EXTRACTION AND PURIFICATION OF CHITOSAN FROM SEA PRAWN (FENNEROPENAEUS INDICUS)

SNEHA PAUL1, AISWARYA JAYAN2, CHANGAM SHEELA SASIKUMAR1, SANJAY M CHERIAN3

1Department of Cellular & Molecular Biochemistry, Frontier Lifeline & Dr. K.M. Cherian Heart Foundation, Mogappair, Chennai - 600 101, India. 2Department of Biotechnology, Karunya University, Coimbatore - 641 114, Tamil Nadu, India. Email: sheelsasic@yahoo.co.in

ABSTRACT

Aim: The main study focuses on the purification of chitosan from chitin isolated from Fenneropenaeus indicus, will be suitable for the pharmaceutical industry.

Objective: Chitosan is an amino polysaccharide prepared by processing prawn waste (shell) which involves the demineralization, deproteinization and deacetylation. Chitosan is a versatile natural polysaccharide, its second most abundant natural polymer. Researchers have found that chitosan as biocompatible, biodegradable and non-toxic, which have made wide applicability in the pharmaceutical field.

Result: The chitosan yield was found to be 57.69%, and it was analyzed for its physiochemical parameters, antibacterial and antifungal activity.

Keywords: Chitosan, Chitin, Natural polymer, Characterization, Purification, Physiochemical parameters, Antibacterial, Antifungal activity.

INTRODUCTION

Chitosan is a very simple substance. It is derived from chitin, a polysaccharide that is found in the cell wall of fungi and also in the exoskeletons of crustaceans. They are then being processed by removing the shells from shellfish such as shrimp, lobster, crabs etc. [1]. It is obtained by the partial deacetylation of chitin; natural polymer composed of β-(1-4)-linked D-Glucosamine, randomly distributed. It consists of two types of monomers; chitin-monomers and chitosan-monomers [2].

Commercially, chitosan is produced between 3800 and 20,000 Daltons, obtained by the partial deacetylation of chitin; natural polymer composed of β-(1-4)-linked D-Glucosamine, randomly distributed. It consists of two types of monomers; chitin-monomers and chitosan-monomers [2].

Chitosan has been used over a wide range of applications, such as wound healing agents, drug carriers, chelating agents, membrane filter for water treatment and bio-degradable coating or film for food packaging. It is also used as a potential biomaterial that can be used for nerve repair. Chitosan is used in water purification by spreading the powder over the surface where any toxic substances such as greases, oils, or dangerous heavy metals are immediately absorbed, and the scum is then easily removed. Chitosan is an antibacterial agent. Chitosan is used in food as a preservative agent to prolong the freshness of the product and makes it taste better. Purified chitosans are used for medicinal purposes where they have contact with skin undergo a strict inspection process, this promotes in healing wounds. Chitosan and its derivatives, such as trimethylchitosan (where the amino group has been trimethylated), have been used in non-viral gene delivery [5].

The novel characteristic features of chitosan not only revile its property in the pharmaceutical industry, but also in drug targeting and delivery. This research is mainly focused on the synthesis of purified chitosan, to determine the various qualities attributes of its physiochemical properties such as molecular weight, viscosity, DD, dry weight, ash value, solubility, pH etc. The confirmation of chitosan was done by FTIR and X-ray diffraction (XRD).

METHODOLOGY

Isolation and extraction of chitosan

The exoskeletons of the prawn waste (shell) were removed separately and was rinsed thrice with tap water and then twice with distilled water. Then they were dried in a hot air oven for about 24 hrs at 55°C. The sample obtained was soaked in boiling 4% sodium hydroxide using 1000 ml beaker for 1 hr. The sample was removed and then allowed to cool at room temperature for 30 minutes. They were then crushed further to small pieces of about 0.5-5.0 mm [6].

Demineralization

The sample obtained was demineralized using 1% hydrogen chloride with 4 times its quantity. They were then soaked for 24 hrs to remove minerals. The above samples were treated with 50 ml of 2% sodium hydroxide for 1 hr. The remains of the sample were washed with deionized water and then drained off [7].

