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Fully Deacetylated Chitosan From Shrimp And Crab Using Minimum Heat Input

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Abstract

This study investigated to explore the ideal conditions achieving fully deacetylated chitosan. Chitosan has been obtained from shrimp and crab shell detritus. The deacetylation method was carried out by heating decolorized chitin at 100 ºC for two, four, and six hours, then cold-shaking at 200 rpm for zero, two, and four hours with a shaking device. The maximum degree of deacetylation (DD) was achieved by heating for 6 hours and stirring for 4 hours. The highest DD by the potentiometric titration method, FTIR, and 1H NMR of shrimp chitosan was 97.2%, 98.8%, and 96.39%, against 99.8%, 100%, and 100%, for crab chitosan, respectively. The physicochemical properties of the chitosan with the highest DD have been achieved by X-ray, FTIR, ^{1}H NMR, and SEM. The results of the X-ray, FTIR, and ^{1}H NMR analysis confirmed the preservation of the structure of the obtained chitosan. Extracted shrimp chitosan had 96.5% solubility, 548.71% WBC, and 499.60% FBC, compared to 99%, 475.88%, and 495.84% for crab chitosan, respectively.

Keywords: Shrimp chitosan; Crab chitosan; Fully deacetylated; X-ray; FTIR; ^{1}H NMR

1. Introduction

Generally, optimizing the preparation conditions of biologically active macromolecules is a useful approach to get the intended product under the least drastic and most favorable conditions for good yield while taking into consideration all the influencing factors **[1, 2]**. Since a variety of processing agents affect chitosan's physical and chemical properties, it appears that producing fully deacetylated chitosan in the shortest possible time without affecting the chitosan chain by a simple and economical method is a hard task.

Chitosan is a linear amino-polysaccharide derived from the alkaline deacetylation of biopolymer-chitin at high temperatures. During deacetylation, N-acetyl-D-glucosamine units are transformed into D-glucosamine units, including free amino groups that have a positive ionic charge. High temperatures may cause polymer structural alterations **[3, 4]**. The free amino groups turn chitosan into a cationic form, with possibly valuable antiviral or antibacterial activities like cationic proteins, being reported as antiviral **[5, 6]** and antibacterial activities **[7, 8]**. The positive ionic charge of chitosan allows it to chemically bind to fats, bile acids, and target the bacterial negative cytoplasmic bacterial membrane. This property is the major factor that makes it a versatile tool for a wide range of applications **[9, 10]**.

Shaking provides some kinetic energy to the reaction system. It slightly increases its temperature and speeds up the reaction rate **[11]**. Many studies have investigated the effects of shaking and shaking time **[12, 13]** and several have employed shaking to isolate and extract vital compounds **[14]**. When Oh et al. **[15]** studied the effects of shaking during hydrolysis on the physicochemical characteristics of short-chain glucan aggregates; they observed a slow forming rate without shaking. Studies conducted by Chen et al. **[16]** showed that shaking speeds of 150–

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200 rpm enhanced the rate and extent of enzymatic hydrolysis of cellulose. Ingesson et al. **[17]** showed that excessively high shaking speeds above 200 rpm could lower the extent of cellulose conversion, while moderate shaking speeds (100–200 rpm) provided a good combination of fast initial hydrolysis rates and high conversion yields. Shaking at 150 rpm yielded the maximum glucose oxidase enzyme activity compared to 250 and 100 rpm **[18]**. Higher mixing speeds of 340 rpm only increased the initial rate of hydrolysis and not the final conversion yield **[19]**.

Every extraction and preparation method has different strengths and weaknesses. Therefore, to find a time-efficient way to extract chitin and chitosan, this study attempted to develop an improved technique that enables a good yield. Since it is difficult to obtain fully de-acetylated chitosan in a short time without affecting the chitosan chain, this study aims to explore the effect of combining different heat-treatments and subsequent cold-shaking periods during the main four steps of chitosan extraction; demineralization, deproteinization, decolorization, and de-acetylation on the extent of chitosan extraction. This may help achieve fully deacetylated chitosan on a commercial scale using the least possible time and least possible heat to avoid the potential adverse effects on the chitosan structure.

2. Materials and methods

2.1. Raw materials

The shrimp shells and crab exoskeletons were collected from seafood restaurants in Zagazig, El-Sharkiya Governorate, Egypt. After removing the residual meat, shells were cleaned with tap water and subsequently distilled water, then left to dry at 65 ºC to a constant weight. The dried material was ground and sifted through a 250 µm sieve, then stored in a freezer until use.

2.2. Chemical reagents

All the chemicals and solvents utilized in this investigation were purchased at the highest analytical grade or extra purity. Hydrochloric acid (HCl) was purchased from SD Fine-Chem Limited, India. Sodium Hydroxide pellets (NaOH), Acetone (CH_3COCH_3), Ethyl alcohol (C_2H_5OH 95%), and commercial chitosan (75%) were purchased from Loba Chemie Pvt. Ltd. for High-Grade Laboratory Reagents and Fine Chemicals Mumbai, India.

2.3. Preparation of chitosan

Chitosan was prepared by deacetylating chitin according to the diagram shown in (Fig. 1).

2.3.1. Extraction of Chitin

2.3.1.1. Demineralization

Shrimp and crab shell dried powders were first treated with 1N HCl at a ratio of 10 ml/g for 2 hours in a water bath at 70 ºC, followed by 1-hour of cold-shaking at 150 rpm using a shaking device. The resultant insoluble fraction was filtered, rinsed with distilled water until reaching a neutral pH, and dried to a constant weight at 65 ºC. The procedure was repeated once.

2.3.1.2. Deproteinization

Demineralized shrimp and crab shells were deproteinized using an alkaline treatment with 1N NaOH at a ratio of 15 ml/g for 5 hours in a water bath at 80 ºC, followed by 1-hour of cold-shaking at 150 rpm. The residue (chitin) was filtrated, washed with distilled water until neutral pH, and then dried at 65 ºC to a constant weight. This step was repeated.

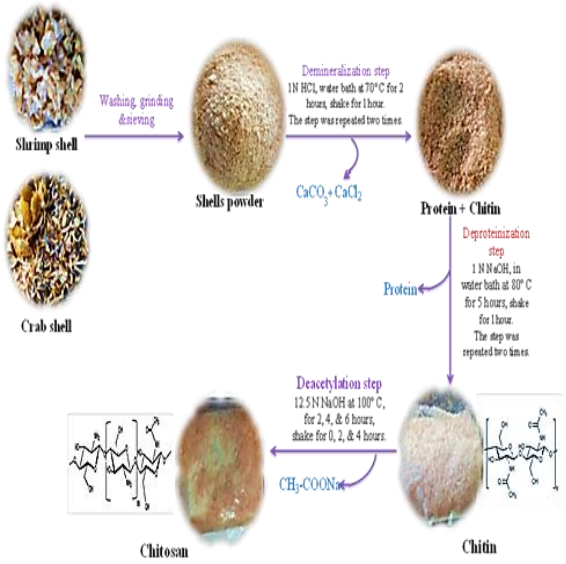
2.3.1.3. Decolorization

Decolorization was performed by immersing chitin in acetone at a ratio of 15 ml/g for 2 hours in a water bath at the acetone boiling point (57 ºC), followed by 1-hour of cold-shaking at 150 rpm to remove oils and pigments. Chitin was submerged in ethyl alcohol 95% at a ratio of 15 ml/g for one hour in a water bath at the ethanol boiling point (79 ºC), followed by 1-hour of cold-shaking at 150 rpm, for further purification.

