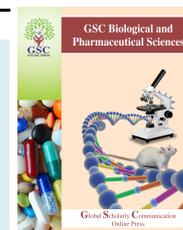


Available online at [GSC Online Press Directory](https://www.gsonlinepress.com/)

GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: <https://www.gsonlinepress.com/journals/gscbps>

(RESEARCH ARTICLE)



Deracination of chitosan from locally sourced Millipede (*Eurymerodesmus* spp.) and its spectroscopic and physio-chemical properties

Ofoegbu Obinna^{1,*}, Umah Nnamdi J.¹ and Aliyu Samuel Jacob²¹ Polymer, Nano materials and Molecular Recognition Research Group, Department of Chemistry, College of Science, Federal University of Agriculture Makurdi, Benue State Nigeria.² Department of Mechanical Engineering, Landmark University, Omu Aran, Nigeria.

Publication history: Received on 05 December 2018; revised on 16 May 2019; accepted on 20 May 2019

Article DOI: <https://doi.org/10.30574/gscbps.2019.7.2.0157>

Abstract

Presently, Chitosan is obtained from chitin extracted from the exoskeleton of Arthropods, fungi, Crustaceans and this limits the availability of this biopolymer despite its very increasing demand due to application versatility. In an effort to expand the sources of obtaining this biopolymer, this work presents the extraction and characterization from a species of millipede *Eurymerodesmus* spp., it was obtained by the conventional demineralization, deproteinization and deacetylation using 5%, 4%, and 3% HCl acid for demineralization. Characterization was done using FTIR spectroscopy, SEM and TEM microscopy. Proximate analysis carried out presents the percentage ash as 3.05 (3% HCl), 2.98 (4% HCl) and 2.63 (5% HCl). Degree of deacetylation obtained are 51.20% for 3% HCl and 59.30% for 4% HCl and 66.30% for 5% HCl. SEM results presented the samples as platelets with spherical configuration and the TEM results confirmed this. The products are in Nano sizes of 100 nm and 200 nm. Chitosan has found applications in areas as cosmetics, agriculture, pharmaceuticals and bioengineering as a result of its biodegradability, non-toxicity, biocompatibility and its eco-friendly nature. Chitosan's potential to form dimers, chelate metal ions, form films, cross link with poly anions and transition metal ions and high mechanical stability, makes it a choice material in Nano fabrications, molecular hierarchical architectures as well as targeted delivery systems. Millipede (*Eurymerodesmus* spp.) can be considered as an alternative source of chitosan to shrimps, prawns, crabs, fungi, and mushroom and obtainable from places that cannot easily access these aquatic raw material resources for chitosan.

Keywords: Chitosan; Demineralization; Deproteinization; Deacetylation; Degree of deacetylation; Millipede (*Eurymerodesmus* Spp.)

1. Introduction

Chitosan is a natural homo polymer/polysaccharide extracted from a wide range of natural sources such as crustaceans, fungi, insects, mush rooms etc. It is gotten from chitin which is a (1, 4) - linked 2- acetamido-2-deoxy-6-D-glucopyranose molecule while Chitosan is a (1 - 4) - linked 2- amino -2 - deoxy-6-D-glucopyranose molecule [1].

Chitin is the second most abundant natural biopolymer on earth after cellulose [2], it is gotten from the exoskeleton of crustaceans, insects as well as fungi and other biological sources by processes of demineralization and deproteinization, its subsequent deacetylation results to the formation of chitosan.

Chitosan is a non-toxic, biodegradable polymer of high molecular weight similar to cellulose, it possesses positive ionic charges which gives it the ability to form bonds with negatively charged moieties such as fats, lipids, cholesterol, proteins [2].

* Corresponding author

E-mail address: oeofoegbu@gmail.com

Chitosan has many applications in areas such as in sciences, engineering, and agriculture and in medicine. This is due to its biocompatibility, biodegradability and other unique properties such as film forming ability, chelation and absorption properties and antimicrobial properties.

It is insoluble in water, organic solvents and aqueous bases but is soluble after stirring in acids such as acetic acid, hydrochloric acids amongst others [3].

Chitin and chitosan has been extracted and isolated from different sources which differ by some percentages and is affected by the source and the place where it is obtained [4].

This work is aimed at extracting chitin and converting it to chitosan from the outer covering of millipede. Many other works have been done on the subject matter from crustacean wastes [5], sea prawn [6], shrimp shell [1, 2, 3, 4, 7], fungi [8], fish scales.

Extraction from all these sources are affected by geographical location but millipede (*Eurymerodesmus*) can be found in moist areas, forests, under decaying logs, in barks of trees, close to streams and creeks, in flower beds, gardens and mulch [9]. This makes millipede (*Eurymerodesmus*) easy to find in almost every geographical location and as such can be harnessed into something useful such as chitosan.

Chitosan is extracted from millipede (*Eurymerodesmus*) via processes of demineralization, deproteinization and deacetylation of millipede (*Eurymerodesmus*) covering as well as to determine the optimal condition to obtain high quality chitosan from this source and to investigate the characteristic properties of the chitosan prepared.

