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FULL LENGTH ARTICLE

Extraction of chitin from prawn shell and preparation of chitosan

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ABSTRACT
Shell waste produced by the seafood industry is one of the most important problems contributing to significant environmental and health hazards. The most frequent method employed for its disposal is burning, which becomes environmentally costly due to the low burning capacity of shells. In such a scenario, conversion of shrimp shell waste to chitosan a commercially valuable product with a myriad of uses, could serve as an effective mode of shell remediation. In the present study, chitosan is obtained from shell wastes of Penaeus monadon, Penaeus merguinesis and Penaeus indicus species by deproteination, demineralization, decolorization and deacetylation processes. The product obtained was analysed for physiochemical parameters like moisture content, pH, viscosity, residue on ignition, degree of deacetylation, solubility and FT-IR studies.
Keywords: *Chitin, Chitosan, Deprotenization, Demineralization, Deacetylation, FTIR spectroscopy*

INTRODUCTION

Chitin is the second most abundant natural polysaccharide after cellulose and is present in the crustacean exoskeleton, insects and fungi [1-5]. The shellfish industry generates a huge amount of shell waste which usually cause environmental nuisance. Alternatively, this waste can be utilized as an economic source of chitin and its derivative chitosan. Chitin and chitosan are considerably versatile and promising biomaterials. Chitosan, the deacetylated chitin derivative, is a more useful and interesting bioactive polymer. Despite its biodegradability, it can be chemically modified to produce derivatives, which have varied applications in the biomedical field. These derivatives are easy to produce and can be made commercially available easily. *Penaeus monadon, Penaeus merguinesis* and *Penaeus indicus* species are used for the present study. The shellfish industry is operative among all the coastal countries and contributes hugely to the food delicacies. During the processing of prawns and shrimps, mostly the meat is taken, while the shell and head portions are generated as wastes. This results in the generation of a huge amount of waste throughout the world. It is estimated that the shellfish industry produces about 60,000-80,000 tons of waste. The disposal of such an enormous amount of waste has become a serious environmental concern. Although these wastes are biodegradable, the rate of degradation of a large amount of waste generated per processing operation is comparatively slow. This results in accumulation over time and adds to environmental problems as they not only produce an obnoxious smell, but also attract pathogenic insects, flies and rodents, thus creating an unhygienic atmosphere. The shell and head wastes of crustaceans contain chitin, proteins and minerals. So, by demineralising and deproteinizing the wastes chitin can be obtained. Chitin can be used for various economical applications. Moreover, the chitin can be further deacetylated to produce chitosan, a valuable chemical substance having a wide range of viable uses. Various derivatives of chitin and chitosan can also be manufactured, which diversify the fields of application of these two chemicals. Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) [6]. It is prepared by treating shrimp and other crustacean shells with the alkali, sodium hydroxide. Chitin is made up of a linear chain of acetyl glucosamine groups while chitosan is obtained by removing enough acetyl groups (CH_3-CO) for the molecule to be soluble in most diluted acids. The actual difference between chitin and chitosan is the acetyl content of the polymer. Chitosan having a free amino group is the most useful derivative of chitin. Chitosan is a non-toxic, biodegradable polymer of high molecular weight and is very much similar to cellulose, a plant fiber [7]. The only difference between chitosan and cellulose is the amine (-NH_2) group in the position C-2 of chitosan instead of the hydroxyl (-OH) group found in cellulose. However, unlike plant fiber, chitosan possesses positive ionic charges, which give it the ability to chemically bind with negatively charged fats, lipids, cholesterol, metal ions, proteins, and

macromolecules. In this respect, chitin and chitosan have attained increasing commercial interest as suitable resource materials due to their excellent properties including biocompatibility, biodegradability, adsorption, and ability to form films, and to chelate metal ions. Chitosan has a number of commercial and possible biomedical uses [8-9]. It can be used in agriculture as a seed treatment and biopesticide, helping plants to fight off fungal infections. In winemaking it can be used as a fining agent, also helping to prevent spoilage. In industry, it can be used in a self-healing polyurethane paint coating. In medicine, it may be useful in bandages to reduce bleeding and as an antibacterial agent; it can also be used to help deliver drugs through the skin.

MATERIALS AND METHODOLOGY

Sample Preparation

The shells were obtained from local market of Mangaluru and Dharwad. Then the samples were washed with tap water and dried under sunlight. Dried samples were subjected to size reduction and sieve analysis. The sample size ranges from 0.3 mm to 0.8 mm.

Deproteinization

The samples were treated with 4 % NaOH solution at room temperature for 24 hours with constant stirring to achieve demineralization. Demineralized samples were washed with distilled water until pH becomes neutral.

Demineralization

Deproteinized shell samples were treated with 4 % HCl solution at ambient conditions. The solution was stirred for 12 hours to remove the minerals [10]. pH of the solution was increased to 7 by treating demineralized samples with distilled water to get raw chitin. Raw chitin was then dried at room temperature.

Purification of chitin

Chitin obtained was further treated with 2 % NaOH and 1 % HCl solutions and then washed with distilled water to get pure chitin.

Decolorization

Decolorization was carried out by treating chitin with 1% potassium permanganate solution about an hour followed by 1% oxalic acid for 30 minutes with stirring.

Deacetylation

Pure chitin samples were subjected to deacetylation to get chitosan by treating with 65 % NaOH solution at ambient conditions with stirring for 72 hours. Then alkali samples were washed with distilled water until pH reached neutral value. Further, chitosan samples were dried at room temperature. The chitosan obtained will be in a creamy-white form [11].

