

Investigation of Biomaterial Characteristics of Chitosan Produced from Crab Shells

Esther O Babatunde ^{1,*}, Joshua O Ighalo ¹ , Sunday A Akolo ^{2,*}, Adewale George Adeniyi ^{1,*} , Lydia Adepoju ³

¹ Chemical Engineering Department, Faculty of Engineering and Technology, University of Ilorin, Ilorin, P. M. B. 1515, Nigeria

² Chemical Engineering Department, Federal University Of Technology, Minna, P. M. B.4000, Nigeria

³ Food Engineering Department, Faculty of Engineering and Technology, University of Ilorin, Ilorin, P. M. B. 1515, Nigeria

* Correspondence: adeniyi.ag@unilorin.edu.ng; babatunde.co@unilorin.edu.ng; Scopus ID: 57203847713

Abstract: Chitosan is a biomaterial that can be obtained from certain parts of aquatic fauna like scales and shells. They are cheap, readily available, and environment-friendly complexing agents for heavy metals. In this study, a crab shell was used as a source of chitosan and compared with commercial chitosan. The yield was 22.75% and 71% degree of de-acetylation. The solubility test showed that it would dissolve within 30 minutes in 0.1 M HCl. The FTIR indicated the presence of –OH functional group at wavelength 1350 cm⁻¹ and R-NH₂ at 3450 cm⁻¹. SEM revealed that the locally developed chitosan has a rough surface characterized with holes, and has a porous, spongy structure. Electron Dispersive Spectroscopy (EDS) was used to examine the presence of elements on the chitosan. Results showed the presence of C, N, O, and Na. Usually, hydrogen is usually present in organic materials, but EDS cannot detect its presence. XRD revealed a low crystallinity of the chitosan obtained.

Keywords: Crab shells; Chitosan; Characterisation.

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1. Introduction

Chitosan has been regarded as a source of potential bioactive material, but it also has several limitations to be utilized in a biological system, including its poor solubility under physiological conditions. Therefore, to overcome these limitations, researchers focused on the derivatization of chitosan by chemical modifications and partially hydrolyzed chitosan by enzymatic actions as it contains various reactive functional groups [1-5]. The main factors which may affect the chitosan properties are its molecular

weight and degree of de-acetylation (DD) [6]. These factors enable the researcher to formulate different grades of chitosan, which differ primarily in molecular weight and degree of de-acetylation.

Chitosan is the de-acetylated form of chitin, which is a linear polymer of acetylamino-d-glucose. Chitosan (poly-1,4-D-glucosamine), which is another polysaccharide biopolymer derived from chitin, also has a high affinity for transition metal ions in a way that the amine groups on chitosan serve as a chelation site for the metal ions [7, 8].



Chitosan is biodegradable, non-toxic, and easily of derivatized, and has many amino and hydroxyl groups that can chelate heavy metal ions[4, 6, 9-12]. Chitosan is less expensive, easily available, and environment-friendly complexing agents for heavy metals. It has been reported as a heavy metal adsorbent in several studies [13-21].

2. Materials and Methods

2.1. Collection of Sample.

Crab shell was obtained from Lagos lagoon (Nigeria). The crab was washed with tap water to remove possible foreign materials present (dirt and sands). The commercial chitosan was from Sigma-Aldrich in Germany (purity 99%).

2.2. Production of Chitosan.

Isolation of chitosan from crab shell wastes involves four traditional steps; de-proteinization (DP), demineralization (DM), de-colorization (DC), and de-acetylation (DA). The wet crab was washed and dried, followed by grinding and sieving to a particle size of 750 μm , and then placed in a plastic bottle for storage at ambient temperature until used. -deproteinization (DP) was then carried out on the crab shells. Four hundred grams of crab shell was placed in a solution of 3.5% NaOH (w/v) for 2 h at 65°C, solid: solvent (1:10, w/v), then the solid was separated from the liquid and washed with distilled water until the absence of color in the medium which represents the absence of protein. The next step was to demineralize the shells. The deproteinized shell was placed in 1 N HCl for 30 minutes at room temperature, solid: solvent (1:15, w/v). Subsequently, the liquid was decanted, and the solid was washed with distilled water until neutral pH. The remaining was dried at 50 C for 12 hr and the product was chitin. The chitin was decolorized with 0.315% sodium hypochlorite (NaOCl) (w/v) for 5 minutes at room temperature solid: solvent (1:10, w/v) was poured into the vessel containing the solid, and the suspension was agitated until the pigmentation of the solid disappeared. The white solid (chitin) was washed and dried at 50 °C for 12 hr in the oven. The de-acetylation of chitin was carried out by mixing chitin with 50% NaOH for 30 min at 121°C, solid: solvent (1:10, w/v). The mixture was washed with distilled water several times to remove residual sodium hydroxide until pH 7 was achieved. The chitosan was dried in an oven at 50 °C for 18 hr.

