TESCAN scanning electron microscopy (SEM). In obtaining the SEM images, the gold thin layer was sputtered on the sample to improve the conductivity of the samples.

2.4.10. Ash Content of Produced Chitosan

Two grams of produced chitosan were loaded in the quartz boat and put in the tube furnace. It was then heated from 25 to 500 °C at the rate of 3 °C/min for 2 h under the nitrogen environment. Afterward, the furnace cooled naturally to 25 °C. At last, the ash content was estimated according to Equation 12.

$$\%\text{Ash content} = \frac{\text{Weight of the ash residue (g)}}{\text{Sample weight (g)}} \times 100\% \quad \text{Eq. 12}$$

3. RESULTS AND DISCUSSION

The production of chitosan from the crab shells in this study was carried out through a simple chemical modification procedure as summarized and shown in Scheme 2. At first, the crab shells were collected from the crab processing industry, which were then processed for deproteinization and demineralization stages. Next, the resulting chitosan was purified prior to characterization. Afterward, the physicochemical properties of as-produced chitosan were investigated for WBC, FBC, solubility, viscosity, MW, and ash content. Further, the FT-IR, TGA, and SEM analyses were also carried out.

3.1. Physiochemical Properties of Produced Chitosan

The physical color and texture of the produced chitosan are shown in Figure **1**, where it demonstrates creamy-white colored powder. Typically, chitosan that
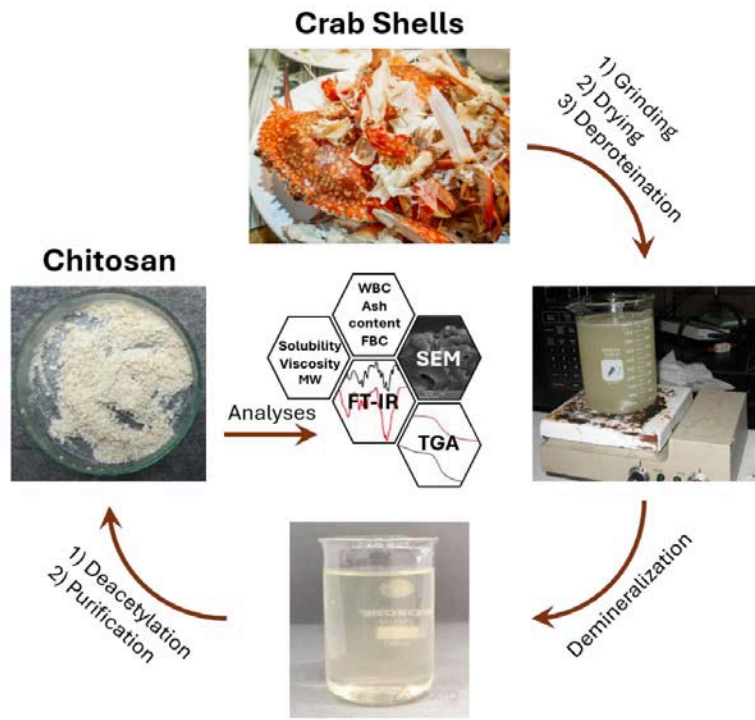
Scheme 2: Schematic representation of the production of chitosan from crab waste.

is in the market appears as a fine, white to off-white powder. In terms of color, the commercial chitosan is generally slightly yellowish. The resulting chitosan in the present study seems in good agreement with the standard product.
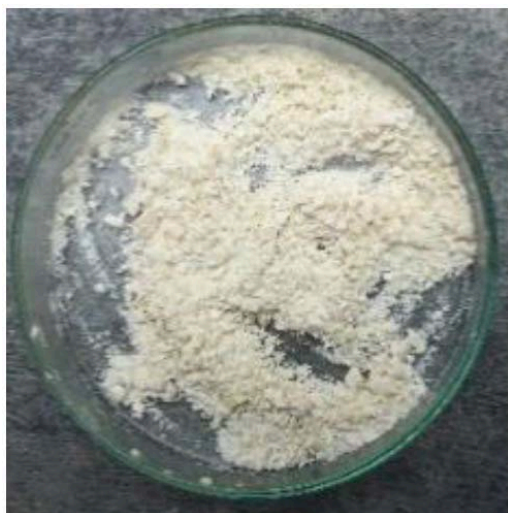
Figure 1: Produced chitosan from the crab (*Scylla serrata*) shell.