2. Material and methods

Using methods from [2, 3, 6, 7], with slight modifications.

Millipede (*Eurymerodesmus_spp.*) samples were obtained locally dried and grinded for use.



Figure 1 Dried millipede sample



Figure 2 Pulverized millipede sample

2.1. Reagents

Hydrochloric acid (HCl) procured from Gunsgdong Guandgwa co. ltd, Shatou Guondghuo China, Sodium Hydroxide pellets (NaOH) produced from central drug house limited, India, Reference sample Chitosan procured from Sigma Aldrich, USA. Methyl orange indicator prepared fresh from analar grade of the powder which was procured from Nice Chemicals Pvt. Limited, Kerala India and the experimental specimen: Millipede (*Eurymerodesmus spp*).

2.2. Methodology for production of chitosan

Production of chitosan involved three processes: demineralization, deproteinization and deacetylation. The dried millipede samples were divided into three portions in poured in sample containers, the samples were weighing 20 g each.

2.2.1. Demineralization

This was done using hydrochloric acid (HCl) of 3%, 4%, 5% concentrations, one portion in 3%, another in 4% and the other in 5% HCl solution in the ratio 1:6 (w/v). It was now allowed to stand for 24 hours before been washed with distilled water and dried in an oven at 60 °C until constant weight was obtained. There was a color change from dark brown to a light brown.

Yield from demineralized samples

- 3% HCl = 12.1 g
- 4% HCl = 11.5 g
- 5% HCl = 12.2 g

2.2.2. Deproteinization

Deproteinization was done using 4% NaOH solution, the squashy samples from demineralization process was soaked in 4% NaOH solution and was heated at 85 C for 20 hours. The residue was washed to a neutral point with distilled water and dried in an oven at 60 °C to a constant weight. The product chitin was obtained.

Yield of deproteinized samples

- 3% HCl = 4.4 g
- 4% HCl = 4.4 g
- 5% HCl = 4.3 g

2.2.3. Deacetylation

In preparing chitosan, the dried chitin samples were soaked in 70% NaOH solution and heated for 12 hours at 70 °C before it was removed, washed and dried under the sun and then was soaked in 70% fresh NaOH solution for 5 days after which it was washed with distilled water, dried in an oven at 60 °C until constant weight was obtained. It was now ready for analysis.

Yield of deacetylated samples

- 3% HCl = 3.6 g
- 4% HCl = 3.6 g
- 5% HCl = 3.5 g

Functional groups characterization was done using Fourier Transformed Infra-Red spectroscopy using FTIR-8400S spectrophotometer (Shimadzu) machine.

2.3. Characterization of samples and physiochemical analysis

2.3.1. Determination of degree of deacetylation

This was calculated using the formula:

$$DD (\%) = 100 - DA$$

Where DA = Degree of acetylation given by the formula

$$DA (\%) = \frac{C:N - 5.14}{1.72} \times 100$$

Where C: N is the percentage Carbon to Nitrogen ratio [10].

Degree of deacetylation (DD) defines the molar ratio of the glucosamine repeating unit to the polysaccharide chain [11], which indicates the amount of the acetyl groups of the Chitin material that has been replaced as found in the resulting chitosan product.

2.3.2. Moisture content determination

This is the quantity of water contained in a material or a given sample of a substance. Determining the moisture content of the produced chitosan was done using gravimetric method adopted from [6], the initial weight of the sample was taken then the samples was dried in an oven at 60 °C and measured until a constant weight was obtained. The moisture content was calculated as follows

$$\text{Moisture content} = \frac{W_0 - W_1}{W_0} \times 100$$

Where, W_0 = weight of wet sample

W_1 = weight of dry sample

2.3.3. Ash value

Ash value was determined using methods from [1], the prepared chitosan sample was placed in a previously ignited, cooled and tarred crucible. The sample was now heated in a muffled furnace at 650 °C for 4 h. The crucibles are now allowed to cool to less 200 °C.

The ash value is now calculated thus

$$\% \text{ Ash} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

Ash is the inorganic residue remaining after water and organic matter has been removed from a sample. A low ash value indicates the effectiveness of the demineralization step [12].

2.3.4. Loss on drying

Loss on drying is a way to determine the amount of volatile matter in a sample not necessarily water. This was done using gravimetric method [1], the weight of the samples was taken before and after drying and calculated as thus

$$\% \text{ loss on drying} = \frac{\text{Wet weight} - \text{Dry weight}}{\text{Wet weight}} \times 100$$

2.3.5. pH measurement

The pH of the samples was measured using a Hanna microprocessor pH meter.

2.3.6. Solubility

The solubility of the samples was demonstrated using various solutions like distilled water, acetone, ethanol, acetic acid and lactic acid. 0.1 g of the samples was poured and stirred in the various solutions and observed for some time. Solubility is an important factor for determining the quality of chitosan produced as a higher solubility will produce a better chitosan [2].