Chitosan can be obtained from wastes of marine fauna such as scales [4, 10, 11, 14, 22-24] and shells [25, 26]. In this paper, we studied the preparation of chitosan from crab shells and compared the characteristics with those of commercial chitosan.

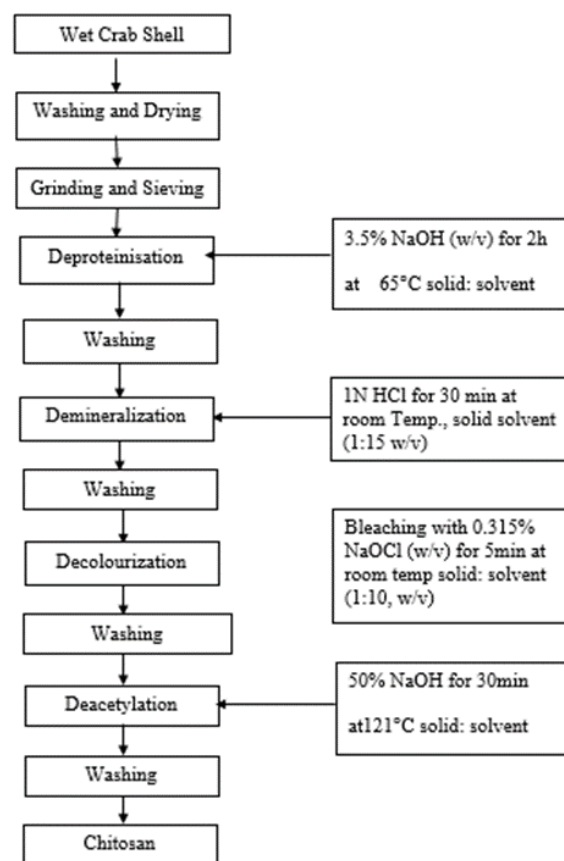


Figure 1. Summary of processes involved in the production of chitosan from crab.

2.3. Characterization of Chitosan.

The solubility test was determined by placing 0.2 g of chitosan in 0.1 M of Hydrochloric acid and stirred till the complete dissolution of chitosan. The chitosan was analyzed by Fourier Transform Infrared Spectroscopy (FTIR) in the wavelength between 4000 cm^{-1} and 400 cm^{-1} and in solid-state using the KBr pellet method. The FTIR spectra were normalized, and major vibration bands were identified associated with the main chemical groups. Room temperature low angle X-ray diffraction (XRD) patterns of the chitosan were studied using a X-ray powder diffractometer using a Ni – filtered Cu K α X-ray radiation source. The relative intensities

were recorded within the range of $10^\circ - 90^\circ$ (2θ) at a scanning rate of 5°min^{-1} . The surface morphology of the chitosan was observed with scanning electron microscopy and fiber metric image and pore histograms. Other characterization tests were Raman

spectroscopy (RS), Scanning Electron Microscopy (SEM), and Electron Dispersive Spectroscopy (EDS).

3. Results and Discussion

3.1. Yields of Locally Produced Chitosan.

During the demineralization process, excessive undesirable foams are produced due to the CO_2 generation ($\text{CaCO}_3 + 2\text{HCl} \rightarrow \text{CaCl}_2 + \text{CO}_2 + \text{H}_2\text{O}$), which was also reported by No and Hur [27]. The demineralized and deproteinized chitin has a light pink color due to the presence of astaxanthin pigment; this pigment was eliminated during the decolorization step to yield cream white chitin powder which was also obtained by No and Meyers [11]. The yield was calculated as the dry weight of chitosan obtained from 400 g of crab shell. Chitosan yield was 22.75%, which is comparatively higher than those reported in the literature. Fernandez-Kim [23] reported 16.7-18.8 % yield of chitosan from crawfish, and No and Meyers [11] reported approximately 23% of chitin from crab shell. Brzeski [26] reported about 14 % yield of chitosan from krill and Alimuniar, and Zainuddin [3] was 18.6% from prawn waste.

3.2 Characterization of Chitosan.

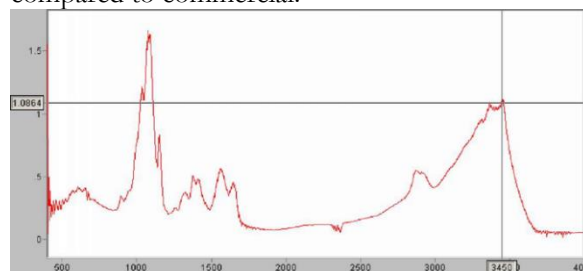
3.2.1. Solubility Test.

