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Study of Extraction and Characterization of Chitosan and Investigation of its Properties

A.B. Janrao[1], M. B. Shinde[2] and S.B. Dahikar[3]

[1&2]Department of Zoology, Sanjivani Arts Commerce and Science College, Kopargaon Ahmednagar, M.S., India
[3]Department of Microbiology, Sanjivani Arts Commerce and Science College, Kopargaon Ahmednagar, M.S., India

ABSTRACT

Chitin (β-(1–4)-poly-N-acetyl-D-glucosamine) is a large generous highly hydrophobic and water-insoluble biomolecule that is a rich form of carbohydrates and has the potential to inhibit the growth of fungus. Chitin is indistinguishable from protein contains amino sugar and naturally occurs in the exoskeleton of crustaceans, mollusacans, coelenterates fungi. Chitosan , where deacetylated form and copolymer of chitin that is partially soluble in water and chitin has greater importance a biomedical and commercial use that is chitin, is used as supplementary diet and also for the production of medicine. Chitin has a great role in plant disease management with the Anti toxicants properties. For the purpose of the study the *Maydelliathelphus alugubris* (crab) was collected from the region of Kopargaon from stagnant water. In this study represents the extraction method of chitin and various chemical methods of Demineralization removal of inorganic salt, Deprotienization (removal of protein), Decolorization (to remove the colour) and the chitosan by Deacetylation for the removal of acetyl group. After grounding 88% of the grounded shell stuff was secured. After various chemical processes such as demineralization 83% stuff was secured, deproteinization 72% and After deacetylation 61% chitosan was obtained. Characterization of its nature that means the purity of chitosan is determined after the detection of its fictional group have done by FTIR (Fourier Transform Infrared) Spectroscopy. The result concluded that the commercially valuable chitosan was obtained from the shell of a crab.

Key words : *Chitin, Chitosan, FTIR-Spectroscopy, Deproteinization, Demineralization, Deacetylation.*

Introduction

All species of all prawn and crab primarily rely on the biopolymer chitin whose structure is nearly comparable to cellulose.

The crustacean shells that are obtained from the seafood industry are of very little commercial worth and are either used as organic manure or animal feed. Because of their high content and ready availability, the shells of crustaceans are the most significant source of chitin for industrial application.

The shells of crustaceans like crabs and shrimps are currently the main industrial sources of raw material for the synthesis of chitin. With aqueous solutions with a pH higher than 7, chitosan is typically insoluble; however, in diluted acids (pH6.0). There isn't a single chemical structure for chitin. Numerous polysaccharide compounds with N-acetyl-B-D-glucasamine units ranging from (50 to 100%) and D-glucasamine units ranging from (0 to 50%) are included. Chitin is a solid with a flocculent look that ranges in colour from pale yellow to brown. Additionally, water cannot dissolve chitin (Tanigava *et al.,* 1992). The freshwater crab (Potamonalgariense)

belongs to family potamidae, which is the largest freshwater crab family and comprises 95 genera and 505 species (Bott R., 1967). Crustaceans shell consists of 30 to 40% Protein and 30 to 50% calcium carbonate. 20 to 30% chitin but it varies in species to species and seasons (Cho *et al.*, 1998). (Limam *et al.*, 2011) have reported on the extraction and characterisation of chitin and chitosan from two species of crustaceans of Tunisian origin. biopolymers means something, that is originated made up from living matters; from the combination of molecules. There are large number of natural polymers considered as biomaterial such as collagen, silk, alginate, starch and elastin (Correlo *et al.*, 2005). The development of efficient and Safe gene carrier system that are capable of transferring DNA in the cells is a major goal of the gene therepy. Several literature shows that chitosan is a suitable material for efficient non-viral gene therepy (Jayakimar *et al.*, 2010). Chitosan exhibits antibacterial properties, and its film has demonstrated tremendous potential for use in food preservation. By lengthening the time between events, lowering the growth rate, or reducing the live counts of microorganisms, the antimicrobial action restricts or prevents microbial development, potentially supporting the use of chitosan film in food packaging and food preservation (Han, 2000).

Materials and Methods

Study area and sampling locations: For the study the crabs *Maydelliathelphus alugubris* were collected securely from the region of Kopargaon from stagnant river water of Godavari kept in cool ice bag to the laboratory.

Separation and cleaning of shells

The shells from the body of crab were separated and wasted with tap water to remove the excess mass. After the removal and washing, the shells were treated with the 1N HCL to remove organic salts and ions, and then the shells were dried in the oven at 60° to 70 °C overnight until they became dried completely. The dried shell then grounded with the help of mortar and pestle the size 600 to 800um.

