International Journal of Environmental Engineering– IJEE
Volume 1 : Issue 4 *[ISSN 2374-1724]*
Publication Date : 27 December,2014

A Simple and Effective Method for Extraction of High Purity Chitosan from Shrimp Shell Waste

Divya K, Sharrel Rebello and Jisha M S

Abstract—***Shell waste produced by the sea food industry is one of the most important problems contributing significant environmental and health hazards. The most frequent method employed for its disposal is burning which becomes environmentally costly due to 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. Chitosan was obtained from shrimp shell waste by deproteination, demineralisation, decolourization and deacetylation processes. It was characterized using FT-IR, SEM and XRD. The physiochemical parameters like moisture content, pH, viscosity, residue on ignition, degree of deacetylation and solubility was also analysed.***

Results: Crude chitin was collected from shell of Penaeus monodon which was then processed to obtain chitosan. The chitosan yield was found to be 46%. Chitosan obtained had 5% moisture content, pH of 8 and 85% degree of deacetylation. Viscosity was 80cps. Residue on ignition was only 2% and was soluble in 1% acetic acid solution. The FT-IR, SEM and XRD data confirms the structure of chitosan.

Conclusion: Biopolymers like chitin and chitosan are important due to their biological and physiochemical properties. These properties offer many potential applications in various fields like environmental protection, agriculture, medicine, pharamaceutics and biotechnology. The current study demonstrated an effective method for extraction of high purity chitosan from shrimp waste.

Keywords—Penaeus monodon, chitin, chitosan.

Introduction

Sea food, a delicacy for many are seen in market in a wide variety of products. The sea food industries process and package the harvested products. During the processing, the meat is only taken, while the head and shells of shell fish are generated as waste. This results in generation of large amount of shell waste globally.

Divya K#, Sharrel Rebello, Jisha M.S.*
School of Biosciences, Mahathma Gandhi University,
Kerala, India.

The shell fish industry which is prominent in all costal countries generates about 60,000 to 80,000 tons of waste [1]. Even though the wastes are biodegradable, the dumping off large quantities makes degradation process slow resulting in accumulation of waste overtime which is a major environmental concern.

A quick and effective solution to this is recycling of shell wastes and extraction of commercially viable substances like chitin from them. Chitin on its own has various applications. This can further be deacetylated to form chitosan which has a wide range of uses [2].

Chitosan is a linear aminopolysacchride of (1→4) linked N-acetyl glucosamine and glucosamine units. It is a white, hard, inelastic and nitrogenous polysaccharide [3]. Chitosan finds a variety of applications due to its high biodegradability, non-toxicity and antimicrobial properties. It is used in biomedical industries, agriculture, genetic engineering, food industry, environmental pollution control, water treatment, paper manufacture, photography and so on [4].

Even though there are different methods for extraction of chitosan from shrimp shell, most are time consuming or low yielding. The present study aims to synthesize chitosan using a simple but effective method for producing chitosan.

Materials and Methods

1. **Sample Preparation**: Shells of *Penaeus monodon* or Giant Tiger Prawn were obtained from the shell fish industries in Cherthala, Kerala. The shells were washed, air dried and refrigerated overnight. This was then oven dried for four consecutive days at 65^0C.

2. **Extraction of Chitosan**: Chitosan was prepared using a combination of three procedures [5-7] . Five gram of shrimp shell waste was treated with 4% NaOH at room temperature for 24hours. The alkali was drained from the shells and washed with distilled water repeatedly till pH dropped to neutral. This process caused deproteinization of shells. The

SEEK DIGITAL LIBRARY

deproteinized shells were treated with 4% HCl at room temperature for 12hours for demineralization to yield chitin. The acid was drained off from chitin, washed with distilled water and finally dried at room temperature. The process was repeated with 2% NaOH and 1% HCl. The chitin obtained still had a slight pink hue. Further decolourisation was achieved by soaking chitin in 1% potassium permanganate for 30 mins followed by 1% oxalic acid for 30 mins to 2hours. The decolourised chitin was deacetylated to form chitosan by treating with 65% NaOH for 3 days at room temperature. Alkali was drained off and washed repeatedly with distilled water till pH was lowered. Chitosan was further dried at room temperature and stored.

3. **Estimation of Chitosan Yield**: The weight of chitosan produced is measured and yield is calculated.

4. **Characterisation of Chitosan**:

Composition Analysis: Moisture content and residue on ignition or ash content were analysed based on methods by Association of Official Analytical Chemists [8].

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

Viscosity: Viscosity of chitosan was determined at room temperature using a Brookfield digital viscometer [9].

