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Extraction and Characterization of Chitosan by Simple Technique from Mud Crabs

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A B S T R A C T

Chitosan is a naturally available biopolymer. It occurs as a component of crustacean shells, insect exoskeletons, fungal cell walls and plankton. In this work, chitin was extracted from mud crab shells waste. Chitosan, a useful biopolymer, was obtained from pulverized shells of crabs via demineralization, deproteination and de-acetylation. These steps were crucial for the elimination of calcium carbonate and other minerals as well as protein which are present in the shells. In this study, the chemical compositions of crab shells waste were analyzed by Fourier transform infrared spectroscopy (FTIR). From the 200 g exoskeleton of mud crab, 52.46 g (26.23%) chitin was extracted. Out of the 52.46 g chitin, 38.68 g (73.73%) chitosan was obtained.

Introduction

Chitosan is a partially deacetylated polymer of glucosamine (2 acetamido-2-deoxy b-1, 4 D-glucan). It is essentially a natural water-soluble derivative of cellulose with unique properties. Chitosan is used as a flocculent, clarifier, thickener, plant disease resistance promoter, anti-cancer agent, wound healing promoting and antimicrobial agent. The crab shell is formed from three basic components chitin, protein and calcium salt. Chitin is a fairly completely acetylated polysaccharide in nature, being only second after cellulose (Gaikwad *et al.*, 2015). Chitosan, which is a natural and linear polysaccharide made from chitin by a chemical process involving deproteinization, demineralization, decolouration and deacetylation, has received considerable attention because of its properties. Due to its fungicidal effects and elicitation of defence mechanisms in plant tissue, chitosan has become useful and highly appreciated as a natural biodegradable high molecular polymer compound that is a nontoxic and bioactive agent. Chitin and

chitosan are both polysaccharides, chemically almost like cellulose from which they only differ by the presence or absence of nitrogen (Banos *et al.,* 2006). Crab shell is additionally a source of chitosan, which is a polysaccharide. Crustacean shell waste consists of 25-30 per cent chitin, 25 per cent protein and 40-50 per cent calcium carbonate (Pandharipande and Bhagat, 2016).

Chitosan nanoparticles (CNPs) induce and augment immune responses in plants. CNPs treated leaves produced significant improvement in the plants innate immune response through induction of defence enzyme activity, upregulation of defence-related genes including that of several antioxidant enzymes as well as elevation of the levels of total phenolics (Chandra *et al.,* 2015). In the present study, attempts were made to extraction of chitosan from mud crab (*Scylla serrata* Forskal) and characterized.

Materials and Methods

Materials and sample collection

The mud crab (*Scylla serrata* Forskal) shells were collected from the Navsari fish market and shells were autoclaved and air-dried under sunlight. Dried shells then ground in small particles and sieved by passing through a 0.3-0.5 mm sieve. Hydrochloric acid(analytical grade) and sodium hydroxide (analytical grade) were used in chitin and chitosan preparation process. Distilled water was also used to prepare desired concentration of chemical solution and to wash the sample.

Extraction of chitin and chitosan

Then mud crabs shell particles were subjected to deproteinization, demineralization, decolourization (Figure 1) and deacetylation for preparation of chitosan powder as given below

Deproteinization

The mud crab shell waste from the carotenoid extraction process was treated with 4.0 per cent of NaOH solution with a ratio of ground shell to the solution of 1:20 (w/v) with constant stirring for 2 h at 90ºC to remove the protein.

The shell particles were then filtered and the filtrates were washed with tap water for 30 min until the pH becomes neutral (7). The deproteinized shells were dried in the oven at 60ºC for 24 h.

Demineralization

The deproteinized mud crab shells were demineralized with 2.5 per cent (w/v) of hydrochloric acid (HCl) at room temperature for 6 h to remove the mineral content with a ratio of ground shell to the solution of 1:20 (w/v).

The samples were then filtered and washed for 30 min with tap water until the pH becomes neutral (7). The demineralized shells were then dried in the oven at 60ºC for 24 h.

Decolouration and Dewatering

Decoloration was achieved by treating the samples with acetone for 10 min and drying for 2 h at ambient temperature and removed the residues of acetone.

The decolourized shell particles were then washed in running tap water, rinsed, filtered, and dried at 60ºC for 24 h in the oven to obtain crab chitin.

Deacetylation of Chitin

The chitin obtained was treated with 50 per cent (w/v) aqueous NaOH with a ratio of chitin to the solution of 1:15 (w/v) at 105ºC

for 2 h. Then, the chitin was filtered and washed with deionized water until the pH becomes neutral (7) to obtain the chitosan.

The chitosan obtained was then dried at 60ºC for 24 h in the oven. Finally, the product was pulverized and packed as chitosan.

FTIR analysis of chitosan

For determination of the functional groups in chitosan powder was measured by Fourier Transform Infrared Spectrometry and the analysis was done at Sophisticated Instrumentation Center for Applied Research and Testing, Vidyanagar, Anand, Gujarat.

