

Extraction and ftir characterization of chitosan from *Portunus pelagicus* shell wastes

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Abstract

In the present study, Chitin a natural polymer is extracted from the *Portunus pelagicus* shell wastes. Its prime derivative chitosan is prepared from chitin groups with a strong alkaline solution. Yield of both chitin and chitosan was determined and yield percentage was calculated. Physico-chemical characterization of chitosan was performed by determining moisture and ash content. Fourier Transform Infrared spectroscopy (FTIR) was done to show the functional groups of extracted chitosan. The characteristic peaks of chitosan appeared confirmed high quality and purity of chitosan. Degree of deacetylation was calculated from FTIR spectrum which showed *Porunus pelagicus* shell wastes can be used to extract chitin and chitosan.

Keywords: Chitosan, Polymer, Moisture, Ash, FTIR spectrum

1. Introduction

Since antiquity, fishing and seafood consumption has played an important role in coastal regions of Tamil Nadu. Utilisation of marine resources for human consumption has increased rapidly worldwide. The shell fish industry is operative among all the costal countries and contributes hugely to the food delicacies. During the processing of prawns, shrimps, crabs and lobsters 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 wastes throughout the world (Tarafdar and Biwas, 2013). It is estimated that the shell-fish 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 but the rate of degradation of a large amount of waste generated per processing operation is comparatively slow (Prashanth and Tharanathan, 2007). This results in accumulation of shell wastes

that leads to obnoxious smell and attract pathogenic insects, flies and rodents, thus creating an unhygienic atmosphere. The immediate solution to this problem seems to be quick recycling of the crustacean shells generated and extraction of commercially viable substances that can be further used in other applications. Massive amounts of shells have been abandoned as wastes from worldwide seafood companies. The processing of human food production also causes 50–70% shells and residual meat (Basosidik *et al.*, 2013). Seafood processing industry in India is contributing tonnes and tonnes of waste materials and among them shrimp and crab waste occupy more than a lakh tonnes every year (Philip and Nair, 2006). In the past two decades, scientists have been researching on the recovery of valuable products from shell wastes.

After cellulose, chitin is the second most abundant natural biopolymer found in nature. Chitin is a linear biopolymer composed of 2-acetamido-2-deoxy-D-glucopyranose units linked by β glycosidic bonds (Subhapradha *et al.*, 2013). Chitin is usually isolated from the exoskeleton of crustaceans and more particularly from shrimps and crabs. It is also found in the cuticle of insects and cell wall of fungi (Ausa Chandumpai *et al.*, 2004). Chitin is insoluble in most of the solvents, because of its compact structure. The most common derivative of chitin is chitosan, which is a natural polysaccharide comprising of copolymers of glucosamine and N-acetylglucosamine (Dutta, 2004), and can be obtained by the partial deacetylation of chitin. When the degree of deacetylation (DDA) reaches higher than 50%, chitosan becomes soluble in acidic aqueous solutions and it behaves as a cationic polyelectrolyte (Nesr *et al.*, 2013). Over the past several years, chitinous polymers, especially chitosan, has received increased attention as one of the most promising renewable polymeric materials for its extensive application in the pharmaceutical and biomedical industries (Barwin Vino *et al.*, 2012).

Chitosan has received extensive applications in industrial and biomedical areas such as paper production, textile finishes, photographic products, cements, heavy metal chelation, waste water treatment and film formation (Ravi Kumar, 1999).

To reduce environmental pollution in the coastal areas and to recover useful by-product out of those biodegradable wastes, the present study aims to extract and characterize chitosan from *Portunus pelagicus* shell wastes collected from Mimisal, Tamil Nadu, India.

2. Materials and Methods

Extraction of chitin and chitosan from crab shell wastes

Demineralization

20gm of sample powder was demineralised with 300ml of 2N HCl at 80 °C for 24 hours with constant stirring in a magnetic stirrer at 800 rpm and then filtered. The filtrate was again and again washed with distilled water and filtered till the liquid showed neutral pH. The filtrate was then dried in hot air oven and weighed following the method of Takiguchi (1991a).

Deproteinization

The filtrate obtained after demineralization) was then deprotenized with 300ml of 1N NaOH at 80 °C for 24 hours with constant stirring in a magnetic stirrer at 800 rpm and then filtered. The filtrate was again and again washed with distilled water and filtered till the liquid showed neutral pH. The obtained filtrate was chitin and it was dried in hot air oven and weighed following the method of Takiguchi (1991b).

