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**Research Article**

# Production and characterization of chitosan extracted from *Penaeus vannamei* (Boone, 1931) shell wastes

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## Abstract

**One of the biggest issues posing serious risks to the environment and public health is shell waste created by the marine food industry. Such a situation may allow for the efficient remediation of shrimp shell waste by turning it into the economically important substance chitosan, which has a wide range of applications. This study seeks to investigate the chitosan production from waste *Penaeus vannamei* shell utilising chemical methods that include demineralization, deproteinization, and deacetylation. The chitosan was characterized through analysis of the physiochemical parameters such as moisture content (3.98±0.65%), pH (7.74±0.13), degree of deacetylation (71.13±0.44%), solubility (95.24±0.51%), antioxidant activity, and antibacterial activity. The synthesis of chitosan from shell waste would increase economic potential, generate employment, and alleviate the environmental impact of shrimp shell waste management.**

**Key words:** Chitin, Chitosan, Environmental impact, *Penaeus vannamei*, Shell waste

## Highlights

- Shrimp shell waste poses serious risks to the environment and public health.
- Shrimp shell waste can be utilized to produce chitosan.
- Production of chitosan from the shell will reduce the environmental impact.

## INTRODUCTION

Aquaculture has expanded recently as most productive and dynamic sector with a result of economic development in many nations. Among the various processing sectors, the fish processing industries produce between 50% and 80% solid and liquid waste all over India (Thirukumaran *et al.*, 2022; Ashokkumar *et al.*, 2022). Such trash is not recycled; instead, it is burnt, disposed of in landfills, or deposited in waterways, posing major environmental hazards. Fish excrement contains nitrates and phosphates, which promote algal blooms and kill aquatic life by lowering dissolved oxygen levels (Sarkar, 2018; Chan, 2019). As a result, both terrestrial and aquatic ecosystems are impacted by these conventional waste disposal techniques, which has a negative effect on the environment. A significant possibility to preserve the environment is the use of trash produced by the fish processing industry. On the other hand, many researchers are taking the challenges of inventing new technology in the food industry targeting well sustainability and economical beneficiary achievement towards consumers (Herrero *et al.*, 2020). It would be a fantastic strategy to end world hunger if wastes produced during the harvest or processing of agricultural and fishery products be converted into commercial goods with a high level of added value rather than producing more food, which would result in more waste. Therefore, the recovery of value-added products from fish waste is becoming a part of the current waste utilization strategy because of the better realization of foreign exchange earnings and high unit value (Runge *et al.*, 2017). Chitin is the second most prevalent biopolymer in nature after cellulose which gives the exoskeletons of live organisms a sturdy structure. The main

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source of chitin for industry is marine resources, namely the shells of crustaceans like shrimp, crab, and lobster. Apart from that, it is also found in most fungi cell wall and insects. It is a linear homo-polysaccharide with a number of n-acetyl D-glucosamine units joined by β-1-4 linkages (Renuka *et al.*, 2019). Because of the acetamide group, chitin does not readily dissolve in water and mild acids. Despite the fact that chitin exhibited a variety of functional characteristics, its applicability in many industries was constrained by its large molecular weight and poor solubility. This problem prompts the discovery of chitosan from chitin in the late 1850s (Crini, 2019). Chitosan, a significant chitin derivative, is a partially deacetylated form of chitin. A rigid linear chain heteropolymer made of D-glucosamine (GlcN) and N-acetyl-D-glucosamine (GlcNAc) linked by a β-(1, 4)-linkage, chitosan is used in food packaging. Chitosan sold commercially ranges in molecular size from 50 to 2000 kDa. The degree of acetylation refers to the proportion of glucosamine to N-acetylglucosamine. The biological properties of the chitosan are determined by this level of acetylation. For commercially produced chitosan, the degree of deacetylation (DD) was typically between 80% and 85%. Due to the positive charges on the amino group, it is a cationic polysaccharide. Mild acids are used to dissolve chitosan. Because of its solubility, chitosan is regarded as the most significant chitin derivative. Due to its unique qualities including bioactivity, biodegradability, chelating ability, absorption capacity, and environmental friendliness, chitosan offers a wide range of applications from agriculture to the pharmaceutical sector. Additional useful qualities of chitosan include its ability to operate as an antioxidant, an antibiotic, a cholesterol-lowering agent, an edible film packaging material, a texturizing and flocculating agent, a binding and emulsifying agent, and a clarifying agent.