Extraction of Chitin

Deproteinization

The grounded shell was treated by 2% KOH with a ratio of 1:10 by continuous stirring with the help of

Fig. 1. Separated crab shells.

Fig. 2. Grounded shell (stuff)

a magnetic stirrer. After that the sample was filtered and washed with deionized water until became naturalized pH. Then the stuff were dried in the oven overnight at 50 °C.

Decolourization

The deproteinized stuff the treated with Acetone to remove its colour untill colour of the chitin became natural. Further sample were filtered with whattman filter paper angain the precipitant kept in oven for drying purpose. The chitin was now ready.

Fig. 3. Decolourized chitin

Fig. 4. Chitosan

Extraction of Chitosan

The extraction of chitosan is performed by the process of deacetylation.

Deacetylation

Deacetylation has often been cited as an important parameter that determines many physiochemical properties of chitosan such as crystanility, hydrophilicity, degradation. The dried chitin was then treated with 20% of 100 ml NaOH solution, boiled at 50°c with continuous stirring for 1 hour. Then with the help of whattman filter paper the mixture was filtered and again cleaned with the deionized water untill pH became natural. The mixture was again filtered and dried in oven. The obtained chitosan was measured.

Results and Discussion

Chemical properties of chitosan

1. Chitosan is a linear polyamine.
2. It has reactive amino groups (-NH2). Consist carboxylic, hydroxyl and amide group
3. The chitosan also consist reactive hydroxyl groups(-OH).
4. It has chelating ability with many metal ions.

Table 1. Obtained stuff from crab shell.

Determination of percentage yield(%):	
Wt. of dried shells	18 gm
Wt of demineralized powder	15 gm
Wt. of deproteinized powder	13.4 gm
Wt.of deacetylated powder	11.2 gm

Table 2. Yield of chitosan (%) from crab shell

Percentage of raw shell stuff	88%
Percentage of demineralised stuff	83%
Percentage of deproteinized stuff	72%
Percentage of deacetylation stuff	61%

Characterization of Chitosan

Fourier transform infrared (FTIR) is one of the important analytical techniques for determination of organic compounds including chemical bond, as well as organic contents. In this study the characterization of chitosan was detected with Perkin Elmer spectrum 10.4.2 type FT-IR.

The infrared (IR) spectra of chitosan from the crab species *Maydelliathelphus alugubris* are shown in Fig. 08. The graph was interpreted on the basis of Shape and intensity of peaks. Hydrogen bonding consists of abroad or sharp peak. The hydroxyl group bears band in 3443.5-3259.5, and carboxylic group is present at the peak of band 1069.2- 876.34 both carboxyl group and carboxyl groups are identified and the percentage occurred of obtained chitosan is 61%. The different species of crab showed different yields of percentages of chitosan.

Conclusion

The current study confirms that the commercially valuable chitosan was obtained from the shell of crab performing various chemical processes. The graph was interpreted on the basis of the Shape and intensity of peaks. Hydrogen bonding consist of the broad or sharp peak. In the graph hydroxyl and car-

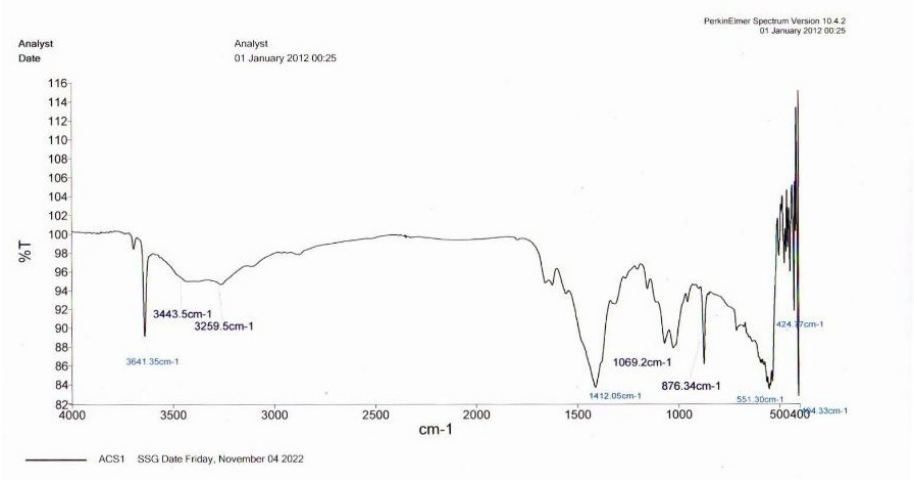

Fig. 5. Characterization of Chitosan obtained from Crab shell by FTIR

boxylic groups are present. The chitosan is useful for plant disease management and dietary supplement and different biological control measures.

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