International Journal of Earth Sciences Knowledge and Applications
journal homepage: http://www.ijeska.com/index.php/ijeska
e-ISSN: 2687-5993

Research Article https://doi.org/10.5281/zenodo.17020651

Extraction and Characterization of Chitosan from the Shells of Indian White Prawn, *Fenneropenaeus indicus* (Edwards, 1837)

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Article history
Received 25 June 2025
Accepted 22 August 2025
Published 31 August 2025

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How cite
Htay, W.L.L., Gyi, K.K., 2025. Extraction and Characterization of Chitosan from the Shells of Indian White Prawn, *Fenneropenaeus indicus* (Edwards, 1837). International Journal of Earth Sciences Knowledge and Applications 7 (2), 201-207.
https://doi.org/10.5281/zenodo.17020651.

Abstract
The shell wastes of Indian white prawn, *Fenneropenaeus indicus*, collected from markets and restaurants, were used as raw material for the extraction of chitin and chitosan. The yields of chitin and chitosan extracted from shrimp were 33.35 ± 2.62% and 29.45 ± 1.77%, respectively. The solubility and water-binding capacity of chitosan were 100% and 830.44 ± 128.36%, respectively. The solubility and water-binding capacity of the extracted chitosan are high enough that it can easily be incorporated into various applications. Moreover, the ash content of chitosan produced was relatively low (0.1 ± 0.03%) from *Fenneropenaeus indicus*, which is indicative of effective demineralization and low impurities in the extracted chitosan. In addition, the functional groups of the chitosan and its surface morphology were identified by Fourier Transform Infrared (FTIR) spectra and Scanning Electron Microscopy (SEM). The featured peaks of the FTIR spectra of the extracted chitosan in this study were comparable to those in other related studies. Furthermore, the SEM micrographs of the surface morphology of chitosan revealed rough and fibrous structures with regular or irregular pores.

Keywords
Acid-base treatment, characterization, chitin, chitosan, FTIR, SEM, shrimp shell

1. Introduction

In Myanmar, a total of 480,000 fish farms is being operated to breed fish and shrimp across the nation. In addition, more than 120 cold storage facilities are being run for the processing of marine products (Myanmar Digital News, 2023). With the production of exportable frozen products, a huge quantity of waste (around 40–80%, depending on species and processing method) is produced by processing plants. In shrimp industries, usual processing only uses shrimp meat and discards the excess, including the shells, heads, legs, appendages, and tails. These wastes are also disposed of from markets and kitchens. The reuse of waste from the fishing industry (Wang and Nguyen, 2019), markets, and kitchens is not a common practice, and a large percentage of the waste is discarded directly into the environment without treatment. Any material that is not used during its production or consumption process due to technological or market limitations, which can cause damage to the environment when not properly managed, is considered waste (Cai et al., 2018; Bedoï et al., 2019). The proper utilization of these shell wastes can reduce the potential for environmental contamination and convert the shell waste into valuable materials such as chitin.

Chitin is produced from hard-shelled marine invertebrates, commonly known as crustaceans (shrimp and crab). The shell composition of crustaceans, such as shrimp, can be slightly different depending on the species, season, and habitat; nevertheless, the main components are calcium carbonate (20–50%), protein (20–40%), chitin (15–40%), and lipids (0–14%) with elevated contents of omega-3 fatty acids and pigments (Šimat et al., 2022). Chitin is a polymer of N-acetylglucosamine and the second most abundant organic polysaccharide compound in nature after cellulose. The chemical formula of chitin is poly-1,4-linked N-acetylglucosamine, and its molecular formula is

$(C_8H_{13}NO_5)_n$. Chitin can be processed into chitosan through the deacetylation process. This process converts N-acetylglucosamine (GlcNAc) units into glucosamine (GlcN) units, which have amino groups (NH_2) that exhibit even greater properties than those of chitin.

