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Extraction, Characterization and Physico Chemical Properties of Chitin and Chitosan from Mud Crab Shell (Scylla Serrata)

KEYWORDS: Scylla serrata, Chitin, Chitosan, FT-IR, NMR

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ABSTRACT *In the present study, Chitin and Chitosan extracted from the shells of Scylla serrate. The chemical process involving demineralization, deproteinization, decolourizations and deacetylation. The process of deacetylation involves the removal of acetyl groups from the molecular chain of chitin. The purpose of this research is to observe the parameters that can enhance the degree of deacetylation of chitosan production to the highest percentage. The parameters such as temperature, concentration of sodium hydroxide and the time required for heating purpose were observed. The physiochemical characteristics and proximate composition were also determined. The yield of chitosan 38.23%, moisture content (0.40±1.63%), water binding capacity (333 ± 12.5%) and fat binding capacity (166±24%), were measured using FT-IR spectra and NMR. Result of the study indicates that crab shells are rich source of chitosan and this chemical constituent very much effects of biological and antimicrobial activities.*

Introduction
Chitin and chitosan hold great economic value due to their versatile biological activities and chemical applications, mainly in pharmaceutical areas (Kato et al., 2003). They are produced from chitin and chitosan, which is a natural carbohydrate polymer found in the exoskeleton of crustaceans, such as crab, shrimp and lobster, as well as in the exoskeleton of marine zooplankton, including coral and jelly fishes (Shahidi 2005). Chitin and chitosan photopolymer of 2-acetamido-2-deoxy-D-glucose (N acetylglucosamin) residues linked by ß-(1-4) bonds is a common constituent of insect exoskeletons, shells of crustaceans and fungal cell walls.Chitosan are used in dietary supplements, water treatment, food preservation, agriculture, cosmetics, textiles paper and medicinal application (Pradip 2004). There has been a large increase in chitosan research during the past decade. This is due to its biocompability, biodegradability, non-toxicity and other unique properties such as film forming ability and adsorption properties and antimicrobial activity (Kumar, 2000).

Different techniques have been proposed to evaluate the average degree of acetylating of chitin to chitosan, including infrared FT-IR (Domszy 1985). ^{13}C solid-state NMR (Raymond 1993) may be also used to determine the average degree of acetylation of chitin and chitosan as these techniques do not require the solubilization of the polymer. However, some experimental difficulties arise in each technique. For instance, the amide bond in the infrared spectrum. Among these techniques, ^{13}C solid-state NMR appears to be the most reliable for the evaluation of the acetyl content. The degree of acetylation is usually calculated by measuring the integral of the carbonyl or methyl group divided by the integral of all the carbon atoms in the backbone,(Raymond 1993). However, chitin is frequently associated with other polysaccharides like, which makes the evaluation of the acetyl content more problematic and in some cases, impossible. The need for a technique which can provide an evaluation of the acetyl content is obvious in the case of complex assembly of natural polysaccharides including chitin or chitosan derivatives.

The aim of the present study was to perform a characterization of the chitin and chitosan extracted from crab shell with chemical methods. Which was found to be more amenable for deacetylation. The chitin and chitosan were characterized by physicochemical parameters using FT-IR, NMR and degree of acetylation.

Materials and Method
The Mud crab shells were collected from local market in Coimbatore, Tamilnadu, India. The shells washed and dried under sun light. The viscera and tissues were carefully removed and placed in hot air oven at 60°C for 24 hours. The samples were weighed and packed into the airtight containers.

Deprotenization
Sagheer et al., 2009 was employed to deproteinization, demineralised and deacetylation shell wastes. The sample was then deprotenized with 300ml of 1N NaOH at 80 °C for 24 hour with constant stirring. The NaOH was exchanged intermittently and the sample was washed with distilled water every time before adding fresh NaOH. After 24 hour the sample was filtered. The sample filtrate was washed as before and dried. The weight was noted.

Demineralization
Demineralization 20gm of sample powder was demineralised with 300ml of 2N HCl or 24 hours with constant stirring and thus filtered. The filterate was again washed with distilled water and filtered till the liquid showed neutral pH. The filterate was then dried in a vacuum dryer and weighed.

Deacetylation
Chitin and chitosan extracted from crab chitin through deacetylated following the method of (Takiguchi 1991b). Briefly, chitin was deacetylated with 40% NaOH, heated for 6hrs at 110°C in constant stirring then 10% acetic acid was added to the sample and stored for 12hrs at room temperature with

Volume : 3 | Issue : 8 | Aug 2013 | ISSN - 2249-555X

constant stirring. The dissolved sample was reprecipatated by adding 40% NaOH to pH 10. The sample was then dialyzed by deionized water to a pH of 6.5 and centrifuged at 10,000 rpm for 10minutes and freeze dried.