2.3.2. Deacetylation of chitin to chitosan

The decolorized chitin was deacetylated by treating with 12.5 N NaOH at 100 ºC at a ratio of 15 ml/g for different times (2,4 and 6 h), followed by cold-shaking at 200 rpm for different times (0.0, 2, and 4 hours). The insoluble fraction was isolated by filtration and washed with distilled water until neutral pH. The resulting chitosan was dried to a constant weight at 55 ºC. The DD of chitosan was determined using the potentiometric titration technique. The extracted chitosan from shrimp and crab was denoted as SHC and CRC, respectively, while the commercial chitosan was denoted as COC.

Fig. 1. Steps of chitosan extraction.



2.4. *Analysis of proximate composition of raw materials and chitin*

2.4.1. *Moisture content (%)*

The moisture content of the shells and chitin was determined by the gravimetric method according to AOAC **[20]**. The water mass loss was calculated by drying the sample to a constant weight in an air oven at 105 °C for 24 hours. The water mass was determined with the following equation as the difference between the weight of the wet and oven-dried samples per gram.

$$\text{Moisture content \%} = \left(\frac{(w1-w2)}{w1}\right) \text{X } 100 \qquad (1)$$

Where, W1 and W2 are the weights of wet and oven dried samples, respectively.

2.4.2. *Ash content (%)*

The ash content was determined by sample incineration (0.5 g) at 650 °C for 4 hours in a muffle furnace according to AOAC **[20]**. The percentage of ash value was calculated from the ratio between the weight of the residue and that of the sample weight using the following equation:

$$\text{Solubility\%} = 100 - \left(\frac{\text{Weight of insoluble fraction X 100}}{\textit{Initial weight of sample}}\right) \quad (4)$$

Where, W1 and W2 are the weights (in grams) of the initial sample of chitosan and residue, respectively.

2.4.3. *Determination of total protein (%)*

The total nitrogen was determined by using the micro Kjeldahl method according to AOAC **[20]**. Total protein was calculated by multiplying the total nitrogen by 6.25.

2.5. *Determination of chitosan Degree of Deacetylation (DD) by potentiometric titration method*

A weighed amount (0.2 g) of chitosan was dissolved in 0.1 N HCL (20 cm^3) and deionized water (25 cm^3). After 30 minutes of stirring, another 25 cm^3 of deionized water was added, and the stirring was repeated for another 30 minutes. The solution was titrated against gradual additions (0.1 ml) of 0.1 N NaOH, allowing it to equilibrate, and recording the pH. This process continued until the deflection points, V1 and V2 (the neuralisation points with 0.1 N NaOH of the free H^+ and protonated NH^+, respectively), were recorded. The DD% was calculated using the equation below **[21]**.

$$DD\% = 2.03\ X\left(\frac{(V_2-V_1)}{M+0.0042\ (V_2-V_1)}\right) \qquad (3)$$

Where,

M denotes the sample weight. 2.03 is the coefficient derived from the chitin monomer unit molecular weight, while 0.0042 is the coefficient derived from the difference between chitin and chitosan unit molecular weights.

2.6. *Characterization of the chitosan with the highest DD*

The SHC and CRC with the highest DD (97.2 and 99.8% respectively) and also COC were chosen for the following analyses.

2.6.1. *Solubility*

In a beaker, 0.2 g of sample was solved in 1% acetic acid (20 ml) for 30 minutes on a magnetic stirrer at 250 rpm. After 30 minutes, the solution was filtered onto filter paper and the insoluble fraction was cleaned with distilled water and dried to a constant weight and. Chitosan solubility was calculated by the following equation **[22]**.

$$\text{Ash \%} = \left(\frac{(w2)}{w1}\right) \text{X } 100 \qquad (2)$$

2.6.2. *Water binding capacity (WBC)*

A weighed sample (0.5 g) and 10 ml distilled water were placed in a centrifuge tube (50 ml), agitated for 1 minute, then shaken for 30 minutes and left at room temperature for 12 hours. The samples were centrifuged for 25 minutes at 232 x g. After decanting the supernatant, the tubes were weighed

again, and WBC was determined using the following equation **[23]**.

$$\text{WBC \%} = \frac{(Final\ weight\ of\ tube) - (Initial\ weight\ of\ tube + chitosan)}{\text{Sample weight}}\ x\ 100 \quad (5)$$

2.6.3. Fat binding capacity (FBC)

A representative sample (0.5 g) of chitosan was mixed with 10 ml olive oil in a 50 ml centrifuge tube for 1 minute, held at room temperature for 12 hours, and then shaken for 30 minutes. The samples were centrifuged at 232 x g. After discarding the supernatant, the tube was re-weighted. The following equation was used to calculate FBC **[24]**.

$$\text{FBC \%} = \frac{(Final\ weight\ of\ tube) - (Initial\ weight\ of\ tube + chitosan)}{\text{Sample weight}}\ x\ 100 \quad (6)$$

2.6.4. Intrinsic viscosity determination

Chitosan samples were dissolved in 1% acetic acid at three different concentrations (0.06, 0.08, and 0.1 g/dl). An Ostwald viscometer measured the flow time for each concentration at room temperature. The intrinsic viscosity was defined by capillary viscometry based on the flow time (t_0) of the same volume of solvent or chitosan solution (t) and calculated from the following equations:

Specific viscosity (η_{sp}) was defined by Eq. (7):

$$\eta_{sp} = (t - t_0)/t_0 \quad (7)$$

The reduced viscosity (η_{red}) was obtained by dividing the η_{sp} to the chitosan concentration (C) in g/dL, Eq. (8):

$$\eta_{red} = \eta_{sp}/C \quad (8)$$

The inherent viscosity (η_{inh}), was defined by Eq. (9):

$$\eta_{inh} = \ln \eta_{red}/C \quad (9)$$

The reduced viscosity and inherent viscosity were plotted versus the chitosan concentration to determine the intrinsic viscosity **[25]**.

2.6.5. Determination of the average molecular weight

By using the Mark-Houwink-Sakurada equation (MHS) (Eq. 10), the molecular weight of chitosan was estimated from its intrinsic viscosity ([η]).

$$[\eta] = K \cdot M^{a} \quad (10)$$

Where,

$k=1.81\times10^{-3}$ ml/g and a=0.93 depending on the kind of solvent and temperature **[26]**.