Chitosan is the N-deacetylated derivative of chitin, which has a high density of amino groups and is soluble in aqueous acidic solvents such as acetic or formic acid. Its solubility mainly depends on two key factors: the degree of deacetylation and the molecular weight. A high degree of deacetylation and low molecular weight increase the solubility (Aranaz et al., 2021; Mukhtar et al., 2021). Based on these two parameters, the physicochemical properties of chitin and chitosan are widely different (Chandumpai et al., 2004). Chitosan has been widely used in diverse fields, ranging from waste management to food processing, medicine, and biotechnology. It has become an interesting material in pharmaceutical applications due to its biodegradability, biocompatibility, and low toxicity. Chitosan has found wide applicability in conventional pharmaceutical devices as a potential formulation excipient. The use of chitosan in novel drug delivery systems, such as mucoadhesive, peptide, and gene delivery, as well as oral bioavailability enhancement, has been reported. Chitosan exhibits numerous biological actions such as hypocholesterolemic, antimicrobial, and wound-healing properties. Moreover, chitosan contains a high percentage of nitrogen (6.89%) compared to synthetically substituted cellulose (1.25%) (Gupta and Kumar, 2000), which makes it a useful agent in agriculture by improving soil fertility and promoting seed germination, plant growth, and productivity.

The present study aims to extract value-added products such as chitin and chitosan from the shell wastes of Indian white prawn, *Fenneropenaeus indicus*; elucidate the properties of chitosan biopolymer such as yield, moisture and ash content, solubility, and water-binding capacity; and examine the surface morphological characteristics and structural components of chitosan using Scanning Electron Microscopy and Fourier Transform Infrared spectroscopy.

2. Materials and Methods

2.1. Sample Collection and Preparation

The shell waste of *Fenneropenaeus indicus* was collected from markets and some seafood restaurants in Mawlamyine Township. The collected shrimp shell wastes were brought to the laboratory of the Department of Marine Science at Mawlamyine University. At the laboratory, attached meat or flesh from the shell wastes was removed and cleaned with tap water to get rid of soluble organic matter, other impurities, and adherent proteins. The cleaned shell wastes were dried in sunlight for 2–3 days. After that, the cleaned and dried shell wastes were crushed into pieces and then packed in air-tight plastic bags for the extraction of chitin and chitosan.

2.1.1. Extraction of Chitin and Chitosan by Chemical Method

A dry weight of 100 grams of *Fenneropenaeus indicus* shells was used for the chitin and chitosan extraction. The extraction was carried out by a chemical method through four main stages: deproteinization, demineralization, decolorization, and deacetylation (Fig. 1).

2.1.2. Deproteinization

The first step in the procedure was the deproteinization of shrimp shell waste, which involved the removal of protein content from the shells. This step was performed using a laboratory-scale 1800 milliliter beaker in which shrimp shells were immersed in an alkaline solution of 5% sodium hydroxide (NaOH) at room temperature for 20 hours, followed by heating for 2 hours to remove protein from the shell structure. The shrimp shells were then washed repeatedly with tap water 5–6 times until a neutral pH of 7 was reached. The alkaline supernatants were removed, and the deproteinized wet samples were dried for a few minutes at room temperature.

2.1.3. Demineralization

Demineralization is the second most important step in the procedure. The deproteinized shells were soaked in a 5% hydrochloric acid (HCl) solution for 20 hours at room temperature and heated for 2 hours to remove minerals. After this step, the obtained samples were washed repeatedly with tap water 5–6 times until the pH of the washing water became neutral. The chitin samples were then dried for a few minutes at room temperature.

2.1.4. Decolorization

Since the crustacean exoskeleton naturally contains pigments such as astaxanthins and carotenoids, it was necessary to carry out a decolorization treatment on the chitinous samples. This was performed by immersing the chitin samples in a 5% sodium hypochlorite (NaOCl) solution for 1 hour at room temperature. The samples were then washed repeatedly with tap water until a neutral pH was reached and dried at room temperature until completely dry.