Yield and Moisture of Chitosan

The chitosan yield was calculated by comparing the weight measurements of the raw material to the chitosan obtained after treatment. Moisture content of the prepared chitosan was determined by the gravimetric method (Black, 1965).

The % of moisture content = A (wet weight, g - dry weight, g ×100/wet weight).

Water binding capacity (WBC) and fat binding capacities (FBC) of chitosan were measured using a modified method of Wang 1976.

WBC (%) = (water bounded (g) /initial sample (g)) ×100

FBC (%) = (Fat bounded (g)/initial sample (g)) ×100

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR characterization of samples was performed with a Perkin Elmer-Spectrum RX1 instrument. For the preparation, 2% w/v chitin and chitosan was dissolved in 2% acetic acid solution, poured on a petridish, and finally dried at 60°C for 16 hours under vacuum. Then the sample is mixed with (Potassium bromide) to make a 13mm diameter pellet. The spectra of chitosan samples were obtained within a frequency range of λ = 500 - 4000 cm^{-1}, each spectrum is an average of 64 scans with a resolution of 2 cm^{-1} Lima et al., 2004.

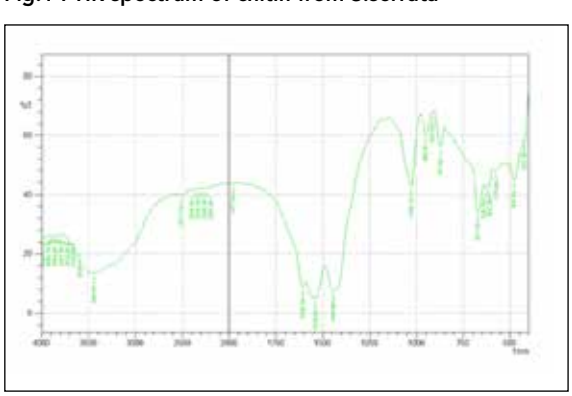
Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR spectra of samples were recorded using BRUKER 500 Ultrashield spectrophotometer in DCl in D2O solution. The experiments were run at room temperature in which the solvent (HOD) peak does not interfere with any chitosan peaks. 1ml of the chitin and chitosan sample solution was transferred to 5mm NMR tube. The sample tube was inserted in the magnet and allowed to reach thermal equilibrium for 10 minutes before performing the experiments (Heux et al., 2000).

Results and Discussion

Chitin and chitosan naturally abundant polysaccharide found particularly in the shell of crustaceans. It is white, hard; inelastic, nitrogenous polysaccharide forms the major source of surface pollution in coastal areas. It is a specially biopolymer having specific properties including biodegradability, biocompatibility and bioactivity. It is interesting not only as an abundant resource but also a novel type of functional material (Kobayashi et al., 1990).

Fig.1 FTIR spectrum of chitin from S.serrata



The crab shell chitosan prepared by using acid and alkaline treatments, the mean yield and moisture content of the extracted chitosan was found to be 38.23% and 40.16% water binding capacity and fat binding capacity of S.serrata shells extracted chitosan were 333.0±12.7% and166.0±24.9%. According to Cho and No, 1988, WBC and FBC of five commercial chitosan products ranged from 38.23% to 40.16% and 314% to 535% respectively.

Fig.2 FT-IR spectrum of chitosan from S.serrata

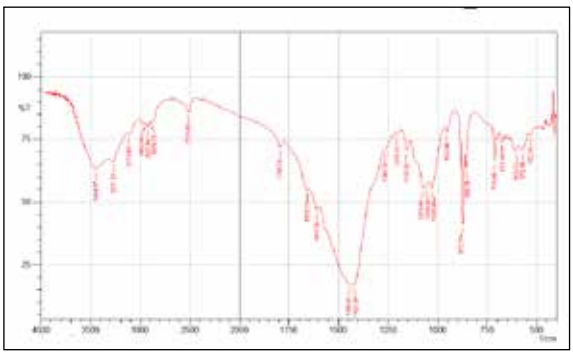


Table 1. FTIR Spectral Peaks of chitin from S.serrata

S.No	Wave Number (cm^{-1})	Possible Assignment of a Absorption Band	Nature of the Peaks
1	3441.01	H- banded OH stretching	Broad
2	2511.32	H- banded OH stretching	Broad
3	1606.7	Aliphatic CH-stretching	Sharp
4	2250.93	Aliphatic CH-stretching	Shoulder
5	1539.2	Amide=O stretching	Strong
6	1444.68	NH bending	Medium
7	1026.13	Pyranose ring bonding	Medium
8	950.91	Pyranose ring bonding	Medium
9	316.19	Amide C=O stretching	Strong
10	871.82-671.23	C-O-C stretching	Weak
11	615.29	C-O-C stretching	Weak
12	628.73- 434.34	Ring skeletal vibration	Very Weak