It is commonly justified that the main physical differences between chitin and chitosan are the ability of chitosan to be soluble in organic acid such as acetic acid or dilute hydrochloric acid. Chitosan with a higher content of protonated amino groups readily forms well-ordered arrangement in Van der Waals force and hydrogen bond, which exceed its tendency for intramolecular chemical bonds [21, 28]. The developed chitosan and the commercial chitosan both dissolved in 0.1 M hydrochloric acid within 30 minutes, demonstrating excellent solubility.

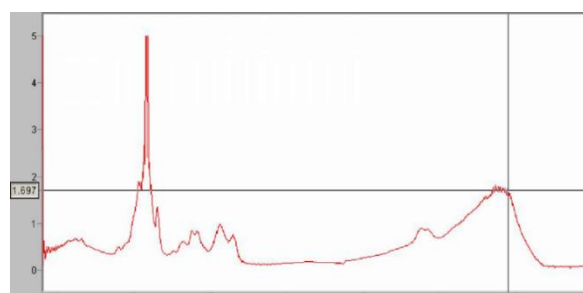
3.2.2. Fourier Transform Infrared Spectrometer (FTIR).

Fourier Transform Infrared Spectrometer (FTIR) was used to probe the surface characteristics of the chitosan. The peak appearing was assigned to a various functional group according to their respective wave number as reported in literature hydroxyl group (OH) peaks appears at a wavelength of 1350 cm^{-1} the Amines group (R-NH_2) peaks appears at $3400-3500\text{ cm}^{-1}$. The FTIR spectrum of Figure 3 and 2 for commercial and locally developed chitosan respectively were relating with FTIR

absorption bands. A wide absorption band at 3438 cm^{-1} for figure 3 and 2 respectively indicates the presence of $-\text{OH}$ stretching while peaks at absorption band featuring bending vibration of N-H from R-NH_2 was observed at 3450 cm^{-1} while C-H was displayed with stretching vibration of 2916.1 cm^{-1} , 2858.3 cm^{-1} and bending vibrations of 1415.7 cm^{-1} , 1375.2 cm^{-1} . It was observed that the peaks were at the same frequency for both commercial and local developed chitosan, but the locally developed chitosan has high absorbance as compared to commercial.



Absorbance /wavenumber (cm^{-1})
Figure 2. FTIR of locally developed chitosan.



Absorbance/Wavenumber (cm^{-1})
Figure 3. FTIR of commercial chitosan.

3.2.3. Raman Spectroscopy (RS).

Raman spectra were obtained for half of the films from deposition A before and after neutralization for determining chitosan's functionality. Raman spectra were analyzed for fingerprint and group frequency peaks. Group frequency peaks tend to occur above 1500 cm^{-1} , while fingerprint modes are unique to the specific molecule and are usually found below 1500 cm^{-1} [29]. Before starting the analysis, the chemical structures of chitosan were examined for the groups



they contained in order to know what to expect. The functional groups identified are presented in tables 1 and 2 for the commercial and locally developed chitosan, respectively. Examining the molecules, chitosan contains 5 methines (C-H) groups per repeat unit and 1 methylene (CH₂) group.

According to Wojtkowiak and Chabanel [29] methyl CH₃ bending can be found at $1460 \pm 10 \text{ cm}^{-1}$ and CH₃ deformation at $1375 \pm 10 \text{ cm}^{-1}$. These peaks are presented in Figures 4 and 5 for both commercial and locally developed chitosan respectively.

Table 1. Wave numbers of the bands observed in the Raman spectra for commercial chitosan and their assignment to the respective normal vibrations.

RS	Assignments
3362w	$\nu(\text{OH})\text{HB}$
3308w	
2932vs	$\nu(\text{CH}_3)$
2885vs	$\nu(\text{CH}_2)$
2818shm	$\nu(\text{CH}_3)$
2743w	$\nu(\text{CH})$
1654w	$\nu(\text{CO})$
1591m	
1458m	$\delta(\text{CH}) + \omega(\text{CH}_2) + \delta(\text{OH})$
1411m	$\delta(\text{CH}_3) + \delta(\text{CH})$
1325	$\nu(\text{CN}) + \delta(\text{CH})$
1263	$\nu(\text{C-O}) + \delta(\text{CH}) + \rho(\text{CH}_2)$

Φ , pyranoid ring; ν , stretching; δ , in-plane bending vibrations; γ , ω , out-of plane bending; HB, hydrogen bond

Table 2. Wave numbers of the bands observed in the Raman spectra for locally developed chitosan and their assignment to the respective normal vibrations.