In the current study, waste from vannamei shrimp (*Penaeus vannamei*) was utilised to create chitin and chitosan. Shrimp consumption is in high demand both domestically and abroad, and the Indian shrimp business has grown significantly in the global seafood trade. Shrimp production in Indian aquaculture is expected to reach 8,43,679 MT in 2020-21, up 12.83% from the production of 7,47,694 MT recorded in the previous year, according to reports from MPEDA, 2020-21. The production of Pacific White Shrimp (*Penaeus vannamei*) increased, which was the key factor driving the growth. The supply of vannamei for the year totaled 8,15,745 MT, an increase of 14.62% over the production of 7,11,674 MT from the previous year. Approximately 96% of the nation's entire farmed shrimp production was derived from *P. vannamei*. The waste from shrimp processing, which makes up around 40-50% of the total, is largely made up of head and shell particles. Shrimp processing facilities in India generate more than one lakh tons of trash from shrimp each year. The production of waste during the processing of seafood, particularly crustaceans, results in environmental degradation and practical disposal challenges. Typically, the body shell makes about 10-15% of a shrimp, while the head makes up 34-45%. These wastes are 35-40% protein, 10-15% chitin, 10-15% minerals, and 10-15% carotenoids. The manufacturer can make money when shrimp shell waste is properly transformed into chitin and chitosan. Additionally, discharging and dumping of trash in an open area may be avoided, thus, lessens the unintentional ecological harm. Therefore, the goal of the current research was to examine the yield and physicochemical characteristics of chitosan made from *Penaeus vannamei* shrimp shell waste.

## MATERIALS AND METHODS

**Collection and preparation of shrimp waste:** From the local "BENFISH" fish processing facility at New Garia in Kolkata, India, shrimp (*Penaeus vannamei*) shell wastes were collected. The wastes from shrimp were cleaned with potable water followed by hot water cleaning respectively for 1 hour to remove the

impurities and dried at 50ºC overnight, ground, and kept in a dry location until extraction.

**Extraction of chitosan from the shrimp shell waste:** Using the process outlined by Benhabiles *et al*. (2012), chitosan was extracted from the shrimp shell with slight modification. 1.5 M HCl (1:10 w/v) was used to demineralize the shell for 30 min at room temperature. After being repeatedly rinsed in water to remove the acid, the decalcified shell was dried at 80ºC. 2N NaOH (1:10 w/v) was used to deproteinize the samples for 2 hours at 40ºC, after which they were rinsed with water and dried 'chitin' was produced. By deacetylating this chitin with 50% NaOH at a chitin to NaOH ratio of 1:10 at 90ºC for three hours, the chitin was transformed into chitosan. After filtering, neutralization with water and 80% alcohol, and overnight drying at 80ºC, the solid 'chitosan' was finally processed. The yield of chitosan was determined by weighting after being dried.

**Moisture, protein, ash content of chitosan:** Moisture content was analyzed as per AOAC method (2000) using a hot air oven. The crude protein content of chitosan was estimated using the Kjeldhal method (AOAC, 2000). Ash content was determined as per the standard method (AOAC, 2000).

**pH of chitosan:** The pH was determined using a digital pH meter as described by Benjakul *et al*. (1997). 0.5 g of pasta sample was centrifuged thoroughly with 4.5 mL of distilled water for 1 min at 1800 rpm, and the homogenate used for pH determination.