2.3.7. Water binding capacity (WBC)

A modified method of [2] was used. A centrifuge tube was weighed and 0.5 g of the sample added alongside 10 ml of water, the solution was mixed well using a vortex mixer for 1 minute to disperse sample. Intermittent shaking was done for 5 seconds for every 10 minute and centrifuged at 3000 rpm (revolution per minute) for 25 minutes and the supernatant decanted.

$$\% \text{ WBC} = \frac{\text{Water bound}}{\text{Initial sample weight}} \times 100$$

2.3.8. Fat binding capacity (FBC)

A modified method of [2] was used. A centrifuge tube was weighed and 0.5 g of the sample added alongside 10 ml of oil (soybean oil), the solution was mixed well using a vortex mixer for 1 minute to disperse sample. Intermittent shaking was done for 5 seconds for every 10 minute and centrifuged at 3000 rpm (revolution per minute) for 25 minutes and the supernatant decanted.

$$\% \text{ FBC} = \frac{\text{Fat bound}}{\text{Initial sample weight}} \times 100$$

2.3.9. Carbon, nitrogen and hydrogen content composition

A Flash-2000 CHNS-O elemental analyzer was used to determine the Carbon, Nitrogen and Hydrogen contents of the samples.

2.3.10. Bleaching of Sample

Chitosan was bleached using method from [13], the chitosan sample was immersed in ethanol solution for 10 minutes and was dried upon removal.

3. Results and discussion

3.1. Yield

The yield obtained at different stages are given in Table 1.

Table 1 Chitosan yield obtained at various stages of chitosan production

Stage	Yield (g)		
	3% HCl	4% HCl	5% HCl
Demineralisation	12.1	11.5g	12.2
Deproteinisation	4.4	4.4	4.4
Deacetylation	3.6	3.6	3.5

3% HCl = 18%, 4% HCl = 18%, 5% HCl = 17.5 %

From the above table, we can see that chitosan obtained (17.8% ave.), is lower compared to that obtained from bat guano - 79% [15], adult potato beetles 72% [10], shrimp 19% [3] but higher than that obtained from cockroach 0.24% [14]. The obtained yield is lower than that obtained by [16] where they got 35.7% from millipede (*Spirobolida*) specie. From [4], chitin yield of 8.9% was obtained when chitosan was extracted from Nigerian brown shrimp. This is lower compared to the yield obtained from chitosan extracted from the specie of millipede (*Eurymerodesmus*) in this work. When compared with [17], chitin produced from Nigerian sources showed that shrimp gave a yield of 8.15%, crab 7.8%, crayfish 2.88%, and periwinkle 0.44% which is way much lower compared to 22% chitosan obtained from millipede (*Eurymerodesmus*).

3.2. Degree of deacetylation

Table 2 Summary of Degree of deacetylation of the various samples

Samples	Degree of deacetylation (%)
Reference material	72.10
5% HCl Sample	66.30
4% HCl Sample	59.30
3% HCl Sample	51.20

From Table 2, 5% HCl used for demineralization gave the best degree of deacetylation of 66.30% followed by that of 4% HCl with 59.30% and the 3% HCl has a 51.20% DD. Their DD though lower than the reference material, is reasonably close to it. The DD obtained from millipede (*Eurymerodesmus*) is lower compared to that obtained from shrimp – 68% [3], beetle – 72% [10], sea prawn – 87% [6], bat guano – 79% [15] but higher than that from Nigerian shrimp – 50.4% [4]. A low DD can be attributed to the conditions used in the extraction process (Alkali concentration, heating time, pressure) and as such, increasing the alkali concentration and heating time may improve the degree of deacetylation.

3.3. Moisture content

The moisture content calculated from the sample is 9%. The moisture content obtained is higher compared to the 5% gotten from potato beetle (*Leptinotarsa decemlineata*) [10], 4% from sea prawn [6]. Isa [4] extracted chitosan from Nigerian brown shrimp and obtained a moisture content of 5.24% which was primarily due to a longer drying time. When compared, Shrimp had a percentage moisture content of 8.70, Crab 6.10 and Crayfish 6.30 lower than that obtained from millipede (*Eurymerodesmus*).

3.4. Bleaching

On bleaching, the samples color became brighter, appearing whitish compared to its color after the deacetylation process.



Figure 3 Bleached chitosan

3.5. Other proximate analysis

Table 3 Summary of other proximate analysis

Sample ID	Moisture (%)	Loss on drying (%)	Fiber (%)	Protein (%)	Lipid (%)	Ash (%)	pH	Solubility in 1% acetic acid (%)	Fat binding capacity (%)	Water binding capacity (%)
Reference material	2.6	2.0	4.5	2.51	1.93	6.31	6.6	Good	2.18	6.59
5% HCl	4.9	2.5	5.98	3.67	2.02	2.63	6.8	Good	7.01	10.01
4% HCl	5.3	2.6	5.89	3.71	2.12	2.98	6.8	Good	6.98	10.98
3% HCl	5.3	2.8	5.89	3.81	2.14	3.05	6.6	Good	6.21	11.23

Table 4 Summary of the organic content of the samples

Sample ID	% Nitrogen	% Carbon	% Carbon: Nitrogen
Reference material	2.58	14.50	5.62
5% HCl	2.21	12.64	5.72
4% HCl	2.76	16.12	5.84
3% HCl	2.67	15.97	5.98

From Table 3 and 4, the loss on drying for all the samples fell between 2% - 3% which is similar to the 2% obtained from sea prawn [6], the fiber content of the millipede sample is higher than that gotten from Nigerian shrimp [4].