RS	Assignments
1146	$\nu(\text{C-O-C}) + \nu(\Phi)$
1114	$\nu(\text{C-OH}) + \nu(\text{C-CH}_2)$
1093	$\rho(\text{CH}) + \rho(\text{CH}_2) + \rho(\text{CH}_3)$
1044shm	$\rho(\text{CH}_3) + \delta(\text{CH}) + \delta(\text{OH})$
991shm	$\nu(\Phi) + \delta(\text{CH})$
936m	$\nu(\text{CN})$
896m	$\nu(\Phi) + \rho(\text{CH}_2)$
703w	$\omega(\text{NH}_2) + \delta(\Phi)$
566shm	$\gamma(\text{NH}) + \gamma(\text{C=O}) + \omega(\text{CH}_3)$
493m	$\gamma(\text{CO-NH}) + \delta(\text{C-CH}_3)$
479m	$\gamma(\text{COC})$
444m	$\gamma(\text{OH} + \gamma(\Phi))$
424m	$\gamma(\text{OH} + \gamma(\Phi))$
285m	$\delta(\text{C-NH-C}) + \gamma(\text{OH})$

Φ , pyranoid ring; ν , stretching; δ , in-plane bending vibrations; γ , ω , out-of-plane bending; HB, hydrogen bond

Raman spectroscopy is very helpful for distinguishing amines from alcohols because the N-H stretch is distinctly stronger than the O-H stretch [29]. Also, hydrogen bonding has less of an effect on amines than alcohol, which changes the spectra completely. There is one primary amine in the chitosan repeat unit the NH bend occurs at 1500 cm^{-1} . The bands from the range $500\text{--}1500 \text{ cm}^{-1}$ can be assigned to the vibrations: $\delta_s(\text{CH}_3, \text{CH}_2)$ at 1429 cm^{-1} , $\delta_s(\text{CH}_3, \text{CH}_2)$ at $1360\text{--}1372 \text{ cm}^{-1}$, $\delta(\text{CH})$ at 1319 and 1336 cm^{-1} , $\nu(\text{C-C})$ and $\nu(\text{C-O})$ in the range $1200\text{--}1300 \text{ cm}^{-1}$, $\delta(\Phi\text{-OH})$ at 1163 cm^{-1} , $\nu_s(\text{C-O-C})$ in the range $1000\text{--}1160 \text{ cm}^{-1}$, $\gamma(\text{CH})$ in the range

$850\text{--}1000 \text{ cm}^{-1}$, and $\delta(\Phi)$ in the range $500\text{--}720 \text{ cm}^{-1}$.

3.2.4. Scanning Electron Microscopy (SEM).

The morphology of commercial and locally developed chitosan from Figure 7 and 6 was obtained using the scanning electron microscope at four different magnifications. The locally developed chitosan has a rough surface characterized with holes and has a porous, spongy structure while commercial chitosan, presented in Figure 8, has the similar characteristic as the locally developed chitosan but with fewer holes and pores. This is further buttressed in Figure 9.

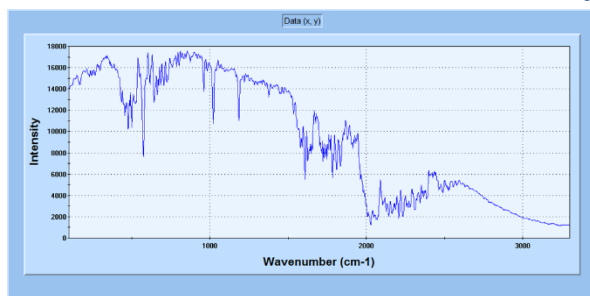


Figure 4. Raman spectroscopy of commercial chitosan.

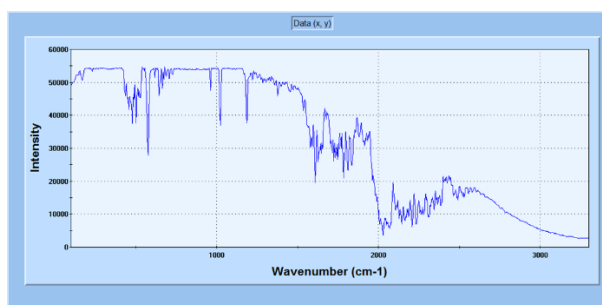


Figure 5. Raman spectroscopy of local developed chitosan.

