Lactic acid demineralization of shrimp shell and chitosan synthesis

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Abstract

The use of lactic acid was compared to hydrochloric acid for shrimp shell demineralization in chitosan synthesis. Five different acid concentrations were considered for the study: 1.5M, 3.0M, 4.5M, 6.0M and 7.5M. After demineralization, the shrimp shell were deproteinized and subsequently deacetylated to produce chitosan using sodium hydroxide solution. The synthesized chitosan samples were characterized using solubility, FTIR, SEM, XRD and viscosity. The SEM, FTIR and XRD analysis indicated that chitosan was synthesized with a high degree of deacetylation (83.18±2.11 when lactic acid was used and 84.2±5.00 when HCl was used). The degree of deacetylation and the molecular weight of the chitosan samples were also estimated. ANOVA analysis (at 95% confidence interval) indicated that acid type and concentration did not significantly affect the solubility, degree of deacetylation, viscosity and molecular weight of the chitosan within the range considered.

Keywords

Chitosan; Demineralization; Lactic acid; Shrimp

Introduction

Chitin is a natural polysaccharide composed of *N*-acetyl-D-glucosamine units and is the second most abundant biopolymer after cellulose [1]. It occurs as a principal structural polymer in the covering of insects and crustaceans and in the cell walls of many fungi. Chitin and chitosan have also been prepared from fish scale [2]. Chitin composition may range from trace quantities up to about 40% of the body weight of the organism [3]. In shrimp processing, between 40 and 50% of the total mass ends up as waste. Crustacean shell waste consists mainly of 30-40% protein, 30-50% calcium carbonate, and 20–30% chitin [4], though these may vary with species and location [5].

Chitosan is a unique natural biopolymer which is commercially derived from chitin and has found numerous applications in food, agriculture, cosmetic, biomedicine, textile, water treatment and pharmaceuticals [6].

In the preparation of chitin from natural sources, chemical treatment step to remove calcium carbonate (demineralization) and protein (deproteinization) was usually required. Hydrochloric acid seemed to be the preferred reagent for demineralization [7]. However, the harsh nature of the chemical treatments could have resulted in an anomerization and hydrolytic effects on the chitin structure [8]. The molecular weight decrease of chitin and chitosan was associated with the use of chemicals for pretreatment [9]. Waste treatment was also a fundamental drawback of the process. As such, substitution with milder and environment-friendly chemicals continues to gain attention [10, 11]. Furthermore, the use of organic acids may have had other benefits such as the fact that they might have been produced from low cost biomass and the resulted organic salts from the demineralization process could have found other applications [12]. The objective of this manuscript was to synthesize chitosan using similar concentrations of lactic acid and hydrochloric acid and investigate the effect of the acid treatments on chitosan property.

Material and method

Shrimp was obtained from Samaru market and taken to the Department of Biological Science, Ahmadu Bello University for identification. The exoskeleton of the shrimp was

manually removed, washed, dried, and ground to pass through a 250μm sieve. Lactic acid (AnalaR, BDH was procured and prepared into five concentrations (1.5, 3.0, 4.5, 6.0, and 7.5M).

8.57ml of 1.5M lactic acid solution was introduced into a conical flask (250ml) with stirring on a magnetic stirrer at room temperature (≈ 27°C). 10g of the prepared shrimp shells were quickly introduced and stirred for 6 hours after which the content of the flask was quickly filtered and washed with deionized water until neutrality as determined using a pH meter (Kent EIL 7055). The demineralized samples were dried and weighed in an oven.

The entire procedure was then repeated for the other lactic acid concentrations of 3.0, 4.5, 6.0 and 7.5M after which similar concentrations and quantities of hydrochloric acid were used to demineralize the shrimp shells. The resulting samples after demineralization were weighed and treated in 0.62M Sodium hydroxide solution at a ratio of solute to solvent of 1:10 (w/v) for 16 hours in a 250ml conical flask at ambient temperature (27°C) for 16 hours to dissolve the proteins and sugars thus isolating the crude chitin.

The samples were then washed thoroughly with deionized water until neutrality then filtered and dried to constant weight using an oven. The extracted chitin obtained from the process of deproteination was weighed and treated in 25M NaOH solution (1:10w/v) for 20 hours at a temperature of 115°C in an autoclave in order to dissolve the remaining proteins and remove acetyl groups from the chitin.

The extracted chitosan was then washed properly with deionized water until neutrality then filtered and dried to constant weight using an oven.

0.2g of dried chitosan samples obtained from Acetic acid demineralization and HCl demineralization was weighed and dissolved in 1% acetic acid followed by mild stirring and then filtered. Solubility was computed as the fraction that dissolved.

