ISSN: 2277-9655
Impact Factor: 4.116
CODEN: IJESS7

IJESRT

INTERNATIONAL JOURNAL OF ENGINEERING SCIENCES & RESEARCH TECHNOLOGY

# REVIEW ON SYNTHESIS, CHARACTERISATION AND BIOACTIVITY OF CHITOSAN

**Nisha Pujari , S. L. Pandharipande**
[*] Chemical Engineering Department, Laxminarayan Institute of Technology, Nagpur, India

**DOI**: 10.5281/zenodo.160867

## ABSTRACT

One of the most widespread biopolymer in nature, after cellulose, is chitin. It can be extracted from sources like crustaceans, microorganisms and insects. However, the main commercial sources of chitin are shells of crustaceans such as prawns, crabs, lobsters and krill that are supplied in large quantities by the shellfish processing industries. Extraction of chitin can be done by two processes, chemical and biological. Chemical method requires acids and bases like sodium hydroxide, hydrochloric acid and acetic acid for deproteination, demineralisation and decolourisation processes respectively and microbial method includes the use of various micro-organisms for the similar steps as chemical process. The chitin obtained is further processed by deacetylation method using sodium hydroxide to convert into chitosan, another product having high industrial significance which possesses valuable properties like biocompatibility, biodegradability, antibacterial nature, film forming and fibre forming ability promoting its use in a variety of interesting applications. Applications of chitosan are found in industries such as textiles, medicine, food, agriculture, paper, cosmetics and wastewater treatment. The paper reviews the methods of synthesis, characterisation of chitosan with analytical methods like FTIR, SEM, NMR etc. and bioactivity determination of Chitosan as anti-microbial, anti-blood coagulant etc. properties.

**KEYWORDS**: Chitin, Chitosan, Characterisation

## INTRODUCTION

Chitin is widely found in insects, fungi, and yeast and marine invertebrates. However, in higher plants and animals, chitin is not present. Generally, the shell of selected crustacean consists of 30-50% calcium carbonate and calcium phosphate, 30-40% protein and 20-30% chitin. The principal source of chitin is shellfish waste such as shrimps, crabs, and crawfish. Although chitin itself is insoluble in water, on deacetylation it yields chitosan, a product having a wide range of viable uses. Chitosan, which is very much similar to cellulose, is a non-toxic, biodegradable polymer of high molecular weight. It is a co-polymer of glucosamine and N-acetylglucosamine and has generated interest due to its biocompatibility, high charge density, non-toxicity and mucoadhesion. The biological properties like biodegradability, adaptability, hemostatic activity and wound healing properties of chitin and chitosan attracted much attention to their biomedical applications. They are also used in water engineering, in the food and nutrients industry, film forming and coagulating ability and many more applications [1].

Until now, chemical, microbiological and enzymatic methods have been used for preparing chitosan from prawn shell powders. The chemical method involves demineralization, deproteinisation, and deacetylation steps using strong acids and/or alkali. However, the use of such chemicals seriously pollute the ecological environment and harm human health, produce abundant waste and can hydrolyse the polymer. With increased demands on environment-friendly culture, more eco-friendly processes have been worked upon by researchers using microbiological and enzymatic methods for producing chitin and chitosan. The enzymatic method consists the use of trypsase, papain, and pepsase. However, the high cost of enzymes and the low extraction are some of the pitfalls of these methods [2].

This paper reviews the methods of synthesis, characterisation and bioactivity determination of Chitosan from prawn shells.

ISSN: 2277-9655
Impact Factor: 4.116
CODEN: IJESS7

Seventeen papers reported in the literature have been studied and the review is presented in three parts: synthesis, characterisation methods and bioactivity determination.

## LITERATURE REVIEW

### Synthesis

#### *Chemical method*

Abhrajyoti Tarafdar [3] & Gargi Biswas [3] performed the extraction of chitosan from prawn shells and its use in successfully carrying out various applications in laboratory. They used inedible parts including head, body shells and tails of P. monodon and P. indica for extraction of chitosan. Experiment were performed using two different methods. In the first, 10 grams of prawn shell waste was used and washed. Demineralization process was carried out by adding 1.5N HCl at room temperature for 1hour followed by de-proteinisation with 0.5% NaOH at 100ºC for 30 minutes. This method helped to weaken the protein tertiary structure of the shells. The process was repeated for decolourisation with 3% NaOH at 100ºC for 30 minutes to obtain chitin slurry. Deacetylation of chitin yielded chitosan which was prepared by treating with 42% aqueous NaOH at 95°C for 1.5 hour and washed and then dried. In second method, biomass of shrimp waste collected was 5grams. It was then de-proteinised in 4% aqueous NaOH at room temperature (25ºC) for 21 hours. The de-proteinised shell was demineralized by 4% HCl at room temperature for 12 hours. The chitin was dried at ambient temperature. The Chitosan was obtained by treating chitin with 50% aqueous NaOH at 40ºC for 3 days. Finally, the chitosan was dried at ambient temperature. It was observed that chitosan produced employing process-II was more readily soluble in 1% acetic acid solution than that produced through process-I. It was confirmed that the chitosan obtained from P. monodon shells had better activity and quality than that obtained from P. indica shells.