2.1.5. Deacetylation

Deacetylation of chitin was carried out by soaking the extracted chitin in 60% sodium hydroxide solution for 20 hours and then heating for 2 hours. After deacetylation, the obtained chitosan was washed with distilled water until a neutral pH was reached and dried overnight at room temperature. The dried chitosan was ground into powder using an electric blender, weighed, and stored in air-tight plastic bags for further analysis.

2.2 Yield and Characterization of Chitosan Biopolymer

2.2.1. Analysis of Chitin and Chitosan Yields

The chitin and chitosan yields (%) were calculated as the dry weight (in grams) of the chitin/chitosan flakes relative to the dry weight (in grams) of shrimp shell wastes by the following equation.

$$Yield\ (\%) = (Dried\ chitin/chitosan\ weight)/(Dry\ weight\ of\ shrimp\ shell\ wastes) \times 100 \quad (1)$$

2.2.2. Ash Content

Ash content was determined by drying 1.0 g of chitosan powder in a pre-weighed crucible and heating it at 575°C continuously for 6 hours in a muffle furnace. After that, the sample was removed and cooled for 15 minutes in the desiccator and weighed. The obtained weights were calculated using the following equation.

$$Ash\ (\%) = (Ash\ weight)/(Initial\ chitosan\ weight) \times 100 \quad (2)$$

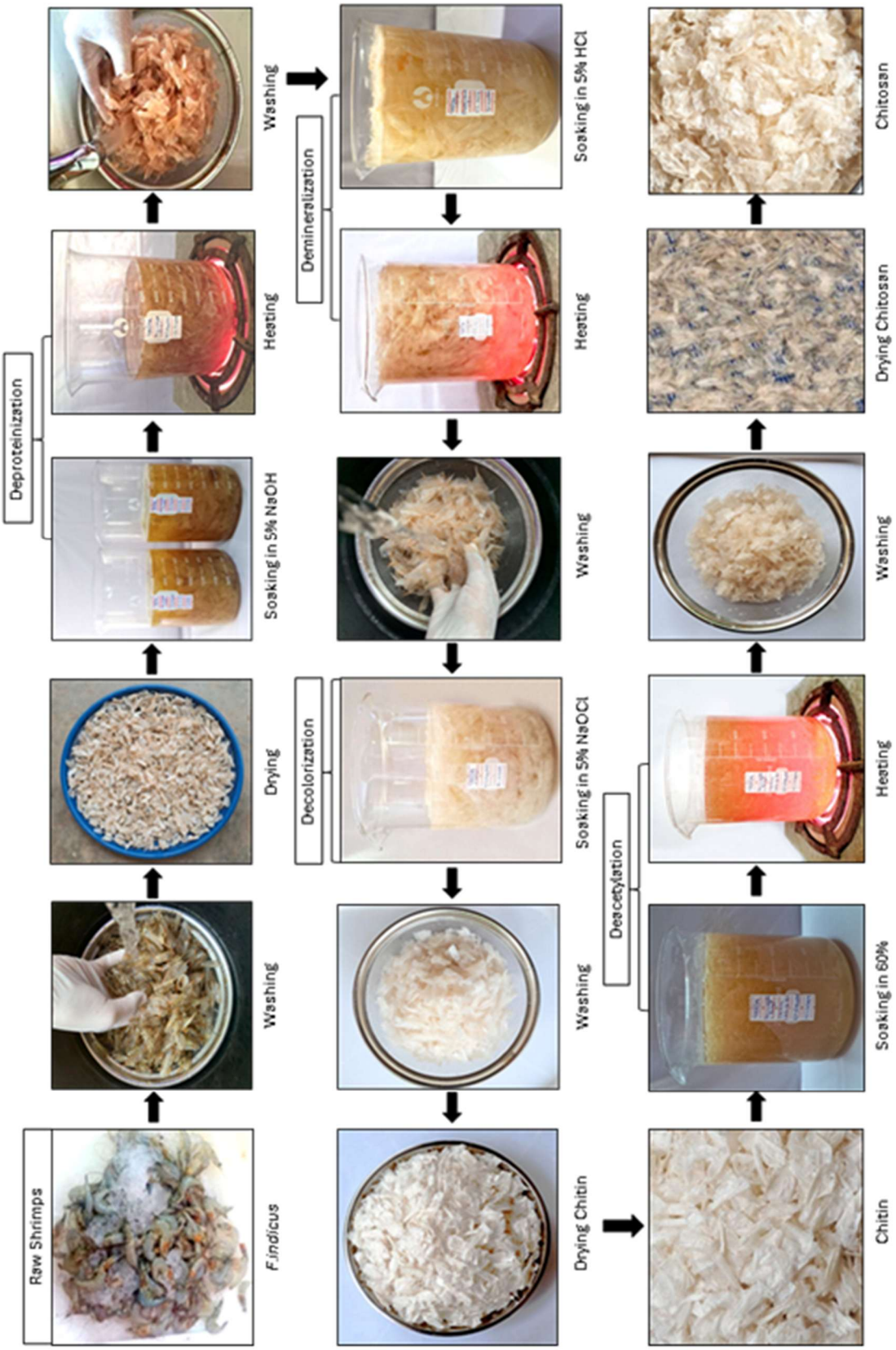

Fig. 1. The extraction procedures of chitin and chitosan from shrimp shell waste

2.2.3. Moisture Content

To determine moisture content, 1.0 g of chitosan powder was placed in a pre-weighed crucible and heated at 105°C for 1 hour in the hot air oven. The percentage of moisture was determined by the weight loss before and after drying:

$$\text{Moisture content (\%)} = (\text{Initial chitosan weight} - \text{Weight after drying})/(\text{Initial chitosan weight}) \times 100 \quad (3)$$

2.2.4. Solubility in 1% Acetic Acid Solution