The viscosity of the solution of the various samples obtained by dissolving 0.2 g of the dried chitosan in 1% acetic acid was measured using a viscometer.

The molecular weights of the various samples were determined using the Mark-Houwink equation:

$$[\eta] = KM \tag{1}$$

where M is the viscosity average molecular weight; K and a are constants, whose values depend on the polymer type and the chosen solvent. As shown in [13] for chitosan and the solvent 0.1M acetic acid these constants are 3.5×10^{-4} and 0.76, respectively, and they do not

depend on the deacetylation degree. In addition, [η] is intrinsic viscosity.

The degree of deacetylation of the various chitosan samples obtained was determined using relationship [14]:

$$DD = 118.883 - [40.1647_{\times}(A_{1655}/A_{3450})] \quad (2)$$

where A_{1655} and A_{3450} are absorbance bands at 1655 and 3450 from FTIR spectra.

The structural differences in the synthesized chitosan was taken for Fourier transform infrared spectroscopy analysis (8400S spectrophotometer, Shimadzu), Scanning Electron Microscopy (JEOL JSM – 630 J) and X-Ray Diffraction (X'Pert PRO MRD XL) analysis.

Results and Discussion

Table 1 shows the yield of Chitosan obtained from 10g of dried shrimp shell powder using the various acid demineralization.

Table 1. Effect of demineralizing acid type and concentration on the yield of chitosan

Acid concentration (M)	Yield for Lactic acid demineralization, %	Yield for HCl demineralization, %
1.5	21	14
3.0	15	19
4.5	12	11
6.0	11	11
7.5	14	13

The results of chitosan yield of Table 1 were within reported values of 15-20% for crabs (*chinoecetes opilio*) and shrimps (*pandalus borealis*) chitosan [15]. Statistical analysis (ANOVA at a confidence interval of 0.05) gave *P-values* greater than 0.05 indicating that acid type and concentration did not significantly affect the yield of chitosan.

Tables 2 and 3 present the properties of extracted chitosan. From Tables 2 and 3, the solubility of the extracted chitosan from the demineralization of both lactic acid and Hydrochloric Acid at the five different concentrations was within the range of 35-45% in 1% acetic acid solution. ANOVA gave *P-values* of 0.161458 and 0.771715 which indicates that the solubility of the extracted chitosan is independent of the type acid used, and the concentration of the acid used in the demineralization of extracted chitosan respectively.

Table 2. Properties of chitosan synthesized after lactic acid demineralization

Concentration (M)	Solubility (%)	Viscosity (cps)	Molecular weight (g/mol)
1.5	25	2.5	1.18×10^5
3.0	30	3.0	1.50×10^5
4.5	15	2.7	1.30×10^5
6.0	15	1.9	0.82×10^5
7.5	20	2.0	0.88×10^5

Table 3. Properties of chitosan synthesized after Hydrochloric acid demineralization

Concentration (M)	Solubility (%)	Viscosity(cps)	Molecular weight (g/mol)
1.5	20	2.0	0.88×10^5
3.0	25	4.5	2.55×10^5
4.5	35	3.0	1.50×10^5
6.0	30	2.5	1.18×10^5
7.5	45	3.0	1.50×10^5

The various samples showed low viscosity using the viscometer within the range of 3.0 - 3.5 cps, this low viscosity is attributed to the poor solubility of the extracted chitosan in 1% solution of acetic acid. However, the viscosity of the extracted chitosan was not significantly affected by the acid used in the demineralization process and the concentration of acid.

Using the Mark-Houwink equation the various molecular weight of the samples was calculated within the range of $1.5 \times 10^5 – 1.83 \times 10^5$g/mol though within the values reported by [15]. However, several factors during production, including high temperature, concentration of alkali, reaction time, previous treatment of the chitin, particle size, chitin concentration, dissolved oxygen concentration and shear stress may influence the Molecular weight of chitosan.

Figures 1 and 2 present typical FTIR spectra obtained. The sample of FTIR spectra of synthesized chitosan presented in Figure 1 and Figure 2 shows the bands typical of all the chitosan samples produced using both lactic acid and HCl demineralization. The spectra indicate the presence of absorption band observed between 1220 and 1020 cm^{-1}, which represents the free amino group ($-NH_2$) at C2 position of glucosamine, a major group present in chitosan.

