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Extraction and Characterisation of Chitin and Chitosan from Mussel Shell

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Abstract
In view of the increasing littering of the river banks by shells of crustaceans, a study was carried out to investigate the extraction and characterization of chitin and chitosan from mussel shell. Chitin and chitosan was extracted and characterized from mussel shell found in banks of the Gubi Dam in Bauchi, Nigeria using the conventional methods of pretreatment, demineralization, deprotienization and deacetylation. The results obtained revealed that carbon nitrogen ratio of the chitosan extracted was 5.9 with a degree of deacetylation of 60.69% and 60.66% calculated from the elemental analysis and the FTIR spectra of chitosan respectively. The FTIR spectra for chitosan gave a characteristic $-NH_2$ band of 3447 cm^{-1} and a carbonyl group band of 1477 cm^{-1}. The mussel shell was discovered to contain a mineral content of 51.62% and a chitin composition was found to be 21.32%.
KEYWORDS: Characterisation, Chitin, Chitosan, Extraction, Mussel.

1.0 Introduction

Chitin, poly (b-(1-4)-N-acetyl-D-glucosamine), is a natural polysaccharide of major importance, first identified in 1884 (Rinaudo, 2006).Among the novel families of biological macromolecules, whose relevance is becoming increasingly evident are chitin and its main derivative, chitosan. Potential and usual applications of chitin, chitosan and their derivatives are estimated to be more than 200 (Aranaz *et al*., 2009). The duo is the second-most abundant high molecular-weight biopolymers and is recognized as versatile, environmentally friendly raw materials (Hsu *et al*., 2004). Chitin is the major component in the shell of the shrimps, and crabs, cartilage of the squid, and outer cover of insects, it also occur as ordered crystalline microfibrils forming structural components in the exoskeleton of arthropods or in the cell walls of fungi and yeast. It is also extracted from a number of other living organisms in the lower plant and animal kingdoms, serving in many functions where reinforcement and strength are required (Rinaudo, 2006). Generally, the shell of selected crustacean was reported to consist of 30-40% protein, 30-50% calcium carbonate and calcium phosphate, and 20-30% chitin (Fernandez-Kim, 2004). Depending on the processing method used to derive the chitin (Khora and Limb, 2003), and also depending on the source, its degree of deacetylation may range from 30% to 95% (Martino *et al*., 2005). Chitosan is a natural polysaccharide comprising of copolymers of glucosamine and *N*-acetylglucosamine, and can be obtained by the partial deacetylation of chitin. In its crystalline form, chitosan is normally insoluble in aqueous solutions above pH7; however, in dilute acids (pH6.0), the protonated free amino groups on glucosamine facilitate solubility of the molecule (Martino *et al.,* 2005). Chitosan can be obtained by deacetylation of chitin through enzymatic or alkaline method during the course of deacetylation; parts of polymer N-acetyl links are broken with the formation of D-glucosamine units, which contain a free amine group, increasing the polymer's solubility in aqueous means (Kalut, 2008).

Chitosan has been widely used in vastly diverse fields, ranging from waste management to food processing, medicine and biotechnology (Kalut, 2008). In agriculture, the use of chitosan has been established to improve the yield of rice and orchid production (Kim, 2010).

A lot of researches have been conducted on the extraction, characterization and application of chitin and chitosan, Chen *et al*., (2007) produced chitin and chitosan from fungal cell wall, a fungus called Ganodermatsugae was used for this purpose. The main components of G. tsugae fruiting bodies are β-1,3-D-glucan and chitin which were found to be safe materials in enhancing wound healing in Wistar rats and accelerate proliferation and migration of fibroblasts and keratinocytes with no cytotoxicity.George *et al*, (2011) carried out a study on chitosan from fungi. He used Aspergillusflavus, Botrydiplodiatheobromae, Cladosporiumcladosporioides, Fusarium Sp. and Phoma Sp. and these were all isolated from stem and leaf segments taken from medicinal plants. Chitosan was also prepared from

shrimp processing waste (shell) using conventional chemical process for the other crustacean species. The physicochemical properties, molecular weight (165394g/mole), degree of deacetylation (75%), ash content as well as yield (15%) of prepared chitosan indicated that shrimp processing waste (shell) are a good source of chitosan. The water binding capacity (50.2%) and fat binding capacity (37.0%) of prepared chitosan are good agreement with the commercial chitosan. FT-IR spectra gave characteristics bands of $-NH_2$ at $3443cm^{-1}$ and carbonyl at $1733cm^{-1}$ (Islam *et al*., 2011). Isa *et al*. (2012) studied the Extraction and Characterization of Chitin from Nigerian Sources, The studies revealed that the shrimp had the highest yield of chitin of 8.15%, crab, crayfish and periwinkle had yields of 7.8%, 2.88% and 0.44% respectively.