**Determination of degree of deacetylation (DD):** The degree of deacetylation was determined by acid-base titration method (Yuan *et al*., 2011). Chitosan (0.3-0.5 g) was dissolved in 30 mL 0.1 M HCl in a 250 mL flask at 20±5°C with stirring. Two drops of methyl orange indicator were added, and 0.1 M NaOH was used to titrate the solution. The end point of titration is indicated by the color changes from pink to orange yellow. A pH meter was also used to make the final titration point determination more precise. The water content of chitosan was also determined by heating 0.5 g of chitosan at 105°C until a constant weight was reached, and water content was calculated accordingly. Three parallel samples were used. The percent of free $NH_2$ groups in chitosan was calculated as follows:

$NH_2\% = [(C1V1 - C2V2) \times 0.016] / [G\,(100 - W)] \times 100$

$DD\% = NH_2\%/9.94\% \times 100\%$

Chitosan theoretic $NH_2$ content % = $(16/161) \times 100\% = 9.94\%$

C1: Concentration of HCl (M);
C2: Concentration of NaOH (M);
V1: the volume of HCl added (mL);
V2: the volume of NaOH added by titration (mL);
G: Sample weight (g);
W: sample water content (%);
0.016: equal to $NH_2$ content (g) in 1 mL of 1 M HCl.

**Solubility of chitosan:** Solubility was assessed using Fernandez-Kim (2004). Chitosan powder samples (0.1 g) were put into a centrifuge tube and then dissolved in 10 mL of 1% acetic acid for 30 min while being shaken in an incubator running at 240 rpm at 25ºC. The solution was then centrifuged at 10,000 rpm for 10 minutes after being submerged in a hot water bath for 10 min. The solution was then cooled to room temperature (25ºC). Decanting of the supernatant was done. After being rinsed in distilled water (25 mL), the undissolved particles were centrifuged at 10,000 rpm. The undissolved pellets were dried at 60°C for 24 hours after the supernatant was removed and weighed. The solubility (%) was calculated as follows:

$$\frac{\text{(Initial weight of tube + chitosan) – (Final weight of tube + chitosan)}}{\text{(Initial weight of tube + chitosan) – (initial weight of tube)}} \times 100$$

**Water binding capacity (WBC) and Fat binding capacity (FBC) of chitosan:** Water binding capacity (WBC) and fat binding capacity (FBC) of chitosan were determined according to the method described by Wang and Kinsella (1976) with slight modification. For WBC or FBC, weight of 50 mL capacity centrifuge tube with 1 g of sample was measured, followed by 20 mL distilled water or vegetable oil was added. Both tube solutions were mixed for 5 s at every 10 min. After one hour, the resulting solutions were centrifuged at 3200 rpm for 25 min at room temperature. The supernatant was removed, and the tube weight was measured. WBC and FBC were determined by using the following formula:

WBC (%) = [water bound (g)/ initial sample weight (g)] x 100
FBC (%) = [fat bound (g)/ initial sample weight (g)] x 100

**Antioxidant properties of chitosan:** The efficacy of the chitosan to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was determined using spectrophotometry method (Shimada *et al.*, 1992) with slight modification. Briefly, after 5 mL of sample solution was added to 5 mL of 0.008% 1,1-diphenyl-2-picrylhydrazyl (DPPH) with 50% ethanol decolorization of DPPH donated $H^+$ was followed by measuring absorbance at 528 nm. DPPH scavenging activity was determined using the following equation. The scavenging activity (%) of different concentrations of chitosan was determined as followed:

$$\frac{(A_{528} \text{ of control} - A_{528} \text{ of sample})}{A_{528} \text{ of control}} X\ 100$$

**Antimicrobial properties of chitosan:** The agar disc diffusion method described by Hafsa *et al.* (2016) with some modifications was used to determine the antibacterial capacity of chitosan. Briefly, the inocula of the bacterial strains prepared from 24 h broth cultures dilution series were taken to meet required bacterial population for seeding by using sterile distilled water. Different concentration of chitosan was aseptically dipped into 10 mm diameter discs and then placed on Mueller Hinton agar (Merck) plates, previously surface spread with 0.1 mL of inoculums containing approximately $10^5$-$10^6$ CFU/mL of tested bacteria (the cell density was measured using McFarland standards). The plates were then incubated at 37ºC for 24 h. The inhibition zone diameter was measured with a caliper in mm accordingly.