Deacetylation

The process was then carried out by adding 50% sodium hydroxide to the obtained sample on a hot plate and boiling it for 2 hrs at 100°C. The sample was then allowed to cool at room temperature for 30 minutes. Then they were washed continuously with 50% sodium hydroxide. They sample obtained is filtered (chitosan is obtained). The sample was then left uncovered, and oven-dried for 6 hrs at 110°C [8].

Purification of chitosan

The obtained chitosan has to be purified to make it suitable for use. The purification process was designed in three steps - removal of insoluble with filtration, reprecipitation of chitosan with 1 N sodium hydroxide, demetallization of retrieved chitosan [9].

Physiochemical parameters

Molecular weight: Intrinsic viscosity by Brookfield viscometer is used to determine the average molecular weight. The average molecular weight was obtained from Mark-Houwink equation:

$$\eta = KM^η$$
Where \([\eta]\) is the intrinsic viscosity, \(M\) is the average molecular weight of the solution, respectively and \(K\) and \(a\) are the Mark-Houwink constants specific for a given polymer [10].

**Moisture content**

Moisture content of the prepared chitosan was determined by the gravimetric method. The water mass was determined by drying the sample to constant weight and measuring the sample after and before drying. The water mass was the difference between the weights of the wet and oven dry samples [11].

Percentage of moisture content = \((\text{wet weight, g} - \text{dry weight, g}) \times 100\) \text{ wet weight, g}

**Loss on drying**

Loss on drying of the prepared chitosan was determined by the gravimetric method. The water mass loss was determined by drying the sample to constant weight and measuring the sample after and before drying. The water mass (or weight) obtained showed the difference between the weights of the wet and oven dry samples [12].

Percentage of loss on drying = \((\text{wet weight, g} - \text{dry weight, g}) \times 100\) \text{ dry weight, g}

**Ash value**

The ash value of chitosan was determined by taking the prepared chitosan sample which was previously ignited, cooled, and tared crucible. The samples were heated in a muffle furnace preheated to 650°C for 4 hrs. The crucibles were then allowed to cool in the furnace to <200°C and then were placed into desiccators with a vented top [13].

Percentage of ash = \((\text{weight of residue, g}) \times 100\) \text{ sample weight, g}

**pH and solubility**

The pH measurement of the chitosan solutions was carried out using a microprocessor pH meter. Solubility: The solubility of chitosan was demonstrated in various solutions like distilled water, acetone, ethanol, acetic acid and lactic acid. The chitosan obtained here got dissolved completely in acetic acid.

**DD**

IR technique was used for determining the DD according to the previously reported methods [14,15]. The percentage of the acetylated amine group was determined by

\[ \text{N-acetyl} \% = 100 - \left( \frac{A_{1652}}{A_{3446}} \right) \times 100 / 1.33 \]

**Characterization of chitosan**

The prepared biopolymer chitosan was analyzed by Shimadzu FTIR 8300 spectrometer in the wavelength between 400/cm and 4000/cm and in the solid state using potassium bromide pellets [16]. This polymer was also analyzed by XRD.

**Antibacterial and antifungal by well diffusion method**

The antibacterial assays were done on human pathogenic bacteria and fungi. The nutrient agar medium was poured into the petri plate. Allow the medium to solidify, 1 ml of inoculums was placed on the plates and spread it with cotton swab. Six wells around 10 mm diameter were cut out aseptically with the help of cork borer. Among six wells, one well was filled with 30 μl of diluted acetic acid solution and another five well with different concentration (10, 30, 60, 90, 120 μl) of chitosan solution. Incubate at 37°C for 24 hrs and note for zone of inhibition [17].

**RESULTS AND DISCUSSION**

An effort had been made to explore the physicochemical properties and antimicrobial activity as well as structural properties of prawn waste (shell) collected from Saiadapet market, Chennai. The results of physicochemical and functional properties of the prepared chitosan are given in the (Table 1) [Fig. 1].