A chitosan sample of 0.1 g obtained from the deacetylation process was dissolved in 10 ml of 1% acetic acid solution and continuously shaken until a homogeneous solution was obtained. The solution was filtered using the Whatman Grade 1 filter paper with a pore size of 11 µm to separate the undissolved particles. The procedure was repeated three times. The percentage of solubility was calculated using the following equations:

$$\text{Insoluble (g)} = \text{final dry weight of filter paper (g)} - \text{initial weight of filter paper (g)} \quad (4)$$

$$\text{Insoluble (\%)} = (\text{Insoluble (g)})/(\text{Sample weight (g)}) \times 100$$
$$\text{Solubility (\%)} = 100 - \text{insoluble (\%)} \quad (5)$$

2.2.5. Water Binding Capacity (WBC)

A centrifuge tube containing 0.1 g of chitosan was weighed. Then, 10 ml of water was added to the sample and mixed for 5 minutes to spread the sample. The content was left at ambient temperature for half an hour, then centrifuged at 4000 rpm for 45 minutes and left overnight. After the supernatant was decanted, the tube was weighed again. The percentage of WBC was measured by the following formula:

$$\text{WBC (\%)} = \text{Water bound chitosan weight (g)}/\text{Initial chitosan weight(g)} \times 100 \quad (6)$$

2.2.6. Scanning Electron Microscopy (SEM) Analysis

The microstructure of the extracted chitosan was analyzed using a JEOL NeoScope JCM-6000Plus Versatile Benchtop SEM. Before imaging, the dried chitosan sample was ground, mounted on a sample holder, and examined under an acceleration voltage of 15 kV to capture surface morphology.

2.2.7. Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy was employed to analyze the structural characteristics of chitosan. Spectra were recorded over a frequency range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} using a PerkinElmer Spectrum Two FTIR Spectrometer.

3. Results and Discussion

3.1. Yield and Characterization of Chitosan

The yields of chitin and chitosan extracted from 100 grams of *Fenneropenaeus indicus* shell waste were 33.35 ± 2.62% grams and 29.45 ± 1.77% grams, respectively. These values are consistent with previous reports, such as the 23% chitosan yield reported by No and Meyers (1989).