2.6.6. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were recorded for chitosans using an FTIR spectrophotometer (4100 Jasco-Japan) with a frequency range of 400-4000 cm^{-1}. The DD% of chitosan could be calculated using the following equation **[27]**.

$$DD\% = 118.883 - (40.1647X\frac{A1655}{A3450}) \quad (11)$$

Where A1655 is the absorbance of the amide band at 1655 cm^{-1}. A3450 is the absorbance of the O-H band at 3450 cm^{-1}. The factor (40.1647) is the ratio of A1655/A3450 for completely N-acetylated chitosan. The number 118.883 was presented as a baseline.

2.6.7. Proton nuclear magnetic resonance spectroscopy (^1H NMR)

^{1}H NMR (Joil spectrometer, USA) (500 MHz) was used to examine chitosan samples (10 mg) dissolved in 1ml CD_3COOD/D_20 (2% v/v) solution. The DD% of chitosan samples were determined using the equation below **[28]**.

$$DD\% = 100 - \left(\frac{2xA_{CH3}}{A_{H2-H6}}\right)X100 \quad (12)$$

Where ACH3= CH3 area in -NHCOCH3 group, AH2-H6= areas of H2, H3, H4, H5 and H6.

2.6.8. X-ray powder diffraction (XRD)

The chitosan crystallinity was determined by XRD analysis. A PANalyticalX`Pert PRO X-ray machine (Netherland) was used to perform the XRD measurements on powder samples. Cu Kα radiation has been used as an X-ray source (45 kV, 30 mA). The samples were scanned at 4 min^{-1} scanning rate and 25 °C from 2θ = 5-40°. Using the following equation, the crystalline index (CI%) was determined from the ratio of the crystal phase to the total of the crystal phase and amorphous phase **[29]**.

$$Crystallinity\ index\ \% = \left(\frac{I_{110} - I_{am}}{I_{110}}\right)X100 \quad (13)$$

Where, I_{110} represents the highest intensity of the

lattice diffraction pattern at 20°. I_{am} represents the intensity of amorphous diffraction at 16°.

2.6.9. Scanning electron microscopy (SEM)

SEM was used to examine the surface morphology of chitosan samples using a High-Resolution Field Emission SEM (Quanta FEG 250-Czechoslovakia). A sputter coater (Edwards S150A-BOC Edwards, UK) was used to coat chitosan samples with gold under a vacuum before the examination to improve contrast. At various magnifications and locations, images of the sample surface were captured.

3. Results and discussion

3.1. Proximate composition of raw materials

The proximate chemical composition of fresh shrimp and crab wastes is presented in Table 1. The moisture content of the shrimp and crab shells was 11.39% and 5.37%, respectively. Chemical analysis revealed that the wastes of the shrimp shell retained more water than the wastes of the crab shell, in agreement with results reported by Olafadehan et al. **[30]**. The crab waste was found to have high ash content (56.08%) compared with the shrimp waste, which was 35.02%. Hamdi et al. **[31]** found 53.8% ash in blue crab shells, and Zhang et al. **[32]** obtained an ash content in shrimp shells of more than 30%. The crude protein obtained showed that the shrimp and crab shell waste were rich in this component at 38.49% and 36.24%, respectively. Rødde et al. **[33]** found that the protein content varied between 33% and 40%, and the ash content varied between 32% and 38% of the dry weight of the shrimp shells. The composition difference may be due to the used species.

		Moisture content (%)	Ash content (%)	Protein content (%)
Shells	Shrimp	11.39	35.02	38.49
	Crab	5.37	56.08	36.24
Chitin	Shrimp	6.19	0.72	1.91
	Crab	5.84	0.64	1.68

Table 1 Moisture, ash, and protein content of shrimp and crab shells and chitin (%).

3.2. Proximate composition of chitin

Eliminating all organic and mineral content in the raw materials is the purpose of any extraction process. The produced chitin quality depends on the extraction conditions such as acid and alkali concentrations and the reaction time with samples **[34, 35]**. To judge whether there are any differences in moisture, ash, and protein content between shrimp and crab shells and chitin prepared under the used method. The ingredients were measured in the produced chitin. The results are shown in Table 1. The moisture content of the extracted chitin from shrimp and crab shells was 6.19% and 5.84%, respectively. The high moisture content may be due to the remaining water in the sample, which was utilized during the deproteinization step, as previously stated by da Silva et al. **[36]**. The content of ash and protein indicates the effectiveness of the demineralization and deproteinization stages. Compared to the precursor, high reductions in the ash and protein content of extracted chitin were observed. The ash content of shrimp and crab chitin was 0.72% and 0.64%, respectively. Samar et al. **[37]** obtained similar results. Chitin is tightly linked with proteins, necessitating a more rigorous treatment to eliminate all related proteins **[38]**. The protein contents of the extracted chitin from shrimp and crab shell wastes were 1.91% and 1.68%, respectively. These percentages were lower than the percentages obtained by Olafadehan et al. **[30]**. Lertsutthiwong et al. **[39]** stated that the linkage between chitin and protein moieties is probably through amide formation. This formation occurs between the free amine groups in chitin and the side-chain carboxylate groups in the protein. In the presence of NaOH, the amide is hydrolyzed and the protein content in the chitin product is reduced. Deproteination at room temperature should be done at 4% NaOH for 21 h to get a low protein content. In this study, repetition of the purification steps (demineralization and deproteinization) contributed to a very low percentage of ash and protein in the resulting chitin, also helping reduce the time it takes to obtain chitin.

3.3. Results of chitosan DD experiment by potentiometric titration method

The chitosan preparation conditions from chitin recovered from crustacean shells are important due to their influence on structural parameters such as molecular weight, degree of deacetylation, and acetyl group distribution **[40]**. The variables for the current