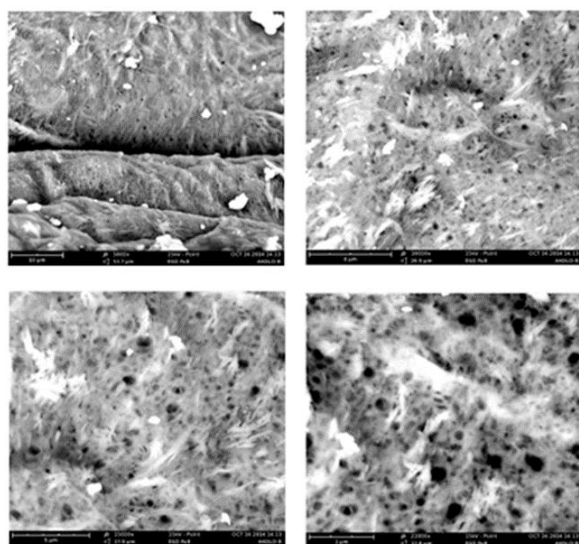


Figure 6. SEM image of locally Developed Chitosan.

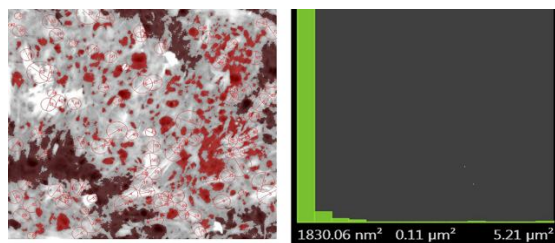


Figure 7(a-b). Fibermetric Image and Pore Histogram of local developed chitosan.

3.2.5. Electron Dispersive Spectroscopy (EDS).

Electron Dispersive Spectroscopy (EDS) is a test to examine the presence of elements through the amplitude of wavelength for the x-ray emitted after the electron was hit by the electron beam. For

the emission of x-ray, the atoms must contain a minimum of K-shell and L-shell where the electron is allowed to dislodge from shell to shell.

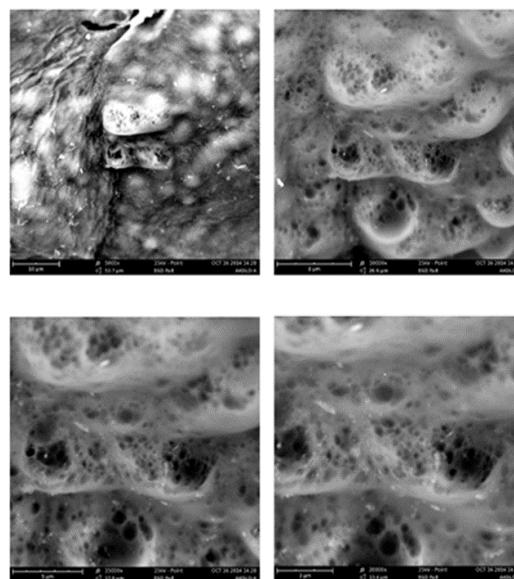


Figure 8. SEM image of commercial chitosan.

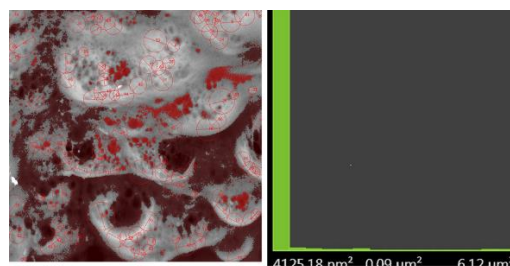
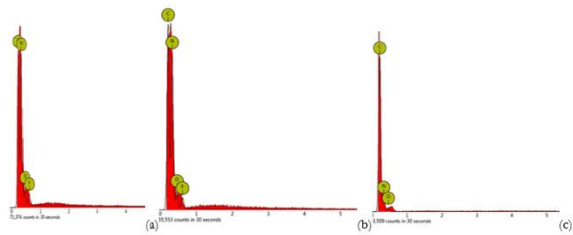


Figure 9(a-b). Fibermetric Image and Pore Histogram of commercial chitosan.

Therefore, hydrogen being the only element in the periodic table with only K shell is not detectable with EDS[30, 31]. Figure 10 is the EDS image of commercial chitosan, the spots represented with + in the Figure shows the points whose elemental composition are presented, Figure 11a represent spot 2 and Table 3 presents the weight composition of the elements. Figures 11b and 11c depict the spectrum of spot 3 and spot 6 respectively, and Table 4 and 5 presents the elemental composition of spot 3 and spot 6 for commercial chitosan. Figure 12 is the EDS image of locally developed chitosan and Figures 13a and 13b represents spot 2 and spot 6, respectively, and tables 6 and 7 presents the elemental compositions for spots 2 and 6. Table 3 and 4 contains Fluorine which is not part of elemental weight composition of chitosan, and also Table 6 and 7 contains Sodium which might be present as a result of inadequate washing during the synthesis stage.