**Statistical analysis:** All the data were checked for normal distribution with normality plots prior to analysis of variance (ANOVA) to determine significant differences among means at $\alpha = 0.05$ level, using statistical tools of Microsoft Office Excel (2007) and R software (Version 2.14.1).

## RESULTS

**Yield of chitosan:** Table 1 displays the chitosan that was extracted from shrimp waste. Out of the shrimp waste, 38.51±0.08% of it was converted into chitosan.

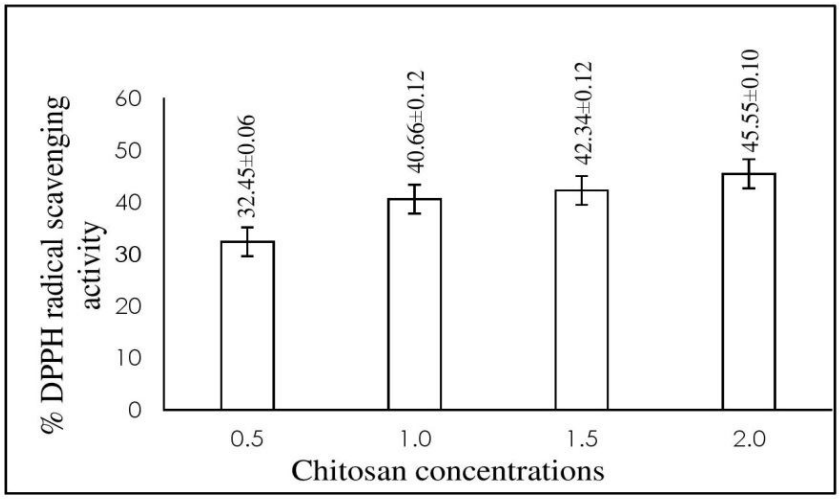
**Degree of deacetylation:** Degree of acetylation (DD) refers to the ratio of D-glucosamine to N-acetyl D-glucosamine. The quantity of D-glucosamine formed during the conversion of chitin into chitosan was measured. Therefore, chitosan is deacetylated to a greater extent, the higher the D-glucosamine level. According to Table 1, the extracted chitosan in this investigation has a degree of deacetylation (DD) of 71.13±0.44%.

**Proximate composition and pH of extracted chitosan:** In this study, moisture content, ash content and protein content of extracted chitosan were found 3.98±0.65%, 2.34±0.40% and 5.42±0.47%, respectively. pH of chitosan in the present study was 7.74±0.13. The results are shown in Table 1.

**1. Physicochemical characteristics of extracted chitosan**

| Parameters | Results |
|---|---|
| Yield (%) | 38.51±0.08 |
| Ash (%) | 2.34±0.40 |
| Protein (%) | 5.42±0.47 |
| Moisture (%) | 3.98±0.65 |
| Degree of deacetylation (%) | 71.13±0.44 |
| pH | 7.74±0.13 |
| Solubility (%) | 95.24±0.51 |
| Water binding capacity (%) | 648±9.97 |
| Fat binding capacity (%) | 386.68±11.55 |

Values are mean of three determinations (n=3) with S. D.