Characterization of chitosan

1 Moisture content determination

Moisture content of the samples were determined on wet basis. The samples were kept in an oven for 1 hour at 100˚C. The percentage moisture content was the difference between the weights of the wet and oven dried samples [12] and expressed as

$$Moisture\ \ content\ \ (\%) = \frac{(wet\ weight - dry\ weight)}{wet\ weight} x\,100 \qquad (1)$$

2 Ash content determination

Ash content of the chitosan was determined by combustion using a constant weight crucible. 1.0 g of chitosan sample was combusted in the crucible in an oven at 550˚C ± 20˚C for 3 hours until constant weight was achieved [13].

Ash content was then calculated in percentage as

$$Ash\ content\ \ (\%) = \frac{(initial\ weight - final\ weight)}{final\ weight} x\,100 \qquad (2)$$

3 pH

The pH measurement of chitosan solutions were carried out using pH meter and pH paper.

4 Viscosity

Viscosity was determined using Ostwald's viscometer by dissolving the chitosan samples in 1% acetic acid.

5 FT-IR

The spectra of the chitosan samples were measured in the spectral range from 400 cm^{-1} to 4000 cm^{-1} using Nicolet Impact-410, USA Fourier Transform-Infrared spectrometer in transmittance mode with a resolution of 4 cm^{-1}.

RESULTS AND DISCUSSIONS

The moisture contents of the chitosan were around 4.1%. The moisture content may vary depending on season, relative humidity and intensity of sunlight. The percentage moisture content values of the species are given in Table 1.

Table 1: Moisture content

Species sample	Moisture content (%)
Penaeus monodon	4.3
Penaeus indicus	3.9
Penaeus merguinesis	4.05

Chitosan is hygroscopic in nature. Hence, it can be affected by moisture absorption during storage. The ash content of chitosan is an indication of the effectiveness of the method employed for removing inorganic materials. The ash content values of chitosan are given in Table 2.

Table 2 : Ash content

Species sample	Ash content (%)
Penaeus monodon	1.37
Penaeus indicus	1.5
Penaeus merguinesis	1.18

Chitosan was soluble in 1% acetic acid at 50˚C. The solubility in acetic acid indicates the purity of chitosan. Chitosan, unlike chitin has high content of highly protonated free amino group that attracts ionic compounds. This is the reason for its solubility in inorganic acid. pH of the chitosan solutions were around 8. Viscosity of chitosan can be used to determine molecular weight. The pH of chitosan solutions were around 12 cP. High molecular weight chitosan yields high viscous solution. Hence low viscosity chitosan is more preferred [14]. The chitosan obtained had low viscosity. The Fourier Transform Infrared Spectroscopy (FT-IR) plot is as shown in Fig. 1 below. The absorption bands are listed in Table 3. The major absorption band is observed between 3438/cm and 1075/cm which represents the free amino group (-NH2) at C2 position of glucosamine, a major group present in chitosan. Further the sample showed the absorption bands at the various peaks 712, 896, 1421, 2856 and 2924 which is similar to standard chitosan. This shows the confirmation of chitosan [15].

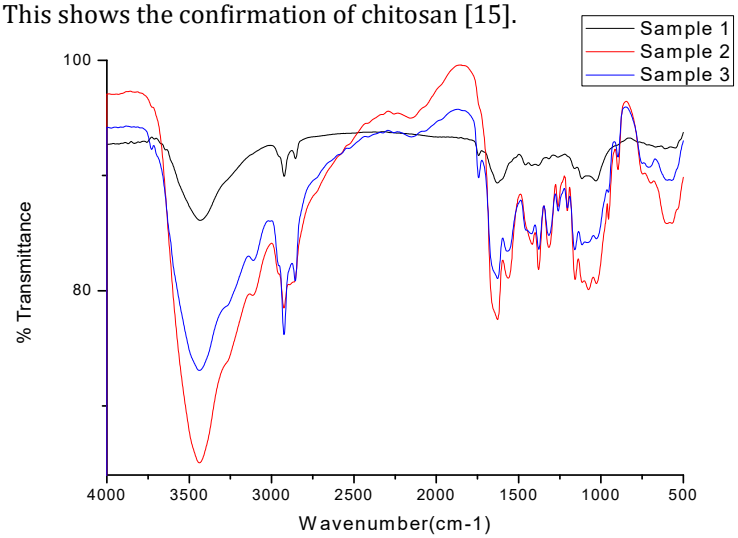

Fig. 1 : FT-IR plot for chitosan

Table 3 : Wavelengths of main bands

Sl. No	Wave number (cm^{-1}) [Expt.]	Wave number (cm^{-1}) [Lit.]	Possible assignment of absorption bond
1.	3438	3444	NH_2 stretching
2.	2924	2915	CH stretching
3.	2856	2879	Aliphatic CH stretching
4.	1421	1421	Amide C=O stretching
5.	1075	1073	CO stretching
6	896	896	Ring stretching
7	712	713	C-O-C stretching

CONCLUSION

Chitosan is prepared by using prawn shell materials by applying an appropriate treatment with dilute HCl and NaOH. The relative content of chitosan in these sources is determined. By employing FTIR study, all functional groups in chitosan macromolecules are elucidated. In the experimentally prepared chitosan, the bands were more pronounced than in the standard one, which proves the higher degree of morphological arrangement (higher degree of crystalline order) in the former.

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