Musarrat H. Mohammed, Peter A. Williams et.al. [4] performed the extraction of chitin and chitosan from prawn shells. Frozen prawn shells were initially washed with boiling water and then dried in an oven at 60ºC. These were crushed and powdered prawn shells were treated with 5% NaOH and refluxed at 60ºC for 2 h followed by treatment with acetone to remove pigments at room temperature for 24 h. These were further treated with a 0.5 or 1% HCl solution for 24 h at 25ºC to dissolve the calcium carbonate. The prawn shells were then washed several times with water to obtain. Deacetylation process was done by treating of extracted chitin with sodium hydroxide (NaOH) solution at elevated temperature and concentration. Effects of various parameters such as temperature, NaOH concentration and reaction times on the deacetylation process were investigated. Further, the chitosan produced was washed several times with distilled water, checked for pH neutrality and dried at 60ºC in a vacuum oven.

F. Nessa, Shah Md. Masum et.al. [5] evaluated the influence of deacetylation process in chitosan production on the physiochemical and functional properties of prawn shell chitosan. The prawn shells obtained were sun dried and ground into course particles through a centrifugal grinding mill. The dried prawn shell (1kg) was demineralized with 10% HCl acid at ambient temperature for 22 hrs. The demineralized shells were deproteinised with 10% sodium hydroxide solution for 24 hrs at 70º C. Samples were decolorized with acetone and dried under vacuum for 2-3 hrs until the powder was crispy. The resultant product was chitin. Deacetylation of chitin was achieved by using 60% NaOH solution. Four samples of prawn shell chitosan were prepared. The duration of deacetylation process was 45 hrs, 55 hrs, 65 hrs and 72 hrs. The resulting chitosan were rinsed to neutrality with distilled water, and dried at 65º C for 40 hrs in the oven. The yield of chitin was 20% and chitosan ranged from 16.4-19.6%. The prawn shell chitosan samples had a moisture content ranging from 0.3% to 0.4%. The nitrogen content of the prawn shell chitosan samples varied between 7.91% and 8.33%. The ash content of prawn shell chitosan range from 0.19% to 0.24%. The degree of deacetylation of prawn shell chitosan samples ranged from 45% to 75%. Three prawn shell chitosan samples B, C, D were found to have excellent solubility ranging from 96.01 to 97.2% with no significant difference but sample A which showed comparatively lower solubility (44.3%), may be due to lower degree of deacetylation (DD). WBC of the samples B, C & D varied from 738.3 % to 748.4% and FBC differed among chitosan products, ranging from 335.5% to 589.6%.

V. Mohanasrinivasan, Mudit Mishra et.al. [6] carried out the demineralization of shrimp shells, obtained from the local market of Vellore, where it was first suspended in 4 % HCl at room temperature and then washed with water to remove acid and calcium chloride. Then deproteinisation of shells was done further by treating it with 5 % NaOH at 90C. After the incubation time the shells were sun dried. The product obtained was chitin. Chitosan was prepared by deacetylating the obtained chitin with NaOH solution at room temperature for 72 h. The residue

ISSN: 2277-9655
Impact Factor: 4.116
CODEN: IJESS7

obtained was washed with running tap water, filtered, sun dried and was finely ground. The resultant whitish flakes obtained after grinding is chitosan. The prepared chitosan had a percentage yield of 17 % and an ash content of 2.28 % which when compared to commercial chitosan had 2 %. The solubilty of chitosan was found to be 1 % acetic acid solution and partially soluble in water and moisture content of chitosan was measured to be 1.25 %. In the present study, DD of the prepared chitosan was found to be 74.82 %. Chitosan prepared from shrimp shells has WBC and FBC of 1,136 and 772 %.

Divya K, Sharrel Rebello and Jisha M S [7] collected five grams of shrimp shell waste and treated it with 4% NaOH at room temperature for 24 hrs. The alkali was drained from the shells and the shells were then washed with distilled water followed by treatment with 4% HCl at room temperature for 12hours to yield chitin. Chitin was then filtered, washed with distilled water and dried at ambient conditions. Same process was repeated with 2% NaOH and 1% HCl. A slight pink colour was observed in chitin thus obtained. Chitin was soaked in 1% potassium permanganate for 30 mins and then in 1% oxalic acid for 30 mins to 2hours for decolourisation to take place. The decolourised chitin was then treated with 65% NaOH for 3 days at room temperature to form chitosan. This alkali was then drained off and washed repeatedly with distilled water till pH was neutral. The Chitosan obtained was further dried at room temperature and stored. The chitosan yield was found to be 46%. The degree of deacetylation was found to be 85% and the concentration of chitosan in acetic acid is 7.7g/L.

Anshar Patria [8] used the randomized design group of 3 x 4 with two factors in this study. Shrimp shells were procured from market of Banda Aceh city, Indonesia, washed, dried in sun light for 24 h and further dried in a furnace at a temperature of 80˚C for 24 h. 20 g samples of shrimp shell powders were left for 1 hour at 90˚C in 3.5 % NaOH. The solution was then filtered and the residue was washed and re-dried in a furnace at a temperature of 60˚C for 4 hours. Thus, chitin powder was formed. This is then added with 2-N-hydrochloric acid and allowed to stand for 1 hour at 90˚C. It is washed and dried in a furnace at 60˚C for 4 hours. The extracted chitin is then treated with acetone for 4 hours in soxhlet and bleached with 0.32 % sodium hypochlorite for 5 min at room temperature. A total of 5 g of chitin were reacted with 50 ml of 50 % sodium hydroxide at various temperatures, then filtered and thus chitosan is formed. Chitosan yield were ranged from 50.39 to 88.25 % with average of 67.42 %, while the chitin yield was 40 %.