[Values are mean of five determinations (n=5) with S. D.; Values of mean do not vary significantly (p>0.05) among the treatments]

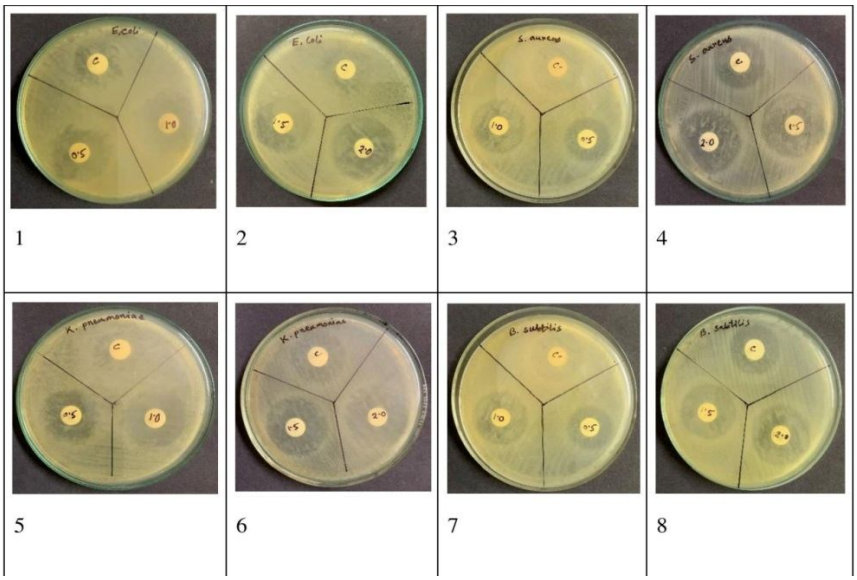
**Fig. 1. Antioxidant activity of the extracted chitosan**

**Determination of solubility of chitosan:** Chitosan quality is mostly determined by its solubility. Higher solubility is a sign of higher-grade chitosan. The synthesized chitosan demonstrated 95.24±0.51% solubility in a solution of 1% acidic acid.

**Water binding capacity (WBC) and Fat binding capacity (FBC) of chitosan:** Water binding capacity (WBC) of the extracted chitosan was 648±9.97%. The fat binding capacity (FBC) of shrimp chitosan was measured using vegetable oil. The result was 386.68±11.55%.

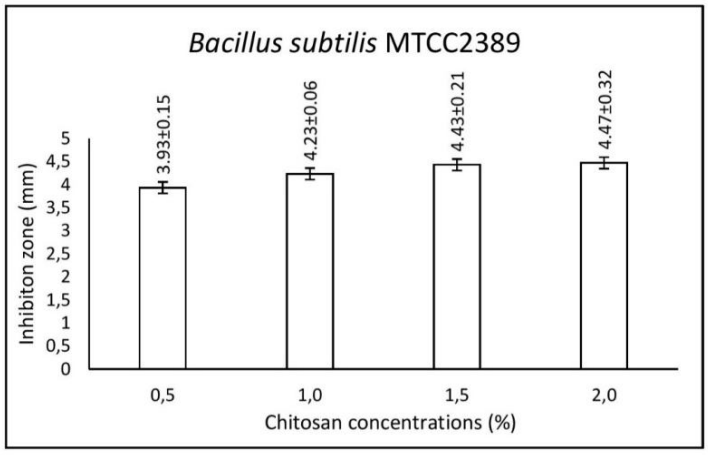
**Antioxidant properties of extracted chitosan:** The Antioxidant property of the extracted chitosan to reduce 1,1-diphenyl-2-picrylhydrazyl (DPPH) was found to be 32.45±0.06%, 40.66±0.12%, 42.34±0.12% and 45.55±0.10% at 0.5%, 1.0%, 1.5% and 2.0% concentrations, respectively (Fig. 1).

**Antimicrobial properties of extracted chitosan:** Antimicrobial activity of chitosan against bacteria (Gram positive: *Staphylococcus aureus* MTCC7443, *Bacillus subtilis* MTCC2389 and Gram negative: *Escherichia coli* MTCC443, *Klebsiella*




[The positive control is denoted as C for acetic acid and 0.5, 1.0, 1.5, 2.0% are chitosan concentrations, respectively]