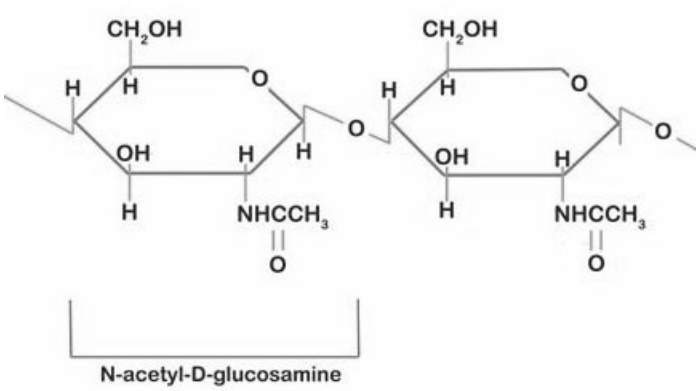

Figure 1.0 Chemical Structure of Chitin (Lertsutthiwong *et al*., 2002)

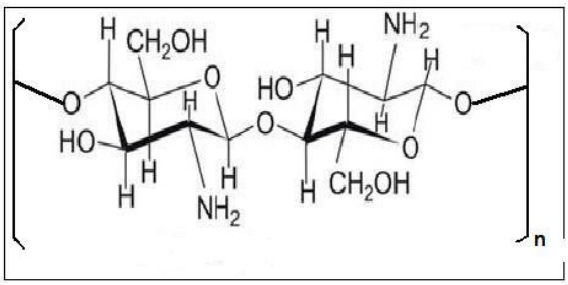

Figure 2.0 Structure of Chitosan (Lertsutthiwong *et al*., 2002).

Although many works have been reported, little work have been recorded on the extraction of chitin from mussel shell which increasingly litters river banks and in areas where they are consumed, disposal of the shell constitutes environmental problem. Thus this work is geared towards the extraction and characterisation of chitin and chitosan.

2.0 Materials and Method

The mussel shells used for the chitosan extraction were gotten from the Gubi Dam bank of Bauchi, north eastern part of Nigeria. The chitosan extraction was done in four steps; pre-treatment, demineralization, deprotienization and the deacetylation steps.

The shells obtained were washed thoroughly with distilled water and dried in an oven to constant weight at a temperature of 35^{o}C. Then 100g of the sample was taken for the extraction process. The dried shells were size-reduced and soaked in 0.68 M HCl (1:10 w/v) at ambient temperature (approximately 30^{o}C) for 6 hours after which it was washed in the acid until no bubbles were seen and no colour change was observed. The sample was then washed with distilled water until a relatively neutral was obtained and then the demineralized shell was dried to constant weight. The demineralized mussel shells were dried weighed and soaked in 0.62 M NaOH solution (1:10w/v) at ambient temperature (approximately 30^{o}C) for 16 hours. After which the shells were then washed thoroughly with water followed with distilled water until a neutral pH was obtained. The chitin was then dried to constant weight and ground and screened with 150µm sieve. The chitin obtained was then deacetylated in 25 M

NaOH (1:10 w/v) for 20 hours at 75°C. After deacetylation, the chitosan is then washed thoroughly with water followed by distilled water. The resulting chitosan was then dried to constant weight and prepared for characterization.

The characterization was done by Fourier transformed infrared (FTIR) spectroscopy (the Perkin Elmer Spectrum 100 FTIR spectrometer was used) in the range of 400 to 4000 cm^{-1} and the plots are shown as Figures 4 and 5 for chitin and chitosan respectively. The proximate analysis on chitin and chitosan was also carried out to determine parameters such as; ash content, moisture content and protein content of the chitin and chitosan. The composition of the mussel shell and the elemental analysis of the chitin and chitosan were conducted using the standard method (AOAC, 1990).
The DDA was evaluated after the characterization using Equation 1 and 2 respectively,