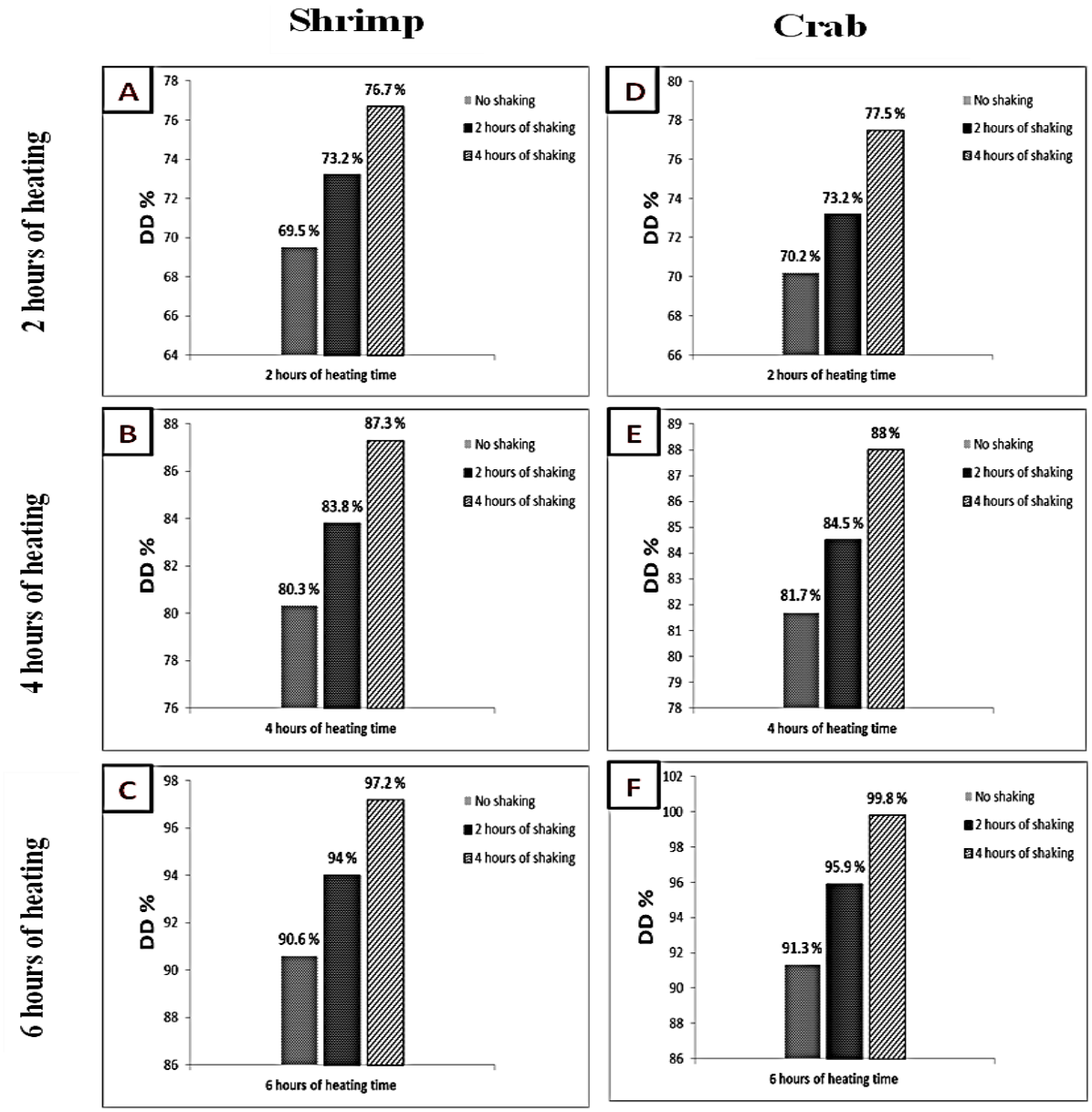
study were heating at 100 °C and shaking at 200 rpm for varying periods with a concentrated sodium hydroxide solution (50%). Shaking was used to limit the time of heat exposure and reduce the detrimental impact on chitosan chains. The vibration speed of 200 rpm was chosen according to the results of Chen et al. **[16]**, which enhanced the rate and extent of enzymatic hydrolysis of cellulose. Chitosan is commonly deacetylated after being treated with a 50% concentrated sodium hydroxide solution at 100 °C to remove some or all of the acetyl groups from the polymer **[23, 41]**. Potentiometric titration appears to be the most straightforward and reliable process for determining DD **[42]**. Fig. 2 shows the results of experimental testing to investigate the effects of heating at 100 °C for different periods (2, 4, and 6 hours) and followed by stirring at 200 rpm for different periods (0, 2, and 4 hours). The results showed that the DD increased by increasing the heating time. The stirring procedure also had a good effect on the DD, indirectly decreasing the necessary quantity of heat energy. According to Figs. 2A and 2D, chitosan has been successfully produced from the dried shrimp shell (Fig. 2A) and crab shell (Fig. 2D) waste by heating for just 2 h with %DD, 69.5%, and 70.2%, respectively. The same figure (Figs. 2A and 2D) shows the effect of shaking for 2 and 4 hours on the %DD of SHC (73.2%, 76.7%, respectively) and (73.2% and 77.5%) for CRC, respectively. This could be because shaking increases the number of collisions between reactant molecules, implying that shaking accelerates the deacetylation process, which reduces the harmful effect of heat. Under deacetylation conditions of 50% NaOH at 100 °C for 8 hours **[43]**, a relatively lower DD (74%) was obtained for shrimp chitosan. The higher DD was obtained by heating for 4 hours and shaking for 0, 2, and 4 hours. The results were 80.3%, 83.8%, and 87.3% for SHC (Fig. 2B), against 81.7%, 84.5%, and 88.0% for CRC (Fig. 2E), respectively. Based on Kumari et al. **[23]** definition that the term chitosan is only used when the DD is greater than 70%, all the prepared materials can classify as chitosans even at the lowest used time. In general, He et al. **[44]** reported that chitosan with DD between 55-70% is considered low; 70–85% means medium; 85–95% means high; and 95–100% means ultrahigh chitosan. The products obtained after 4 hours of heating followed by 4 hours of shaking can consider as a high-DD chitosan. Ultrahigh-DD crab (95.9%) chitosan could be obtained after a 6-hour heating reaction followed by 2 hours of stirring. The maximum degree of deacetylation (DD) was achieved by heating for 6 hours and stirring for 4 hours, recording 97.2% for SHC and 99.8% for CRC. The last crab chitosan is virtually entirely deacetylated and very close to 100% DD. The highest DD of chitosan obtained by Soon et al. **[45]** was 81.06%, using 50% NaOH at 90 °C for 30 h and 100 rpm rotation. The intensifying of purification steps to obtain chitin has contributed to the possibility of getting fully deacetylated chitosan. This study has confirmed the possibility of producing fully deacetylated chitosan using a conventional heating approach and inexpensive with appropriate modifications, i.e., reducing the time of heat exposure at 100 ºC by incorporating a stirring step at room temperature (2-4 h). As a result of this experience, the SHC and CRC with the highest DD (97.2 & 99.8%, respectively) were chosen for the rest of the analyses.

3.4. Characterization of the chitosan with the highest DD

3.4.1. Solubility

Chitosan solubility is one of the most important properties to comprehend and regulate, as it determines the bioactivity of chitosan **[42]**. The findings in Table 2 indicate that the obtained chitosan has a higher solubility than commercial chitosan in 1% acetic acid, registering 96.5% and 99% for SHC and CRC, respectively, against only 68.5% for COC. Kumari et al. **[23]** found that the solubility of chitosan from crab and shrimp was 60% and 70%, with DD% of 70% and 78%, respectively, when using 40% KOH at 90 ºC for six hours. The obtained solubility ratio may also confirm the degree of deacetylation for shrimp and crab chitosan. Huang et al. **[46]** stated that lower solubility values suggest incomplete deacetylation. It was observed that the solubility of chitosan samples suits the DD. These findings are consistent with earlier ones reported by Agarwal et al. **[22]**. The highly protonated free amino groups NH^{3+} on the C2 atom of the GlcN unit, correlated with the high chitosan DD, may attract ionic compounds and increase chitosan solubility, in agreement with Weißpflog et al. **[47]**. This explains why the solubility of the obtained chitosan, with high DD and free amino groups, was higher than the solubility of COC and chitosan obtained by previous

Fig. 2. DD% of extracted shrimp and crab chitosan as assayed by the potentiometric titration method.



studies as stated by Mohan et al. **[48]**, with lower DD and free amino groups content.