K. Kamala, P. Sivaperumal and R. Rajaram [9] collected P. stylifera shrimp shell wastes from the Versova landing centre, Mumbai. Shells were thoroughly washed with running tap water and then placed in hot air oven at $60^0$C for 24 hours. 100 grams of shrimp shell powder was left in 1000 ml of 7% (w/w) HCl at room temperature for 24 h. Acid was washed with distilled water to neutral. Deproteination was carried out by immersing the residue in 1000 ml of 10% (w/w) NaOH at 60°C for 24 h for. To wash the residue to neutral, distilled water was used. To remove ethanol-soluble substances from the obtained chitin, 250 ml of 95% and absolute ethanol were used. Chitin was dried overnight in an air oven at 50°C. Chitosan was prepared by putting chitin in 50% NaOH for 8h at 60°C to prepare crude chitosan. After filtration, washings were given with hot distilled water at 60°C for three times. The crude chitosan was dried in an air oven at 50°C overnight. 1 gram of obtained chitosan was added in 20 ml of 2% (w/w) acetic acid. After reaction, to adjust the solution to neutrality, 10% NaOH was used. After filteration, two times the volume of ethanol were added to the filtrate. After incubation at ambient condition overnight, the crystal of water-soluble chitosan was liberated and dried in an air oven at 50°C. The yield of water soluble chitosan and crude chitosan was 87.8% and 54.3 %.

Sneha Paul, Aiswarya Jayanthe et. al. [10] removed the exoskeletons of the prawns separately, washed with tap water and distilled water and dried at 55°C for about 24 hrs in a hot air oven. The sample obtained was immersed in boiling 4% NaOH for 1 hr and then allowed to cool at ambient conditions for 30 minutes. Demineralisation was carried out using 1% hydrogen chloride in ratio of 1:4 for 24 hrs to remove minerals. Further deproteination was carried out by treating the samples with 50 ml of 2% NaOH for 1 hr. The remains of the sample were washed with deionized water. Deacetylation process further carried out by adding 50% NaOH at 100°C to the sample for 2 hrs. The sample was then cooled at ambient conditions for 30 minutes and then washed with 50% NaOH. The sample obtained is filtered, left uncovered, and oven-dried for 6 hrs at 110°C. The purification process of obtained chitosan was carried out in three steps - removal of insoluble with filtration, reprecipitation of chitosan with 1 N NaOH, demetallization of retrieved chitosan. The degree of deacetylation of the prepared chitosan was found to be 87% and yield upto 67%.

Arafat A., Sabrin A Samad et. al.[11] dried shrimp shells in sun for 2 days. After sun drying, the shrimp were grounded into powder. This powdered shrimp shell was demineralized with HCl at room temperature with 5%

ISSN: 2277-9655
Impact Factor: 4.116
CODEN: IJESS7

HCl for 24 hours with a ratio of 1:6. To remove acid and calcium chloride, these shells were rinsed with water and dried in an oven to 60°C. Demineralized shells were deproteinized with 5% NaOH solution for 48 hours at 60-70°C at a ratio of 1:10 (w/v). The residue was washed with distilled water to neutral pH and dried for 2 days. The product found is called chitin. Deacetylation process was carried out by treating samples with 60% NaOH solution and heated for 2 hours. After rinsing and drying, the deacetylated chitin i.e. chitosan was ready. The degree of deacetylation and yield of prepared chitosan was found to be 87% and 19% respectively.

Md. Monarul Islam, Shah Md. Masum et. al. [12] collected shrimp shell waste materials from Khulna, Bangladesh. Shrimp shells were scraped free of loose tissue, washed with cold water and sundried for 2 days. The shells were then suspended in 4% HCl at room temperature in the ratio of 1:14(w/v). After 36 hours, to remove acid and calcium chloride, the shells rinsed with water. Further deproteination was carried out by treating samples with 5% NaOH at 90°C for 24 hours with a ratio of 12:1(v/w). The washed to neutrality in running tap water. This obtained chitin was dried in sun. Deacetylation of chitin was achieved by using 70% NaOH solution with a ratio of 1:14 (w/v) at room temperature for 72 hours. The resulting chitosan was rinsed neutrality in running tap water and distilled water and dried in sun. The degree of deacetylation was 75% and yield was upto 15%.

Yateendra Shanmukha Puvvada et. al. [13] collected the shrimps exoskeletons from Suryalanka coast. To dissolve the proteins and sugars, crushed shrimps exoskeletons were soaked in boiling NaOH (2 and 4% w/v) for 1 hour. After this, the samples were allowed to cool for 30 minutes at room temperature and the exoskeletons were crushed to pieces of 0.5-5.0 mm. The grounded exoskeleton was demineralized using 1% HCl with four times its quantity for 24 h to remove the minerals. To decompose the albumen into water soluble amino-acids, the demineralized shrimp shell samples were subjected to deproteination process for 1 hour with 50 ml of 2% NaOH solution. This chitin is washed with deionized water, which is then drained off. The deacetylation process is carried out by adding 50% NaOH and then boiled at 100°C for 2 h. Afterwards the samples were washed continuously with the 50% NaOH and filtered in order to retain the solid matter, which is the chitosan. The samples were then oven dried at 110°C for 6 h. The obtained chitosan was purified in three steps, namely, removal of insoluble with filteration, reprecipitation of chitiosan with 1 N NaOH, demetallisation of retrieved chitosan. The chitosan yield was found to be 35.49%.