The produced chitosan was soluble in 1% acetic acid attaining the same feat as that from sea prawn [6] and shrimp [2], its pH is similar to that of chitosan produced from sea prawn [6]. The ash value is between 2% - 3% lower than that gotten from Nigerian shrimp (3.83%) [4] but higher than that obtained from sea prawn (1.86%) [6]. The carbon content of the chitosan samples is lower compared to that of chitosan from Bat guano 47.52% [15], that of the 5% HCl is higher than others thus giving it a high percentage carbon: nitrogen ratio and a lower degree of deacetylation.

3.6. FTIR Analysis

The characteristic functional groups and their respective percent transmittances for the respective samples are presented in Table 5.

Table 5 characteristic functional groups of commercial/reference, crude and the 3%, 4% and 5% HCl treated samples.

Samples	Functional groups and transmittance (cm ⁻¹)			
	NH ₂	Absorbance	OH	Absorbance
Standard sample	Present	3525.99	Present	3626.29
Crude sample	Present	3433.41	Present	3610.88
3% HCl acid sample	Present	3340.82	Present	3634.01
4% HCl acid sample	Present	3525.99	Present	3634.01
5% HCl acid sample	Present	3525.99	Present	3634.01

Table 6 A comparative summary of the functional groups found in the reference chitosan, crude sample and the 3%, 4% and 5% HCl samples.

Functional groups	Transmittance (cm ⁻¹)				
	Standard	Crude	3% HCl	4% HCl	5% HCl
NH stretching	3525.99	3433.41	3340.82	3634.01	3525.99
Amide band I	1658.84	1651.12	1681.98	1681.98	1681.98
Amide band II	1504.53	1527.57	-	1489.10	1489.10
Ring stretching	887.28	871.85	854.14	871.85	871.85
CH ₂ bending	1427.37	1442.80	1404.22	1411.94	1419.66

Table 7 presents a comparative summary of FTIR spectroscopic analysis of Chitosan obtained from American cockroach [14], Bat guano [15], Sea prawn [6], Shrimp shell [3], standard chitosan from Sigma Aldrich USA, and produced chitosan of 5%, 4% and 3% HCl

Functional groups	Transmittance (%)							
	Cockroach	Bat guano	Shrimp	Prawn	Standard	5% HCl	4% HCl	3% HCl
O-H stretching	3400	3437	3454	3446.4	3525.99	3525.99	3634.01	3340.82
C-H stretch	2928	2921	2926	2927	2885.6	2893.32	2885.6	2885.6
C=O stretch	1650	1656	1627	1652.8	1658.84	1681.98	1681.98	1681.98
CH ring stretching	894.91	899	-	880.6	887.28	871.85	871.85	864.14
C-O asymmetric stretching	1095	1068	1021	1026	1026.16	1026.16	1026.16	1026.16
N-H bend, C-N stretch	-	1554	1554	1576.2	1504.53	1489.1	1489.1	1404.22