Puvvada et al. (2012) also reported that the chitosan yield was found to be 34% after the purification of shrimp shells. Furthermore, the yields of chitin and chitosan are influenced by the source of the shell, environmental conditions, and the extraction methods employed.

In the chemical isolation of chitosan, a successful demineralization process can be measured by the level of the remaining ash after undergoing an anaerobic combustion process through a muffle furnace (William and Wid, 2019). The higher the ash content, the less successful the demineralization process, and vice versa (Pădureţu et al., 2018).

In this study, a relatively low ash content of chitosan was observed at 0.12 ± 0.03%. This low value of ash content in chitosan suggests that substantial levels of inorganic materials in the raw shrimp shells were removed by the acid applied in the purification processes, and that a high-purity chitosan was produced. The moisture content of chitosan was 12.0 ± 1.0%.

According to Rege et al. (1999), commercial chitosan typically exhibits a moisture content range of 7% to 11%. In this study, the moisture content of chitosan was slightly higher than the commercial chitosan range.

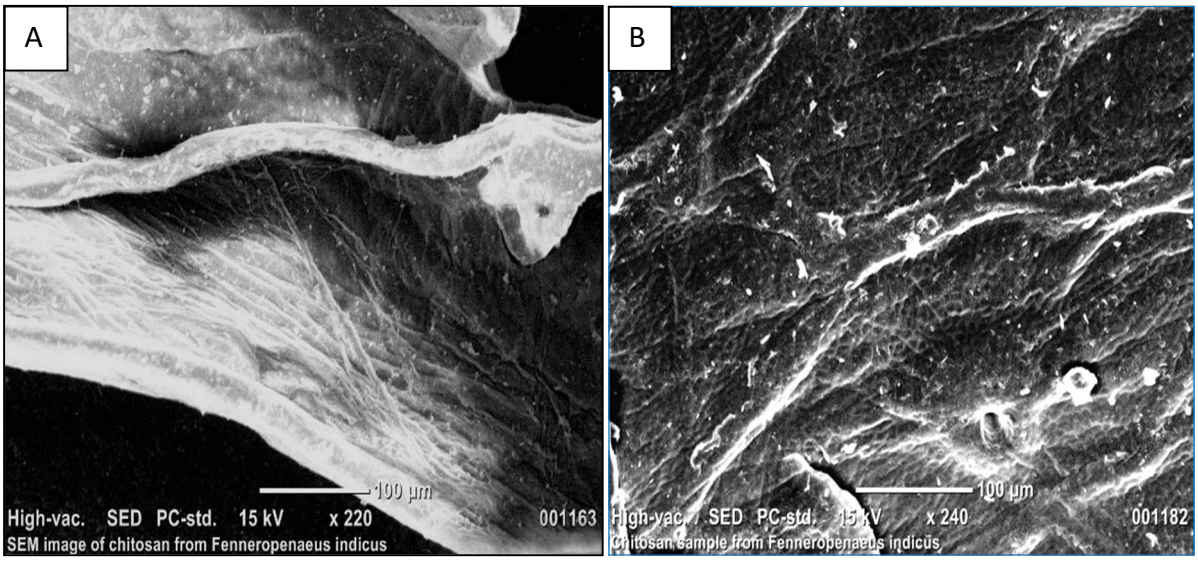

Fig. 2. SEM images of chitosan: A) Chitosan has layer flakes (220×) and B) porous and fibrous structures (240×) (Micrographs taken by Dr. Khin Khin Gyi)