***Microbial method***

M. Khorrami, G. D. Najafpour et.al. [1] purchased Lactobacillus plantarum 1058 from Persian Type Culture Collection (PTCC). The MRS medium was used. The medium was autoclaved at 101 kPa for 20 min at 121 °C. The inoculated culture was cultivated in an incubator shaker at 30 °C and agitation rate of 180 rpm for 24 hours. The 100 mL shrimp shell powder broth was sterilized, cooled and inoculated with 5% of seed culture. In the incubator shaker, batch fermentation was carried out at 30°C and 180 rpm. This medium was incubated for 6 days. The raw chitin obtained, after fermentation was treated with 0.5 mol L–1 HCl for 2 hours at ambient conditions and then washed. This was further treated with 0.5 mol L–1 NaOH for 2 hours at room temperature and washed. The purified chitin was put into a flask with 55 % NaOH solution for 4 hours in a water bath at 95 °C, followed by washing and drying. The final product, chitosan, had a degree of deacetylation of about 83 %.

Hongcai Zhang, Yafang Jin et. al. [2] to extract chitin, fermented shrimp shell powders by successive two-step fermentation of Lactobacillus plantarum ATCC 8014 and Serratia marcescens B742 was used. The one step fermentation, identified optimal fermentation conditions were 2% SSP, 4 d of culture time, 2 h of sonication time, 10% incubation level and while that of using L. plantarum ATCC 8014 fermentation conditions were 2% SSP, 2 d of culture time, 10% incubation level and 15% glucose. Successive two-step fermentation resulted in chitin yield of 18.9% with the final deproteinization rate of 94.5% and demineralization rate of 93.0%. Results showed that the chitin prepared by the later method exhibited similar structural and physicochemical properties to those of the commercial one, while using less chemical reagents.

Jag Pal, Hari Om Verma et.al. [14] took lactobacillus cell and transferred it in 5 ml MRS broth and incubated it at 30ºC for 24 hrs. 2 ml of starter culture was taken and transfer in 100 ml sterile MRS broth and incubated at 30 °C for a further 24 hrs, thus making them ready for fermentation. Shell fish waste was ground properly and 10% of any carbon source followed by 10% culture inoculums was added to it. This was then incubated for 180hrs followed by filtering and the solid cake obtained was dried in hot air oven. In addition, deproteinisation can be made by adding exo-proteases or by proteolytic bacteria. Lactic acid producing bacteria carries out the calcium carbonate separation through the conversion of an added carbon source. Thus, chitin was extracted. The advantage

ISSN: 2277-9655
Impact Factor: 4.116
CODEN: IJESS7

of this technique is production of liquid fraction which is rich in protein and can be used for animal feed and humans.

Sumathi, S., Hamsa, D., et.al. [15] focused on the isolation of the prawn shell waste and its conversion to chitosan using microbial method. Latex milk of Euphorbia neriifolia, which has potent medicinal properties, was attempted to incorporate with chitosan and check its antimicrobial activity. The prawn fish shell waste was collected from the Ukkadam fish market in Coimbatore. The prawn shell waste were further washed thoroughly with tap water to remove the soil particles, adherent protein, soluble organics, and other impurities and then dried for 24 hours in a forced air oven at 60°C. Chitin was extracted from the prawn shell by deproteinisation and demineralisation. Chitosan was further prepared from chitin through deacetylation process. Its results proved the antimicrobial activity exhibited by the samples on test organisms.

Islem Younes, Olfa Ghorbel-Bellaaj et. al.[16] obtained the shrimp shells in fresh condition from a shrimp processing plant located in Sfax, Tunisia. Shell waste were washed thoroughly with tap water, and cooked with distilled water at a ratio of 1:2 (w/v) for 20 min at 90°C. The cooked sample was drained and kept at −20°C until further use. Two commercial enzymes, bromelain and alcalase were chosen as control for deproteinization experiments. Shrimp waste homogenate was mixed with distilled water in ratio of 1:3. The pH and temperature of the mixture, for each enzyme, were adjusted to the optimum conditions. To inactivate enzymes, the reaction was stopped by heating the solution at 90°C for 20 min. Demineralization was carried out in a dilute HCl solution of 1.5 M HCl in 1:10 (w/v) ratio for 6 h at 50◦C under constant stirring. The chitin product was filtered and washed to neutrality with deionized water and then dried for 1 h at 60°C. To deacetylate the purified chitin, it was treated with 12.5 M NaOH in 1:10 (w/v) ratio at 140°C for 4 h. This residue was filtered and washed with deionized water, and the crude chitosan was obtained by drying in a dry heat incubator at 50°C overnight. Results showed that chitosan dissolved at 50 mg/ml.

Dilyana Zvezdova [17] used different local resources like shrimp shell and crab to extract chitosan. Demineralization was carried out in all species by treating it with 7% HCl solution at room temperature with a ratio of 10 (v/w). Distilled water was used to wash the residue till neutral pH. Then the demineralized samples were dried and weighed. The demineralised shells were subjected to deproteinization using 10% NaOH at 60 °C. This was repeated several times. Then the resulting solution was washed with water till pH was neutral. The purified chitin was dried at 50 °C to constant weight. Deacetylation was prepared but putting the chitin obtained into 50% NaOH for 8 h at 60°C to prepare crude chitosan. After filtration, the residue was washed with hot distilled water repeatedly. The chitosan was obtained by drying at 50°C in an air oven for overnight. This chitosan gives higher degree of morphological arrangement than that of standard chitosan.

**Characterisation**

The chitosan synthesised is analysed for its quality using analytical methods like FTIR, XRD, SEM etc. for determination of functional groups, crystallinity, Surface morphology respectively. The details have been presented in Table 1 to 9.