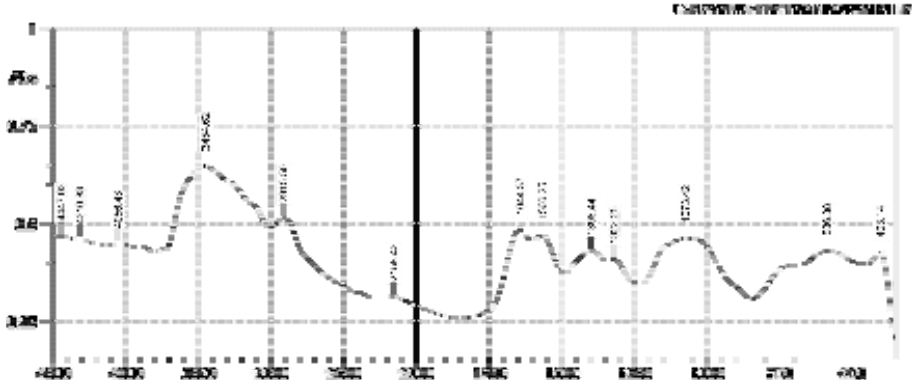

Figure 1. *FTIR spectra of extracted chitosan after demineralization using 3.0M Lactic Acid*

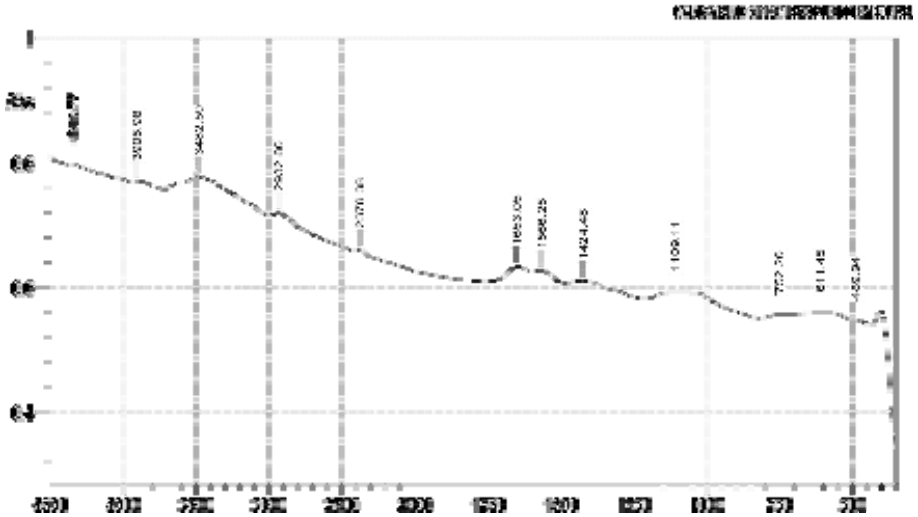

Figure 2. *FTIR spectra of extracted chitosan after demineralization using 4.5M HCl*

Furthermore, the sample showed the absorption bands for the free amino group between 1026 and 1259 cm^{-1} while the peak at 1374 cm^{-1} represents the –C-O stretching of primary alcoholic group (-CH_2 - OH). The absorbance bands of 3268, 2930,2878,1563, and 1418 cm^{-1} indicated the N-H stretching, symmetric CH_3 stretching and asymmetric CH_2 stretching, CH stretching, C=O stretching in secondary amide (amide I) and C–N– stretching in secondary amide (amide II) respectively. Absorbance bands were observed at 3283, 2921, 2865,1643,1552,1421, 1022, 893and 752 cm^{-1} which confirms the structure of chitosan. Table 4 shows the various degree of deacetylation of the chitosan derived after Lactic acid and HCl demineralization using various acid concentrations.

Having confirmed that the synthesized samples contained chitosan using FTIR, the degree of deacetylation (DD) was calculated using Equation (2).The values obtained were within 83.18±2.11 when lactic acid was used and 84.2±5.00 when HCl was used. Demineralization using 3.0M and 4.5M gave the highest DDs for lactic acid demineralization

(86.2%) and HCl demineralization (89.2%) respectively. However, statistical analysis using ANOVA indicated that the effects of acid type and concentration did not significantly affect the DD as they recorded *P-values* of 0.97 and 0.32 respectively (at $\alpha = 0.05$).

The DD is an important parameter affecting solubility, chemical reactivity, and biodegradability. Depending on the source and preparation procedure, DD may range from 30% to 95 % [16]. It is rare however to have the production of chitosan with 100% degree of deacetylation. Therefore, commercial chitosan with various degree of deacetylation in the range of 75–85% is commonly found.

Table 4 compares the degree of deacetylation for the treatment methods used. XRD patterns of extracted chitosan are illustrated in Figure 3.

