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Bioconversion Of Seafood Waste Into Chitosan: A Step Towards Sustainable Biomaterials

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Abstract:
Chitosan from crustacean shell waste, providing a reproducible approach to produce high-quality biopolymer for environmental applications. The sequential treatment of raw shell material—comprising demineralization with dilute hydrochloric acid, deproteinization with sodium hydroxide, and thermal deacetylation—yielded chitosan with a high degree of deacetylation. Fourier Transform Infrared (FTIR) spectroscopy confirmed the structural conversion of chitin to chitosan, while solubility and yield analyses further validated its quality. The optimized chitosan demonstrated properties that make it suitable for heavy metal remediation studies, particularly for copper and zinc ions. Its high density of amino and hydroxyl groups enhances its affinity toward metal ions, supporting its application as a low-cost, biodegradable, and eco-friendly adsorbent. The findings indicate that shell waste, often discarded as an environmental burden, can be converted into a valuable resource for water treatment technologies. Overall, this work highlights the dual benefit of waste valorisation and pollutant removal, underscoring the potential of optimized chitosan extraction as a sustainable approach for heavy metal bioremediation.
Keywords: *Chitosan, Crustacean shell waste, Biopolymer, Deacetylation, Fourier Transform Infrared Spectroscopy (FTIR)*

INTRODUCTION

Chitin is the second most abundant natural polysaccharide after cellulose and plays a crucial role in providing mechanical strength to the exoskeleton of many living organisms. Marine resources, particularly the shells of crustaceans such as shrimp, crab, and lobster, serve as the primary commercial source of chitin. Chemically, chitin is a linear homopolysaccharide made up of several repeating units of N-acetyl-D-glucosamine linked through β-(1→4) glycosidic bonds (*Younes & Rinaudo, 2015*). The presence of the acetamide group renders chitin insoluble in water and weak acids, thereby limiting its direct applications despite its wide range of functional properties. This limitation led to the discovery and development of chitosan, a partially deacetylated derivative of chitin, during the mid-19th century (*Crini, 2019*).

Chitosan is a linear heteropolymer composed of D-glucosamine (GlcN) and N-acetyl-D-glucosamine (GlcNAc) units linked by β-(1→4) bonds. It is characterized as a cationic polysaccharide due to the presence of positively charged amino groups, which make it soluble in dilute acids. The ratio of GlcN to GlcNAc, referred to as the degree of deacetylation (DD), plays a key role in determining the physicochemical and biological properties of chitosan. Commercially available chitosan typically exhibits a molecular weight range of 50–2000 kDa and a DD of approximately 80–85%. Owing to its solubility, biodegradability, biocompatibility, chelating ability, and high adsorption capacity, chitosan has become one of the most valuable derivatives of chitin (*Hamed et al., 2016*). It has found applications across multiple sectors, including agriculture, food processing, pharmaceuticals, water treatment, and biotechnology. Moreover, chitosan demonstrates several functional activities such as antioxidant and antibacterial properties, as well as uses as a cholesterol-lowering agent, flocculant, texturizer, emulsifier, and clarifying agent. India is one of the largest producers and exporters of shrimp globally, with a significant share in the international seafood market. A total of 85 shrimp species have been recorded in Indian waters, of which about 55 are of commercial importance. During 2017–2018, India exported approximately 13.77 lakh tons of marine products, with frozen shrimp contributing a major portion of this export volume (*MPEDA, 2019*). Shrimp processing, however, generates substantial waste in the form of heads and shells, which account for nearly 40–50% of the total shrimp biomass. The by-products from shrimp processing plants in India exceed one lakh tons annually (*Kumar & Suresh, 2014*). These wastes are rich in valuable biomolecules such as protein (35–40%), chitin (10–15%), minerals (10–15%), and carotenoids (*Sachindra & Bhaskar, 2008*). Improper disposal of shrimp shell waste contributes to environmental pollution and poses a challenge for waste management. Converting this waste into

chitosan not only offers an environmentally sustainable solution but also provides an additional source of income for seafood processors. Therefore, the present study aims to extract chitosan from shrimp shell waste and investigate its structural, physicochemical, and functional properties to evaluate its potential for commercial and industrial applications.