3.1.1. Degree of Deacetylation

The degree of deacetylation (%DD) of produced chitosan from crab (Scylla serrata) was determined by the titration method according to Equation 1. The results showed that the produced chitosan was deacetylated at about 76.46 ± 1.6%. It obviously indicated the efficiency of the removal of the acetyl group from the chitin. Also, it showed the content of the amino group in this biopolymer. In a study, it was reported that the %DD of commercial chitosan is in the range of 56.0-99.0% [30]. As %DD of the produced chitosan in the present work is in the %DD range of commercial chitosan, it means that the corresponding titration method was successfully carried out.

3.1.2. Yield of Produced Chitosan

The produced chitosan's yield was calculated using Equation 2 as the dry weight of chitosan obtained from 10 g of dried crab (Scylla serrata) shell sample. From 10 g of dried crab shell powder, about 2.7 ± 0.09 g of purified dry chitosan was found after purification. The chitosan yield was estimated to be approximately 26.8 ± 0.9%. Some recent studies reported the yield of chitosan from Scylla serrata ranges from 13.1 to 27.8% [31-33].

3.1.3. Water Binding Capacity

The water binding capacity (WBC) was determined by using Equation 3. Based on the measurement and calculation, the produced chitosan from the crab (Scylla serrata) waste has a WBC of approximately 680.9 ± 8.8%. As reported in the literature, the WBC of commercial chitosan is about 458-805% [34]. The results showed that the WBC of the resulting chitosan was in the range of the WBC of the commercial chitosan, indicating that the current work was successfully done.

3.1.4. Fat Binding Capacity

The fat-binding capacity (FBC) of extracted chitosan from crab waste was determined using three types of oil, including soybean oil, mustard oil, and olive oil. The FBC values differ depending on the types of oil. The FBC was estimated according to Equation 4, where the olive oil showed the lowest capacity (205.9 ± 2.1%), whereas the mustard oil showed the highest capacity (298.7 ± 3.3%). Meanwhile, the FBC value of soybean oil was observed around 222.1 ± 2.4%. According to the reported literature [35], the chitosan's FBC ranges from 217 ± 7 to 403 ± 6%. In this study, the FBC of the produced chitosan falls within the commercial range. As for the variance, it can happen due to the differences in crystallinity and salt forming unit.

3.1.5. Solubility of Produced Chitosan

The solubility of produced chitosan was determined by the spectrophotometric method. It was measured for methanol, acetone, sodium hydroxide, water, and acetic acid. The solubility was also measured in the
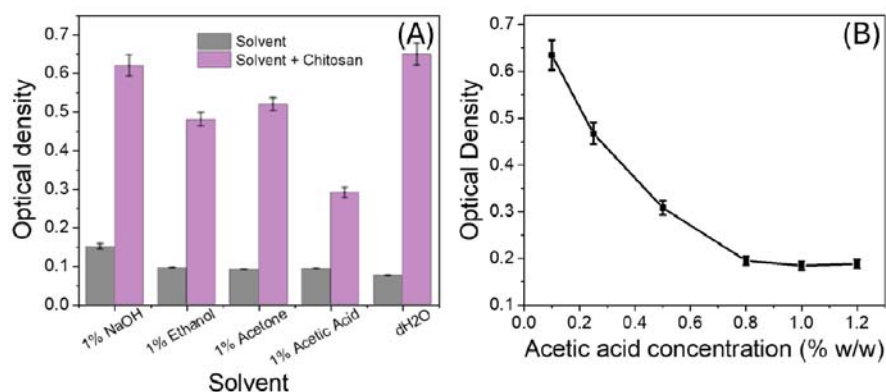
Figure 2: (**A**) Various solvent vs. optical densities to compare the solubility of chitosan in different solvents, and (**B**) Concentration of acetic acid vs. optical density to observe the solubility of chitosan in different concentrations of acetic acid.

different concentrations of acetic acid, viz. 0.1, 0.5, 0.8, 1.0, and 1.2%, following established procedures. According to the principle, a clear solution absorbs low light and shows a lower optical density (OD) value, whereas a dark solution shows a higher OD value. The solubility of chitosan in different solvents as shown in Figure **2A** was measured based on this principle. It can be seen that chitosan in 1.0% acetic acid absorbs the minimum light compared to other solvents. So, a decision could be taken that the produced chitosan from crab shells was the most soluble in acetic acid among others. Since chitosan is highly soluble in acidic conditions, the same experiment was done using various concentrations of acetic acid (1.2, 1.0, 0.8, 0.5, and 0.1%) as solvent. Also, it can be seen in Figure **2B** that the produced crab chitosan was more soluble in 1.0% acetic acid. From this analysis, chitosan in 1.0% acetic acid absorbs nethermost light than other acetic acid concentrations because it could dissolve more crab chitosan than others did.