Figure 10. EDS image for the commercial chitosan.



Figures 11a (spot 2), 11b (spot 3) and 11c (spot 6): EDS spectrum for commercial chitosan.

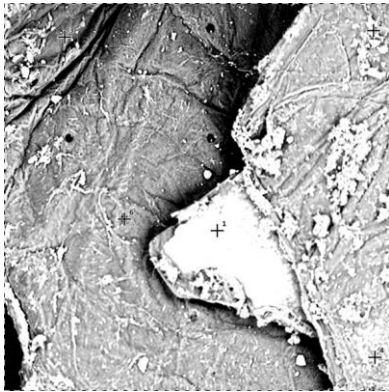


Figure 12. EDS image for locally developed chitosan.

3.2.6. X-Ray Diffraction (XRD).

The XRD spectrum of chitosan has low crystallinity. X-ray diffraction pattern for pure chitosan has peaks at $2\theta=10^\circ$, and 20.09° for pure chitosan confirms the semi-crystalline nature[32].

The XRD pattern of commercial and locally developed chitosan is shown in Figures 14 and 15, respectively. Both showed broad diffraction at $2\theta = 20^\circ$, and that symbolizes semi-crystalline chitosan. This was supported by by Yen, Yang, and Mau [25] as the two characteristic crystalline peaks of chitosan at $9-10^\circ$ and $19-20^\circ$ with comparable crystallinity.

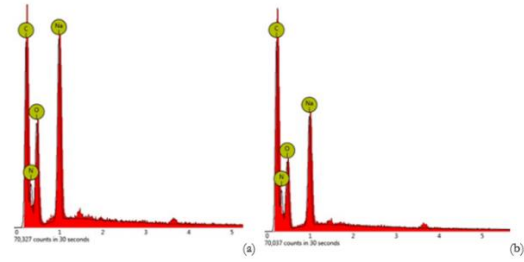


Figure 13a (spot 2) and 13b (spot 6): EDS spectrum for locally developed chitosan.

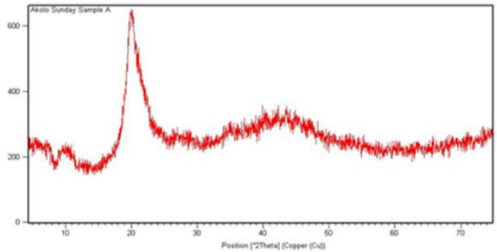


Figure 14. XRD for commercial chitosan.

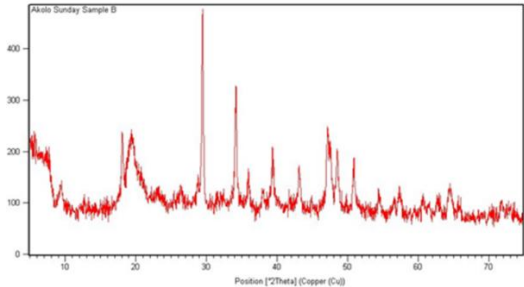


Figure 15. XRD for locally developed chitosan.

Table 3. Elemental weight composition of spot 2 for commercial chitosan.

Atomic number	Element symbol	Element name	Confidence level	Concentration percentage	Certainty percentage	Error percentage
7	N	Nitrogen	100	47.8	99.2	0.8
8	O	Oxygen	100	27.6	97.4	2.6
9	F	Fluorine	100	14.7	96.7	3.3
6	C	Carbon	100	9.9	99.2	0.8

Table 4. Elemental weight composition of spot 3 for commercial chitosan.

Atomic number	Element symbol	Element name	Confidence level	Concentration percentage	Certainty percentage	Error percentage
7	N	Nitrogen	100	49.6	99.1	0.1
8	O	Oxygen	100	26.6	97.3	2.7
9	F	Fluorine	100	12.8	96.4	3.6
6	C	Carbon	100	11	99.2	0.8

**Table 5.** Elemental weight composition of spot 6 of commercial chitosan.

Atomic number	Element symbol	Element name	Confidence level	Concentration percentage	Certainty percentage	Error percentage
7	N	Nitrogen	100	40.3	97.5	2.5
6	C	Carbon	100	31.6	93.4	0.7
8	O	Oxygen	100	28.1	95.3	4.6

Table 6. Elemental weight composition of spot 2 for locally developed chitosan.

Atomic number	Element symbol	Element name	Confidence level	Concentration percentage	Certainty percentage	Error percentage
8	O	Oxygen	100	39.7	98.3	1.5
11	Na	Sodium	100	38	99	1
6	C	Carbon	100	12	93.4	1.1
7	N	Nitrogen	100	10.3	97.5	2.4

Table 7. Elemental weight composition of spot 6 for locally developed chitosan.