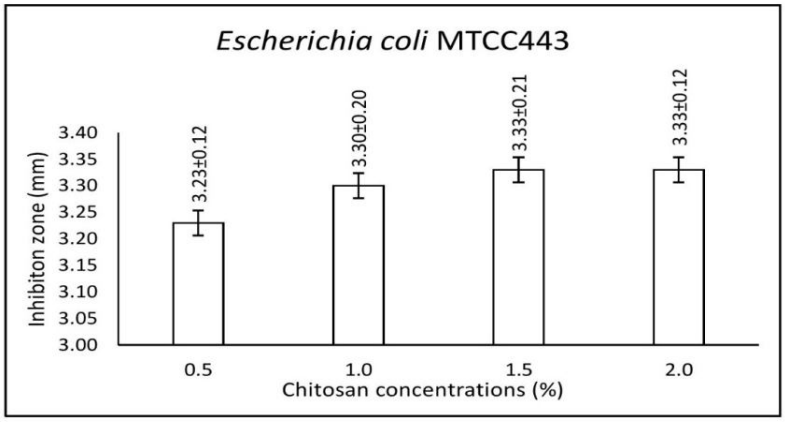
**Fig. 2. Plates showing inhibition zones (1-8) of chitosan against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae***




[Values are mean of five determinations (n=5) with S. D.; Values of mean do not vary significantly (p>0.05) among different concentrations]

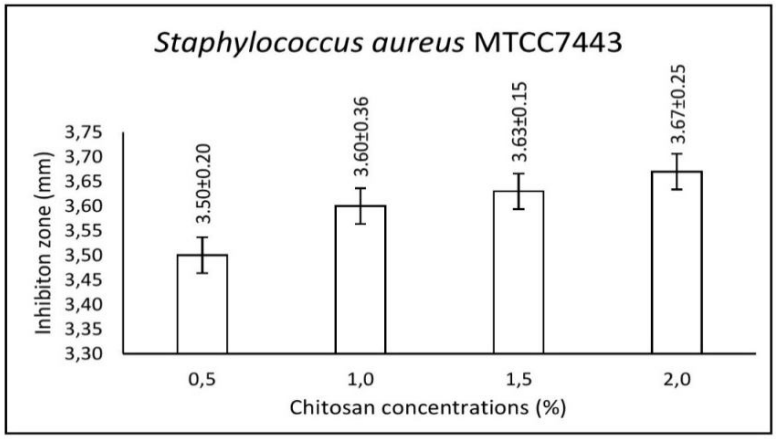
**Fig. 3. Antimicrobial activity of chitosan against *Bacillus subtilis* MTCC2389**

*pneumoniae* MTCC618) was assessed in the present study. The inhibition zones were 3.93±0.15 mm (0.5% chitosan), 4.23±0.06 mm (1% chitosan), 4.43±0.21 mm (1.5% chitosan) and 4.47±0.32 mm (2% chitosan) against *Bacillus subtilis* MTCC2389; 3.23±0.12 mm (0.5% chitosan), 3.30±0.20 mm (1% chitosan), 3.33±0.21 mm (1.5% chitosan) and 3.33±0.12 mm (2% chitosan) against *Escherichia coli* MTCC443; 3.50±0.20 mm (0.5% chitosan), 3.60±0.36mm (1% chitosan), 3.63±0.15 mm (1.5% chitosan) and 3.67±0.25 mm (2% chitosan) against *Staphylococcus aureus* MTCC7443; 3.33±0.21 mm (0.5% chitosan), 3.43±0.06 mm (1% chitosan), 3.60±0.17 mm (1.5% chitosan) and 3.73±0.21 mm (2% chitosan) against *Klebsiella pneumoniae* MTCC618 (Fig. 3,4,5,6).