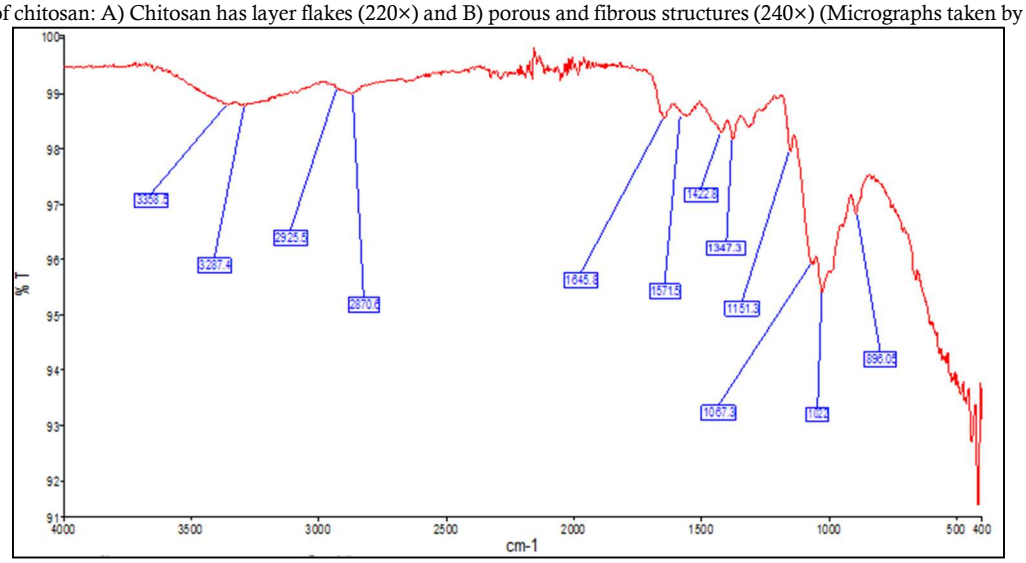

Fig. 3. FTIR analysis of chitosan extracted from *Fenneropenaeus indicus*

Chitosan solubility in 1% acetic acid solution was 100%, which was higher compared to the previous studies conducted by Paul et al. (2014), where the solubility of chitosan was 90–95%. Solubility characterization is one of the most important parameters for determining the quality of chitosan, where higher solubility indicates better-quality chitosan (Ahing and Wid, 2016).

The water-binding capacity of chitosan can be primarily attributed to its polycationic nature, which facilitates interaction with water molecules through hydrogen bonding. In this study, the water-binding capacity of chitosan was found to be 830.44 ± 128.36%. Similarly, Gadgey and Bahekar (2017) reported that commercial chitosan exhibited a water-binding capacity of 812.67 ± 7.64%.

The extracted chitosan was characterized by a pale-yellow color, reflecting variations in purity and residual pigments due to the source material and extraction methods (Kurita, 2001), and an average particle size of 859.0 ± 44.64 micrometers, indicating a relatively coarse material. Larger particle sizes have a positive effect in agricultural applications, as they can lead to improved plant growth and disease resistance (Dash et al., 2011). This slower degradation is advantageous in agriculture, where prolonged action is often required.

3.2. Scanning Electron Microscopy (SEM) Analysis of Chitosan

SEM was utilized to investigate the surface morphology of chitosan, revealing distinct fibril and porous structures at varying magnifications. Chitosan displayed a more regular porous pattern (Fig. 2). These morphological characteristics, including enhanced water retention, nutrient availability, and microbial protection, directly contribute to improved seed germination and early seedling growth (Badawy and Rabea, 2011; Dago-Serry et al., 2024).

Table 1. Characteristic absorption bands (wave number, cm-1) in the FTIR spectra of extracted chitosan and standard chitosan

Bonds	Functional groups	Extracted chitosan	Standard chitosan	Reference range*
O-H	Hydroxyl groups	3358.5	3441.0	3200-3600
N-H	Primary amines	3287.4	3259.7	3250-3400
C-H	CH_3 and CH_2 (alkanes)	2925.5, 2870.6	2927.9, 2880.0	2850-2950
C=O	Carbonyl (Amide I)	1645.8	1662.6	1600-1800
N-H, C-N	(Amide II)	1571.5	1554.6	1550-1650
C-H, C-N, N-H	Combination of C-H modes, N-H bending, and C-N stretching (Amide III)	1347.3	1322.0, 1262.0	1250-1350
C-O-C	Glycosidic linkage band (alkenes)	1151.3, 1067.3, 1022.0	1155.0, 1077.0, 1031.0	1040-1155
	ω β-1, 4-glycosidic bond	896.1	897.0	897