Based on the above results, the solubility of the obtained chitosan is considered very high, which means we could employ it in many useful applications in the future.

3.4.2. Water binding capacity analysis

WBC is one of the functional properties of chitosan and expresses the tendency of water to bind to hydrophilic substances **[48, 49]**.

The ultra-high DD CRC and SHC exhibited higher WBC values, i.e., 775.89% and 748.72%, respectively, than the COC (673.76%), as shown in Table 2. The increase in WBC for CRC and SHC compared to the COC is 15% and 11%, respectively. It is noticeable that with the increase in the DD, the ability of the resulting chitosan to bond with water increased, which resulted in an increase in binding sites for -NH_2 groups. This allows hydrogen bonds to form with water molecules. In a similar study, the WBC of crawfish chitosan varied from 660.6% to 745.4% **[50]**, and *Metapenaeus stebbingi* chitosan had a WBC of 712.99% **[51]**. On the other hand,

Marei et al. **[43]** reported a low value of 538% WBC for shrimp chitosan. Compared to the results of previous studies, chitosan produced in the manner used in this study is distinguished by its high affinity for water. The water retention capacity of the obtained chitosan will significantly help in using it in many applications.

3.4.3. Fat binding capacity analysis

FBC indicates the amount of oil absorbed per unit weight **[48]**. The FBC of the obtained chitosan was measured using olive oil. As exhibited in Table 2, CRC and SHC recorded higher FBC (499.41% and 495.65%) than the COC (387.82%). Chitosan from crab legs demonstrated varying FBC values ranging from 217%–403% **[52]**, and about 326% for shrimp chitosan in the research by Marei et al. **[43]**. Accordingly, extracted chitosan has a high ability to bind or absorb fat. Perhaps because the highly deacetylated extracted chitosans are more electron-attractive due to the more released free amino groups. The FBC values of the prepared chitosan were generally lower than the WBC of the same samples. The higher WBC value than FBC can be ascribed to the fact that water has a higher polarity than oil, causing greater water attachment to the chitosan chain's amine and hydroxyl groups. This conclusion is consistent with the findings of Huang and Tsai **[53]**. They stated that chitosan with a low molecular weight is more effective than chitosan with a high molecular weight at binding fat. FBC is one of the most important functional parameters of chitosan, determining its appropriateness for various applications, and its values vary depending on the preparation procedure **[23]**. The ability of chitosan to bind fat leads to the adsorption of fat and cholesterol in the diet, preventing fat loss during cooking **[53]**. Chitosan with a high FBC can be used as a functional food or nutritional component for biological activities **[49]**.

3.4.4. The intrinsic viscosity and average molecular weight analysis

The data in (Fig. 3) represents the evolution of a linear fit of the reduced and inherent viscosity as a function of chitosan concentration, which was used to calculate the intrinsic viscosity, as present in (Table 2). The intrinsic viscosity was decreased as the DD of chitosan increased. The intrinsic viscosity of SHC and CRC was 4.457 and 3.593, respectively, compared to 39.672 for the COC, corresponding to DD values of 97.2%, 99.8%, and 74.6%, respectively. This demonstrates how chitosan deacetylation affects the viscosity and flow parameters of the solutions **[54]**.

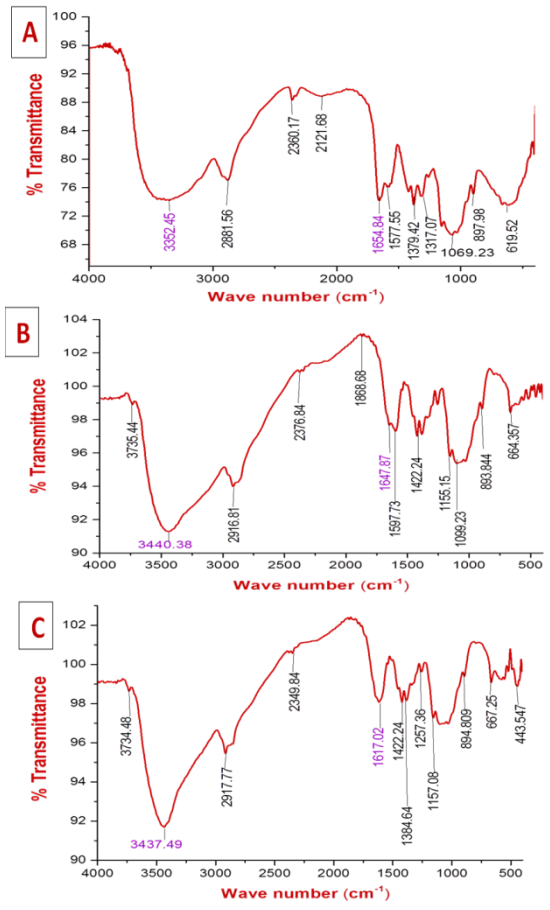
Substituting the intrinsic viscosity values in equation (10), gave the average molecular weight of SHC and CRC as 4.265 and 3.515 kDa, respectively, against 44.66 kDa for COC. The complete isolation of the acetyl groups obviously resulted in a decrease in the molecular weight of the resultant chitosan, which confirms the resulting DD%. The values of SHC and CRC are lower in the present study than those reported by Kumari et al. **[23]** (6.27 kDa and 6.27 kDa, respectively) due to the lower DD (78% DD and 70%, respectively). The molecular weight of chitosan extracted from Colorado potato beetle adults **[55]**, larvae, and grasshoppers **[56]** was 2.722 kDa, 2.676 kDa, and 4.5 kDa, respectively. Generally, both the DD and the various chitosan sources have an impact on the variance in molecular weight. Furthermore, the molecular weight of chitosan can be affected by numerous parameters, such as prior chitin treatment, alkaline concentration, and reaction duration **[40]**. The low molecular weight chitosan from shrimp and insects has excellent antiseptic and anticancer properties useful for drug development **[48]**.

3.4.5. FTIR spectrum analysis

The FTIR spectra of COC, SHC, and CRC (Fig. 4) affirm their general characteristic chemical composition. The extent of the absorption peaks of the chitosan derived from the tested species with FTIR yielded ranges similar to those obtained in the studies conducted earlier by Kaya et al. [57] and Kim [58] as presented in Table 3. Their spectra exhibited the amide I band (C=O in -NHCOCH3) at 1654.84 cm^{-1}, 1647.87 cm^{-1}, 1617.02 cm^{-1}, and the amide II (amine $-NH_2$) at 1597.73 cm^{-1}, 1590.98 cm−1, and 1577.55 cm-1, respectively, in agreement with Kaya et al. [57]. The bending vibration of amine groups in the amide II band supports the deacetylation of chitin in consistence with Rasweefali et al. [49].