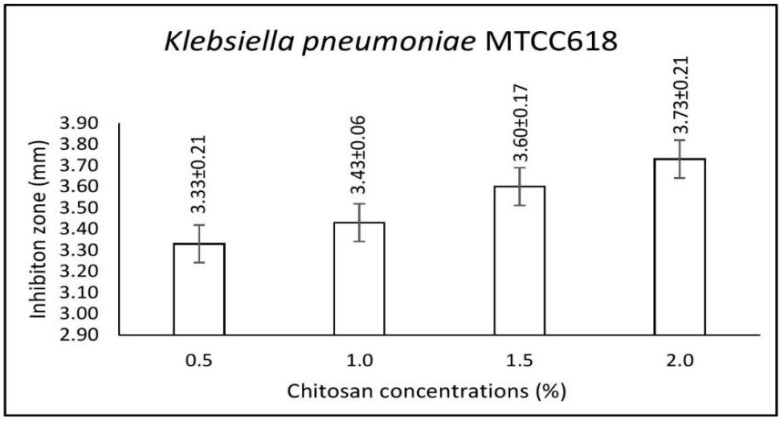

[Values are mean of five determinations (n=5) with S. D.; Values of mean do not vary significantly (p>0.05) among different concentrations]

**Fig. 4. Antimicrobial activity of chitosan against *Escherichia coli* MTCC443**




[Values are mean of five determinations (n=5) with S. D.; Values of mean do not vary significantly (p>0.05) among different concentrations]

**Fig. 5. Antimicrobial activity of chitosan against *Staphylococcus aureus* MTCC7443**




[Values are mean of five determinations (n=5) with S. D.; Values of mean do not vary significantly (p>0.05) among different concentrations]

**Fig. 6. Antimicrobial activity of chitosan against *Klebsiella pneumoniae* MTCC618**

## DISCUSSION

The major procedure for extraction of chitosan from *P. vannamei* shells includes the extraction based on the alkaline deacetylation of chitin with a strong alkaline solution via deproteinization, demineralization, and deacetylation of shrimp shells powder. The current work is an effort to look into the physicochemical characteristics of chitosan obtained from *P. vannamei* shells. Table 1 depicts the yield, moisture, ash, deacetylation level, solubility and water and fat binding capabilities of *P. vannamei* shell-extracted chitosan.

The increased yield of chitosan in this study was achieved by demineralization and deproteinization being repeated two times, which removed minerals and proteins. Chitosan yield might be influenced by the length of the process and the concentrations of acid and alkali during demineralization and deprotonation (Samar *et al*., 2012). The chitosan yield of *P.vannamei* shell*s*, being about 38.51±0.08%, justifies its use as an economic as well as environmentally friendly way of producing chitosan on an industrial scale due to the large-scale production of *P. vannamei*. Divya *et al.* (2014) reported chitosan yield was found to be 46% in shells of *Penaeus monodon*. Similar to the present study, a chitosan yield of 30-36.7% was reported from the crab shell (Yen *et al.*, 2009). It might be the reaction time which has a significant impact on the different yields.

The biochemical study confirmed the effectiveness of all the steps involved in making chitosan from shrimp *P. vannamei* shell wastes, including demineralization, deproteinization, and deacetylation. Since water was removed from the chitin before chitosan was produced, Lare *et al*. (2022) reported that the moisture content (6.79%) of chitosan was lowered. Due to the presence of the acetyl group in the chitin sample, the ash content (6.41%) of chitosan was higher than chitin. It is important to remember that ash is the inorganic residue left over from a sample after water and organic stuff have been eliminated. After the chitin was deproteinized, the chitosan sample was found to have a significant protein level (6.16%), which was likely caused by the chitin's insufficient deacetylation. Similarities in moisture (6.24 %) and ash levels (0.39%) of extracted chitosan were discovered by Renuka *et al*. (2019). According to Renuka *et al*. (2019) pH of commercial chitosan is around 8 and in extracted chitosan prepared from shrimp shell was 7.9 that was quite similar to the present study.

The chitin derivative chitosan is completely or partly N deacetylated. 71.13±0.44% of the present sample was found to be deacetylated. The greater DD value is basically increasing the solubility of chitosan. Chitosan's purity may be determined by how easily it dissolves in acetic acid. In contrast to chitin, chitosan has a significant amount of strongly protonated free amino groups that draw ionic substances. Its solubility in inorganic acid results from this. Renuka *et al*. (2019) investigated that extracted chitosan had 98.4% solubility in 1% acidic acid solution, which in the study was 95.24±0.51%.