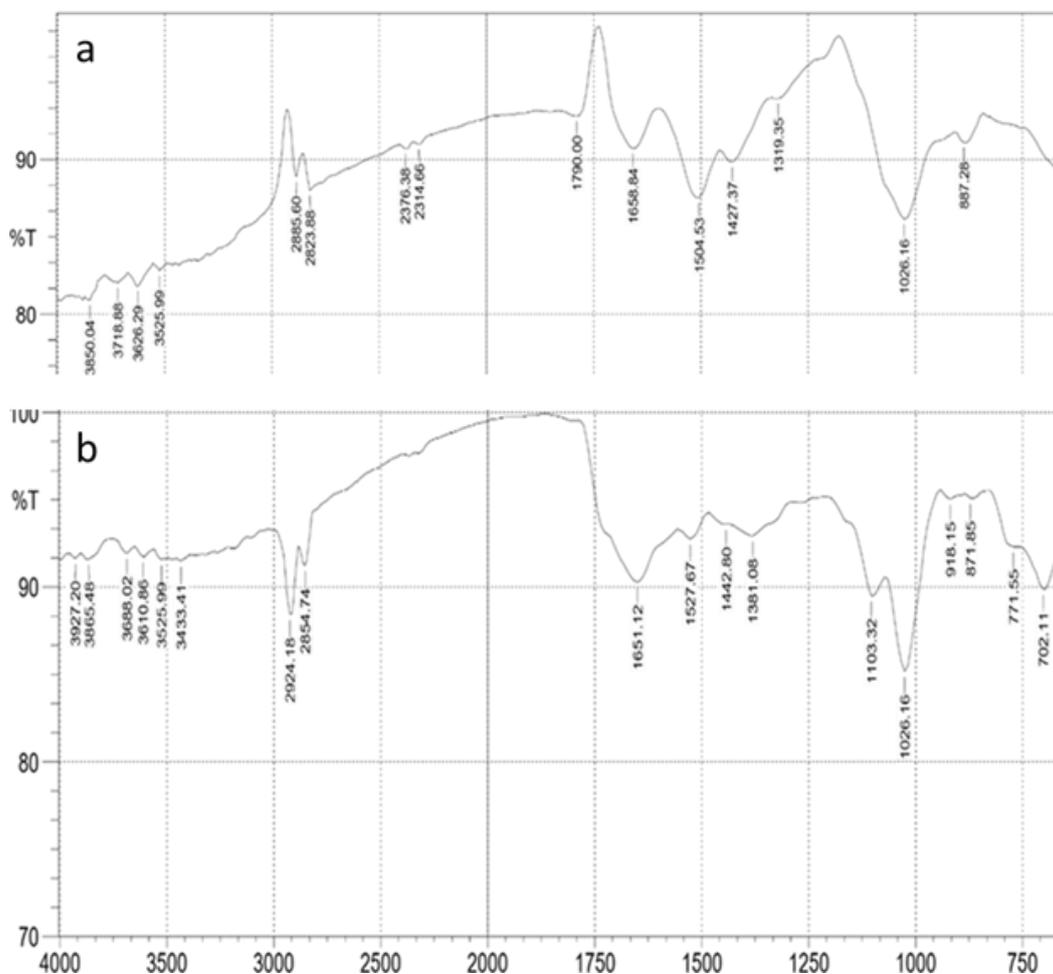


Figure 4 FTIR spectra of standard chitosan (a) and the crude millipede (*Eurymerodesmus*) sample (b)

