

## Extraction and Characterization of Chitin and Chitosan from *Penaeus Monodon* and its Application for Water Purification: An Approach to Utilize Waste

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### Abstract

This study was conducted to elucidate the uses of chitosan extracted from *Penaeus monodon*. Chitosan is a natural polysaccharide that shapes structural additives inside the exoskeleton of crustaceans. In this research, chitin and chitosan had been extracted from the *Penaeus monodon* exoskeleton via the chemical system through a series of steps which include demineralization, deproteinization, and deacetylation. The chitosan received by the deacetylation system turned into analyzed for biochemical parameters like protein, lipid, carbohydrate, ash, moisture, degree of deacetylation, water binding capacity (WBC), fats binding capacity (FBC), and solubility. The statistics show that chitin contains 3.82% protein, 1.24% lipid, 68.45% carbohydrate, 2.9% ash, 9.6% moisture content, 67.60% DD, 640% WBC, 420% FBC, and 99% solubility which were higher than chitosan. The textile effluent was treated with extracted chitosan and chitosan membrane for 30 days. Maximum decolourization (55.56 to 95.75%) of the effluent occurs with 2 g of chitosan. This study concluded that chitosan is a promising absorbent for removing colour from textile effluent.



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### Keywords

Characterization; Chitin;  
Chitosan; Decolourization;  
Demineralization;  
*Penaeus Monodon*.

### Introduction

In marine Arthropods, the largest group of Crustaceans contains about 30,000 species. Shells of marine crustaceans along with shrimp, crabs, and lobsters are biowastes that make contributions to the pollution of coastal areas. The crustacean shell wastes contain 8-10% chitin, 30-65% protein, and

10-20% calcium.<sup>1</sup> The preparation of chitin, chitosan, and their components from shrimp shells takes place in three stages: demineralization, deproteinization, and deacetylation.<sup>2</sup>

Chitin is white, hard, elastic, natural polysaccharide compound, and insoluble in water. It is the second

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most excessive organic polysaccharide compound. It is commonly present in fungi, yeasts, arthropods, molluscs, and annelids.<sup>3</sup> Chitin is a biopolymer that contains repeating units of  $\beta$ -(1 $\rightarrow$ 4 linked residues)-2-acetamido-2-deoxy-D-glucopyranose semi-crystalline components. Chitin is composed of linear chains of acetylglucosamine groups. The differentiation between chitin and chitosan is the presence of acetyl groups.<sup>4</sup> Chitin has excellent biocompatibility, adsorption capacity, nontoxicity, antibacterial, immunological activity, drug delivery, and chelating properties.

In the enzymatic method, chitosan is obtained by cleaving the N-acetyl units of the polymer to form D-glucosamine units. Chitosan is non-toxic, insoluble in water, and dissolves after mixing with acids.<sup>6,7,8</sup> Chitosan is characterized by biocompatibility, biodegradability, and adsorption activity.<sup>9</sup> Chitin, chitosan, and its residues are mainly used in the food industry, pharmacy, cosmetics products, biochemistry, biotechnology, and polluted water treatment.<sup>10</sup>

Chitin, chitosan, and its derivatives are organic coagulants and do not create secondary pollutants. The functional groups of  $-\text{NH}_2$  and  $-\text{OH}$  can induce the adsorbing capacity of dyes.<sup>11</sup> They could be used as coagulating or flocculating agents for polluted textile wastewater.<sup>12</sup> The shrimp shells chitosan can be used as a bio adsorbent for the removal of colour from textile wastewater.<sup>13</sup> In the present study, the shrimp shell, chitin, chitosan, and membrane were prepared, and their Physico-chemical and functional properties were elucidated. The chitosan applied in the field of water purification had also been worked.

## Materials and Methods

### Collection of Samples

The *Penaeus monodon* exoskeletons were collected from the local fish market in Colachel, Tamil Nadu during December. The shrimp shells were thoroughly washed with tap water and cleaned. Then, the shrimp exoskeleton shells were dried under sunlight for 2-3 days.