One of the functional characteristics of chitosan is water and fat binding capacity. Chitosan's ability to bind fat and water was somewhat similar to the results of Renuka *et al*. (2019). Commercial chitosan's WBC ranges from 581 to 1150%, while its ability to absorb fat varies from 315 to 170% (Knorr, 1982; Rout, 2001). The differences in crystallinity, the presence of salt-forming groups, and the remaining protein in the chitosan all had an impact on WBC (Knorr, 1982).

Ibrahim *et al*. (2019) reported that the antioxidant properties (DPPH radical activity) of chitosan prepared by different techniques i.e., traditional, commercial, microwaved and autoclaved were 41.86% (0.5% chitosan) and 47.67% (1.0% chitosan), 42.72% (0.5% chitosan) and 58.53% (1.0% chitosan), 45.87% (0.5% chitosan) and 59.16% (1.0% chitosan), 26.82% (0.5% chitosan) and 33.92% (1.0% chitosan), respectively. DPPH radical scavenging activity for the chitosan extracted from vannamei used in the present study were

32.45±0.06% (0.5% chitosan), 40.66±0.12% (1% chitosan), 42.34±0.12% (1.5% chitosan) and 45.55±0.10% (2% chitosan), respectively (Fig. 1).

The antimicrobial activity of chitosan against bacteria is expressed as diameters (mm) of the growth inhibition zone (Fig. 2) and found similar trends to Cao *et al*. (2009). Antibacterial activity for the chitosan extracted from vannamei used in the present study was shown respectively by inhibition zones. 3.93±0.15 mm (0.5% chitosan), 4.23±0.06 mm (1% chitosan), 4.43±0.21 mm (1.5% chitosan) and 4.47±0.32 mm (2% chitosan) was found against *Bacillus subtilis* MTCC2389 (Fig. 3). 3.23±0.12 mm (0.5% chitosan), 3.30±0.20 mm (1% chitosan), 3.33±0.21 mm (1.5% chitosan) and 3.33±0.12 mm (2% chitosan) was found against *Escherichia coli* MTCC443 (Fig. 4). 3.50±0.20 mm (0.5% chitosan), 3.60±0.36mm (1% chitosan), 3.63±0.15 mm (1.5% chitosan) and 3.67±0.25 mm (2% chitosan) was found against *Staphylococcus aureus* MTCC7443 (Fig. 5). 3.33±0.21 mm (0.5% chitosan), 3.43±0.06 mm (1% chitosan), 3.60±0.17 mm (1.5% chitosan), and 3.73±0.21 mm (2% chitosan) was found against *Klebsiella pneumoniae* MTCC618 (Fig. 6).

Bioactive chitosan can be successfully produced using crustaceans (Vannamei shells and heads) from Indian fish markets and fish processing businesses, and its physical, chemical and biological properties were also evaluated. Chitosan was produced by deacetylating the isolated chitin. The chitosan that was made had qualities that were acceptable to the market. The end product had a high DD. Chitosan offers a vast array of industrial and commercial uses because of its special properties (antioxidant and antimicrobial activity). As suggested in the literature, more study can be done to increase the degree of deacetylation, most likely by chitin size reduction, longer reaction durations, and higher deacetylation temperatures. Shrimp shell wastes produced by companies that process shrimp contain valuable materials including chitin, chitosan and pigments. These materials are typically dumped into the environment as garbage, which causes serious environmental issues. Utilization of shrimp wastes can contribute to a cleaner environment by using these wastes to make chitin or chitosan.

**Conflicts of interests:** Authors have no conflict of interest in this study.

**Author's contribution:** AI: Involved in investigation, data generation, and preparing original draft; SC: Engaged in conceptualization, data curation, supervision and final editing; AB, PM, SN: Involved in final checking, minor correction and editing.

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