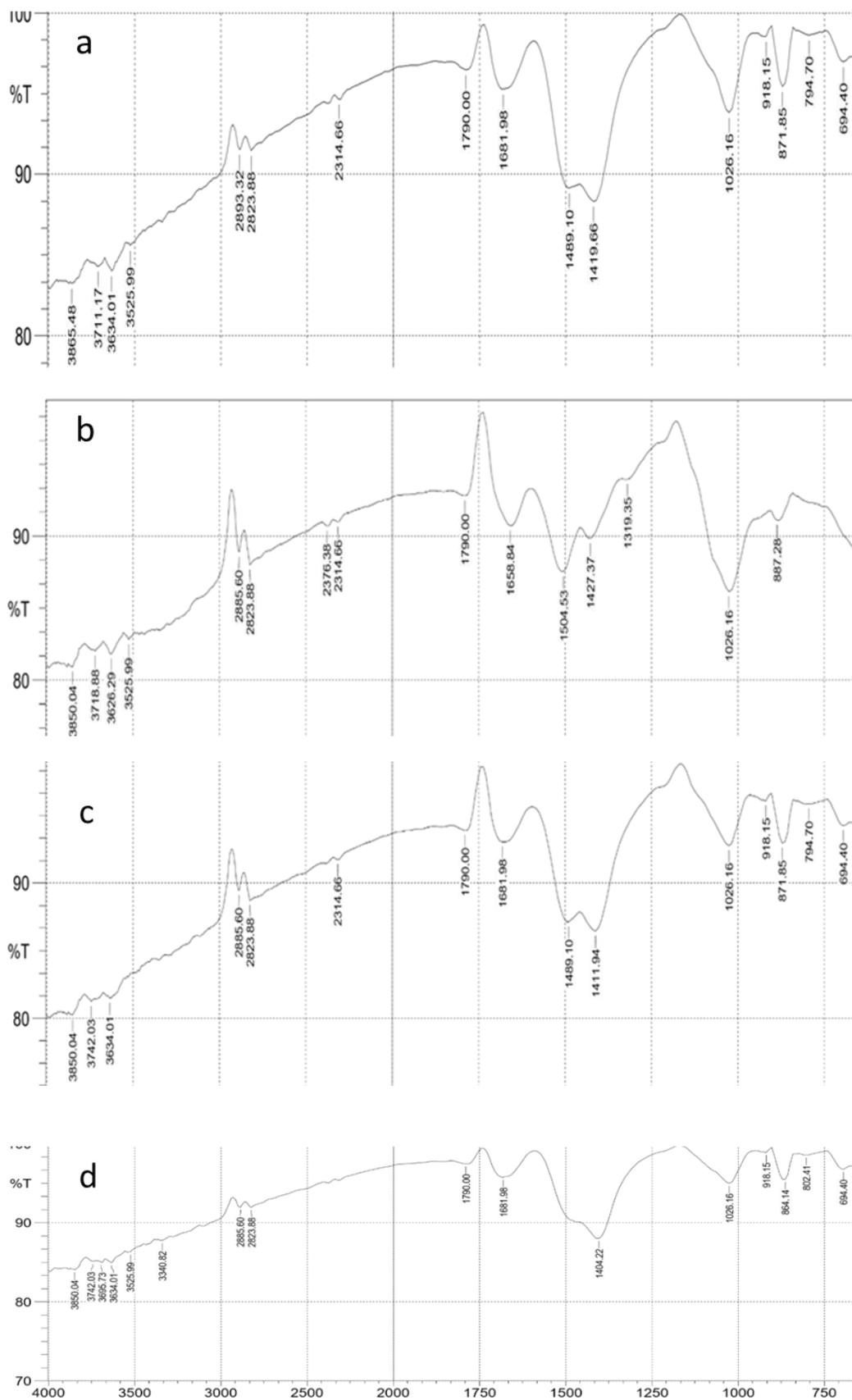


Figure 5 FTIR spectra of (a) Reference material, (b) 5% HCl demineralised Chitosan, (c) 4% HCl demineralised Chitosan, (d) 3% HCl demineralised Chitosan

Fourier transform infrared spectroscopy (FTIR) has been a means of identifying and also authenticating chitin and chitosan produced via different means by the presence of their unique functional groups.

Peaks at 3340 cm^{-1} indicates the symmetric stretching vibration of OH and amine N-H, the peak is only seen in (Figure 5d) above indicating improper demineralization of the sample unlike in b and c as supported by the absence of the same peak as seen in the reference material (Figure 5a). Peaks between 2200 cm^{-1} and 2900 cm^{-1} indicates the presence of CH stretch, four peaks are appearing in the reference material, three in 5%, three in 4%, two in 3% and two for the crude sample. Peaks at 1600 cm^{-1} indicates C=O stretching of amide I which has a higher percentage transmission in the prepared chitosan than in the crude and reference material, between 1095.49 cm^{-1} and 1033.77 cm^{-1} indicates C-O stretch which can be found at 1026.16 cm^{-1} for all samples.

Peaks at 894 cm^{-1} shows ring stretch characteristics band of β -1, 4-glycosidic linkage which is found at 887.28 cm^{-1} for the reference material, 875.81 cm^{-1} for 5% HCl, 871.85 cm^{-1} for 4%, 864.14 cm^{-1} for the 3% HCl and 871.85 cm^{-1} for the Crude sample in the Figures above. Within the range of 2921 cm^{-1} - 2879 cm^{-1} appeared peaks showing methylene groups on NHCCH_3 and CH_2OH which is found at 2885.6 cm^{-1} for reference material, 2893.32 cm^{-1} for 5% HCl sample, 2885.6 cm^{-1} for 4% and 3% HCl samples and 2924.18 cm^{-1} for the crude sample. A lower transmittance for the produced samples with respect to the crude sample is a proof that deacetylation has occurred.

Peaks at 1026.16 cm^{-1} , 1790 cm^{-1} , 2823.88 cm^{-1} , 2885.6 cm^{-1} , 3525.99 cm^{-1} , 3626.29 cm^{-1} , 3850.04 cm^{-1} on both the reference material and also the prepared chitosan samples show that chitosan has been produced.

3.7. Scanning Electron Microscopy (SEM)

This analysis showed the surface morphology of the samples which gives further insight into the nature of the products obtained.