Collection of Shrimp Shell Waste

Shrimp shell waste was collected from the Thoothukudi district. The shells were carefully removed and thoroughly washed with running tap water to eliminate sand, salts, and adhering organic matter. The cleaned exoskeletons were shade-dried for two days for further processing.

Sample Preparation

The dried shrimp shells were made brittle by sun drying for 2 days, after which they were ground into fine powder using a grinder. The powdered shells were stored in opaque plastic bottles at ambient temperature until use.

Extraction of Chitin and Preparation of Chitosan

Chitosan preparation was carried out in three consecutive steps: **demineralization, deproteinization, and deacetylation**.

1. Demineralization of Shrimp Shells

The finely powdered shrimp shells were subjected to demineralization using **5% HCl solution** (solid-to-solvent ratio 1:6 w/v) at room temperature for 24 hours. After treatment, the shells became soft and squashy. They were rinsed with distilled water until neutral pH to remove residual acid and calcium chloride, then oven-dried at 60 °C.

To ensure complete demineralization, a small portion of the treated shells was reacted with **10% HCl**. The absence of bubble generation confirmed the removal of calcium carbonate. (*Arafat et al., 2015)*

2. Deproteinization

The demineralized shells were treated with **5% NaOH solution** (solid-to-solvent ratio 1:10 w/v) at 60–70 °C for 48 hours (Arafat et al., 2015). After treatment, the residue was thoroughly washed with distilled water until neutral pH was achieved. The product obtained after drying for 2 days was **chitin**.

3. Deacetylation (Preparation of Chitosan)

Chitin was converted to chitosan through deacetylation by treating it with **60% NaOH solution**. The reaction mixture was heated in a domestic microwave oven for 2 hours. The deacetylated chitin (now **chitosan)** was rinsed thoroughly with distilled water until neutral pH and dried at 60 °C. The final dried product was **chitosan**, ready for further studies



Characterization (pH, ash, moisture, solubility, FTIR, Degree of Deacetylation).

Chitosan Characterization *(Rashmi et al., 2016)*

Moisture Content Determination

Moisture content of the chitosan samples was determined on a wet basis. The samples were kept in a hot-air oven at 100 °C for 1 hour. The percentage moisture content was calculated

Moisture content (%) = (Wet weight – Dry weight) / Wet weight × 100

This parameter is important because excess moisture can promote microbial contamination and reduce storage stability *(Rashmi et al., 2016).*

Ash Content Determination

Ash content of chitosan was determined by combustion in a constant weight crucible. A 1.0 g chitosan sample was combusted in a muffle furnace at 550 °C ± 20 °C for 3 hours until constant weight was

achieved.

Ash content (%) = **(Initial weight – Final weight) / Initial weight × 100**

Ash content represents the inorganic residue (e.g., calcium carbonate, salts, minerals) present in the sample and is an indicator of the purity of chitosan *(Rashmi et al., 2016; Ghorbel-Bellaaj et al., 2012).*

pH

The pH of chitosan solution (prepared in 1% acetic acid) was measured using a pH meter and verified with pH indicator paper. The pH value influences the solubility and biological compatibility of chitosan *(Rashmi et al., 2016).*

Viscosity

Viscosity was determined using an Ostwald's viscometer by dissolving the chitosan samples in 1% acetic acid. Viscosity is directly related to the molecular weight of chitosan and significantly affects its functional applications, such as film-forming ability and chelation capacity *(Kumirska et al., 2010).*

Degree of Deacetylation (DA) of Chitosan

Titration Method

Dried chitosan (0.2 g) was dissolved in 20 cm^3 of 0.1 M hydrochloric acid and 25 cm^3 of deionized water. The solution was stirred continuously for 30 minutes, after which another 25 cm^3 of deionized water was added and stirring continued for an additional 30 minutes.

Once the chitosan was completely dissolved, the solution was titrated with 0.1 M sodium hydroxide solution using an automatic burette with 0.01 cm^3 accuracy.