(Ji et al. 2020; McMurry 2023)

3.3. FTIR Analysis of Chitosan

FTIR spectrum of the extracted chitosan showed absorption bands with several characteristic peaks at 3358.5, 3287.4, 2925.5, 2870.6, 1645.8, 1571.5, 1347.3, 1151.3, 1067.3, 1022-, and 896.1-centimeters inverse (cm^{-1}) (Table. 1; Fig. 3).

The spectra showed peaks around 3358.5- and 3287.4-centimeters inverse, indicating the stretching vibrations of hydroxyl (O–H) and amine (N–H) bands. The stretching vibration of the methyl group (-CH_3) was observed at 2925.5 centimeters inverse. For the methylene groups (-CH_2), peaks were detected at 2870.6 centimeters inverse in the extracted chitosan samples. The peak at 1645.8 centimeters inverse in the spectra corresponds to the vibrations of the carbonyl group (amide band I). The peak at 1571.5 centimeters inverse represents the N–H bending (amide band II). Amide I and

Amide II are known as the characteristic bands for chitosan and were observed at around 1645.8- and 1571.5-centimeters inverse, respectively. These characteristic bands are commonly attributed to the stretching of the carbonyl group hydrogen bonded to the amide group of the neighboring intra-sheet chain. The peak at 1347.3 centimeters inverse represents the C–N stretching vibrations. The spectra also featured peaks at 1151.3, 1067.3-, and 1022-centimeters inverse, which are related to the presence of a C–O–C bond in the chitosan structure. A characteristic band at 896.1 centimeters inverse is attributed to β-1,4 glycosidic bonds.

Moreover, the peak at 896.1 centimeters inverse further confirms the presence of β-1,4 glycosidic linkages. The characteristic bands and peaks identified in the FTIR spectra of chitosan in this study are consistent with the values reported in previous literature (Vino et al., 2015; Song et al., 2013). Chitosan amides have been reported to act as natural growth promoters, improving germination rates, seedling vigor, and stress tolerance, ultimately leading to healthier and more resilient plants (Orzali et al., 2017; Chakraborty et al., 2020; Dutta et al., 2022).

4. Conclusions

In this study, the extraction procedure included an acid-base alternating treatment for shell remediation. A lower ash content (less than 1%) was observed, which revealed that the extracted chitosan underwent efficient demineralization, indicating high purity and good quality. Moreover, the high solubility and water-binding capacity of chitosan make it a versatile material suitable for a wide range of applications. Furthermore, the porous and fibrillar nature, corroborated by SEM images, confirmed that the extracted chitosan is consistent with standard chitosan in terms of morphological appearance. In addition, the observation of the FTIR spectra revealed three prominent bands—Amide I (carbonyl stretching), Amide II (amine bending and carbon-nitrogen stretching), and Amide III (a combination of amine bending, carbon-nitrogen stretching, and carbon-hydrogen bending)—which proved that the resultant products exhibited the characteristic features of chitosan. In conclusion, the extraction procedure used in this study is effective in achieving standard chitosan quality, which can be applied widely across various fields.

Acknowledgements

We wish to express our sincere gratitude to Dr. Chit Sein, Rector of Mawlamyine University, as well as Dr. Khing Khing San, Dr. Myint Thida, and Dr. Thidar Htun, Pro-Rectors of Mawlamyine University, for their kind permission to undertake this research for the Master of Science degree in Marine Science. We are also deeply thankful to the committee chair, Dr. Nyo Nyo Tun, Professor and Head of the Department of Marine Science, Mawlamyine University, and to the committee members for their invaluable comments and suggestions. The authors also acknowledge all contributors who provided technical assistance and valuable insights that enriched this research.

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