Furthermore, the three mentioned chitosan samples have absorption peaks at 3352.45 cm^{-1}, 3440.38 cm^{-1}, and 3437.49 cm^{-1}, respectively, corresponding to the stretching vibrations of the hydroxyl group –OH in agreement with Marei et al. [43]. The distinctive absorption peak, corresponding to the –CH stretching regions of COC, SHC, and CRC appeared at 2881.56 cm^{-1}, 2916.81 cm^{-1}, and 2917.77 cm^{-1}, and the absorption peaks caused by the "C–O–C stretching vibration" appeared at 1069.23 cm−1, 1099.23 cm^{-1}, and 1101.15 cm^{-1}, respectively. The existence of a peak at roughly 1155 cm^{-1} to 1165 cm-1 indicates the distinctive bands of polysaccharide structure (the C-O-C glycosidic linkage) in the chitosan, which is consistent with Kim [58]. This confirms the preservation of the structure of the obtained product. Globally, the FTIR chitosan absorption peaks included in the FTIR spectrum match those documented by Weißpflog et al. [47].

Fig. 3. Evolution of reduced and inherent viscosity as a function of chitosan concentration: A) COC, B) SHC, and C) CRC.



Using the band absorption intensity at 1655 cm-1 (amide I band) with the band and at 3450 cm-1 (-OH band) as an internal standard according to equation (11) estimated the DD% as shown in Table 5.

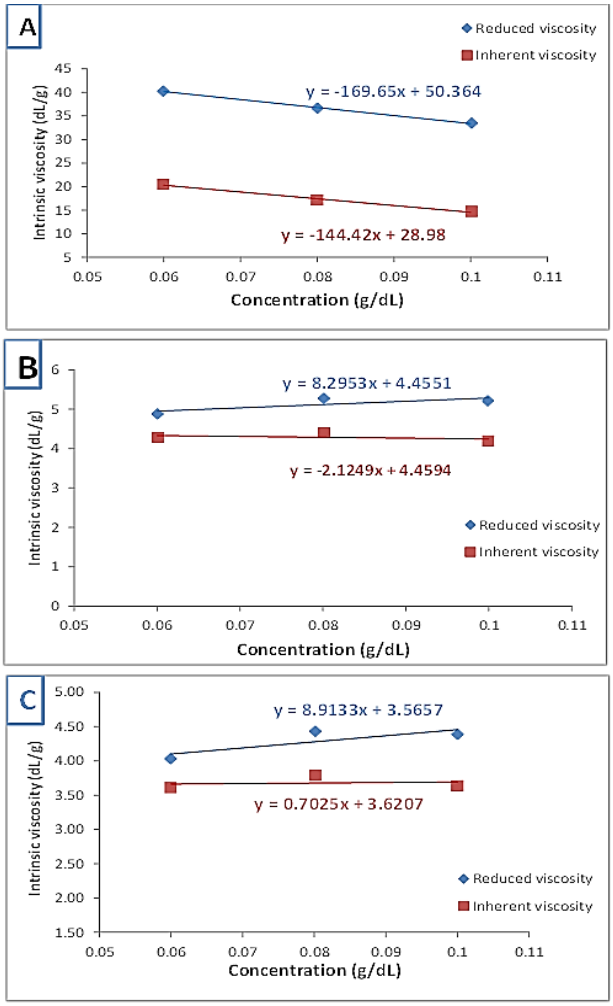


Fig. 4. FTIR spectra of chitosan and their corresponding wavenumber: A) COC, B) SHC, and C) CRC.

3.4.6. ^{1}H NMR analysis

The ^{1}H NMR spectra of chitosan samples the slight differences in chemical shifts between peaks (about 0.15 ppm), may be due to experimental errors **[60]**. The chemical shift of methyl protons in the acetamide group for COC, SHC, and CRC appeared at 1.98, 2.00, and 2.01 ppm, respectively. Estimating the DD% of chitosan from the areas of the signals at 1.95-2.09 ppm (methyl) and the sum of the areas from 3.18 to 4.34 ppm (H2, H3, H4, H5, and H6) in the ^{1}H NMR according to equation (12) gave the values in Table 5.

Table 2, Solubility, WBC, FBC, Intrinsic viscosity, average molecular weight, and CI% for SHC, CRC, and COC.

Chitosan Type	Solubility (%)	WBC (%)	FBC (%)	Intrinsic viscosity	Molecular weight (kDa)	CI%
COC	68.5	673.76	387.82	39.672	44.66	76.34
SHC	96.5	748.72	495.65	4.457	4.265	30.33
CRC	99	748.72	499.41	3.593	3.515	28.80

Table 3, FTIR bands and their corresponding wave numbers of the commercial and extracted chitosan.

Functional group	Wavenumber (cm^{-1}) frequency			
	Range of standard wavenumber (cm^{-1}) *	COC	SHC	CRC
Primary amines NH_2 and pyranose OH	3450–3455	3352.45	3440.38	3437.49
(C-H) in pyranose ring	2850–2950	2881.56	2916.81	2917.77
Amide I (C=O)	1620–1660	1654.84	1647.87	1617.02
Amide II (NH_2)	1590–1610	1577.55	1597.73	1590.98
(CH_2) in CH_2OH group	1420–1430	1420.96	1422.24	1422.24
(CH_3) in $NHCOCH_3$ group	1375–1382	1379.42	1383.68	1384.64
(C-O-C) glycosidic linkage	1155–1165	1154.09	1155.15	1157.08
C–O–C stretching	1070–1075	1069.23	1099.23	1101.15
Pyranose ring	890–900	897.98	893.844	894.809

*The range of standard wavenumbers (cm^{-1}) is similar to that obtained in the studies conducted earlier by Kaya et al. **[57]** and Kim **[58]**.

Table 4 Chemical Shifts for protons in chitosan determined by ^{1}H NMR.

Types of proton	Position (**δ**, ppm)			
	Ranges of position (**δ**, ppm)	COC	SHC	CRC
H_1 (H_1 of $GluNH_2$)	4.85-4.97	4.88	4.86	4.89
H_2 (H_2 of $GLuNH_2$)	3.18-3.24	3.22	3.21	3.22
H_3,H_4, H_5,H_6	3.74-4.34	3.84	3.92	3.86
HN-$COCH_3$	1.95-2.09	1.98	2.00	2.01

Table 5 DD% for COC, SHC and CRC by potentiometric titration method, FTIR, and ^{1}H NMR.