The FTIR spectra were collected at a resolution of 4 cm^{-1} in the transmission mode (4000–400 cm^{-1}) on instrument make Spectrum GX, Perkin Elmer, the U.S.A. having range 10,000 cm^{-1} to 370 cm^{-1} and reflectance measurement were carried out by IR Quant spectrum search software.

Solubility test

Solubility of chitosan was calculated by solution of 10 mL of 1% acetic acid containing 0.1 g of produced chitosan was put in a centrifuge tube.

The sample was centrifuged at 10,000 rpm for 30 min. After the supernatant was poured away, the undissolved part of chitosan was washed with 25 mL of distilled water and then centrifuged at 6,000 rpm.

The supernatant liquid was poured away and the undissolved solid was dried at 60 °C for 24 h in an oven. The amount of the dried solid was weighed and the percentage of solubility was calculated (Demir *et al.,* (2016).

Results and Discussion

Yield of Chitin extraction and chitosan production

The yield of chitin extraction from 200 g exoskeleton of mud crab shells was 52.46 g (26.23%).The yield of chitosan produced from extracted chitin was 38.68 g (73.73%), which was similar to the results that was accordance with the finding of Gaikwad *et al.,* (2015), Demir *et al.,* (2016), Aung *et al.,* (2018), Bernabe *et al.,* (2020) and Sumaila *et al.,* (2020) who reported that the quality of chitosan produced from the crab was superior to fish chitosan. The yields were above average in these studies and our study indicated that crabs are one of the major resources of chitin and chitosan.

FTIR of chitosan

The FTIR spectrum (Figure 2) of extracted chitosan from crab shells showed a peak at 3441.26 cm^{-1} that indicated stretching vibration of hydroxyl (-OH) group, -NH group of amines and hydrogen bonding. 1644.91 cm^{-1} peak in extracted chitosan indicated the vibrations of (-C=O)) carbonyl group (amide band I). The peak at 1422.80 cm^{-1} showed the presence of amide II (N-H) bending band of -CONH-. For -CH group in CH_2OH peak were observed at 2924.64 cm^{-1} (-CH_2 Stretching), -CH_3 group of NHCOCH was shown at 1382.40 cm^{-1} and oxygen stretching of glycoside linkage was 1073.21 cm^{-1} (C-O-C stretching bridge of O-H groups). The pyranose ring was found at 896.46 cm^{-1}. The presence of the entire band stretching in the extracted chitosan compared with reported literature and from FTIR patterns of previous workers Demir *et al.,* (2016), Mythili and Aysha (2017) and Aung *et al.,* (2018) confirmed extracted material as chitosan**.**