***Table 1. Details of characterisaton method of chitosan***

| SR. NO. | TITLE | AUTHOR | REF. NO. |
|---|---|---|---|
| 1. | Production of Chitin and Chitosan from Shrimp Shell in Batch Culture of Lactobacillus plantarum. | M. Khorrami, G.D. Najafpour,H. Younesi and M. N. Hosseinpour | 1 |
| **CHARACTERISATION METHOD** | | | |
| **A). FTIR:** OH stretching: 3490-3489; C–H symmetrical stretching: 2920-2925; C=O in amide groups: 1644-1644; NH2 bending vibration: 1548-1548; C–O group in amide group: 1030-1027. | | | |

***Table 2. Details of characterisaton method of chitosan***

| SR. NO. | TITLE | AUTHOR | REF. NO. |
|---|---|---|---|

ISSN: 2277-9655
Impact Factor: 4.116
CODEN: IJESS7

| | | | |
|---|---|---|---|
| 2. | Production of chitin from shrimp shell powders using Serratia marcescens B742 and Lactobacillus plantarum ATCC 8014 successive two-step fermentation. | Hongcai Zhang, Yafang Jin, Yun Deng, Danfeng Wang, Yanyun Zhao. | 2 |

**CHARACTERISATION METHOD**

**A). SEM:** The smashed prawn shell powder displayed smooth microfibrillar crystalline structure, while the SEM images after demineralisation showed more slightly fracture than that of prawn shell powder after deproteinsiation with sonication treatment.

**B). FTIR:** Vibrations of –NH group: 1577 $cm^{-1}$; vibrations of -C-O group: 1654 $cm^{-1}$; vibrations of -CO-CH3 group: 2932 $cm^{-1}$; polysaccharide structures: 890 and 1156 $cm^{-1.}$

**C). The wide-angle X-ray diffraction (WAXD):** Purified chitin had WAXD pattern and showed two crystalline peaks at 2θ=9.3° and 19.1°.

***Table 3. Details of characterisaton method of chitosan***

| SR. NO. | TITLE | AUTHOR | REF. NO. |
|---|---|---|---|
| 3. | Extraction of chitin from prawn shells and conversion to low molecular mass chitosan. | Musarrat H. Mohammed, Peter A. Williams, Olga Tverezovskaya | 4 |

**CHARACTERISATION METHOD**

**A). FTIR:** Splitting of the amide I band: 1658 $cm^{-1}$; Intramolecular hydrogen bond: 1628 cm-1; NH stretching: 3262 cm-1 and 3114 cm-1; Mineral CaCO3: 1798, 1420-1430 and 876 cm-1; Protein has been removed: absence of peak at 1540 cm-1; Primary amine groups: 1606-1566 cm-1; NH2 deformation of primary amines: 1632 cm-1; NH2stretching: 3398 cm-1.

**B). NMR Spectroscopy:** DD was calculated by using integrals at 3.11 ppm and at 1.99 ppm.

**C).Gel Permeation Chromatography:** The polymer molecules eluting at peak 1 for 2 h have a Molecular weight of $1.3*10^6$ while those corresponding to peak 2 have a Molecular weight of $3.5*10^3$. At the longer reaction times of 5 h and 10 h, peak 1 elutes at higher elution times, corresponding to Mw values of $9.6*10^5$ and $3.1*10^5$ respectively. At a reaction time of 10 h average Mw values ranging from $0.6\text{-}2.1*10^5$.

***Table 4. Details of characterisaton method of chitosan***

| SR. NO. | TITLE | AUTHOR | REF. NO. |
|---|---|---|---|
| 4. | Studies on heavy metal removal efficiency and antibacterial activity of chitosan prepared from shrimp shell waste. | V. Mohanasrinivasan, Mudit Mishra et.al. | 6 |

**CHARACTERISATION METHOD**

**A). XRD:** The XRD pattern illustrates two characteristics broad diffraction peaks at 2θ = 10° and 20°.

**B). FTIR:** OH stretching vibrations: 3,450.65 $cm^{-1}$; Asymmetric stretching of $CH_3$ and $CH_2$: 2,924.09 and 2,852.72; Bending vibration of $NH_2$: 1,629.85 $cm^{-1}$.

**C). SEM:** The micrographs showed non-homogenous and non-smooth surface.

ISSN: 2277-9655
Impact Factor: 4.116
CODEN: IJESS7

***Table 5. Details of characterisaton method of chitosan***

| SR. NO. | TITLE | AUTHOR | REF. NO. |
|---|---|---|---|
| 5. | A Simple and Effective Method for Extraction of High Purity Chitosan from Shrimp Shell Waste. | Divya K, Sharrel Rebello and Jisha M S. | 7 |
| **CHARACTERISATION METHOD** | | | |
| **A). XRD:** The XRD pattern showed characteristic peaks at $2\theta = 9.28^0$ and $20.18^0$. **B). SEM:** Chitosan had a long thin crystal structure on a smooth surface. Non-smooth and Non-homogenous surface structure of chitosan was also reported. **C). FTIR:** Deformation to CH3group: 1375 $cm^{-1}$ corresponds to symmetrical. N-H deformation of amide II: 1552 $cm^{-}1$; Vibration of amide I band: 1618 $cm^{-1}$; Amide I stretching of C=O: 1654 $cm^{-1}$; Free amino group at C2 position of glucosamine:1029 $cm^{-1}$; C-O starching of primary alcoholic group: 1375 $cm^{-1}$. | | | |