3.1.6. Determination of Viscosity

Viscosity is a crucial factor in determining the MW of chitosan. A higher MW of chitosan usually provides highly viscous solutions, which is undesirable for industrial handling. The viscosity of produced chitosan decreases with an increased time of demineralization. In acetic acid, it tends to increase with decreasing pH but decreases with decreasing pH in hydrochloric acid. Intrinsic viscosity is an essential rheological parameter that is frequently used to characterize the hydrodynamic properties of polymers and to calculate the average MW of polymers using Mark-Houwink's equation. The inherent viscosity of chitosan depends on both the degree of ionization and the strength of the ions [36]. The intrinsic viscosity and relative viscosity of the resulting chitosan from the crab shell in this study are 0.30 ± 0.01 and 3.15 ± 0.10, respectively.
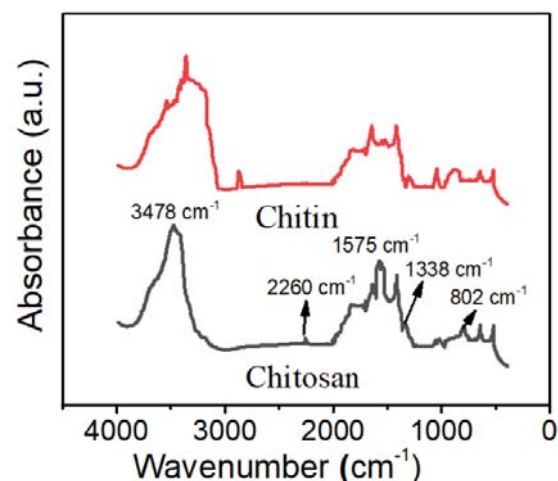

Figure 3: FT-IR spectroscopy of the produced chitosan.

3.1.7. Molecular Weight of Produced Chitosan

Chitosan is a biopolymer with high MW and varies in the sources and the extraction methods. The native chitin's MW is usually larger than one million Daltons, while some researchers determine the MW of chitosan from 2.0×10^5 to approximately 1.4×10^6 Daltons [37]. It was also reported in a study that the MW of chitosan can be in the range of 100 kD to thousands of KDs [38]. Meanwhile, the MW of crab chitosan obtained in this study was 9.9×10^5 Da.

3.1.8. Fourier Transform-Infrared Spectroscopy

As shown in Figure **3**, the -OH stretching peak shifted from 3363 cm^{-1} in chitin to 3478 cm^{-1} in chitosan. This shift could be due to the increased hydrogen bonding from additional -NH$_2$ groups in chitosan. The presence of the nitrile group at 2260 cm^{-1} in chitosan was not found for chitin, indicating a chemical difference likely arising from the processing or modification of chitin to chitosan. The amide I band was slightly shifted from 1646 cm^{-1} in chitin to 1639 cm^{-1} in chitosan. Amide II and III bands (related to protein content) were present in chitosan at 1575 cm^{-1} and

Table 1: Spectrum Peaks Comparison between the Produced and Standard Chitosan

Vibration mode	Standard chitosan (cm^{-1})	Produced crab chitosan (cm^{-1})
(NH$_2$) associated in primary amines (OH) associated in pyranose ring	3420	3478.1
(C≡N) nitrile group	2344.05–2346.5	2260
(C=O) in NHCOCH$_3$ group (Amide I)	1654	1639
Amide II band (N-H bending)	1580	1575
(CH$_2$) in CH$_2$OH group	1422	1413
(CH$_3$) in NHCOCH$_3$ group	1380	1409
Amide III band (C-N stretching)	1320	1338
(C-O) in the secondary OH group	1075	1053
(C-O) in the primary OH group	1029	1020.3
Pyranose ring stretching	895	802