Deacetylation (Conversion of Chitin into Chitosan)

Chitosan was extracted from chitin through partial deacetylation process following the method of Takiguchi, 1991b. Chitin was deacetylated with 40% NaOH, heated for 6 hours at 110°C with constant stirring in a magnetic stirrer at 800 rpm and then filtered. To this filtrate 10% acetic acid was added and stored for 12 h at room temperature with constant stirring. The dissolved sample was reprecipitated by adding 40% NaOH, adjusting to pH 10. The obtained filtrate chitosan was dialyzed by deionized water to a pH of 6.5 and centrifuged at 10,000 rpm for 10 min and freeze dried.

Physicochemical characterization of extracted chitosan

Determination of yield of chitin and chitosan

The yield of the chitosan was obtained by comparing the weight of the raw material to the weight of the obtained chitin and chitosan after treatment (Mohanasrinivasan et al. 2013).

$$\text{Yield \%} = \frac{\text{Weight of the sample}}{\text{Weight of the raw material}} \times 100$$

Determination of Moisture and Ash Content in Chitosan

The moisture content of extracted chitosan was determined following the method of AOAC, (1990). The water mass of the sample was estimated by drying of 1g of the sample in a pre-weighed container and measured. The initial weight was noted. The container was placed in a hot air oven at 600° for 6 hours until constant weight was obtained and final weight was also noted.

$$\text{Moisture (\%)} = \frac{\text{Wet weight} - \text{Dry weight}}{\text{Wet weight}} \times 100$$

Ash content of the sample was estimated following the method of AOAC (1990) by incinerating 1g chitosan in pre-weighed tarred crucibles. The sample was heated in the muffle furnace at 600°C for a period of 6 hours. The crucibles were allowed to cool and weights of the residues were noted.

$$\text{Ash (\%)} = \frac{\text{Wet weight} - \text{Dry weight}}{\text{Wet weight}} \times 100$$

Fourier Transform Infrared Spectroscopy (FTIR) analysis

The FTIR spectrum of the extracted chitosan was determined according to the method of Mohammed et al. (2013). For the preparation, 1% w/v of chitosan was dissolved in 1% acetic acid and dried. The fine powder was thoroughly mixed with potassium bromide and made into KBr pellets. The FTIR spectra were recorded using a Thermo Nicolet Avatar 370 spectrophotometer in the frequency range of 400-4000 cm⁻¹. Each spectrum is an average of 64 scans with a resolution of 2 cm⁻¹.

Degree of Deacetylation

The DD of extracted chitosan was determined using FTIR spectrum with frequency of 4,000 to 400 cm⁻¹ following (Baxter et al., 1992; Abouzeed et al. 2015) equation.

$$DA \% = 100 - (A_{1637}/A_{2930}) \times 100$$

3. Results

Chitin was extracted from the shell wastes of *Portunus pelagicus* followed by N-deacetylation to obtain chitosan. The yield percentage was given in Table. 1. In our study, from the 20 g of the raw material yielded 7.41g chitin and the yield percentage was calculated as 62.95%. From chitin, 3 g chitosan was extracted and the yield percentage was calculated as 85%. Moisture and ash percentage of chitosan was 32.9% and 1.6% respectively (Table. 2). Physicochemical characterization of chitosan by FTIR technique was carried out to identify the functional groups present in it and to calculate the degree of deacetylation process.

The FTIR showed effective major peaks at the range of 3430.48, 3268.53, 3110.11, 2928.17, 2892.45, 2515.09, 1794.88, 1627.43, 1457.03, 1323.65, 1261.84, 1204.76, 1156.73, 1071.37, 1034.04, 954.53, 873.36, 751.50 cm^{-1} . The infrared characteristic peaks and the functional groups were presented in Table. 3 and Figure. 1. In the present study, extracted chitosan showed the alcohol O-H stretching band at 3430.48 cm^{-1} and the N-H stretching band of free amine groups was depicted in the range of 3268 cm^{-1} and 3110.11 cm^{-1} . The band observed at 2,928.17 and 2,892.45 cm^{-1} corresponds to asymmetric stretching of CH₃ and CH₂. The intensive peak appeared at 1457.03 cm^{-1} corresponds to NH₂ bending vibration of amine group is a characteristic feature of chitosan polymer. In addition, other bands in the region of 1323.65–1457.03 cm^{-1} , are attributed to the bending vibrations of the methyl groups. The band at 1071.37 cm^{-1} was identified as C–O stretching band. The out plane bending –OH vibrations are observed at 712.45 cm^{-1} .