Degree of Deacetylation: Chitosan homogenous solution was prepared using dil. HCl containing 0.01 mol/L and titrated against 0.1M NaOH. The end point was detected by the inflections of p^H values. Two inflections were mainly noted. First one corresponds to HCl neutralization and second to neutralization of ammonium ions of chitosan. The difference between two points give the amount of amino groups in chitosan chain also called the degree of deacetylation **DD % = 100- DA%** [10].

Solubility of Chitosan: Chitosan dissolves completely in 1% acetic acid. Weigh a few grams of chitosan and add 35ml 1% acetic acid. It was kept in a magnetic stirrer for 30mins. The sample was taken out and insolubles were removed by filtration through Whatmann No.1 filter paper and weighed.

X ray Diffraction spectrometer: XRD analysis of chitosan was used to detect its crystallinity. A Bruker AXS D8 Advance diffractometer was used for the purpose.

Scanning Electron Micrograph: The structure of chitosan was examined using scanning electron microscope (SEM/EDAX JEOL (Japan), Model JSM 6390.

FT-IR Studies of Chitosan: The chitosan and chitin samples were characterized from 4000 to 400 cm^{-1} using infrared spectrophotometer (Shimadzu IR Prestige 21 FT-IR – ATR attached).

Results and Discussion

Extraction of chitosan from shrimp shell requires harsh chemical treatments. The shrimp shell even though contains majority of chitin, also has proteins and minerals. Proteins are removed by deproteinization and carbon and other salts are removed by demineralization. Chitin is a linear polymer of 2-acetamido-2-deoxy-β-D-gluco-pyranose (Glc NAc). This is deacetylated to form chitosan. It is a linear binary polymer of Glc NAc and 2-amino-2-deoxy-β-D-gluco-pyranose (Glc N) [3].

The current method of chitosan extraction is superior to other available methods based on the higher yield of pure quality chitosan. Deproteinization and demineralization steps were repeated twice. This aided in higher yield of chitin from the shells. The final deacetylation of chitin at room temperature for 3 days gave a longer reaction time which resulted in higher yield of chitosan.

The chitosan yield was found to be 46%. The yield reported by [5] was 34%. Extraction of chitosan from crab shell reported a yield in the range 30-36.7%. The difference in yield is due to reaction time which has a positive effect on the yield [11].

The physiochemical parameters are tabulated in **Table 1.** The ash content of chitosan is an indication of the effectiveness of the method employed for removing inorganic materials. The ash content of 2% is due to the presence of calcium carbonate which is found in large amount in shrimp shells. It is lowest in squid pen chitosan, about 0.17% [12].

SEEK DIGITAL LIBRARY

The moisture content was 5%. Even though previous studies reported much less moisture content, permitted level is below 10%. The moisture content may vary depending on season, relative humidity and intensity of sunlight [13]

Viscosity of chitosan can be used to determine molecular weight. High molecular weight chitosan yields high viscous solution. Hence low viscosity chitosan is more preferred [3]. The chitosan obtained had low viscosity. Chitosan is fully or partially N-deacetylated derivative of chitin [2]. The degree of deacetylation was found to be 85%. The higher DD value is due to high amount of protein [5].

The solubility of chitosan in acetic acid is a mark of its purity. The concentration of chitosan in acetic acid is 7.7g/L. This indicates that the obtained chitosan was 77% pure. 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 [14].

The XRD pattern of shrimp chitosan (**Fig. 1**) showed characteristic peaks at $2\theta = 9.28^0$ and 20.18^0. The sharper peaks are an evidence of denser crystalline structure. The characteristic peaks of chitosan is reported in range of $2\theta = 9.9$- 10.7 and 19.8- 20.7 [15].

SEM analysis showed that chitosan had a long thin crystal structure on a smooth surface (**Fig 2**). This was in accordance with previous data [16]. Non-homogenous and non smooth surface structure of chitosan was also reported by [17]

The shrimp sample showed peaks at 1375 cm^{-1}, 1552 cm^{-1}, 1618 cm^{-1}, 1654 cm^{-1}. Peak at 1375 cm^{-1} corresponds to symmetrical deformation to CH_3 group. Peak at 1552 cm^{-1} corresponds to N-H deformation of amide II. 1618 cm^{-1} corresponds to vibration of amide I band and 1654 cm^{-1} corresponds to amide I stretching of C=O [18]. The bands can be seen in the **Fig. 3**. Chitosan spectra shows peak at 1029 cm^{-1} corresponding to free amino group at C2 position of glucosamine. It is a major peak of chitosan. The peak at 1375 cm^{-1} corresponds to C-O starching of primary alcoholic group [19]. The bands are seen in **Fig. 4.**

Table 1: Characteristics of chitosan extracted from *P.monodon*.