Calculating the percentage degree of deacetylation using the Kasaai Equation (Abdou *et al.*, 2008)

$$DDA\% = \frac{6.857 - \left(\frac{C}{N}\right)}{1.7143} \times 100 \quad (1)$$

$$DDA\% = 60.69\%$$

Using the FTIR analysis figure 2.0,

$$DDA = 100 - \frac{A_{1655}}{A_{3450}} \times 115 \quad (2)$$

But $A_{1655} = -\log\left(\frac{Transmittance_{1655}}{100}\right)$ also $A_{3450} = -\log\left(\frac{Transmittance_{3450}}{100}\right)$

$$DDA = 60.66\%$$

3.0 Results and Discussion

Tables 1, 2 and 3 present the results of mussel shell compositions, proximate analysis and carbon nitrogen content of chitin and chitosan respectively.

As shown in Table 1, the mussel cuticle was found to contain 23.25% of chitin which is close to 21.53% as reported by Abdou *et al.*, (2008) for shrimp shell, this may be due to variation in mineral content of different water bodies in which this crustaceans strive in.

The mineral content ($CaCO_3$) of the shell was found to be 51.62% which is slightly higher than the range in literature for crab as 40-50%, and higher than that of shrimp which is 20-30% but lower than that of oyster cuticle which is 85-90% (Kurita, 2006), thus it can be said that the mineral content of the cuticle varies in different members of the crustaceans and also varies with location.

Table 1.0 Mussel shell composition

Component	Composition (%)
Protein	9.99
Mineral	51.62
Chitin	23.25
Chitosan	15.14

The moisture content of chitin was very much higher than that of the chitosan (Table 2). This was expected since water the chitin was dried to constant weight before the deacetylation process. The ash content of chitin was lower than that of chitosan this could be attributed the presence of the acetyl group in the chitin sample as also reported by

Isa *et al* 2012. Protein content of the chitosan sample was considered high after deprotienization of the chitin and this could be attributed to the low degree of deacetylation of the chitin.

Table 2.0 Proximate analysis of chitin and chitosan

S/N		Moisture Content (%)	Ash Content (%)	Fat (%)	Protein (%)
1	**Chitin**	12.90	26.45	0.63	4.93
2	**Chitosan**	1.40	36.87	0.05	2.29

The carbon-nitrogen analysis with a chitosan degree of deacetylation of 60.69% which is higher than that reported by Isa *et al.,* (2012), of 50.64% for shrimp shell with almost 10%, but the result is still lower than the reported degree of deacetylation of 98.38-98.79% achieved by Kalut (2008) this may be attributed to the nature of the raw material used, its immediate environment and also the methods applied during the processes. There is an indication that the chitosan has been extracted in this work, since the necessary condition as stated by some literature is that the degree of deacetylation should be about 50% and it should be soluble in acidic media (Honarkar and Barikani, 2009). The chitosan was found to dissolve completely in 1M acetic acid after 53 minutes with initial vigorous stirring and later allowed to stand.

Table 3.0 Carbon Nitrogen content of chitin and chitosan

S/N		Carbon (%)	Nitrogen (%)	DDA (%)	Carbon Nitrogen ratio
1	**Chitosan**	13.32	2.29	60.69	5.82
2	**Chitin**	26.23	4.02	19.37	6.52