Atomic number	Element symbol	Element name	Confidence level	Concentration percentage	Certainty percentage	Error percentage
8	O	Oxygen	100	39.6	98.3	1.6
11	Na	Sodium	100	38	99	1.2
6	C	Carbon	100	14.9	93.4	0.9
7	N	Nitrogen	100	13.5	97.5	2.2

4. Conclusions

Chitosan has been successfully prepared from a crab shell with a yield of 22.75% and 71% degree of de-acetylation. The commercial and locally developed chitosan was characterized by a solubility test, which dissolves within 30 minutes in 0.1 M HCl. The FTIR indicates the presence of –OH functional group at wavelength 1350 cm⁻¹ and R-NH₂ at 3450 cm⁻¹. SEM revealed that the locally developed chitosan has a rough surface

characterized with holes, and has a porous, spongy structure. Electron Dispersive Spectroscopy (EDS) was used to examine the presence of elements on the chitosan. Results showed the presence of C, N, O and Na. Usually, hydrogen is usually present in organic materials, but EDS cannot detect its' presence. XRD revealed a low crystallinity of the chitosan obtained.

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Conflicts of Interest

The authors declare no conflict of interest.

References

- Chen, Y.L. Preparation and Characterization of Water Soluble Chitosan Gel for Skin Hydration. *University Sains Malaysia* **2008**.
- Mourya, V.K.; Inamdar, N.N. Chitosan-modifications and applications: Opportunities galore. *Reactive and Functional Polymers* **2008**, *68*, 1013-1051, <https://doi.org/10.1016/j.reactfunctpolym.2008.03.002>.
- Alimuniar, M.; Zainuddin, C. *An economical technique for producing chitosan*. Elsevier Applied Science 1992.
- Johnson, E.L.; Peniston, Q.P. *Utilization of shellfish waste for chitin and chitosan production*. AVI Publishing 1982.
- Moorjani, M.N.; Khasim, D.I.; Rajalakshmi, S.; Puttarajappa, P.; Amla, B.L. Chitosan of high viscosity and protein as a valuable by-product from squilla. In: *Book Chitosan of high viscosity and protein as a valuable by-product from squilla*. MIT Sea Grant Program, 1978; pp. 210-216.
- Dutta, P.; Duta, J.; Tripathi, V.S. Chitin and Chitosan: Chemistry, properties and applications. *Journal of Scientific and Industrial Research* **2004**, *63*, 20-31.
- Guibal, E. Interactions of metal ions with chitosan-based sorbents: a review. *Separation and Purification Technology* **2004**, *38*, 43-74, <https://doi.org/10.1016/j.seppur.2003.10.004>.
- Varma, A.J.; Deshpande, S.V.; Kennedy, J.F. Metal complexation by chitosan and its derivatives: a review. *Carbohydrate Polymers* **2004**, *55*, 77-93, <https://doi.org/10.1016/j.carbpol.2003.08.005>.