### Preparation of Chitin and Chitosan

Shrimp exoskeleton obtained from *Penaeus monodon* were soaked in 0.05M acetic acid solution at 30°C for 24 hours. The soaked exoskeletons were thoroughly cleaned with water and air-

dried. The particles were deacetylated using HCl (0.68 M) at 30°C for 6 h. The deacetylated particle was separated and cleaned with water and pH was adjusted at 6.5-7.5. Protein contents were removed from the dried materials using 0.62M NaOH solution at 30°C for about 16 h. The deproteinized residues were then separated and cleaned with distilled water until a pH was maintained at 6.5 - 7.5. The particles were dried, ground, and sieved through a 150  $\mu\text{m}$  sieve (mesh). Chitin became deacetylated using NaOH solution (25 M) at 65°C for approximately 20 h. Afterward, the residues were separated, and the pH was maintained at 6.5-7.5.

### Preparation of Chitosan Membrane

Five grams of dried chitosan powder was dissolved in a 3% acetic acid. The chitosan solution was spread on a Petri dish and was heated at 60°C. Afterward, with a 50% reduction, the dish was kept at 30°C for 24h. Then, the dried membrane was soaked in NaOH solution for 24h. The membrane was cleaned with double distilled water and dried by natural drying.

### Estimation of Protein

The protein content was determined by the Foltch method.<sup>14</sup> Sample (0.1 g) was added with 1 ml of distilled water and centrifuged at 5000 rpm for 10 min. Solution (A) was prepared by adding 75 ml of NaOH (0.1 N) solution and adding 2 g of sodium carbonate. Solution (B) was prepared by adding 0.05 g of copper sulphate and dissolved in 5 ml double distilled water, to which 0.1 g sodium potassium tartrate was added. Solution (C) was prepared by taking 49 ml solution (A) to which 1 ml solution (B) was added. Three test tubes were taken and labelled as blank, test, and standard. 2.5 ml of solution (C) was added to all test tubes. The blank tube was kept in 0.1 ml double distilled water. In test one, 0.1 ml of sample was taken. The standard solution of 0.1 ml of bovine serum albumin was poured into the standard tube. Finally, 0.6 ml folin reagent was taken to each tube and thoroughly mixed. All tubes were maintained at 30°C for 20 min and the OD (optical density) was determined at 625 nm.<sup>14</sup>

$$\text{Protein content} = \frac{\text{OD of sample}}{\text{OD of standard}} \times 0.1$$

### Estimation of Lipids

Total lipids were extracted by a chloroform methanol method.<sup>15</sup> The dried sample powder was

homogenized with chloroform (2 ml) and methanol (1 ml). Then, the residues were mixed in a vortex at 2800 rpm. The extract was equilibrated with about 20 % of sodium chloride solution. The extracted contents were weighed and calculated.<sup>12</sup>

Lipid content = Petri dish with sample – Petri dish without sample × 100

### Estimation of Carbohydrates

The carbohydrate content was extracted by the Dubois method<sup>16</sup>. One milliliter of the sample was dissolved with 1 ml phenol (5 %), and H<sub>2</sub>SO<sub>4</sub> (5 ml) and maintained at 30°C for 20 min. Finally, the OD of sample was recorded at 490 nm.

$$\text{Carbohydrate content} = \frac{\text{OD of sample}}{\text{Std OD}} \times \text{std conc}$$

### Ash Content

The contents of ash were measured by the laboratory muffle furnace method.<sup>17</sup> The 1 g sample was sealed with a lid, kept in a furnace, and maintained at 575°C for 6 h. After chilling, the weight was obtained.

$$\text{Ash (\%)} = \frac{\text{Ash weight(g)}}{\text{Initial chitosan weight (g)}} \times 100$$

### Moisture Content

Moisture content was measured by the gravimetric method.<sup>18</sup> A half-gram sample powder was taken and maintained at 110°C for 3 h. After chilling, the weight was estimated, and the percentage of moisture content was determined using the given formula.<sup>15</sup>

$$\text{Moisture content (\%)} = \frac{\text{Chitosan initial Weight} - \text{Weight after drying}}{\text{Chitosan initial Weight}} \times 100$$

### Degree of Deacetylation (DD)

The DD was determined by the titration method of acid-base.<sup>19</sup> Sample (0.1 g) was dissolved in HCl solution (0.1 mol/l) and methyl orange (5 drops) was added. Finally, the dissolved chitosan solution was titrated with NaOH solution (0.1 mol/l)

$$\text{DD (\%)} = \frac{C_1V_1 - C_2V_2}{M \times 0.0994} \times 0.016$$

Were,

HCl concentration (C<sub>1</sub>)

The volume of HCl solution (V<sub>1</sub>)

The concentration of standard NaOH (C<sub>2</sub>)

The Volume of NaOH solution (V<sub>2</sub>)

Dosage of a sample (M)