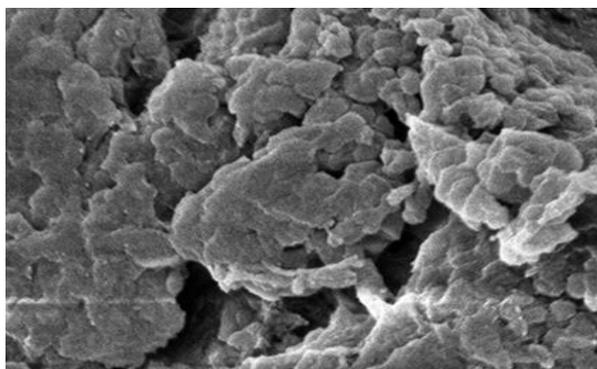


Figure 6 Micrograph of Chitosan obtained from the 5% acid treatment

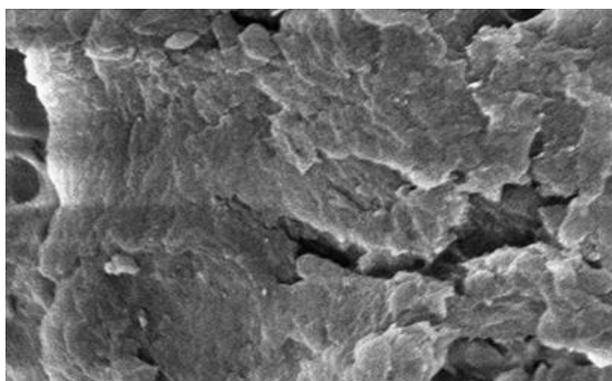


Figure 7 Micrograph of Chitosan obtained from the 4% acid treatment

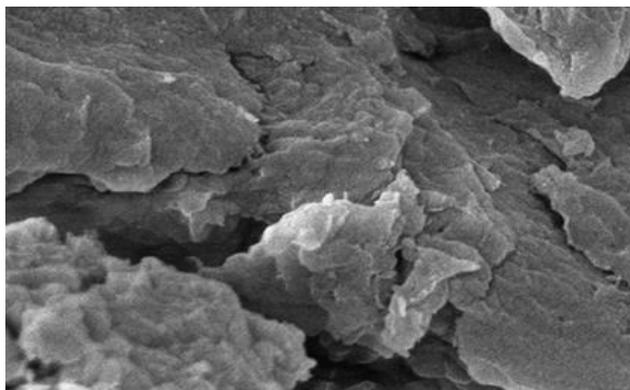


Figure 8 Micrograph of Chitosan obtained from the 3% acid treatment

The analysis as presented as Figs. 6-8, shows the chitosan materials as platelets with spherical shape. The platelets overlay on each other and is possibly so because of some inter molecular hydrogen bonding between the chitosan structures. This identification is in agreement with [18] and [19].

3.8. Transmission Electron Microscopic Analysis (TEM).

This was carried out using JEOL, JEM-2010 Electron Microscope. The analysis supports the result obtained from the scanning electron microscopy (SEM), Figures 9-11.

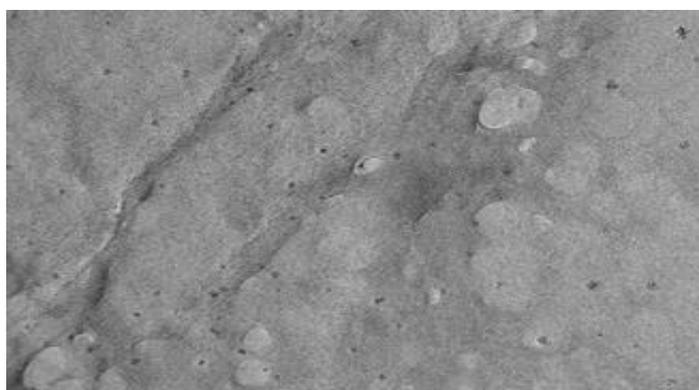


Figure 9 Temograph of Chitosan obtained from the 5% acid treatment

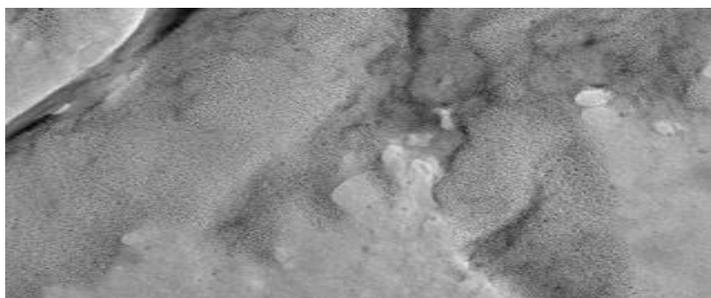


Figure 10 Temograph of Chitosan obtained from the 4% acid treatment

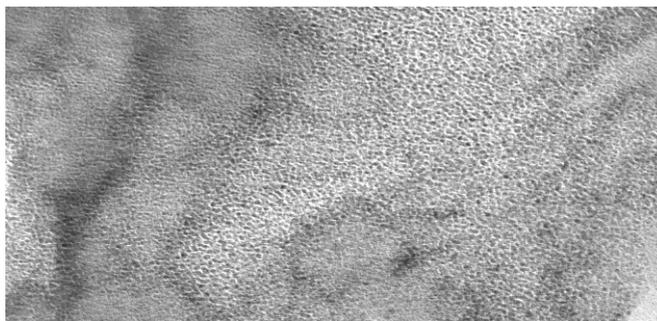


Figure 11 Temograph of Chitosan obtained from the 3% acid treatment

The result of the TEM supports the spherical nature of the chitosan and clearly showed the plate like surface topography.

4. Conclusion

Biopolymers still have unexploited potentials which can effectively substitute the hazardous and non-biodegradable synthetic polymers. Chitosan over the years has been extracted mainly from aquatic sources and this has limited the sourcing of the material from non-aqua-endowed areas, but the optimized utilization of millipede in this activity expands the resource environment with the advantage of an enlarged manufacturing potential for chitosan.

This study shows the feasibility of extracting Chitin and conversion to chitosan from millipede (*Eurymerodesmus*). The FTIR studies confirm the presence of characteristic functional groups and their conformation to that of commercially available product. The deacetylation was optimal with the 4% HCl treatment with a degree of deacetylation of 30%. The proximate analysis confirmed the presence and percentage composition of various contents of the millipede used. Further work can be done to improve on the degree of deacetylation probably through size reduction of the chitin, increase in concentrations of the reagents, reaction time and increase in temperature of deacetylation.

Comparing literature reports millipede gave a higher yield of chitin and chitosan than that of other locally (Nigerian) sourced materials. This gives it an advantaged utility status.