***Table 6. Details of characterisaton method of chitosan***

| SR. NO. | TITLE | AUTHOR | REF. NO. |
|---|---|---|---|
| 6. | Extraction and Characterization of Water Soluble Chitosan from Parapeneopsis Stylifera Shrimp Shell Waste and Its Antibacterial Activity. | K. Kamala, P. Sivaperumal, R. Rajaram | 9 |
| **CHARACTERISATION METHOD** | | | |
| **A). FTIR**: Polysaccharide structure: 1155, 1078, 1032, and 899 $cm^{-1}$; amide I and III bands: 3425, 1651, and 1321 $cm^{-1}$; vibration of OH and CH: 1418 $cm^{-1}$; OH group: 3411 $cm^{-1}$; C–H stretching vibration: 2919 $cm^{-1}$; Bound water: 1610 $cm^{-1}$; the sugar units in polysaccharide: 842 and 877 $cm^{-1}$ | | | |

***Table 7. Details of characterisaton method of chitosan***

| SR. NO. | TITLE | AUTHOR | REF. NO. |
|---|---|---|---|
| 7. | Isolation and Characterization of Chitin From Prawn Shell Waste and Incorporation into Medical Textiles. | Sumathi S., Hamsa D., et.al. | 15 |
| **CHARACTERISATION METHOD** | | | |
| **A). FTIR:** The IR spectrum of the standard chitosan contained 15 major peaks whereas the IR spectrum of the sample from prawn waste also recorded 13 peaks. | | | |

***Table 8. Details of characterisaton method of chitosan***

| SR. NO. | TITLE | AUTHOR | REF. NO. |
|---|---|---|---|
| 8. | Chitin and chitosan preparation from shrimp shells using optimized enzymatic deproteinization | Islem Younes, Olfa Ghorbel-Bellaaja et. al. | 16 |
| **CHARACTERISATION METHOD** | | | |
| **A). CP/MAS-NM**: C1-C6 carbons of N-acetyl glucosamine monomeric: 50 and 110 ppm. The carbonyl group: 173 ppm; methyl group: 23 ppm; CH3: 23 ppm; C-O: 173 ppm | | | |

ISSN: 2277-9655
Impact Factor: 4.116
CODEN: IJESS7

*Table 9. Details of characterisaton method of chitosan*

| SR. NO. | TITLE | AUTHOR | REF. NO. |
|---|---|---|---|
| 9. | Synthesis and characterization of chitosan from marine sources in Black Sea | Dilyana Zvezdova | 17 |
| **CHARACTERISATION METHOD** | | | |
| **A). FTIR:** N-H in $NH_2$: 3425-2881 $cm^{-1}$; $CH_3$ group: 2921-2879 $cm^{-1}$; $NH_2$groups: The band at 1597 $cm^{-1}$; Amide II: 1559 $cm^{-1}$; N–H bending vibrations: 1600–1400 $cm^{-1}$; N–H bending mode of –$NH_2$: 1600.9 $cm^{-1}$; N–H bending mode of amide 1 band: 1647.19 $cm^{-1}$ | | | |

Comparative studies of FTIR spectra show that the –OH and symmetrical NH stretching vibrations are indicated by the wavenumber of around 3454 $cm^{-1}$ which may be indicative of OH group in chitosan. $CH_3$ and $CH_2$ stretching's indicated by the peaks of approximately 2924 and 2852$cm^{-1}$ showed the extent of conversion of chitin to chitosan. Bending vibrations of NH2 was observed at 1629 $cm^{-1}$. The mineral matter remains were indicated by the peaks 1798, 1420-1430, and 876 $cm^{-1}$. Complete removal of protein was indicated by absence of peak at 1540 $cm^{-1}$. Sugar units in polysaccharides were shown by the peaks at 842-877 $cm^{-1}$. XRD analysis showed characteristic peaks for chitosan at approximately $2\theta = 10^o$ and $20^o$. Comparative study SEM method on chitosan showed its thin crystalline, non - homogenous and non-smooth surfaces. Gel permeation chromatography showed molecular weights ranging from $0.6*10^5$ to $1.3*10^6$ depending on the reaction times.

**Bioactivity Measurements**

The antimicrobial and shelf life properties of chitosan have been reviewed and summarised. The details are presented in Table 2.

*Table 10. Details of Antimicrobial and Shelf-life studies*

| SR. NO. | TITLE | AUTHOR | REF NO. |
|---|---|---|---|
| 1. | Extraction of Chitosan from Prawn Shell Wastes and Examination of its Viable Commercial Applications | Abhrajyoti Tarafdar and Gargi Biswas | 3 |

| AREA OF APPLICATION | REMARKS |
|---|---|
| **A). Anti-Bacterial Activity:** The degree of deacetylation plays an important role in determining the antibacterial activity of chitosan as well. It was also observed that chitosan has profound antagonistic activity against gram negative bacteria than gram positive.<br>**b). Anti-Fungal Activity:** Inhibited the growth of fungi and that the anti-fungal activity of Chitosan enhanced with the degree of deacetylation.<br>**c). Chitosan As Food Preservative And Shelf-Life Enhancer:** The experimental sets, which were coated by chitosan showed no signs of spoilage after 1 week of storage and looked as fresh. The experimental cucumbers showed no microbial or fungal attack, though they had stated to ripen on the twelfth. The experimental set of tomato which were coated by chitosan were unchanged & looked as fresh without any sign of spoilage even after seven days. | The experiments carried out by the authors showed chitosan to be an anti-bacterial and anti-microbial product. It also can be used as a food preservative upto a limited number of days and increases the scavenging activity and also acts as an anticlotting agent for blood. |