NH<sub>2</sub> group equivalent weight (0.016)

NH<sub>2</sub> compound proportion (0.0994)

### Water Binding Capacity (WBC)

The 0.5 g of sample was dissolved with 10 ml of water and mixed on a vortex for 5 min and maintain at 29°C for 30 min. After, the bound particles were read at 3000 rpm for 30 min.<sup>3</sup>

$$\text{WBC (\%)} = \frac{\text{Bound of water (g)}}{\text{Initial chitosan dosage (g)}} \times 100$$

### Solubility

The sample was dissolved in acetic acid (1 %) for 30 min and cooled at 30°C. Then, the mixed particles were read at 10000 rpm for 10 min. The undissolved particles were separated and centrifuged at 10000 rpm and dried at 60°C for one day. Then, the residues were measured and calculated.<sup>20</sup>

$$\text{Solubility (\%)} = \frac{(\text{Initial tube weight} + \text{Chitosan}) - (\text{Final tube weight} + \text{Chitosan})}{(\text{Initial tube weight} + \text{Chitosan}) - (\text{Initial tube weight})} \times 100$$

### Fat Binding Capacity (Fbc)

Chitosan (0.5 g) was put in a tube with 10 ml oil and mixed on a vortex for 5 min. The residues were read at 3000 rpm for 25 min. After centrifuged, the tube was measured and calculated.<sup>3</sup>

$$\text{FBC (\%)} = \frac{\text{Bound of Fats(g)}}{\text{Initial chitosan dosage (g)}} \times 100$$

### Effluent Sampling

The textile effluent was collected from a cotton spinning mill at Tirupur, Tamil Nadu, South India (Lat. 11.11°N, Long. 77.34°E). The effluent was collected in a sterile plastic can from the discharge tank of the textile mill. The pH and temperature of the sample were determined on the site by using a pH meter and a thermometer. The effluent was stored in a refrigerator and used for further analysis.<sup>21</sup>

### Decolourization Experiment

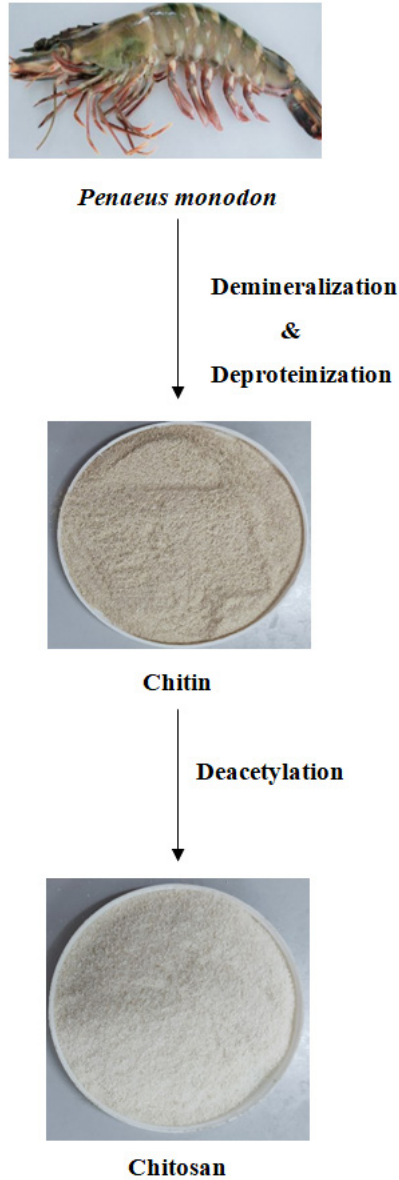
Sample (0.5, 1.0, 2.0 g) was added to varying concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90, 100%) of the textile effluent. The initial absorbance was noted, and the absorbance was measured periodically at a time interval of 24 h at 500 nm for 30 days.

$$\text{Decolourization (\%)} = \frac{\text{OD (zero day)} - \text{OD (sample)}}{\text{OD (zero day)}} \times 100$$

**Fourier Transform Infrared Spectroscopy (Ftir)**

The spectra of FTIR were analyzed utilizing the software of Perkin Elmer Spectrum. A few milligrams

of chitosan were placed in the ATR head from 4000 to 450  $\text{cm}^{-1}$ . For each spectrum, 16 scans were collected and averaged.