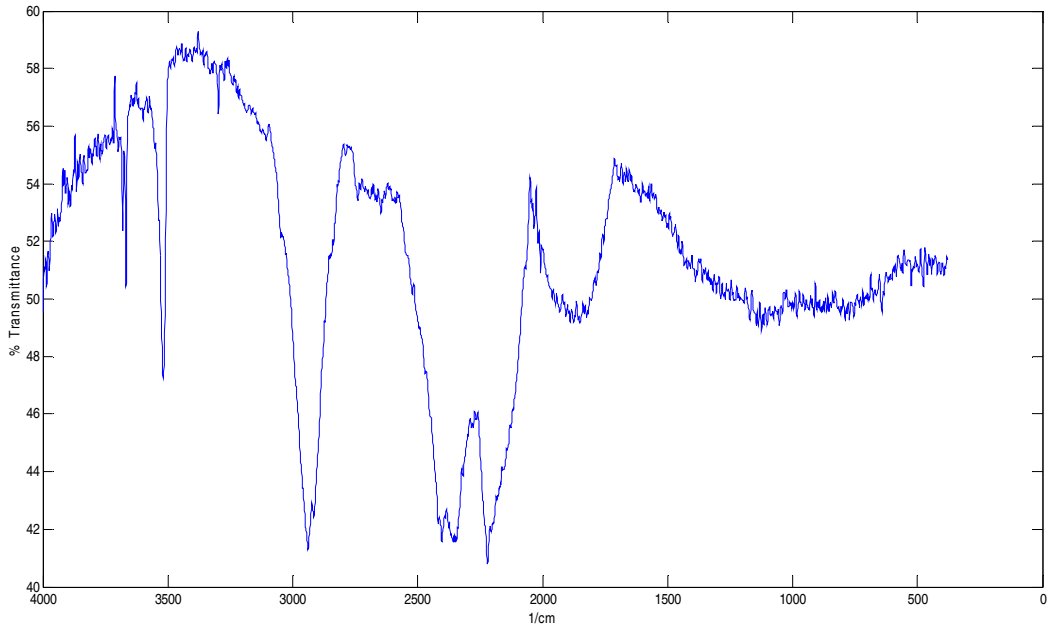

Figure 3.0: FTIR spectra of chitin

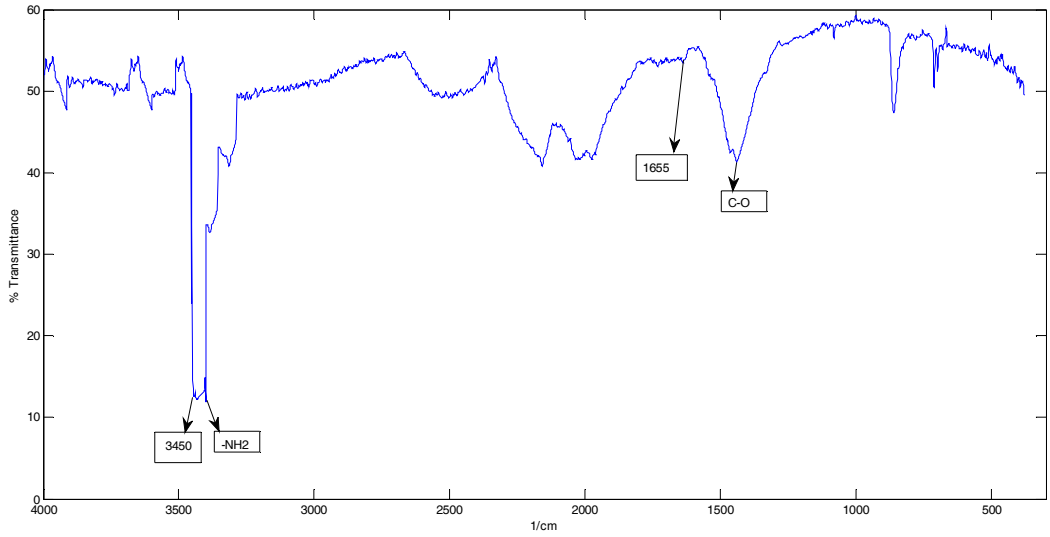

Figure 4.0: FTIR Spectra for Chitosan

Figures 3 and 4 are the FTIR spectra for the chitin and chitosan respectively; the FTIR spectra gave characteristics bands of $-NH_2$ at 3447cm^{-1} and carbonylgroup band at 1477cm^{-1}. It should be noted that the frequency ranges for the different classes of carbonyl compound overlap, and the carbonyl frequency alone is not sufficient to characterize the functional group (Coates, 2000).The degree of deacetylation was calculated using the baseline equation as used by Jiao *et al.,* (2010), the wavelengths 1655 and 3450 cm^{-1}were the baseline bands used in calculating the degree of

deacetylation. It is interesting to know that both the degree of deacetylation calculated using the carbon-nitrogen ratio and that from the FTIR spectra are almost similar, this further confirms the fact that the mussel cuticle can be used as a raw material for chitin and chitosan extraction.

4.0 Conclusion

Chitin and chitosan has been extracted and characterized from mussel shell found in banks of the Gubi Dam in Bauchi, Nigeria. The carbon nitrogen ratio of the chitosan extracted was 5.9 with a degree of deacetylation of 60.69% and 60.66% calculated from the elemental analysis and the FTIR spectra of chitosan respectively. The FTIR spectra for chitosan gave a characteristic $-NH_2$ band of 3447 cm^{-1} and a carbonyl group band of 1477 cm^{-1}. The mussel shell was discovered to contain a mineral content of 51.62% and a chitin composition was found to be 21.32%.

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