Table 2. FTIR Spectral Peaks of chitosan from S.serrata

S.no	Wave Number(cm^{-1})	Possible Assignment of a Absorption Band	Nature of the Peaks
1	3444.87	H-bonded NH2 &OH stretching	Broad
2	2519.03	Aliphatic CH stretching	Sharp
3	2879.72	Aliphatic CH stretching	Shoulder
4	1421.54	Amid C=O stretching	Sharp
5	1070.49	NH bending	Medium
6	1058.92	Ring banding	Medium
7	1029.99	Ring banding	Medium
8	8737.5	C-O-C stretching	Sharp
9	856.39	N=Q=N bending vibration	Strong
10	713.66	C-O-C stretching	Sharp
11	673.16-532.35	Pyranose bending vibration	Medium

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Fig 3. ^{13}C NMR spectrum of chitin from S. serrata

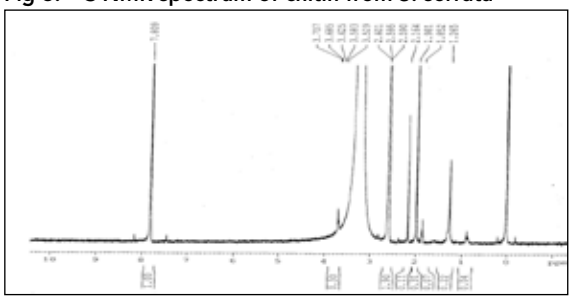
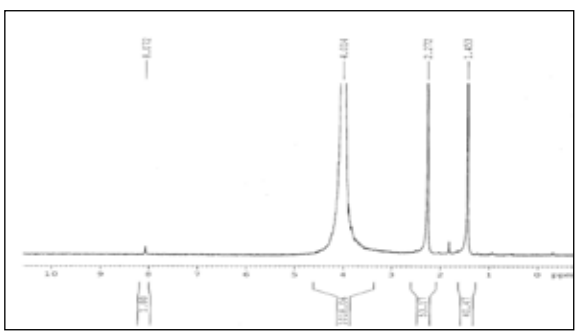


Fig 4. ^{13}C NMR spectrum of Chitosan from S.serrata



In the present study, the yield of chitosan from the S.serrata (38.23%) higher that of in P.sumisulatus, M.affinis (Al Sagheer 2009) and the silk worm, B.mori (Zhang 2000). However, the yield 40.032% was reported Yen and Yang, 2008 for the extraction of chitosan from the crab shells. The FT-IR spectrum of the chitin and chitosan recorded major peaks lying between 578.64cm^{-1} and 3668.78cm^{-1}; whereas the FT-IR spectrum of chitin and chitosan from S.serrata recorded peaks between (Fig.1). The wave number, possible assignment of absorption band and their nature of peaks of chitin and chitosan from S.serrata are given in (Table 2 &3).

In the present investigation, it is revealed that chitosan spectral data are in complete accordance with the compound structure expected. The structure of ^{13}C NMR spectrum of Chitin and Chitosan is presented in Figure 3 the solvent proton resonates at 7.809 and 8.072 ppm. In the present study, ^{13}C NMR spectra were recorded in D_2O/DCL at room temperature. All the chemical shift of the protons is shifted 1ppm downfield when compared to the NMR spectra observed in the literature (Lavertu 2003). FT-IR characterization of the chitin and chitosan was performed with FT-IR- 8400S instrument with a frequency range of 4000 to 500 cm^{-1}. It is clearly seen from the absorption patterns of the spectrum is similar to that of the literature and suggesting good quality of chitin and chitosan biopolymers has been obtained. Absorption peak is from Aliphatic C-H vibration of 2250.93 and 2519.03. Absorption band at represents the stretching vibration of the carbonyl group, C=O from acetamide (-NHCOCH3). Other characteristic absorptions for chitin are at 1539.92 and 1421.72 indicating the bending vibration of –NH and stretching vibration of –CN from acetamide group, respectively. The IR spectrum agrees with that reported in the literature Lima et al., 2004 for chitin and chitosan.

The present study chitosan was isolated from chitin Deacetylalion was determined by using two methods FTIR Spectrometer, Solid State ^{13}C NMR methods. This study concluded that FTIR, NMR method is one of the best methods of determination of Deacetylalion.

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