More so, the production of chitosan from locally and widely distributed resources is economically impacting as it would reduce the amount of forex from its importation particularly for under-developed and developing economies.

Compliance with ethical standards

Acknowledgments

The authors wish to acknowledge the assistance of Mr. Emmanuel Ali of National research Institute for chemical technology Zaria, Kaduna State Nigeria; who helped with the FTIR analysis.

Disclosure of conflict of interest

No conflict of interest exists between the authors or any funding body.

References

- [1] Puvvada YS, Vankayalapati S and Sukhavasi S. (2012). Extraction of chitin and chitosan from exoskeleton of shrimp for application in the pharmaceutical industry. *International Current Pharmaceutical Journal*, 1, 19.
- [2] Hossain MS and Iqbal A. (2014). Production and characterization of chitosan from shrimp waste. *Journal of the Bangladesh Agricultural University*, 12(1), 153-160.
- [3] Arafat A, Samad SA, Masum SM and Moniruzzaman M. (2015). Preparation and characterization of chitosan from shrimp shell waste. *International Journal of Scientific and Engineering Research*, 5(3), 1.
- [4] Isa MT, Ameh A, Tijjani M and Kadama KK. (2012). Extraction and characterization of chitin and chitosan from Nigerian shrimps. *International Journal of Biological and Chemical Sciences*, 6(1), 446-453.

- [5] Gagne N. (1993). Production of chitin and chitosan from crustacean waste and their use as a food processing aid. M.Sc thesis, McGill University, Montreal Canada, 190-198.
- [6] Paul S, Jayan A, Sasikumar CS and Cherian SM. (2014). Extraction and purification of chitosan from chitin isolated from sea prawn (*Fenneropenaeus indicus*). Asian Journal of Pharmaceutical and clinical research, 7(4), 201-204.
- [7] Toan NV. (2009). Production of chitin and chitosan from partially autolyzed shrimp shell materials. The Open Biomaterials Journal, 1, 21-24.
- [8] Nadarajah K, Kader J, Mazmira M and Paul DC. (2001). Production of chitosan by fungi. Pakistan Journal of Biological Sciences, 4(3), 263-265.
- [9] Enghoff H. (2018). Subterranean millipedes, some patterns and adaptations Arab Public Health Association. Conference Abstracts, 1. e29273.
- [10] Kaya M, Baran T, Erdoğan S, Menteş A, Özüsağlam MA and Çakmak Y. (2014). Physicochemical comparison of chitin and chitosan obtained from larvae and adult Colorado potato beetle (*Leptinotarsa decemlineata*). Materials Science and Engineering, 45, 72-81.
- [11] Wang C, Yuan F, Jin L, Du X and Cai H. (2015). Evaluation of the deacetylation degree of Chitosan with Two-Abrupt-Change Coulometric Titration. Electroanalysis, 28(2), 401-406.
- [12] Islam MM, Masum SM, Rahman MM, Molla MA, Shaikh AA and Roy SK. (2011). Preparation of chitosan from Shrimp Shell and Investigation of Its Properties. International Journal of Basic and Applied Sciences, 11(1), 77-80.
- [13] Antonino DQR, Fook LB, Lima DOV, Rached DFR, Lima E and Lima RDS. (2017). Preparation and characterization of chitosan obtained from shells of shrimp (*Litopenaeus vannamei* Boone). Marine Drugs, 15(5), 141-153.
- [14] Wanule D, Balkhande JV, Ratnakar PU, Kulkarni AN and Bhowate CS. (2014). Extraction and FTIR analysis of chitosan from American cockroach, *Periplaneta americana*. International Journal of Engineering Science and Innovative Technology, 3(3), 229-304.
- [15] Kaya M, Seyyar O, Baran T and Turkes T. (2014). Bat guano as new and attractive chitin and chitosan source. Frontiers in Zoology, 11(1), 59-69.
- [16] Raghu HS, Raghavendra SN and Rajeshwara NA. (2018). Isolation and characterization of chitin from Millipede (Spirobolida). The Journal of Basic and Applied Zoology, 79, 30.
- [17] Isa MT, Ameh AO, Gabriel JO and Adama KK. (2012). Extraction and characterization of chitin from Nigerian sources. Leonardo Electronic Journal of Practices and Technologies, 1583-1078, 73-81.
- [18] Kumar S and Koh J. (2012). Physiochemical, optical and biological activity of chitosan-chromone derivative for biomedical applications. International Journal of Molecular Sciences, 13(5), 6102-6116.
- [19] Huang K, Wang L, Wang C, Yang C, Hsieh C, Chen S, Shen C and Wang J. (2015). Synthesis and anti-fungal effect of silver nanoparticles and ash; chitosan composite particles. International Journal of Nanomedicine, 2685.

How to cite this article

Ofoegbu O, Umah NJ and Aliyu SJ. (2019). Deracination of chitosan from locally sourced Millipede (*Eurymerodesmus* spp.) and its spectroscopic and physio-chemical properties. GSC Biological and Pharmaceutical Sciences, 7(2), 95-107.