Formula for Degree of Deacetylation

Formula for Degree of Deacetylation

$$DA(\%) = \frac{(V_2 - V_1) \times C_{NaOH} \times 2.03}{m \times 0.0042} \times 100$$

Where:

- **m** = weight of sample (g)
- **V_1, V_2** = volumes of 0.1 M NaOH corresponding to deflection points (mL)
- **2.03** = coefficient from molecular weight of chitin monomer unit
- **0.0042** = coefficient from the difference between molecular weights of chitin and chitosan monomer units

CNaOH = concentration of NaOH (mol/dm^3)

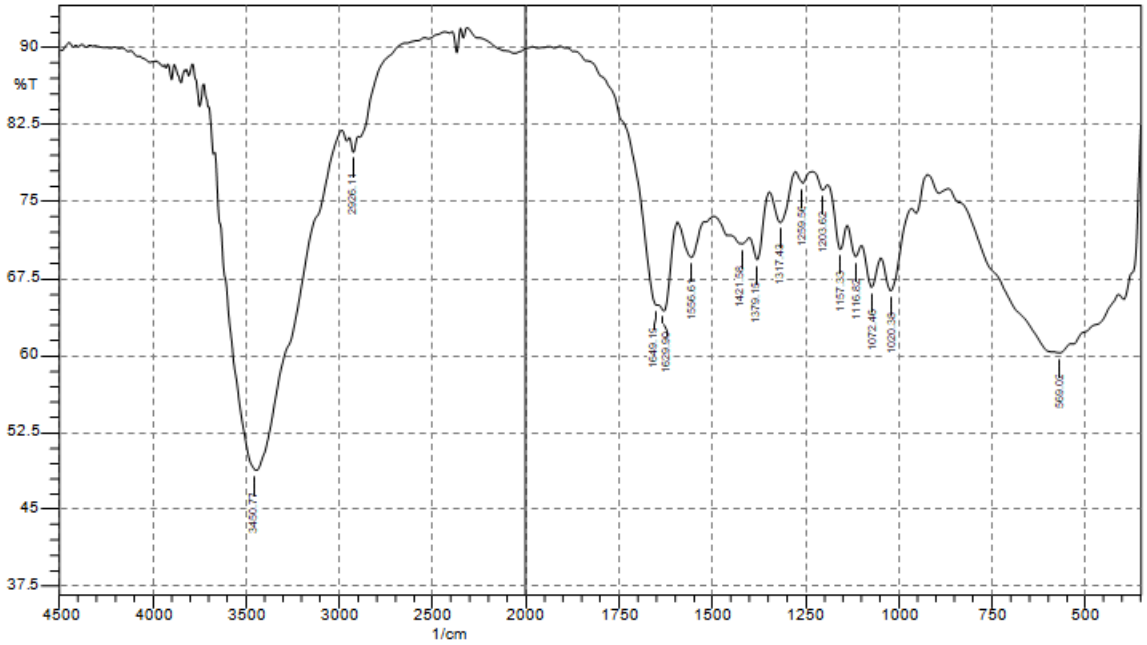
RESULTS:

Characterization of Chitosan:

Parameters analysed for chitosan extracted from shrimp shell wastes

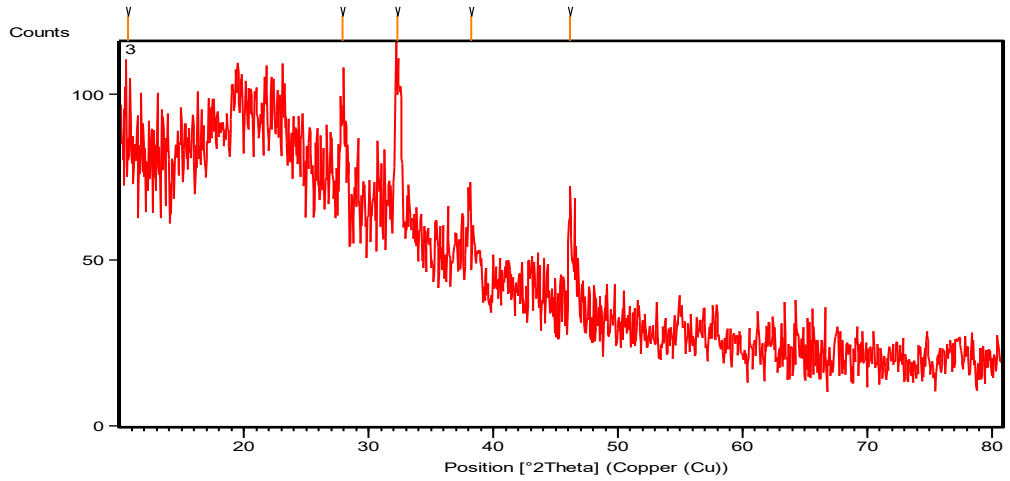
Parameter	Mean Value
Moisture Content (%)	7.8 %
Ash Content (%)	0.65%
pH of Solution	6.2%
Viscosity (cP)	310 cP
FTIR – Broad O–H / N–H stretch	~3450 cm^{-1}
FTIR – C–H stretch	~2926 cm^{-1}
FTIR – Amide I (C=O stretch)	~1650 cm^{-1}
FTIR – Amide II (N-H bend)	~1556 cm^{-1}
FTIR – C–N / C–O stretch	1000–1200 cm^{-1}
Degree of Deacetylation (DA, %)	85.7 %