9. Merrifield, J.D. *Synthesis and characterization of thiol grafted chitosan beads for mercury removal*. University of Maine 2002
10. Rout, S.K. Physicochemical, Functional, and Spectroscopic analysis of crawfish chitin and chitosan as affected by process modification. Dissertation, 2001
11. No, H.K.; Meyers, S.P. Crawfish chitosan as a coagulant in recovery of organic compounds from seafood processing streams. *J Agric Food Chem* **1989**, *37*, 580-583, <https://doi.org/10.1021/jf00087a002>.
12. Struszczyk, H. Microcrystalline chitosan. I. Preparation and properties of microcrystalline chitosan. *Journal of Applied Polymer Science* **1987**, *33*, 177-189, <https://doi.org/10.1002/app.1987.070330115>.
13. Mota, J.A.; Chagas, R.A.; Vieira, E.F.S.; Cestari, A.R. Synthesis and characterization of a novel fish scale-immobilized chitosan adsorbent—Preliminary features of dichlorophenol sorption by solution calorimetry. *Journal of Hazardous Materials* **2012**, *229-230*, 346-353, <https://doi.org/10.1016/j.jhazmat.2012.06.010>.
14. Iqbal, J.; Wattoo, F.H.; Wattoo, M.H.S.; Malik, R.; Tirmizi, S.A.; Imran, M.; Ghangro, A.B. Adsorption of acid yellow dye on flakes of chitosan prepared from fishery wastes. *Arabian Journal of Chemistry* **2011**, *4*, 389-395, <https://doi.org/10.1016/j.arabjc.2010.07.007>.
15. Liu, X.; Cheng, Z.; Ma, W. Removal of copper by modified chitosan adsorptive membrane. *Frontiers of Chemical Engineering in China* **2009**, *3*, 102-106, <https://doi.org/10.1007/s11705-009-0123-7>.
16. Rojas, G.; Silva, J.; Flores, J.A.; Rodriguez, A.; Ly, M.; Maldonado, H. Adsorption of chromium onto cross-linked chitosan. *Separation and Purification Technology* **2005**, *44*, 31-36, <https://doi.org/10.1016/j.seppur.2004.11.013>.
17. Chassary, P.; Vincent, T.; Guibal, E. Metal anion sorption on chitosan and derivative materials: a strategy for polymer modification and optimum use. *Reactive and Functional Polymers* **2004**, *60*, 137-149, <https://doi.org/10.1016/j.reactfunctpolym.2004.02.018>.
18. Qi, L.; Xu, Z. Lead sorption from aqueous solutions on chitosan nanoparticles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2004**, *251*, 183-190, <https://doi.org/10.1016/j.colsurfa.2004.10.010>.
19. Ng, J.C.Y.; Cheung, W.H.; McKay, G. Equilibrium studies for the sorption of lead from effluents using chitosan. *Chemosphere* **2003**, *52*, 1021-1030, [https://doi.org/10.1016/S0045-6535\(03\)00223-6](https://doi.org/10.1016/S0045-6535(03)00223-6).
20. Dambies, L.; Vincent, T.; Guibal, E. Treatment of arsenic-containing solutions using chitosan derivatives: uptake mechanism and sorption performances. *Water Research* **2002**, *36*, 3699-3710, [https://doi.org/10.1016/S0043-1354\(02\)00108-2](https://doi.org/10.1016/S0043-1354(02)00108-2).
21. Tianwei, T.; Xiaojing, H.; Weixia, D. Adsorption behaviour of metal ions on imprinted chitosan resin. *Journal of Chemical Technology & Biotechnology* **2001**, *76*, 191-195, <https://doi.org/10.1002/jctb.358>.
22. Pandharipande, S.; Jana, R.; Ramteke, A. Synthesis and Characterization of Chitosan from Fish Scales. *International Journal of Science, Engineering and Technology Research* **2018**, *7*, 287-291.
23. Fernandez-Kim, S.O. *Physicochemical and functional properties of crawfish chitosan as affected by different Processing protocols*. Louisiana State University 2004.
24. Eletta, A.A.O.; Ighalo, J.O. A Review of fish scales as a Source of Biosorbent for the Removal of Pollutants from Industrial Effluents. *Journal of Research Information in Civil Engineering* **2019**, *16*, 2479-2510
25. Yen, M.T.; Yang, J.H.; Mau, J.L. Physicochemical characterization of chitin and chitosan from crab shells. *Carbohydrate Polymers* **2009**, *75*, 15-21, <https://doi.org/10.1016/j.carbpol.2008.06.006>.
26. Brzeski, M.M. Concept of chitin/chitosan isolation from Antarctic Krill (*Euphausiasuperba*) shells on a technique scale. In: *Book Concept of chitin/chitosan isolation from Antarctic Krill (Euphausiasuperba) shells on a technique scale*. The Japan Society of Chitin and Chitosan, 1982.
27. No, H.K.; Hur, E.Y. Control of Foam Formation by Antifoam during Demineralization of Crustacean Shell in Preparation of Chitin. *J Agric Food Chem* **1998**, *46*, 3844-3846, <https://doi.org/10.1021/jf9802676>.
28. Zhang, H.; Jin, Y.; Deng, Y.; Wang, D.; Zhao, Y. Production of chitin from shrimp shell powders using *Serratia marcescens* B742 and *Lactobacillus plantarum* ATCC 8014 successive two-step fermentation. *Carbohydrate Research* **2012**, *362*, 13-20, <https://doi.org/10.1016/j.carres.2012.09.011>.
29. Wojtkowiak, B.; Chabanel, M. *Spectrochimie Moléculaire*. PWN 1984.
30. Swapp, S. *Scanning Electron Microscopy (SEM)*. 2012.
31. Goldstein, J. *Scanning electron microscopy and x-ray microanalysis*. Kluwer Academic: Plenum Publishers, 2003.
32. Monteiro, O.A.C.; Airoidi, C. Some studies of crosslinking chitosan–glutaraldehyde interaction in a homogeneous system. *International Journal of Biological Macromolecules* **1999**, *26*, 119-128, [https://doi.org/10.1016/S0141-8130\(99\)00068-9](https://doi.org/10.1016/S0141-8130(99)00068-9).