Table 4. Chitosan Degree of Deacetylation (DD)

Concentration (M)	Lactic Acid demineralization	HCl demineralization
1.5	80.5	84.4
3.0	86.2	79.2
4.5	82.1	89.4
6.0	83.7	79.1
7.5	83.4	88.9

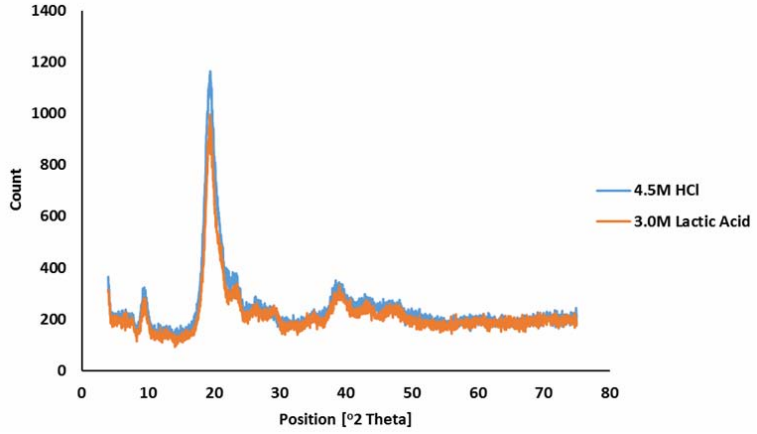

Figure 3. *XRD Diffractogram of prepared chitosan*

XRD patterns of extracted chitosan are illustrated in Figure 3, indicating a striking similarity between the lactic acid and the hydrochloric treated samples. The XRD pattern of chitosan exhibits broad diffraction peaks at $2\theta = 10^{o}$ and 21^{o} which are typical fingerprints of semi-crystalline chitosan [17]. Yen and Mau [18], found that fungal chitosan showed two crystalline reflections at 9.7^{o} and 19.9^{o}. It has been reported that the XRD patterns of shrimp chitosan showed two major characteristic peaks at $2\theta = 9.9\text{-}10.7^{o}$ and $19.8\text{-}20.7^{o}$ [19]. It is

also reported that the two characteristic crystalline peaks with slightly fluctuated diffraction angles found in the XRD patterns indicated that two types of α- and γ-chitosan exhibited comparable degree of crystallinity and had two consistent peaks of 9-10º and 19-20º.

Figure 4 and 5 presents the SEM micrographs for the treatment methods used.

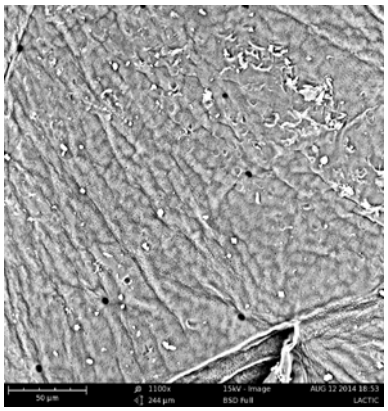

Figure 4. *The SEM Morphology of extracted chitosan after 3.0M lactic acid demineralization*

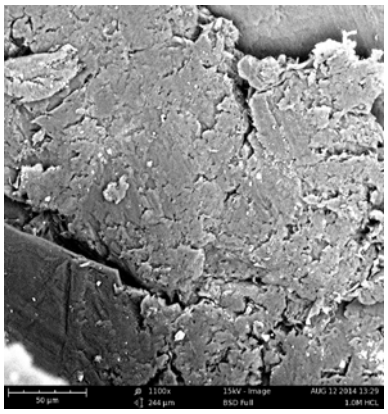

Figure 5. *The SEM Morphology of extracted chitosan after 4.5M Hydrochloric acid demineralization*

Figure 4 and 5 presents the SEM micrographs illustrating the morphology of chitosan prepared after lactic acid and hydrochloric acid demineralization respectively. Under the electron microscopic examination, chitosan showed non homogenous and non-smooth surface with straps and shrinkage which corresponds to the SEM morphology of extracted chitosan [20].

Conclusions

The following conclusion may be drawn from the results of this work: chitosan was synthesized from shrimp exoskeleton after demineralization using lactic acid and hydrochloric

acid. The properties of the synthesized chitosan where found to be independent of acid type and concentration as indicated by ANOVA analysis. Lactic acid which is friendlier to the environment can replace hydrochloric acid in chitosan synthesis.