FT-IR Analysis of Chitosan: The FTIR analysis clearly demonstrated the successful conversion of chitin into chitosan. A broad absorption band at approximately 3450.77 cm^{-1} corresponded to O–H and N–H stretching vibrations, confirming the polysaccharide structure and the presence of primary amine groups characteristic of chitosan (*Younes & Rinaudo, 2015*). The peak near 2926.11 cm^{-1} was attributed to aliphatic C–H stretching, indicating that the polysaccharide backbone was retained after deacetylation. A noticeable reduction in the intensity of the amide I band (C=O stretching) around 1649.19 cm^{-1} suggested significant removal of acetyl groups, while the amide II band (N–H bending) at 1556.61 cm^{-1}



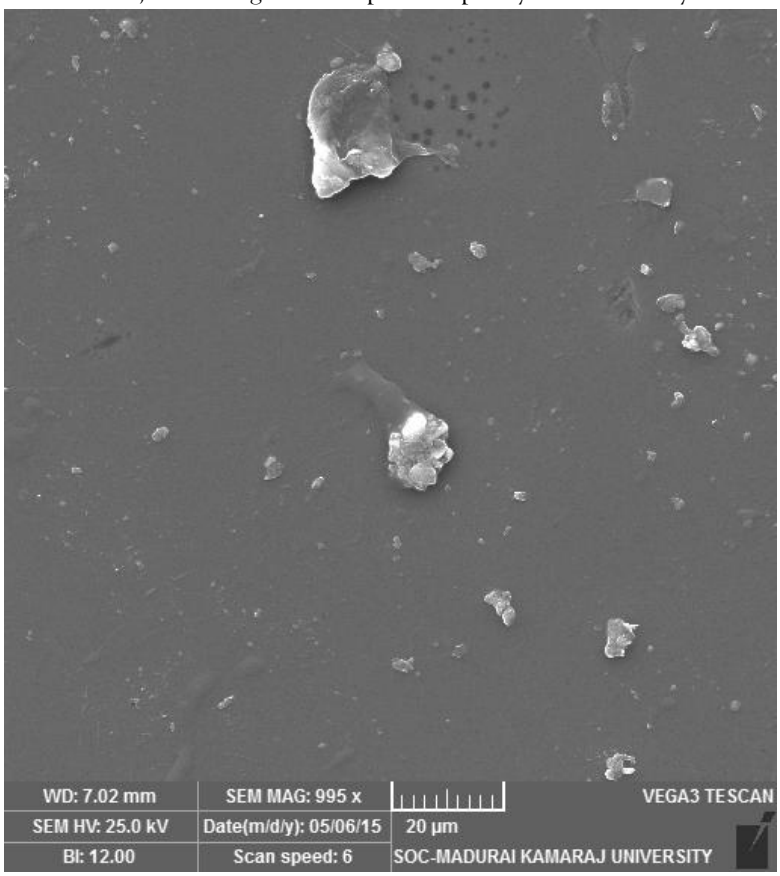
showed lower absorbance, confirming partial deacetylation (*Hamed et al., 2016*). Similarly, the decreased intensity of the amide III band (~1379 cm^{-1}) and C–N stretching (~1317 cm^{-1}) provided further evidence of deacetylation. Strong absorption peaks in the saccharide fingerprint region (1020–1157 cm^{-1}) confirmed the presence of β-(1→4) glycosidic linkages, indicating that the polysaccharide framework remained intact (*Crini, 2019*). The degree of deacetylation was calculated to be about 82.5%, which falls within the standard commercial range (70–85%) and ensures improved solubility in weak acids and enhanced cationic behavior, making the prepared chitosan suitable for diverse industrial, biomedical, and environmental applications (*Kumirska et al., 2010*). The FTIR spectrum confirms that the chitin extracted from shrimp shells was successfully converted to chitosan

XRD Analysis of Chitosan: The X-ray diffraction (XRD) analysis of the sample revealed distinct diffraction peaks at 2θ values of 10.73°, 27.92°, 32.30°, 38.20°, and 46.17°, corresponding to interplanar d-spacings of 8.25 Å, 3.20 Å, 2.77 Å, 2.36 Å, and 1.97 Å, respectively. The peak at 32.30° exhibited the highest intensity, indicating that this crystallographic plane is the most dominant in the sample.