<table>
<thead>
<tr>
<th>Physiochemical parameters</th>
<th>Chitosan</th>
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<tbody>
<tr>
<td>Yield</td>
<td>57.69%</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>159653 g/mole</td>
</tr>
<tr>
<td>Moisture content</td>
<td>4%</td>
</tr>
<tr>
<td>Ash value</td>
<td>1.86%</td>
</tr>
<tr>
<td>Loss on drying</td>
<td>2%</td>
</tr>
<tr>
<td>pH</td>
<td>6.7</td>
</tr>
<tr>
<td>Solubility</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>Degree of deacetylation</td>
<td>87%</td>
</tr>
</tbody>
</table>

The prepared Chitosan from chitin was confirmed as reported data [18]. Chitosan [19] from shrimp shell contains moisture in the range 1.0-1.30% depending upon the season, relative humidity and intensity of sunlight. There is no significant difference in the percentage of moisture content between the reported data elsewhere 1-1.30%. The report generated by KFDA [20] the moisture content of chitosan powder should be <10%. The moisture content obtained was in the range of 4%. Chitosan has very low ash value, 1.86%; indicates the efficiency of demineralization step followed in the preparation of the chitosan sample by removing the minerals. Commercial chitosan [21] is reported to have ash value about 1.18%.

The molecular weight of the prepared chitosan was variable due to various factors such as high temperature, alkali concentration, time of reaction, chitin concentration, dissolved oxygen deliberation, shear stress, etc. and the determined molecular weight is 159653 g/mole [22,23].

The solubility of chitosan was checked with five different solvents such as water, ethanol, NaOH, acetic acid and lactic acid. It was not soluble in alkaline or neutral solution, but was soluble in acidic condition, whereas you compare with lactic acid, it was more soluble in acetic 90-95% solubility was seen. The pH value of chitosan also varies from the range 6.2 to 8.0.

The DD is an important parameter to be noted affecting solubility, chemical reactivity and biodegradability. DD may range from 30% to 95% [24] depending on the available source and procedure. It is calculated by using the equation and FTIR (infrared spectroscopic) analysis of the prepared chitosan [25]. This study (Table 1) revealed that; DD of the prepared chitosan is 87%, 100% DD is very rarely obtained. Commercial chitosan with various DD in the range of 75-85% is generally found.

Chitosan was extracted from chitin got from prawn shell and further purified and confirmed by FTIR - Fig. 2, Table 2 and XRD - Fig. 3. The FTIR studies of the Chitosan from *Fenneropenaeus indicus* species.
Characteristics

Chitosan yield 67%. Then the major absorption band is observed between 1220/cm and 1020/cm which represents the free amino group (-NH$_2$) at C2 position of glucosamine, a major group present in chitosan. Further the sample showed the absorption bands at various peaks 712, 880.6, 1026, 1432, 1576.2, 1652.8, 2927.0, 3446.4, which is similar to standard chitosan. This shows the confirmation of chitosan [26].

XRD has also done to confirm the formation of chitosan and to determine the nature of chitosan powder. XRD patterns chitosan are illustrated in Fig. 3. The XRD pattern of chitosan shows broad diffraction peaks at 2θ = 10° and 21° which are typical fingerprints of semi-crystalline chitosan [27,28] found that fungal chitosan showed two characteristic reflections at 9.7° and 19.9°. Prashanth et al. in the year 2002 found that the wide-angle X-ray diffraction (WAXD) patterns of the shrimp chitosan showed two major characteristic peaks at 2θ = 9.9-10.7° and 19.8-20.7°. It is also reported that [29] the two characteristic crystalline peaks with slightly fluctuated diffraction angles found in the WAXD patterns indicated that two types of α- and γ-chitosans exhibited a comparable degree of crystallinity and had two consistent peaks of 9-10° and 19-20°, where as chitosan extracted from F. indicus showed three consistent peaks at 11.3, 19.0, and 33.0.

The antimicrobial properties of chitosan were done by varying the concentration and showed an inhibition toward Staphylococcus aureus and Candida albicans in various fields like pharmaceutical industry, food packaging, water treatment, drug delivery, etc. The source made from waste (chitosan) shows an excellent antimicrobial activity against human pathogens. Thus, it can be used as good potent source against the infectious pathogens.

REFERENCES

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