1338 cm^{-1}, which indicated the deacetylated nature of chitosan compared to chitin. There was a unique peak at 802 cm^{-1} for chitosan, corresponding to the pyranose ring, which was not highlighted in the chitin spectrum. Further, the FT-IR spectrum wavelength bands obtained from the produced chitosan were compared with the commercial chitosan as presented in Table **1**, where the FT-IR spectrum wavelength bands for standard chitosan were referred to in the literature [39].
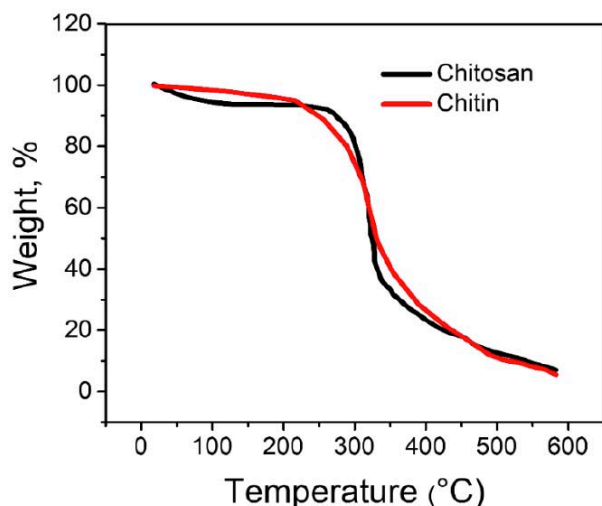
Figure 4: Thermogravimetric analysis for chitin (red line) and chitosan (black line).

3.1.9. Thermogravimetric Analysis

Thermogravimetric analysis is the measurement of a material's thermal stability including polymers/biopolymers. In the present study, the TGA was carried out at the temperature of 18-600 °C (shown in Figure **4**). The mass losses for chitosan are significant later from 270 °C, where the increase in bulk loss after 270 °C was seen in an increasing direction, and more rapidly. It was a consequence that these degradation rates would not negatively impact the reaction when the reaction conditions were considered.

3.1.10. Scanning Electron Microscopy

The SEM micrograph displays the morphological structure of the pure chitosan obtained in this work with 22kx and 49Kx magnifications, where the observation confirmations that the surface of the pure chitosan is platelet-like, nonporous, microfibrils and crystallite, and heterogeneous as displayed in Figure **5**.

3.1.11. Ash Content of Chitosan

Among the most important criteria for assessing the chitosan quality is its ash content. In this investigation, the ash content was determined by pyrolysis employing a tube furnace, where the ash content was estimated using Equation 12. Based on the measurement, the as-prepared chitosan biopolymer exhibited an ash content of approximately 33.7%. Meanwhile, the chitosan derived from mussel shells had an ash content of around 36.9% as reported in the literature [40].

4. CONCLUSION

We demonstrated a low-cost and simple extraction of chitosan biopolymer from mud crab shells, which was aimed at converting seafood waste into a valuable material for various applications. The produced chitosan has a similar color and texture as the commercial grade chitosan. The physicochemical characteristics of the resulting chitosan were compared with commercial-grade chitosan and with the literature data. In general, it can be concluded that the prepared chitosan has the same quality as the commercially available one. The present work paved the way for the low-cost and simple production of chitosan biopolymer. This synthesis route will also help keep the environment clean by recycling valueless wastes into a valued product. Eventually, the product can be utilized as a scaffold for controlled drug release, an antimicrobial agent, and an immobilization matrix in
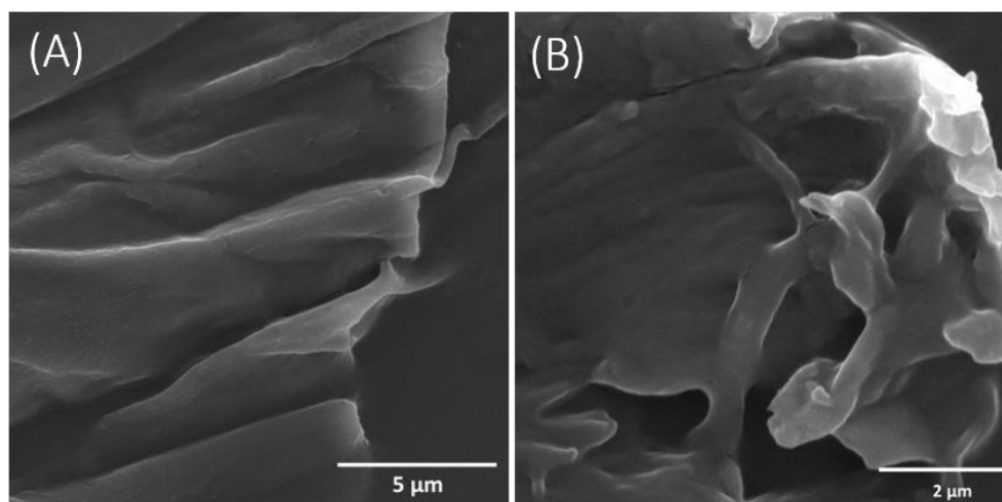
Figure 5: SEM images of produced pure chitosan. (**A**) 22kx and (**B**) 49kx magnifications.

electrode surface engineering of chemical sensor technology.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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