Fig.1

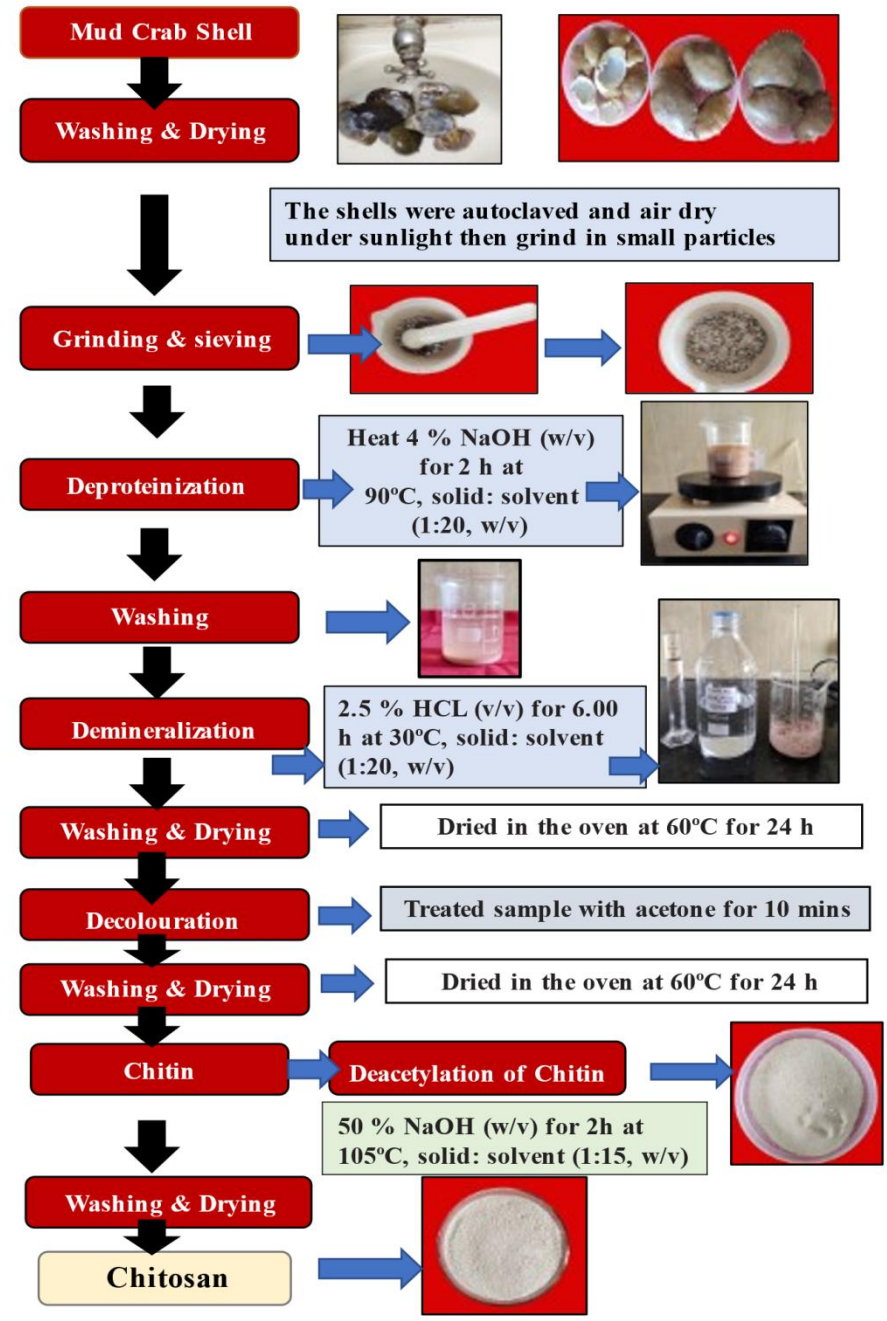
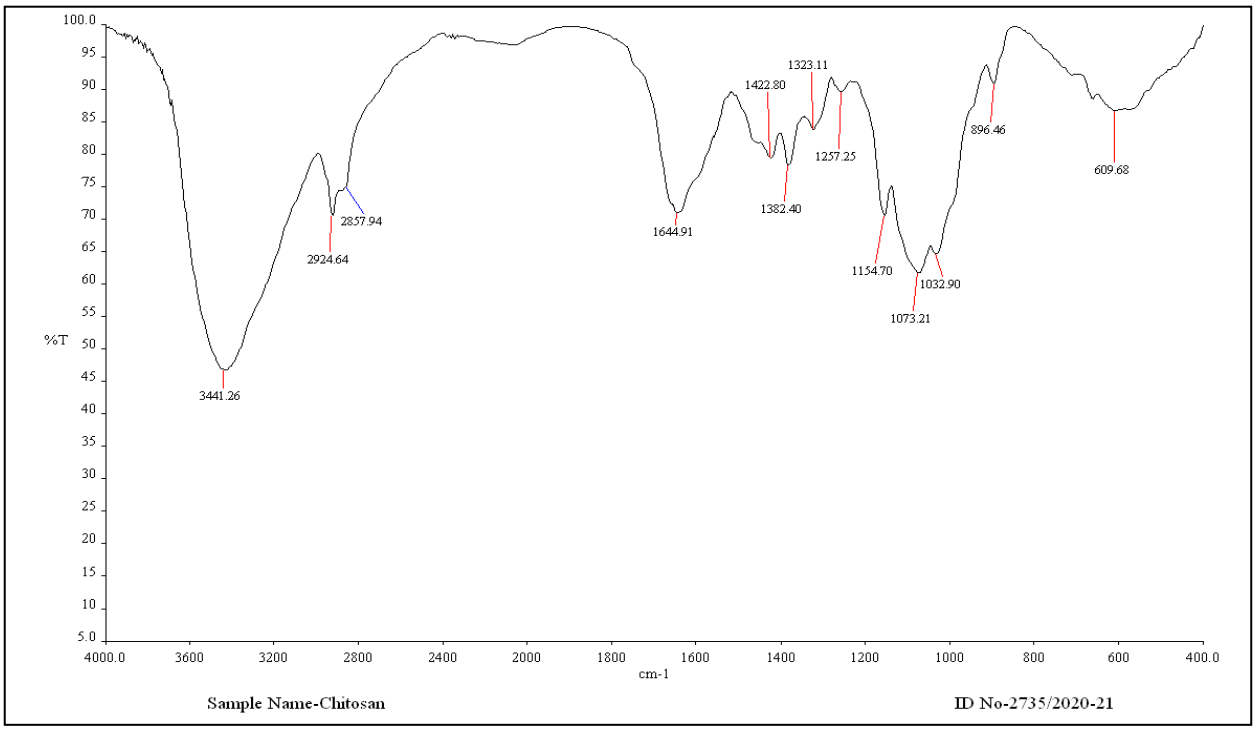


Figure 1 : Extraction of chitin and chitosan from mud crab shells

Fig.2 FTIR spectrum of Chitosan



Solubility

In the present study, mud crabchitosan, demonstrated an excellent solubility of 90.21% in 1% acetic acid solution. The high solubility of produced chitosan was due to the process conditions in deacetylation step (Demir *et al.,* (2016).

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