ISSN: 2277-9655
Impact Factor: 4.116
CODEN: IJESS7

| | |
|---|---|
| **d). Blood Anti-Coagulant Activity:** For set-I, even after 1 hour 30 minutes, test-tube containing 7 ml chitosan showed no signs of clotting. For set-II, test tubes containing 5ml & 7ml chitosan-II solution, even after 1 hour showed no signs of blood clot formation.<br>**E). DPPH Radical Scavenging Activity:** The scavenging activity increased with increase in chitosan concentrations but the degree of deacetylation of chitosan affected the rate of radical scavenging. | |

***Table 11. Details of Antimicrobial and Shelf-life studies***

| SR. NO. | TITLE | AUTHOR | REF NO. |
|---|---|---|---|
| 2. | Studies on heavy metals removal efficiency in industrial effluents | V. Mohanasrinivasan, Mudit Mishra et.al. | 6 |

| AREA OF APPLICATION | REMARKS |
|---|---|
| **A). Heavy metals removal efficiency in industrial effluents**: The results indicated that the ability to adsorb the metal ions present in industrial effluents was possessed by the prepared chitosan. Out of all the metal ions Cu (II) was best absorbed showing removal of 98.97%.<br>**B). Inhibitory activity of chitosan:** The chitosan in the liquid medium inhibited the growth of Xanthomonas species and very less turbidity in the test flask was observed. | Experimental study of the authors proved chitosan to remove metal ions efficiently from industrial effluents and to possess inhibitory activity against Xanthomonas species. |

***Table 12. Details of Antimicrobial and Shelf-life studies***

| SR. NO. | TITLE | AUTHOR | REF NO. |
|---|---|---|---|
| 3. | Isolation And Characterization Of Chitin From Prawn Shell Waste And Incorporation Into Medical Textiles | Sumathi, S., Hamsa, D. et.al. | 15 |

| AREA OF APPLICATION | REMARKS |
|---|---|
| **A). Antibacterial Activity:** Among the three, higher concentration of chitosan showed maximum zone of inhibition followed by higher concentration of latex milk and the chitosan + latex milk.<br>**B). Antifungal Activity:** The percentage of spore germination was drastically reduced in Aspergillus flavus, Aspergillus fumigatus and Mucor. | These authors showed chitosan to be antibacterial and antifungal in nature. |

***Table 13. Details of Antimicrobial and Shelf-life studies***

| SR. NO. | TITLE | AUTHOR | REF NO. |
|---|---|---|---|
| 4. | Extraction And Purification of Chitosan from Chitin Isolated from Sea Prawn (Fenneropenaeus Indicus) | Sneha Paul, Aiswarya Jayan, Changam Sheela Sasikumar et. al.[] | 10 |

ISSN: 2277-9655
Impact Factor: 4.116
CODEN: IJESS7

| AREA OF APPLICATION | REMARKS |
|---|---|
| **A). Antimicrobial activity:** Chitosan showed an inhibition towards Staphylococcus aureus and Candida albicans types of tests microorganism. More prominent zone was seen in higher concentration of chitosan. | This study showed chitosan to be antimicrobial in nature. |

***Table 14. Details of Antimicrobial and Shelf-life studies***

| SR. NO. | TITLE | AUTHOR | REF NO. |
|---|---|---|---|
| 5. | Extraction and Characterization of Water Soluble Chitosan from Parapeneopsis Stylifera Shrimp Shell Waste and Its Antibacterial Activity | K. Kamala, P. Sivaperumal, R. Rajaram | 9 |

| AREA OF APPLICATION | REMARKS |
|---|---|
| **A). Antibacterial activity:** The water soluble chitosan showed higher zone of inhibition range as compared to crude chitosan. It was indicated that both chitosans might have the antibacterial inhibition mechanism. | This study showed chitosan to be antibacterial in nature. |

***Table 15. Details of Antimicrobial and Shelf-life studies***

| SR. NO. | TITLE | AUTHOR | REF NO. |
|---|---|---|---|
| 6. | Chitin and chitosan preparation from shrimp shells using optimized enzymatic deproteinization | Islem Younes, Olfa Ghorbel-Bellaaja, Rim Nasria et. al.[] | 16 |

| AREA OF APPLICATION | REMARKS |
|---|---|
| **A). Antimicrobial activity:** Chitosan obtained by deproteinisation with B. mojavensis proteases having low degree of acetylation showed higher inhibition activity when compared with the commercial one with higher degree of acetylation. | This study showed chitosan to be antimicrobial in nature. |

## CONCLUSION

Chitin and chitosan, both are natural aminopolysaccharides having unique structures and with properties such as biocompatibility, biodegradability, non-toxicity, with a wide range of applications. The raw material sources for their production included crustaceans' shells. An effort has been made in this paper to take a review of recently published papers on synthesis of chitosan from variety of natural resources of prawns and shrimps. Two types of synthesis methods have been reviewed that include chemical and microbial methods. The details of the process parameters employed by various researchers which include quantity and concentration of HCl and NaOH in Demineralisation, Deproteinisation and Deacetylation step with respective temperature and time conditions. In microbial methods details about the medium, temperature, quantities and microbial species with respective time duration have been reviewed and summarised. The paper also reviews the various characterisation methods employed by researchers in evaluation of quality of chitosan synthesised. These analytical methods include FTIR for determination of functional groups, SEM and XRD for surface morphology and surface crystallinity and NMR for magnetic properties of certain atomic nuclei. The paper also covers the findings of various researchers that studied the antimicrobial property of chitosan. Condensed form of summary of characterisation methods and antimicrobial properties along with interpretation has been given in tabular form that would help the prospective researchers.