Chitosan type	DD%		
	Potentiometric titration	FTIR	^{1}H NMR
COC	74.6	78.7	73.2
SHC	97.2	98.8	96.39
CRC	99.8	100	100

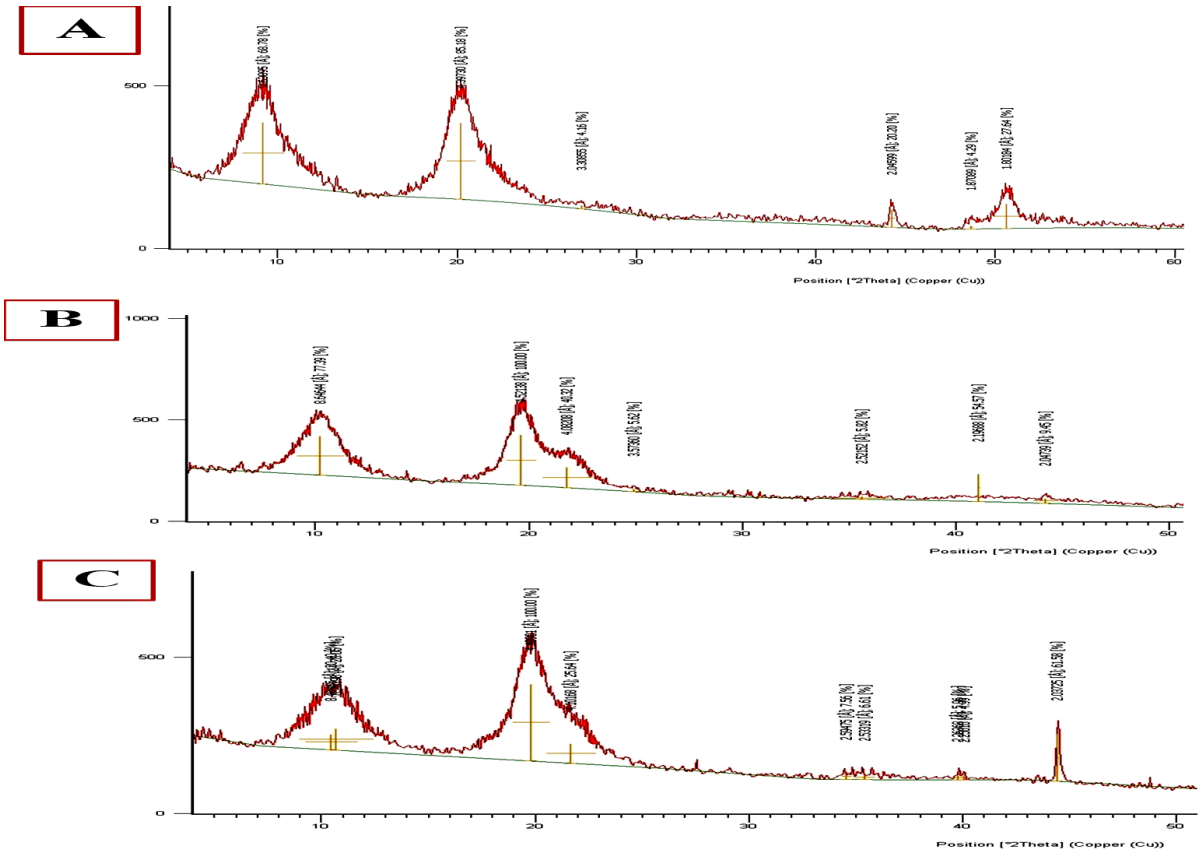


Fig. 5. Chemical shifts for protons of chitosan that were determined by ^{1}H NMR: A) COC, B) SHC, and C) CRC.

3.4.7. XRD analysis

XRD analysis was performed to examine the crystallinity of the chitosan samples (Fig. 6), ant the values of crystallinity index (CI%) are recorded in Table 2. Fig. 6 indicates two separate crystalline peaks for COC, SHC, and CRC, the first at 2θ = 9.17°, 10.22° and 10.7° and the second at 2θ = 20.18°, 19.618° and 19.76°, respectively, similar to the results of Ibitoye et al. **[61]** & Rasweefali et al. **[49]**. Peaks at 2θ around 9° –11° refer to amine I (–N – CO – CH_3) and peaks at 2θ around 19° –20° refer to amine II (–NH_2) of chitosan **[62]**.

The CI% for SHC and CRC was 30.33% and 28.80%, respectively, against to 76.34% in COC. Rasweefali et al. **[49]** reported similar results using CI% value assessments.

The X-ray data represents an average of molecules arranged in a periodic crystal lattice. In addition, the technique is not easily applicable to oligosaccharides.

ngle crystals from

s the preservation of the structure of the obtained chitosan.

SEM analysis

Applying SEM at 800x and 24000x magnification powers produced the surface morphological images of chitosan samples presented in (Fig. 7). At the lower magnification power (800x), the prepared chitosans showed flaky layers, a dense structure, and a lamellar organization, and these agree with Ibitoye et al. **[61]** & Kucukgulmez et al. **[51]**.

Increasing the magnification power to the higher level (24000x), showed highly smooth surface morphologies of chitosan derived from shrimp and crab. The crab chitosan (with high DD) showed a smoother surface than the prepared shrimp chitosan, while the surface morphology of the commercial chitosan showed a highly rough surface.

These findings may relate to the higher number of free amine groups (-NH_2) in high deacetylated chitosan compared to low deacetylated chitosan. The surface roughness in low deacetylated chitosan may

be due to the presence of intervening monomers containing acetamide groups (acetylated monomer "Glu-NHCOCH$_3$") in the chitosan chain. Alternatively, when the quantity of free amine groups rises, the chitosan surface becomes smoother, which is consistent with earlier results from Agarwal et al. **[22]**; Divya et al. **[64]** & Rasweefali et al. **[49]**. Generally, shell waste from crustaceans had been used to produce bio-compatible, non-toxic chitosan with potential applications as antibacterial, antifungal, and antioxidant agents. This study has confirmed the possibility of producing fully deacetylated chitosan using a conventional heating approach with appropriate modifications, i.e., reducing the time of heat exposure at 100 ºC by incorporating a stirring step at room temperature (2-4 h). At the same time, intensifying the purification steps has contributed to the possibility of getting fully deacetylated chitosan.

CRediT author contribution statement

MS and AA conceptualized the research concept. AE has done the data curation; Formal analysis and investigation. AA has achieved the Funding acquisition, revised the manuscript and administered the project. MS has supervised the Investigation and validated the methodology. RS has contributed to the Methodology; Project administration; Resources. AS has contributed to the Supervision, validation and Visualization. AE has written the draft manuscript and MS has written the final manuscript.