References

1. Choorit W., Patthanamanee W., Manurakchinakorn S., *Use of Response Surface Method for the Determination of Demineralization Efficiency in Fermented Shrimp Shells*, Bioresource Technology, 2008, 14(99), p. 6168-6173.
2. Kumari S., Rath P. K., *Extraction and Characterization of Chitin and Chitosan from (Labeo rohit) Fish Scales*, Procedia Materials Science, 2014, 6, p. 482-489.
3. Khanafari A., Marandi R., Sanatei Sh., *Recovery of chitin and chitosan from shrimp waste by chemical and microbial methods*, Iranian Journal of Environmental Health Science and Engineering, 2008, 5(1), p. 19-24.
4. Zhang H., Li R., Liu W., *Effects of Chitin and Its Derivative Chitosan on Postharvest Decay of Fruits: A Review*, International Journal of Molecular Sciences, 2011, 12, p. 917-934.
5. Cho Y. I., No H. K., Meyers S. P., *Physicochemical Characteristics and Functional Properties of Various Commercial Chitin and Chitosan Products*, Journal of Agricultural Food Chemistry, 1998, 46, p. 3839-3843.
6. Kim W. J., Lee W. G., Theodore K., Chang H. N., *Optimization of Culture Conditions and Continuous Production of Chitosan by the Fungi, Absidia Coerulea*, Biotechnology and Bioprocessing Engineering, 2001, 1(6), p. 6-10.
7. Allan G. G., Fox J. R., Kong N., Marine polymers. Part 8. *A critical evaluation of the potential sources of chitin and chitosan*. In: Muzzarelli R.A.A. and Pariser E.R. (eds.). Proceeding of 1st International Conference on Chitin/Chitosan. 1978 MIT Sea Grant Rep. MITSG, USA, p. 64-78.
8. Ng C. H., Chandrkrachang S., Stevens W. F., *Effect of the rate of deacetylation on the physico-chemical properties of cuttlefish chitin*, Advances in Chitin Science, 2000, 4, p. 50-54.
9. Myint K. T., Ng C. H., Chandrkrachang S., Stevens W. F., *Optimal demineralization of crab shells wastes for chitin production*. In: Scchiva K., Chandrkrachang S., Methacanon

P., Peter M. G. (eds.), 2002, p. 15-18.

10. Ameh A. O., Isa M. T., Adeleye T. J., Adama K. K., *Kinetics of demineralization of shrimp exoskeleton in chitin and chitosan synthesis,* Journal of Chemical Engineering and Materials Science, 2013, 4(3), p. 32-37.
11. Ameh A. O., Abutu D., Isa M. T., Rabiu U., *Kinetics of Demineralization of Shrimp Shell Using Lactic Acid*, Leonardo Electronic Journal of Practices and Technologies, 2014, 24, p. 13-22.
12. Mahmoud N. S., Ghaly A. E., Arab F., *Unconventional Approach for Demineralization of Deproteinized Crustacean Shells for Chitin Production,* American Journal of Biochemistry and Biotechnology, 2007, 3(1), p. 1-9.
13. Muzzarelli R. A. A., Peteri R., *Determination of the degree of deacetylation of chitosan by first derivative ultraviolet spectrophotometry*. Carbohydrate Polymers, 2006, 5, p. 461-472.
14. Rout S. K., *Physicochemical, functional, and spectroscopic analysis of crawfish chitin and chitosan as affected by process modification*. PhD Dissertation. Louisiana State University: 2001, Baton Rouge, LA, USA.
15. Shahidi F., Synowiecki J., *Isolation and characterization of nutrients and value-added products from snow crab (Chinoecetes apilio) and shrimp (Pandalus borealis) processing discards*. Journal of Agricultural Food Chemistry, 1991, 39, p. 1527-1532.
16. Martino A. D., Sittinger M., Risbud M. V., *Chitosan: A versatile biopolymer for orthopaedic tissue engineering*, Biomaterials, 2005, p. 5983-5990.
17. Bangyekan C., Aht-Ong D., Srikulkit K., *Preparation and properties evaluation of chitosan-coated cassava starch films,* Carbohydrate Polymers, 2006, 63(1), p. 61-71.
18. Yen M.-T., Mau J.-L., *Physico-chemical characterization of fungal chitosan from shiitake stipes*, LWT-Food Sci. Technol., 2007, 40, p. 472-479.
19. Prashanth K. V. H., Kittur F. S., Tharanathan R. N., *Solid state structure of chitosan prepared under different N-deacetylating conditions,* Carbohydrate Polymers, 2002, 50, p. 27-33.
20. Md. Monarul Islam, Shah Md. Masum, M. Mahbubur Rahman, Md. Ashraful Islam Molla, Shaikh A. A., Roy S. K., *Preparation of Chitosan from Shrimp Shell and Investigation of Its Properties,* International Journal of Basic and Applied Sciences, 2011, 11(1), p. 77-80.