## REFERENCES

ISSN: 2277-9655
Impact Factor: 4.116
CODEN: IJESS7

[1] M. Khorrami, G. D. Najafpour, H. Younesi and M. N. Hosseinpour, "Production of Chitin and Chitosan from Shrimp Shell in Batch Culture of Lactobacillus plantarum" in Chem. Biochem. Eng. Q.26(3) 217–223 (2012).
[2] Hongcai Zhang, Yafang Jin, Yun Deng, Danfeng Wang and Yanyun Zhao, "Production of chitin from shrimp shell powders using Serratia marcescens B742 and Lactobacillus plantarum ATCC 8014 successive two-step fermentation," in Carbohydrate Research, Volume 362, 15 November 2012, Pages 13–20.
[3] Abhrajyoti Tarafdar and Gargi Biswas, "Extraction of Chitosan from Prawn Shell Wastes and Examination of its Viable Commercial Applications," in International Journal on Theoretical and Applied Research in Mechanical Engineering (IJTARME), ISSN : 2319 – 3182, Volume-2, Issue-3, 2013.
[4] Musarrat H. Mohammed, Peter A. Williams and Olga Tverezovskaya, "Extraction of chitin from prawn shells and conversion to low molecular mass chitosan," in Food Hydrocolloids 31 (2013) 166-171.
[5] F. Nessa, Shah Md. Masum, M. Asaduzzaman, S. K. Roy, M. M. Hossaina and M. S. Jahan, "A Process for the Preparation of Chitin and Chitosan from Prawn Shell Waste," in Bangladesh Journal of Scientific and Industrial Research, 45(4), 323-330, 2010.
[6] V. Mohanasrinivasan• Mudit Mishra, Jeny Singh Paliwal, Suneet Kr. Singh, E. Selvarajan, V. Suganthi and C. Subathra Devi, "Studies on heavy metal removal efficiency and antibacterial activity of chitosan prepared from shrimp shell waste," in 3 Biotech DOI 10.1007/s13205-013-0140-6.
[7] Divya K, Sharrel Rebello and Jisha M S, "A Simple and Effective Method for Extraction of High Purity Chitosan from Shrimp Shell Waste," in Proc. of the Intl. Conf. on Advances In Applied Science and Environmental Engineering - ASEE 2014, ISBN: 978-1-63248-004-0 doi: 10.15224/ 978-1-63248-004-0-93.
[8] Anshar Patria, "Production and characterization of Chitosan from shrimp shells waste" in AACL Bioflux, 2013, Volume 6, Issue 4.
[9] K. Kamala, P. Sivaperumal and R. Rajaram, "Extraction and Characterization of Water Soluble Chitosan from Parapeneopsis Stylifera Shrimp Shell Waste and Its Antibacterial Activity," in International Journal of Scientific and Research Publications, Volume 3, Issue 4, April 2013.
[10] Sneha Paul, Aiswarya Jayan, Changam Sheela Sasikumar and Sanjay M Cherian, "Extraction and Purification of Chitosan from Chitin Isolated from Sea Prawn (fenneropenaeus Indicus)," in Asian Journal of Pharmaceutical and Clinical Research, Vol 7, Issue 4, 2014.
[11] Arafat A., Sabrin A Samad, Shah Md. Masum, Mohammad Moniruzzaman, "Preparation and Characterization of Chitosan from Shrimp shell waste," in International Journal of Scientific & Engineering Research, Volume 6, Issue 5, May-2015.
[12] Md. Monarul Islam, Shah Md. Masum, M. Mahbubur Rahman, Md. Ashraful Islam Molla, A. A. Shaikh and S. K. Roy "Preparation of Chitosan from Shrimp Shell and Investigation of its Properties," in International Journal of Basic & Applied Sciences IJBAS-IJENS Vol: 11 No: 01.
[13] Yateendra Shanmukha Puvvada, Saikishore Vankayalapati and Sudheshnababu Sukhavasi, "Extraction of chitin from chitosan from exoskeleton of shrimp for application in the pharmaceutical industry," in International Current Pharmaceutical Journal 2012, 1(9): 258-263.
[14] Jag Pal, Hari Om Verma, Vijay Kumar Munka, Satyendra Kumar Maurya, Deepayan Roy and Jitendra Kumar, "Biological Method of Chitin Extraction from Shrimp Waste an Eco-friendly low Cost Technology and its Advanced Application," in International Journal of Fisheries and Aquatic Studies 2014; 1(6): 104-107.
[15] Sumathi, S., Hamsa, D., Dharani, B., Sivaprabha, J., Malathy, N., Radha, P and Padma P.R, "Isolation and Characterization of Chitin from Prawn Shell Waste and Incorporation into Medical Textiles," International Journal of Recent Scientific Research, Vol. 3, Issue, 8, pp.676 - 680, August, 2012.
[16] Islem Younes, Olfa Ghorbel-Bellaaja, Rim Nasria, Moncef Chaabouni, Marguerite Rinaudo and Moncef Nasri, "Chitin and chitosan preparation from shrimp shells using optimized enzymatic deproteinization," in Process Biochemistry 47 (2012) 2032–2039.
[17] Dilyana Zvezdova, "Synthesis and characterization of chitosan from marine sources in Black Sea," in НАУЧНИ ТРУДОВЕ НА РУСЕНСКИЯ УНИВЕРСИТЕТ - 2010, том 49, серия 9.1.