Characteristics	Chitosan
Chitosan yield	46%
Moisture content	5%
pH	8
Residue on ignition	2%
Viscosity	80cps
Degree of Deacetylation	85%
Solubility	Soluble in 1% acetic acid

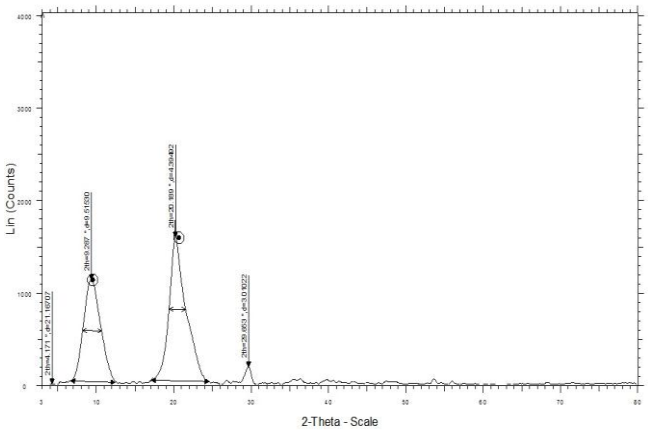

Figure 1: XRD pattern of chitosan extracted from *P.monodon*.

Conclusion

Chitosan was obtained using modified process of previous studies. The characteristics of produced chitosan were in accordance with the commercial standard. The obtained chitosan had low viscosity, high DD and a denser crystalline structure. Chitosan with such properties have many commercial applications and greater scope of industrial applications [20].

SEEK DIGITAL LIBRARY

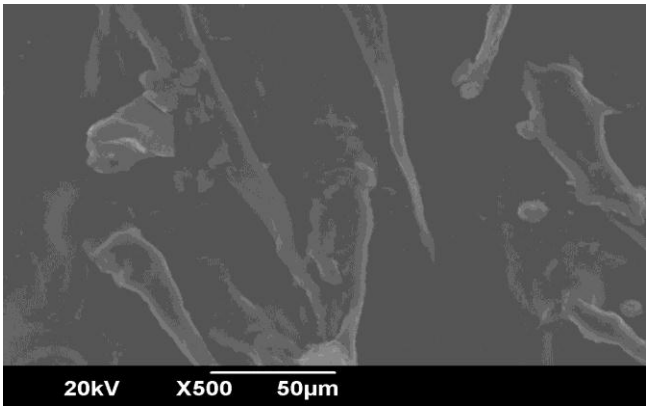

Figure 2: SEM micrograph of chitosan extracted from *P.monodon*.

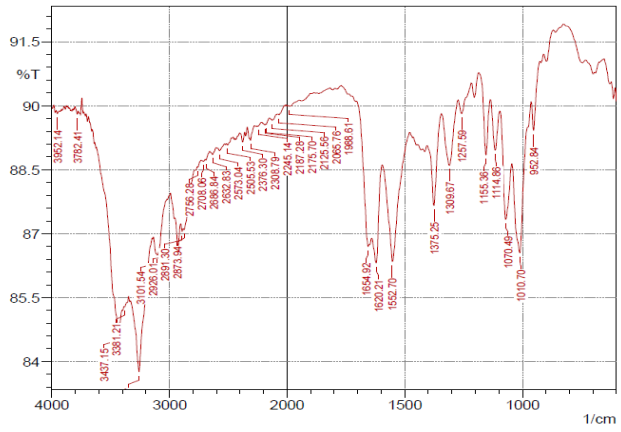

Figure 3: FT-IR spectra of chitin extracted from *P.monodon*.

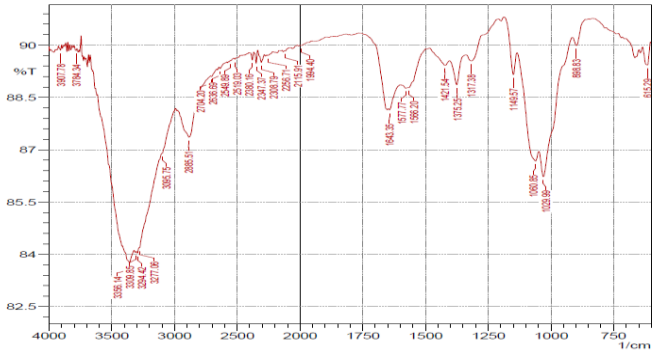

Figure 4: FT-IR spectra of chitosan extracted from *P.monodon*.