Table. 1 Yield % of chitin and chitosan

Shell wastes (g)	Chitin (g)	Chitin Yield %	Chitosan (g)	Chitosan Yield %
20	7.41 ± 0.44	62.95	3 ± 0.29	85

Values are expressed as Mean ± S.D (n = 6)

Table. 2 Physicochemical characterization of Chitosan

Parameters	Values
Moisture (%)	32.9 ± 0.01
Ash (%)	1.6 ± 0.01
Degree of deacetylation (%)	84.13



Fig. 1 FTIR Spectra of Chitosan

Table. 3 FTIR spectral peaks of extracted chitosan

S.No	Wave Number (cm^{-1})	Absorption Bands	Nature of the Peaks
1.	3430.48	Hydroxyl OH stretching	Broad
2.	3268.53	H bonded NH ₂ stretching	Broad
3.	2928.17	Aliphatic CH ₃ stretching	Sharp
4.	2892.45	Aliphatic CH ₂ stretching	Medium
3.	1627.43	Amine I C=O stretching	Sharp
4.	1457.03	Amine II NH ₂ bending	Sharp
5.	1261.84	CH ₃ stretching asymmetrical	Sharp
6.	1204.76	CH ₂ stretching asymmetrical	Sharp
7.	1071.37	NH bending	Medium
8.	1034.04	Ring bending	Medium
9.	954.53	Ring bending	Medium
10.	873.36	N=O=N bending vibration	Sharp
11.	751.50	C-O-C stretching	Sharp
12.	607.55 – 578.42	Pyranose bending vibration	Sharp

4. Discussion

According to Cauchie, (1997) concerning the production of predominant biopolymer of the sea chitin produced by crustaceans, molluscs, insects were 100 billion tones every year. The yield% of chitosan from *Scylla serrata* was 38.23% (Kiruba et al. 2013) and *Scylla tranquebarica* was 10.74% (Thirunavukkarasu and Shanmugam, 2009) which were less when compared with our chitosan yield% from *Portunus pelagicus*. Yen et al., 2009 have showed crab chitosan% were in the range of 30-32%. Islam et al. 2011 showed that yield% was 15.21% and Mohanasrinivasan et al. 2013 as 17% only from shrimp shell wastes. Our results have proved the

highest yield of chitosan from *Portunus pelagicus* shell wastes. Thus economically and biologically valuable product chitosan was effortlessly extracted from low cost raw material. Moisture content less than 10% is more suitable for commercial purposes. The lower value of the ash content indicates the purity of the sample that complete demineralization and deproteinization have taken place (Mohanasrinivasan et al. 2013). Moisture and ash content were relatively high when compared with our results (Felicity Burrows et al. 2007; Sarbon et al. 2014; Walke et al. 2014). Chitosan is produced from chitin by partial N-deacetylation process with a strong alkaline solution like sodium hydroxide (Acharyalu et al. 2013).

The FTIR spectrum of extracted chitosan showed a larger intensity at 1457.03 cm^{-1} than at 1627.43 cm^{-1} which revealed effective deacetylation has taken place by removing acetamido (CH_3CONH) groups and forming more amine (NH_2) groups (Limam et al. 2011; Zvezdova and Uzov, 2012). Our FTIR spectrum are in accordance with the results of Chakraborty and Ghosh, (2010), Walke et al. (2014), Gokilavani et al. (2014), Sakthivel et al. (2015) and Thirunavukkarasu and Shanmugam, (2009).

5. Conclusions

Chitin is enormously available in the exoskeleton of crustaceans and in our study chitosan is extracted from *Portunus pelagicus* shell wastes with high yield%. This can be recommended for large scale production of chitosan of commercial standard. Environmental pollution in the coastal areas created due to operating of many small scale seafood processing industries can be greatly reduced by extracting this biopolymer from it. Chitosan, which is one of the main derivative of chitin can be obtained by N-deacetylation process from the shell wastes. This extracted chitosan may be widely used in biomedical and pharmaceutical companies due to its versatile properties.

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