**Fig 1: Extraction of chitosan**

**Results and Discussion**

In the current study, the protein contents of raw shell, chitin, and chitosan were  $38.8 \pm 0.6$ ,  $3.82 \pm 0.60$ , and  $2.94 \pm 0.05$  respectively. In a similar report the protein content of shell wastes and chitin from shrimp shells were  $32.13 \pm 0.08$  and  $0.80$

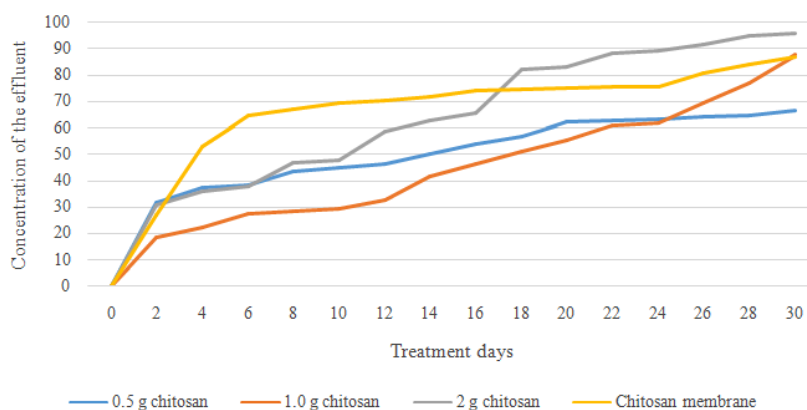
$\pm 0.15$  respectively.<sup>22</sup> Generally, the raw shrimp shell has high protein content (38.81%), and after deproteinization, the chitin had a trace amount (3.82%) of protein. Deproteinization could not remove 100 % of proteins from the shrimp shells.<sup>23</sup>