Acknowledgment

Authors convey thanks to Sophisticated Analytical Instrumentation Facility, CUSAT for providing XRD. We would like to thank School of Chemical Sciences Mahathma Gandhi University Kerala for providing SEM and we also thank DBT-MSUB, School of Biosciences, MG University for providing FT-IR.

References

1. **Muzzarelli R A A, J.C., Gooday G W *Chitin in Nature and Technology*. 1986, Plenum Publishing Corporation: New York.**
2. **Ravi Kumar, M.N.V., *A review of chitin and chitosan applications.* Reactive and functional polymers, 2000. 46(1): p. 1-27.**
3. **Badawy, M.E.I. and E.I. Rabea, *A biopolymer chitosan and its derivatives as promising antimicrobial agents against plant pathogens and their applications in crop protection.* International Journal of Carbohydrate Chemistry, 2011. 2011.**
4. **Cheba, B.A., *Chitin and chitosan: marine biopoly-mers with unique properties and versatile applications.* Global Journal of Biotechnology &. Biochemistry, 2011. 6(3): p. 149-153.**
5. **Puvvada, Y.S., S. Vankayalapati, and S. Sukhavasi, *Extraction of chitin from chitosan from exoskeleton of shrimp for application in the pharmaceutical industry.* International Current Pharmaceutical Journal, 2012. 1(9): p. 258-263.**
6. **Biswas, A.T. and Gargi, *Extraction of Chitosan from Prawn Shell Wastes and Examination of its Viable Commercial Applications.* 2013.**
7. **Annadurai, K.C.a.G., *Synthesis and Characterization of dye coated fluorescent chitosan nanoparticles.* J. Acad. Indus. Res, 2012. 1(14): p. 4.**
8. **Helrich, K.C., *Official methods of Analysis of the AOAC. Volume 2*. 1990: Association of Official Analytical Chemists Inc.**
9. **Mirzadeh, H., et al., *Preparation of chitosan derived from shrimp's shell of Persian Gulf as a blood hemostasis agent.* Iranian Polymer Journal, 2002. 11: p. 63-68.**

SEEK DIGITAL LIBRARY

10. **Zhang, Y., et al., *Determination of the degree of deacetylation of chitosan by potentiometric titration preceded by enzymatic pretreatment.* Carbohydrate polymers. 83(2): p. 813-817.**
11. **Yen, M.-T., J.-H. Yang, and J.-L. Mau, *Physicochemical characterization of chitin and chitosan from crab shells.* Carbohydrate polymers, 2009. 75(1): p. 15-21.**
12. **Shepherd, R., S. Reader, and A. Falshaw, *Chitosan functional properties.* Glycoconjugate journal, 1997. 14(4): p. 535-542.**
13. **Islam, M., et al., *Preparation of Chitosan from Shrimp Shell and Investigation of Its Properties.* International Journal of Basic & Applied Sciences. 11(1).**
14. **Mohammed, M.H., P.A. Williams, and O. Tverezovskaya, *Extraction of chitin from prawn shells and conversion to low molecular mass chitosan.* Food Hydrocolloids. 31(2): p. 166-171.**
15. **Prashanth, K.V.H., Kittur, F. S., & Tharanathan, R. N., *Solid state structure of chitosan prepared under different N-deacetylating conditions.* Carbohydrate Polymers, 2002. 75: p. 7.**
16. **Hwang, J.W.S.L., *Synthesis and characterisation of chitosan from shrimp shell*, UTAR.**
17. **Mohanasrinivasan, V., et al., *Studies on heavy metal removal efficiency and antibacterial activity of chitosan prepared from shrimp shell waste.* 3 Biotech: p. 1-9.**
18. **Limam, Z., et al., *Extraction and characterization of chitin and chitosan from crustacean by-products: Biological and physicochemical properties.* African journal of biotechnology. 10(4): p. 640-647.**
19. **Muhammed, R., et al., *Development and characterization of chitosan nanoparticles loaded with isoniazid for the treatment of tuberculosis.* Res J Pharm Biol Chem Sci, 2010. 1(4): p. 383-390.**
20. **Rinaudo, M., *Chitin and chitosan: properties and applications.* Progress in polymer science, 2006. 31(7): p. 603-632.**

About Author:

Chitosan, a natural polymer with a wide array of applications.

SEEK DIGITAL LIBRARY