The presence of sharp and well-defined peaks, with relatively low full-width at half-maximum (FWHM) values, suggests a high degree of crystallinity. The observed diffraction pattern confirms the formation of a well-ordered crystalline structure, and the relative intensities of the peaks provide insight into the preferred orientation of the crystallites. These results are consistent with typical XRD patterns for materials of similar composition and indicate that the sample possesses a stable and crystalline phase suitable for further material characterization.

Scanning electron microscope (SEM) Analysis: Chitosan prepared from shrimp shell waste was examined using Scanning Electron Microscopy (SEM) at a magnification of 995× and an accelerating voltage of 25.0 kV. The SEM micrograph, as shown in Figure 1, reveals the surface morphology of the extracted chitosan. The image illustrates a non-uniform, rough surface with irregularly shaped particles dispersed across the field. These particles exhibit varied sizes and shapes, ranging approximately between 5 to 20 µm, indicating the heterogeneous nature of the material. The surface appears non-smooth and porous, which is characteristic of chitosan derived from natural sources. Such morphology may influence the functional properties of chitosan, including its adsorption capacity and reactivity in various applications.



CONCLUSION:

The present study successfully converted chitin extracted from shrimp shell waste into chitosan through standard deacetylation processes. The study successfully demonstrated the extraction and characterization of chitosan from shrimp shell waste collected from the Thoothukudi district. The preparation involved a systematic sequence of demineralization, deproteinization, and deacetylation steps, which effectively removed calcium carbonate, proteins, and acetyl groups from the original chitin structure. The deacetylation process, carried out using 60% NaOH and microwave-assisted heating, resulted in the formation of high-quality chitosan with a degree of deacetylation (DA) of 85.7%, which falls within the commercially acceptable range. This high DA value ensures improved solubility, bioactivity, and functional performance of chitosan in various applications. Characterization studies confirmed the successful conversion of chitin to chitosan. FTIR analysis revealed the presence of characteristic peaks associated with hydroxyl, amine, and glycosidic functional groups. The disappearance or weakening of the amide I and amide II bands confirmed the deacetylation of chitin, while the presence of peaks in the saccharide fingerprint region further supported the retention of the β-(1→4) glycosidic linkages in the polymer backbone. These findings verified the structural integrity and functional transformation of chitin into chitosan. Physicochemical properties of the prepared chitosan were within acceptable limits: the moisture content (7.8%) was low enough to ensure stability during storage, while the ash content (0.65%) indicated high purity with minimal residual inorganic matter. The pH of the chitosan solution (6.2) was close to neutral, which is favourable for biomedical and environmental uses, and the relatively high viscosity (310 cP) suggests a moderate molecular weight, suitable for applications such as film formation, water treatment, and drug delivery. XRD analysis revealed sharp and well-defined peaks, particularly at $2\theta = 32.30°$, confirming the semi-crystalline nature of the prepared chitosan. The presence of multiple

diffraction peaks with low FWHM values further supported the structural stability and crystalline order of the sample, highlighting its potential for use in advanced material applications. SEM analysis of the chitosan sample displayed a non-homogeneous and porous surface morphology with irregular particles ranging between 5–20 µm in size. This rough texture is typical of chitosan derived from biological waste and may enhance its functional properties, such as surface area for adsorption, reactivity, and interaction with biological or environmental agents. Overall, the results indicate that shrimp shell waste can be efficiently valorised into high-quality chitosan through eco-friendly and cost-effective processing methods. The physicochemical, structural, and morphological characteristics of the prepared chitosan confirm its suitability for a wide range of applications in bioremediation, biomedicine, agriculture, food packaging, wastewater treatment, and pharmaceutical formulations.

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