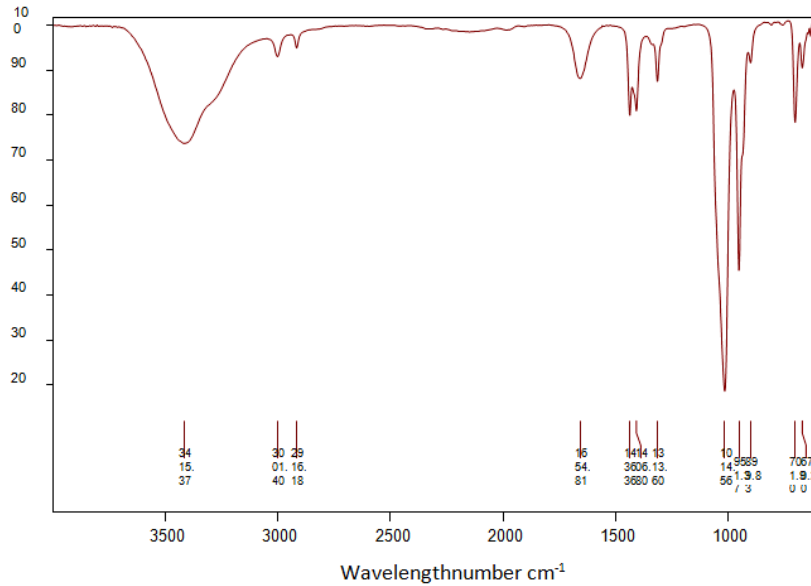
**Fig 2: Chitosan Membrane**

**Table 1: Physico-chemical and functional properties of *P. monodon* shell, chitin, and chitosan**

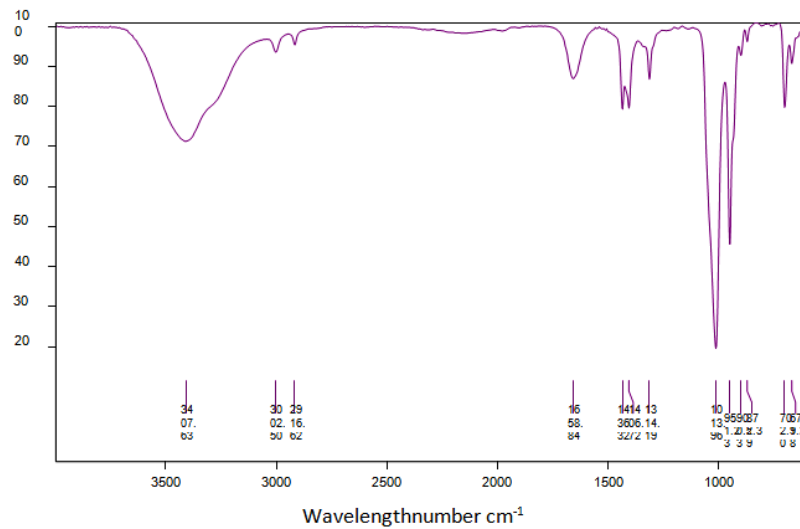
S. No	Physicochemical / functional parameters (%)	Raw shell	Chitin	Chitosan
1.	Protein content	38.8 ± 0.6	3.82 ± 0.60	2.94 ± 0.05
2.	Lipid content	5.1 ± 0.08	0.9 ± 0.25	1.3 ± 0.08
3.	Carbohydrate content	75.42 ± 0.18	68.45 ± 0.45	73.42 ± 0.04
4.	Ash content	17.2 ± 0.16	2.9 ± 0.08	1.5 ± 0.081
5.	Moisture content	21.26 ± 0.52	9.6 ± 1.63	8.2 ± 0.16
6.	Deacetylation	-	67.60 ± 0.26	79.03 ± 0.13
7.	Water binding capacity	170 ± 8.16	640 ± 16.32	580 ± 16.32
8.	Fat binding capacity	140 ± 16.32	420 ± 16.32	420 ± 8.16
9.	Solubility	33 ± 2.44	97.3 ± 0.28	97.6 ± 0.53



**Fig 3: Decolourization of the textile effluent**



**Fig 4: FTIR spectrum of extracted chitosan**



**Fig 5: FTIR spectrum of chitosan after adsorption of textile effluent**

**Table 2: FTIR analysis (functional groups) of extracted chitosan**

Wavelength	Functional groups	Structure
670.20	Alkyl halides	R-Br
701.90	Amines	RNH <sub>2</sub> , R <sub>2</sub> NH
899.83	Amines	RNH <sub>2</sub> , R <sub>2</sub> NH
951.37	Misc.	P-H phosphine
1014.56	Misc.	P-H phosphine
1313.60	Carboxylic acids	RCO-OH

1406.80	Misc.	S=O sulfate
1436.36	Misc.	S=O sulfate
1654.81	Alkenes	8-ring
2916.18	Alkanes	RCH <sub>2</sub> CH <sub>3</sub>
3001.40	Aromatics	Ar-H
3415.37	Phenols	ArO-H bonded

**Table 3: FTIR analysis of chitosan after adsorption of textile effluent**

Wave length	Functional groups	Structure
671.28	Alkyl halides	R-Br
702.90	Misc.	S-OR esters
872.39	Misc.	S-OR esters
900.53	Misc.	P-OR esters
951.23	Misc.	= NOH oxime
1013.96	Misc.	P-H phosphine
1314.19	Esters	RCOOR'
1406.72	Misc.	S=O sulfate
1436.32	Misc.	S=O sulfate
1658.84	Alkenes	8-ring
2916.62	Alkanes	RCH <sub>2</sub> CH <sub>3</sub>
3002.50	Aromatics	Ar-H
3407.63	Phenols	ArO-H bonded

The lipid content of raw shell, chitin, and chitosan was recorded as  $5.1 \pm 0.08$ ,  $0.9 \pm 0.25$ , and  $1.3 \pm 0.08$  respectively. Similarly, the lipid contents of the chitin, raw particles of chitosan, and extracted chitosan from shrimp shells' exoskeleton and recorded them as 1.50, 2.00, and 2.60%.<sup>24</sup> The crude lipid content was 10%, which revealed the relationship with the crude protein contents of the shrimp shells.

The carbohydrate contents of raw shell, chitin, and chitosan were  $75.42 \pm 0.18$ ,  $68.45 \pm 0.45$ , and  $73.42 \pm 0.04$  respectively. In a similar work, the carbohydrate content of the extracted chitin, raw particles of chitosan, and extracted chitosan from the shell as 76.20, 79.18, and 77.55 %.<sup>24</sup>

In the current study, the ash content of the raw shell, chitin, and chitosan was noted as  $17.2 \pm 0.16$ ,  $2.9 \pm 0.08$ , and  $1.5 \pm 0.081\%$  respectively. Based on the presence of ash content, pure chitosan was measured. Ash is a highly-frequency parameter that affects characteristics such as viscosity and solubility.<sup>25</sup> The final process of demineralized

chitosan resulted having 31-36% ash.<sup>26</sup> The chitosan having <1% ash is a good grade of chitosan.<sup>2, 27.</sup>