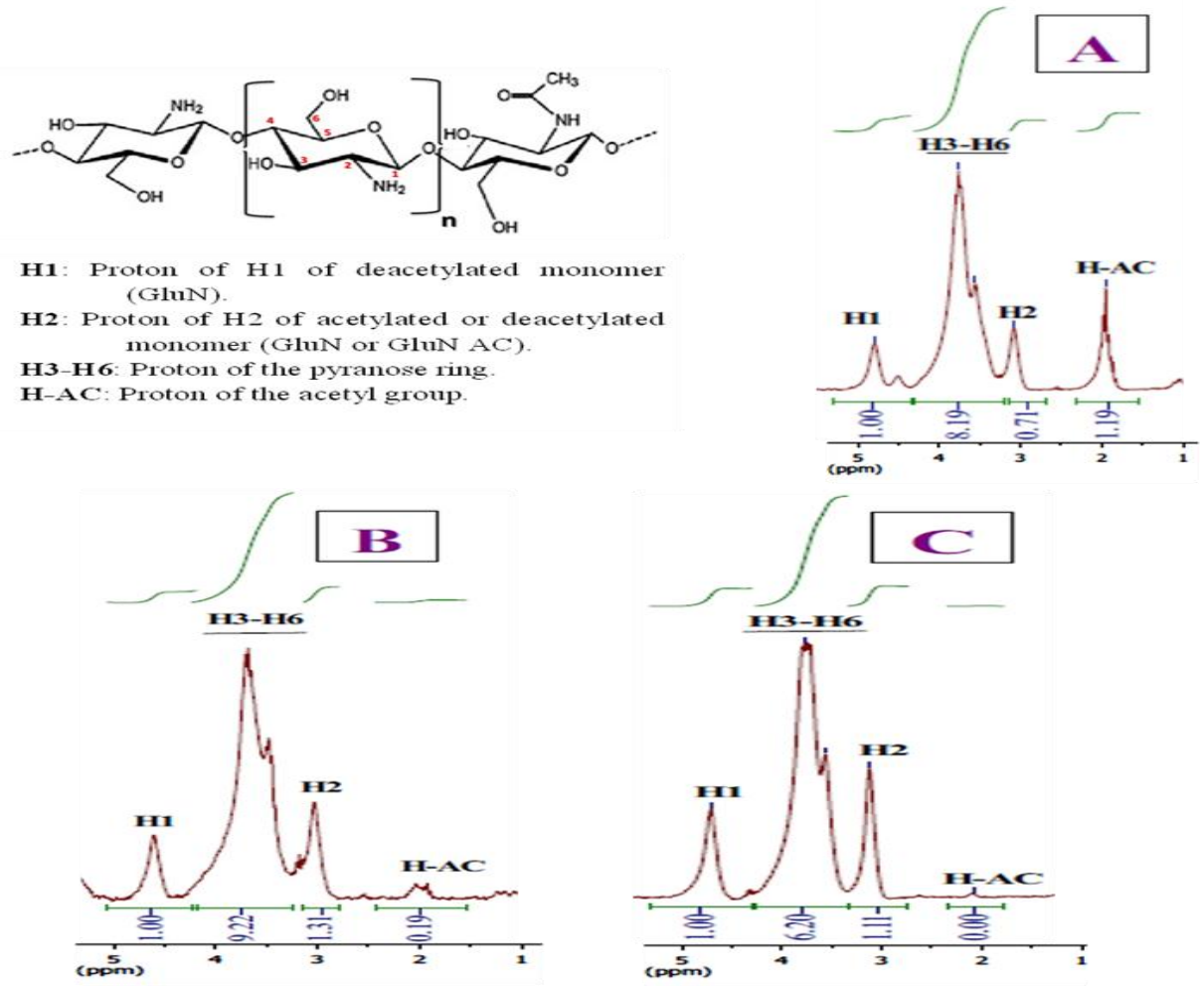


Fig. 6. XRD of chitosan: A) COC, B) SHC, and C) CRC.

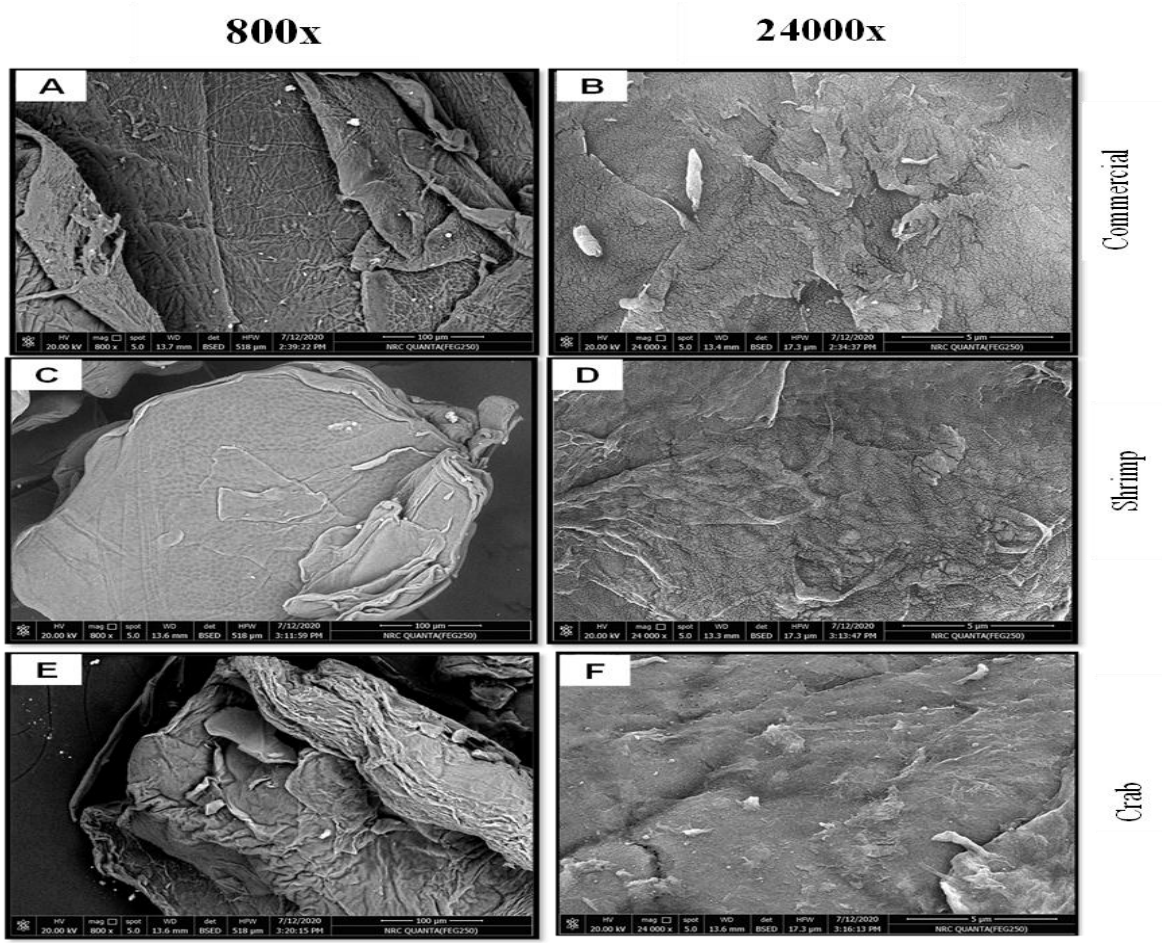


Fig.7. SEM images of COC (A: 800x, B: 24000x), SHC (C: 800x, D: 24000x), and CRC (E: 800x, F: 24000x).

Data availability

Any data associated with this work are available on request.

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