The study described that the moisture contents of raw shell, chitin, and extracted chitosan were  $21.26 \pm 0.52$ ,  $9.6 \pm 1.63$ , and  $8.2 \pm 0.16\%$  respectively. In a related study the moisture content of shrimp chitosan was 9.34%.<sup>28</sup> The commercial chitosan has a 10% moisture content.<sup>29</sup> Chitosan is hygroscopic, which is affected by low moisture absorption during storage.<sup>20</sup>

The DD of the extracted chitin, chitosan was  $67.60 \pm 0.26$  and  $79.03 \pm 0.13\%$ . In a similar work, the degree of deacetylation as 80% which is considered high-quality chitosan.<sup>30</sup> In chitin, the amount of DD depends on the raw material, deproteinization, and demineralization.<sup>31</sup>

Water binding capacity (WBC) of the raw shell, chitin, and chitosan were  $170 \pm 8.16$ ,  $640 \pm 16.32$ , and  $580 \pm 16.32\%$  respectively. Likewise, the WBC of chitosan ranged from 581 to 1150%<sup>32</sup> The commercial, shrimp, and crab chitosan wherein the WBC ranged from 458 to 805%.<sup>33</sup>



The FBC of raw shell, chitin, and extracted chitosan were  $140 \pm 16.32$ ,  $420 \pm 16.32$ , and  $420 \pm 8.16\%$  respectively. In a similar report, FBC in the range from 314 to 535%.<sup>34</sup> The level of FBC depends on deproteinization and deacetylation.<sup>35</sup>

The solubility of raw shell, chitin, and chitosan was observed as  $33 \pm 2.44$ ,  $97.3 \pm 0.28$ , and  $97.6 \pm 0.53$  respectively. In a related work, the shells treated with 50 and 60% NaOH solution gave high solubility ranged from 96.01- 97.2%. The chitosan solubility is influenced by temperature, period of deacetylation, concentration of alkaline and yield of chitin, various methods applied to chitin separation, and size.<sup>36</sup> Chitosan solubility increases proportionally with deacetylation degree.

In this study, figure 3 shows that the rate of decolourization increased from the 2<sup>nd</sup> to 18<sup>th</sup> days of observation (from 81 to 13%). In a similar study, the textile dye was decolourized by prawn shell waste from 93 to 70%.<sup>37</sup> This result indicates that the percentage of decolourization increased when the chitosan dosage was increased. In the chitosan membrane, the optimum decolourization of the effluent occurs at 86.82 to 41.54%. The adsorption sites are less available at high concentrations of textile effluent. So, the percentage of decolourization is highly dependent on the textile effluent concentration.<sup>38</sup>

The absorption efficiency of extracted chitosan was determined by spectrometric assay. Figure 2 & 3 shows the comparison of the FTIR spectrum of extracted chitosan (before treatment) with textile effluent absorbed (after treatment) chitosan. The presence of similar groups were alkyl halides, P-H phosphine, alkenes, alkanes, aromatics, and phenols in the position of a peak at  $600 \text{ cm}^{-1}$  to  $3500 \text{ cm}^{-1}$ . New peaks were recorded at  $872.39 \text{ cm}^{-1}$  which indicated the S-OR esters. The carboxylic

acids group at the wavelength of  $1313.60 \text{ cm}^{-1}$  was modified into the esters group at the wavelength of  $1314.19 \text{ cm}^{-1}$ . The P-H phosphine group was converted into = NOH oxime. In this study, the presence of an amine group at  $899.83 \text{ cm}^{-1}$  was noticed. In a similar study, chitosan oligomers polysaccharide structure at  $899 \text{ cm}^{-1}$ .<sup>39</sup> The functional groups of alkenes were found at  $1654.81 \text{ cm}^{-1}$ . Similarly, the bands of bending vibration of  $\text{NH}_2$  groups were found at  $1656.55$  and  $1658.48 \text{ cm}^{-1}$ .<sup>40</sup> The presence of a full band stretch in the prepared chitosan compared to the standard band stretch indicates that adsorption is occurring on the chitosan oligomers.

### Conclusion

Chitosan has been prepared from *Penaeus monodon* the steps including demineralization, deproteinization, and deacetylation process. The results of FTIR concluded that the shrimp shell chitosan can be used as a good bio-remediating agent for removing colour from textile effluent. Above all, the method employed in the study to prepare chitosan was economical and it could be a way to use the shrimp exoskeleton waste which would otherwise go unutilized as a mere waste polluting the environment.

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### Conflict of Interest

The authors declare no conflict of interest.

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